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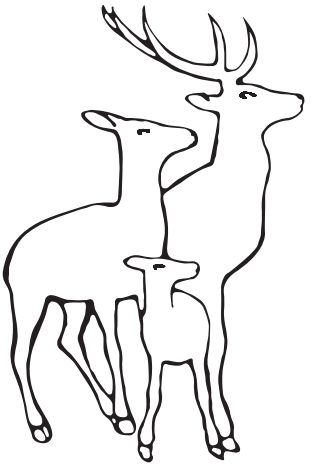

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Original Article

Pre- and postoperative levels of serum brain-derived neurotrophic factor in neonates with congenital heart defects

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ABSTRACT

Background. The objectives of this study were to assess the preoperative and postoperative serum brain- derived neurotrophic factor (BDNF) levels in neonates undergoing surgery for congenital heart defects (CHD). Also to explore the relationship between changes in BDNF levels and the impact of perioperative factors including intraoperative body temperature, aortic cross-clamp time, perfusion time, operation time, inotropic score, vasoactive inotropic score and lactate levels.

Methods. Forty-four patients with CHD and 36 healthy neonates were included in the study. Blood samples for serum BDNF levels were collected three times: preoperatively, and at 24 and 72 hours postoperatively from each patient in the operated group. Additionally, samples were collected once from each individual in the non-operated case group and the control group. Serum BDNF levels were analyzed using the Elabscience ELISA (Enzyme-Linked Immunosorbent Assay) commercial kit. Cranial ultrasonography (USG) was performed on all infants with CHD. Following cardiac surgery, patients underwent second and third cranial USG examinations at 24 and 72 hours postoperatively, respectively.

Results. Forty-four consecutive patients with CHD were divided into two groups as follows: the operated group (n=30) and the non-operated group (n=14). Although there were no differences in the baseline serum BDNF levels between the case and control groups, the preoperative serum BDNF levels were significantly lower in the patients operated compared to the non-operated patients. The serum BDNF levels at the 24th hour postoperatively were higher than the preoperative levels. However, no significant correlation was found between the serum BDNF levels at 24 and 72 hours postoperatively as well as the cranial USG findings at corresponding times.

Conclusions. Serum BDNF levels were initially lower in neonates with CHD who underwent surgery, but increased during the early postoperative period. These results suggest that serum BDNF levels are influenced by CHD and the postoperative period.

Key words: brain derived neurotrophic factor, cardiac surgery, congenital heart defects, neonates.

Congenital heart defects (CHD) are a prominent cause of birth defects in neonates, associated with significant morbidity and mortality. The ongoing advancements in prenatal fetal echocardiography and ultrasonography, along with postnatal pulse oximetry measurement for early detection of CHD, have been directly linked to an increased frequency in the diagnosis of CHD.

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The determination of perioperative damage to the central nervous system in infants with CHD who undergo cardiac surgery and cardiopulmonary bypass (CPB) remains an area of ongoing research. In these patients, the clinical and biochemical markers that can predict outcomes are not well established. Furthermore, there is growing evidence that perioperative management should be individualized based on the specific characteristics of CHD. This is especially evident in infants with cyanotic CHD, who exhibit differences in organ damage, inflammation, and neurobiomarkers following cardiac surgery or bypass.[1]

Ischemia-reperfusion associated tissue damage is a well-known factor following CPB. Despite advancements in CPB equipment and procedures, acute brain injuries related to cardiac surgery still occur. While the pathophysiology of brain damage is not fully understood, recent studies suggest that hypoxia and inflammation play significant roles.[2]

Cardiopulmonary bypass ensures more stable cerebral blood flow, cardiac output, and organ oxygenation. Although this perioperative management reduces neurological morbidity, brain injury remains a significant postoperative complication. Intraoperative interventions, such as CPB and circulatory arrest techniques, along with adverse events from surgical procedures (such as thromboembolism, strokes, and intracranial hemorrhage), and uncorrectable postoperative hypoxia are principal factors influencing neurodevelopmental outcomes in these patients.[3,4] The mechanisms underlying the development of brain injury during and after cardiac surgery primarily involve hypoxic-ischemic injury, the reperfusion phase, and a third phase characterized by gliosis, persistent inflammatory receptor activation, and epigenetic modifications.[5]

Numerous neuromarkers have been investigated to predict neurodevelopmental outcomes in patients undergoing cardiac surgery for CHD. The most commonly studied neuromarkers include S100 calcium-binding protein B (S100B), neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP).[6-12] These markers are frequently analyzed due to their roles in indicating neural damage or stress. GFAP has been proposed as a potential marker for assessing perioperative brain damage.[6]

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that influences the survival, growth, and functions of neurons in both the central and peripheral nervous systems. It plays a crucial role in stabilizing synapses and regulating axon and dendrite branching.[13] Several studies have demonstrated that serum BDNF levels are altered in neonates with conditions such as intracranial hemorrhage (ICH), retinopathy of prematurity (ROP), and hypoxic-ischemic encephalopathy (HIE), as well as in those receiving antenatal and postnatal steroids.[14-17]

Our hospital is a tertiary referral academic institution that provides early diagnosis and management of CHD from the fetal period through to 18 years of age. The objectives of this study were to assess the preoperative and postoperative serum BDNF levels in neonates undergoing surgery for CHD and to explore the relationship between changes in BDNF levels as well as the impact of perioperative factors including intraoperative body temperature, aortic cross-clamp time, perfusion time, operation time, inotropic score, vasoactive inotropic score and lactate levels.

Materials and Methods

In this prospective-controlled study, neonates hospitalized due to CHD in the neonatal intensive care unit (NICU) and the pediatric cardiovascular surgery intensive care (CICU) unit of Başkent University Faculty of Medicine between August 2021 and February 2023 were included. The study received approval from the Başkent University Institutional Review Board and Ethics Committee (Project no: KA21/320, dated August 13, 2021). Written informed consent was obtained from the parents of all participating neonates.

Forty-four patients with cyanotic CHD affecting pulmonary blood flow, as well as acyanotic CHD, including obstructive lesions, that would require interventional or surgical treatment were included in this study. All neonates with CHD who met the above criteria were included in this period. CHD patients were divided into two groups as follows: the operated group, consisting of 30 out of the 44 patients who required surgery, and the non-operated group, comprising 14 out of the 44 patients who did not require surgery during the neonatal period. For the control group, 36 healthy neonates who roomed-in with their mothers during hospitalization following delivery, underwent routine blood tests, including bilirubin and thyroid function tests, at the outpatient clinic. They had no history of illness or congenital heart defects and were included in the study.

The exclusion criteria for the study were as follows: premature neonates with a gestational age of less than 36 weeks; neonates diagnosed with HIE, chromosomal abnormalities, congenital metabolic disorders, or ICH before cardiac surgery; and infants born to mothers diagnosed with preeclampsia, gestational diabetes mellitus, or chorioamnionitis. Additionally, neonates born to mothers with major depressive disorder or mental health issues were excluded from the study due to the potential influence of antidepressant treatment on BDNF levels.

The sample size was determined to be a total of 52 neonates, with 26 undergoing surgery for CHD and 26 serving as healthy controls, based on the results of the power analysis. The test power was estimated to be approximately 80% with an alpha (α) level of 0.05.

The decision to operate on the patients was made by the joint council of pediatric cardiology and pediatric cardiovascular surgery. All cardiac surgeries were performed by the same pediatric cardiovascular surgeon. The preoperative and postoperative follow-ups were conducted by a consistent team of neonatal, pediatric cardiovascular surgery, and pediatric cardiology specialists in the NICU and pediatric CICU. The recorded data for the neonates included gestational age, gender, birth weight, mode of birth, inborn or outborn status, presence of fetal heart diseases, and APGAR scores. Infants categorized as small, appropriate or large for gestational age (SGA, AGA, and LGA , respectively) were defined as having birth weights below the 10th percentile, between the 10th and 90th percentile, and above the 90th percentile for gestational week, respectively, according to the Fenton preterm growth charts.[18] Additional details documented were the age on the day of surgery, length of stay in both the intensive care unit and hospital, cardiac surgery technique (such as arterial switch, aortic coarctation repair, truncus arteriosus repair, Norwood procedure, or shunt operation), CPB usage, aortic cross-clamp time, perfusion time, intraoperative body temperature, and total operation duration. Preoperative, postoperative 24-hour, and postoperative 72-hour lactate levels were measured from venous blood gas samples. Any lactate level ≥ 2.5 mmol/L was considered elevated, indicating potential tissue hypoxia. The inotropic score (IS) was calculated using the formula: 1 X dopamine dose (μg/kg/min) + 1 X dobutamine dose (μg/kg/min) + 100 X adrenaline dose (μg/kg/min). The vasoactive inotropic score (VIS) was calculated as: IS+ 10 X milrinone dose (μg/kg/min) + 10000 X vasopressin dose (U/kg/min) + 100 X norepinephrine dose (μg/kg/min). Prostaglandin E1 infusion was administered at doses ranging from 0.01 to 0.1 μg/kg/min as needed.

In terms of additional anomalies and the presence of ICH, cranial ultrasonography (USG) was performed by the same specialist from the department of radiology for all infants with CHD. Following cardiac surgery, patients underwent second and third cranial USG examinations at 24 and 72 hours postoperatively, respectively. Ultrasonographic findings were categorized into stages according to the Volpe intraventricular hemorrhage (IVH) classification[19]: Grade 1: germinal matrix hemorrhage with no IVH, or IVH occupying

less than 10% of the ventricular area on the parasagittal view; Grade 2: IVH occupying 10-50% of the ventricular area on the parasagittal view; Grade 3: IVH occupying more than 50% of the ventricular area on the parasagittal view, possibly with periventricular echodensities and periventricular venous hemorrhagic infarction (PVHI); this category may also include cystic periventricular leukomalacia.

Blood sampling and serum BDNF assay

Blood samples for serum BDNF levels were collected three times: preoperatively, and at 24 and 72 hours postoperatively from each patient in the operated group. Additionally, samples were collected once from each individual in the non-operated case group and the control group. Two mililiters of blood were drawn into sterile tubes with yellow caps while other blood samples were being checked. These samples were then centrifuged at 3500 rpm for 10 minutes to separate the serum. The serum samples were transferred into sterile Eppendorf tubes and stored at -80°C until analysis.

BDNF levels in the serum samples were analyzed in the Biochemistry Laboratory of Başkent University using the Elabscience ELISA (Enzyme-Linked Immunosorbent Assay) commercial kit (Catalog No: 201-12-1303, Human BDNF). Concentrations in the samples were automatically calculated from a graph generated by the device, utilizing the standards provided in the kit.

Statistical analysis

Data analysis was conducted using the IBM® SPSS 25.0 statistical software package. Descriptive statistics were presented as frequencies and percentages for categorical variables and as means ± standard deviations or medians with minimum-maximum (min-max) ranges for continuous variables. The adherence of continuous variables to normal distribution was assessed through both visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). For categorical variables, comparisons between independent groups were performed using the $\chi 2$ or Fisher's exact tests. The Cochran's Q test was applied for analyzing three or more dependent categorical groups. In the case of continuous variables, the Mann-Whitney U test was utilized for comparing two independent groups, while the Kruskal-Wallis or Friedman tests were employed for comparing three or more groups. Spearman's rank correlation test was used for correlation analysis between two continuous variables, at least one of which was non-parametrically distributed. A p-value of 0.05 was set as the threshold for statistical significance in all analyses.

Results

This prospective-controlled study involved 44 neonates with CHD and a control group of 36 healthy neonates. All but 5 neonates (11.4%) in the CHD group and 1 neonate (2.8%) in the control group were delivered at our hospital. Demographic and clinical characteristics are summarized in Table I.

The comparison of diagnostic and clinical data for operated and non-operated patients is presented in Table II. Out of the 30 neonates who underwent cardiac surgery, corrective surgery was performed on 24 (80%) patients, while palliative surgery was conducted on 6 (20%) patients (shunt operation in 4 patients and Norwood surgery in 2 patients). CPB was utilized in 27 (90%) patients. The median (min-max) aortic cross-clamp time was 87.5 (27-150) minutes, the perfusion time was 140 (106-228) minutes, the operation time was 210 (150-315) minutes, and the intraoperative body temperature was maintained at 25 (22-31) °C.

There was no significant difference in the serum BDNF levels between AGA, SGA and LGA groups ($p>0.05$). The baseline median (min-max) serum BDNF levels were 2.1 (0.8-8.6) ng/mL in the case group and 2.1 (0.6-6.6) ng/mL in the control group, showing no significant difference ($p = 0.92$). However, the preoperative

Table I. Demographic and clinical characteristics of the study and control groups.

	CHD (n= 44)	Control (n= 36)	P
Gestational week, median (min-max)	38.4 (36.0-39.5)	38.3 (36.0-40.3)	0.92
Birth weight, g, median (min-max)*	3175 (1800-4100)	3200 (2200-3800)	
AGA, n (%)	35 (81.4)	27 (75.0)	0.77
SGA, n (%)	4 (9.3)	7 (19.4)	
LGA, n (%)	4 (9.3)	2 (5.6)	0.38
Gender, n (%)			0.87
Male	24 (54.5)	19 (52.8)	
Female	20 (45.5)	17 (47.2)	
Mode of delivery, n (%)			0.01
Vaginal delivery	0 (0.0)	5 (13.9)	
Cesarean section	44 (100.0)	31 (86.1)	
APGAR scores, median (min-max)			
1st min	8 (6-9)	9 (7-9)	<0.001
5th min	9 (7-10)	10 (8-10)	<0.001
Postnatal age (day), median (min-max)	7.2 (1.4-25.6)	2.9 (1.5-7.2)	<0.001

*One patient's birth weight data could not be accessed (missing data) AGA: appropriate for gestational age, CHD: congenital heart defects, LGA: large for gestational age, SGA: small for gestational age.

Table II. Comparison of diagnostic and clinical data of operated and non-operated patients.

	CHD, n (%)		*P*
	Operated (n=30)	Non-operated (n=14)	
Diagnostic classification			
Transposition of the great arteries[&]	19 (63.3)	0 (0.0)	
Pulmonary atresia	3 (10.0)	2 (14.3)	
Coarctation of aorta	1 (3.3)	4 (28.6)	
Hypoplastic left heart syndrome	2 (6.7)	2 (14.3)	
Hypoplastic right heart syndrome	1 (3.3)	3 (21.4)	0.002
Tetralogy of Fallot	1 (3.3)	1 (7.1)	
Taussig–Bing anomaly	2 (6.7)	0 (0.0)	
Truncus arteriosus	1 (3.3)	0 (0.0)	
DORV	0 (0.0)	2 (14.3)	
Cardiac catheterization	9 (30.0)	3 (21.4)	0.72
Prostaglandin E1 infusion	22 (73.3)	2 (14.3)	<0.001
Antenatal diagnosis	27 (90.0)	12 (85.7)	0.64
Length of stay in NICU and pediatric CICU			
median (min-max) (day)	14 (5-36)	5 (2-49)	0.008
Total hospitalization period			
median (min-max) (day)	18 (10-48)	5 (2-49)	0.002

[&] Difference between groups in post-hoc analysis is statistically significant
CHD: congenital heart defects, CICU: cardiovascular surgery intensive care unit, DORV: double outlet right ventricle, NICU: neonatal intensive care unit.

median (min-max) serum BDNF levels were significantly lower in the operated group at 1.9 (0.8-7.8) ng/mL compared to 2.7 (1.4-8.6) ng/mL in the non-operated group (p = 0.04). In the operated group (n=30), the use of prostaglandin E1 was associated with higher preoperative serum BDNF levels (2.1 ng/mL and 1.2 ng/mL, p=0.01). However, no correlation was found between the use of prostaglandin E1 and baseline serum BDNF levels in the case group (n=44) (p=0.58).

The preoperative and postoperative data of the operated patients is presented in Table III. The post-hoc analysis revealed that the statistical difference was attributable to the increase between the preoperative serum BDNF levels and the levels at the 24th postoperative hour. Both IS and VIS scores peaked at the postoperative 24th hour, indicating the highest level of inotropic support during this time. No statistically significant correlation was found between the preoperative, postoperative 24th-hour, and 72nd hour serum BDNF levels and IS, VIS, or lactate levels in the operated patients. Additionally, there was no significant correlation between the serum BDNF levels at the postoperative 24th and 72nd hours and the intraoperative body temperature, aortic cross-clamp time, perfusion time, or operation time (p>0.05).

There was no difference median serum BDNF levels at the postoperative 24th and 72nd hours between in patients who underwent CPB (n=27) compared to those who did not (n=3) (p=0.052 and p=0.14, respectively). No significant correlation was found between lactate levels ≥2.5 mmol/L at the postoperative 24th hour and serum BDNF levels (p=0.77). However, at the postoperative 72nd hour, serum BDNF levels were significantly lower in patients with lactate levels ≥2.5 mmol/L (2.3 ng/mL vs 1.1 ng/mL, p=0.005).

The cranial USG results for the 44 patients were normal before surgery. After cardiac surgery, IVH was detected in 7 patients (23.3%) at the postoperative 24th hour and in 8 patients (26.7%) at the 72nd hour (p=0.002). The cranial USG findings, classified according to the Volpe grading system, identified periventricular hemorrhagic infarction in one patient, grade 3 IVH in one patient, grade 2 IVH in one patient, and grade 1 IVH in five patients. There was no significant correlation between the serum BDNF levels at the postoperative 24th and 72nd hours and the cranial USG findings at these corresponding times (p=0.17 and p=0.56, respectively), nor with the surgical technique used (p=0.34 and p=0.60, respectively).

Discussion

In this study, we investigated serum BDNF levels in neonates with CHD during both the preoperative and postoperative periods, as well as in healthy neonates. The serum BDNF levels measured at the 24th hour postoperatively were higher than the preoperative levels. Additionally, the preoperative serum BDNF levels in the operated neonates were lower compared to those in the non-operated group. We hypothesize that the decrease in serum BDNF levels observed in patients requiring surgery in the early neonatal period may be attributed to alterations in cerebral blood flow and oxygen delivery. The subsequent increase in BDNF levels during the postoperative reperfusion period lends support to this hypothesis. To the

Table III. The preoperative and postoperative data of the operated patients.

	Preoperative	Postoperative 24- hour	Postoperative 72-hour	p
Serum BDNF (ng/mL)	1.9 (0.8-7.8)	2.3 (0.9-15.6)	2.3 (1.0-9.3)	0.04
Inotropic score	0 (0-7)	20.5 (5-50)	5 (0-22)	<0.001
Vasoactive inotrope score	0 (0-7.5)	27.5 (10-57.5)	7.5 (0-27)	<0.001
Lactate (mmol/L)	2.8 (1.4-9.8)	2 (1-14.8)	1. 4 (0.7-7)	<0.001

BDNF: brain-derived neurotrophic factor.

best of our knowledge, this is the first study in the literature to evaluate serum BDNF levels in neonates undergoing surgery for CHD during the neonatal period.

Surgical trauma, ischemia and reperfusion injury are all potential trigger factors of inflammation following CPB.[2] The endothelial damage caused by oxygen free radicals during myocardial hypoxia, ischemia and reperfusion may influence the response to BDNF secretion. Inflammation and oxidative stress have been demonstrated to increase BDNF levels after adult CPB.[20] Our results could not be compared as there are not enough studies in the literature investigating the effect of congenital heart defects and surgery on BDNF levels in newborns.

Brain damage often does not manifest clinical signs in neonates following CHD surgery, making evaluation during this period challenging. Therefore, there is a need for postoperative neuromarkers that can provide insights into both acute and long-term developmental outcomes. The most frequently investigated neuromarkers are S100B protein, NSE and GFAP. To date, only two studies have been published on serum BDNF levels in children with CHD.[7,21] In the study of Sanchez-de-Toledo et al., inclusion criteria were age between 1 day and 17 years. Forty-eight children were included, of whom 15 (31.5 %) were under 2 months of age. [7] In this study, serum BDNF levels were measured at three different time points in patients undergoing pediatric cardiac surgery: preoperatively, immediately after CPB, and 16 hours postoperatively. While they noted a decrease in serum BDNF levels immediately after CPB and an increase at the 16th postoperative hour, there were no significant differences in levels at the three measured time points. The key distinctions between their study and ours include the age range of the patients and the specific timing for postoperative sample collection. In the study by Chiperi et al, the cyanotic and acyanotic CHD included in this study were different from ours. Inclusion criteria were age from the neonatal period until 5 years. Additionally, there was no control group in this study.[21] In contrast, our study assessed serum BDNF levels following cardiac surgery during the early neonatal period. In their study, the results showed lower postoperative BDNF values in both cyanotic and non-cyanotic groups.

In an animal study aimed at determining the effects of hypoxic-ischemic injury, BDNF levels measured in the brain and serum at the outset and 4 hours after the hypoxic event were found to be elevated compared to those in healthy controls.[22] However, it has been demonstrated that intermittent hypoxia can impair hippocampal neuronal excitability and decrease BDNF release in mice.[23] Furthermore, it was observed that administering BDNF to newborn rats after a hypoxic-ischemic injury could reduce brain tissue loss.[24]

BDNF levels are elevated in the cerebrospinal fluid of asphyxiated newborns.[17] Liu et al. found no significant differences in serum BDNF levels at the 24th hour, 72nd hour, and 7th day post-event in newborns with HIE. However, in the moderate-severe HIE group, serum BDNF levels were higher at the 24th hour and 7th day compared to the mild HIE group. Additionally, when comparing the control group with the HIE group, the HIE group exhibited significantly higher serum BDNF levels.[25] This underscores the importance of BDNF in neuroprotection, as decreased levels could render neonates more vulnerable to brain injury.

The half-life of BDNF in the brain and serum remains unknown. In numerous human studies, biomarkers were collected at intervals ranging from 16 hours to 7 days following cardiac surgery or hypoxic ischemic encephalopathy in neonates.[7,8,25] In our study, blood samples for serum BDNF levels were taken at three different times: preoperatively, and at 24 and 72 hours postoperatively.

In the study by Amoureux et al., involving adult patients undergoing CPB, blood samples for serum BDNF were collected before

CPB initiation, 15 minutes before the aortic clamp was released (ischemia sample), and 15 minutes after the aortic cross-clamp was opened (reperfusion sample). They observed that serum BDNF levels during the reperfusion period after CPB were significantly higher than the basal levels, indicating that inflammation and oxidative stress could elevate BDNF levels.[20] The key differences between this study and ours are the patient demographics (adults versus neonates) and the specific timings for serum BDNF measurement, which in their case were 15 minutes before and after the aortic cross-clamp was opened.

In our study, we observed no correlation between the use of prostaglandin E1 and the basal serum BDNF levels in the case group. However, within the operated group, the use of prostaglandin E1 was associated with higher preoperative serum BDNF levels. We speculate that the increase in serum BDNF levels could be attributed to changes in perfusion following the administration of prostaglandin E1 in these patients.

Elevated lactate levels in the early postoperative hours were found to be associated with increased mortality and morbidity.[26] In neonates and children who underwent cardiac surgery, it was discovered that those with newly developed neurological deficits (such as stroke, seizures, ICH, or brain atrophy) had higher lactate levels in the first 24 hours postoperatively. [11] In our study, no significant correlation was found between lactate levels ≥2.5 mmol/L at the postoperative 24th hour and serum BDNF levels. When evaluating levels at the 72nd postoperative hour, it was noted that serum BDNF levels were significantly lower in patients with lactate levels ≥2.5 mmol/L. An increase in lactate levels indicating tissue hypoxia and a decrease in BDNF values were found to be noteworthy.

Vergine et al. indicated that GFAP levels could predict long-term neurodevelopmental outcomes. They found positive correlations between GFAP levels and the duration of surgery and CPB, while noting a negative correlation with intraoperative body temperature. It has been suggested that temperature fluctuations during CPB represent a critical period for neurological damage development.[10] However, in our study, no significant correlation was observed between serum BDNF levels and intraoperative body temperature.

There is abundant evidence indicating preoperative neuroimaging abnormalities in neonates with CHD. [27,28] Mild unilateral ventriculomegaly, marked by increased cerebrospinal fluid (CSF) spaces due to reduced brain volume, stands out as the most prevalent structural brain abnormality. The expansion of these spaces is attributed to delayed brain growth. [28] In our study, neither ventriculomegaly nor ICH was detected in any patient during the preoperative cranial USG examination. Additionally, we did not find any significant correlation between serum BDNF levels at the 24th and 72nd postoperative hours and the cranial USG findings during the same time intervals.

When comparing term infants with and without CHD, research has demonstrated that 32% of infants with critical CHD exhibit white matter damage on preoperative MRI imaging.[27] This type of damage is believed to be associated with alterations in fetal brain blood flow and oxygen delivery.[29] However, we speculate that ischemic lesions and white matter damage may not have been detectable in our study due to the utilization of cranial USG as the imaging modality for both preoperative and postoperative assessments of the brain.

This study has several limitations. Firstly, serum BDNF levels during and immediately after the intraoperative period were not assessed, which hindered the determination of the effect of ischemia and reperfusion before and after aortic cross-clamping. Nevertheless, the rise in baseline serum BDNF levels at the postoperative 24th hour indicates a sustained effect of reperfusion. Secondly, while our study compared early cranial ultrasound findings

with serum BDNF levels, further investigations incorporating long-term neurological outcomes alongside serum BDNF levels could be considered for future studies.

In conclusion, serum BDNF levels were initially lower in neonates with CHD who underwent surgery, but increased during the early postoperative period. These results suggest that serum BDNF levels are influenced by CHD and the postoperative period. Given the significance of hypoxia and ischemia in the neurodevelopmental prognosis following cardiac surgery in neonates, utilizing serum BDNF values as a biomarker for early outcomes and prognosis warrants further investigation. Future studies should involve larger patient cohorts and compare serum BDNF levels with other biomarkers to enhance their clinical utility and reliability.

Ethical approval

The study was approved by Başkent University Institutional Review Board and Ethics Committee (Project no: KA21/320, August 13th, 2021). Written informed consent was obtained from the parents of patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: FK, TO, EI, EAN; data collection: FK, TO, CT, EI, OM, AM[2]; analysis and interpretation of results: KF, TO, OM, AM[6], CT, AID; draft manuscript preparation: FK, TO, AID, EAN. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Effects of palivizumab prophylaxis on respiratory syncytial virus (RSV) infections in Montenegro

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ABSTRACT

Background. Respiratory syncytial virus (RSV) is one of the most common pathogens causing severe lower respiratory tract disease in infancy and childhood. In newborns, young infants, and in infants with co-morbidities, the risk of severe infection is increased. Current protection against severe RSV infection is immunoprophylaxis with the monoclonal antibody palivizumab. The study aimed to assess the effects of palivizumab prophylaxis in the Republic of Montenegro in comparison to the pre-prophylaxis period.

Methods. The study was conducted in prospective/retrospective single center format in Montenegro in the Clinical Center of Podgorica, for the period 2009-2019.

Results. A total of 104 high-risk infants in the palivizumab prophylaxis program (2014-2019 RSV seasons) and 168 high-risk children without palivizumab prophylaxis (2009-2013 RSV seasons) were enrolled. A total of 51 children (49.0%) received prophylaxis for prematurity, 33 (31.7%) for bronchopulmonary dysplasia (BPD), 13 (12.5%) for hemodynamically significant heart disease/defect (HSCHD), and 7 (6.8%) for "miscellaneous" indications. In the control group most children had prematurity (101, 60.1%), followed by BPD (59, 35.1%), HSCHD (3, 1.8%), and "miscellaneous" (5, 3.4%) conditions. Readmission to the pediatric intensive care units (PICU) due to RSV infection was significantly lower in prophylaxis group (0.0 vs 16.1%, $p<0.001$). No lethal outcomes were observed in high-risk children with palivizumab prophylaxis compared to 2.4% in the control group.

Conclusions. The introduction of RSV immunoprophylaxis as well as other new protective treatment strategies for high-risk newborns led to significant improvements in infant and childcare in Montenegro. This is the first report on palivizumab prophylaxis in Montenegro, demonstrating the effectiveness and safety of palivizumab use in clinical settings.

Key words: palivizumab, respiratory syncytial virus (RSV), high-risk children, Montenegro.

Respiratory syncytial virus (RSV) is one of the most common pathogens causing severe lower respiratory tract disease in infancy and childhood.[1] In Europe, it is the most frequent cause of lower respiratory tract infections in infants up to 2 years of life. When it comes to hospitalization, most children hospitalized for bronchiolitis are infected by RSV.[2] On the other hand, RSV has proven to be a major cause of death in children in developing countries.[3]

While clinical presentation is usually very mild in adults and older children (rhinitis or coughing), in newborns, young infants, and in infants with specific comorbidities (e.g., prematurity, bronchopulmonary dysplasia/chronic lung disease [BPD/CLD], hemodynamically significant congenital heart disease [HSCHD], airway anomalies, cystic fibrosis, neurological

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impairments, immunocompromised) the risk of severe RSV infection is increased.[4] During the first two years of life, this high-risk group of children, which is specific in many ways, is characterized by a high sensitivity to respiratory syncytial virus infection. A difficult course and an adverse outcome of the disease can be often expected, which reduces the overall progress of the newborns.

Other infant risk factors of importance for severe RSV manifestations are age <6 months during RSV season, multiple births, male gender, siblings in kindergarten and school, passive smoking, close domestic conditions, malnutrition, lack of breastfeeding, and a family history of allergic diseases or asthma.[2]

Currently, there are no anti-RSV drugs available.[5] In September 2023, United States and European Union medical authorities approved a bivalent vaccine for active immunization of pregnant individuals at 32 through 36 weeks gestational age for the prevention of lower respiratory tract disease (LRTD) and severe LRTD caused by RSV in infants from birth through 6 months of age. This vaccine is also approved for active immunization in individuals 60 years of age and older.[6]

The most common immunoprophylaxis available is a monoclonal antibody palivizumab, which has proven to be safe and efficacious against RSV during the epidemic season.[7]

Palivizumab is a humanized monoclonal antibody that binds to the antigenic A site of the F protein on the surface of RSV, which is involved in viral attachment and the process of fusion between the virus and cell membranes, as well as between infected cell membranes, leading to syncytium formation. Palivizumab neutralizes RSV by blocking virus-to-cell and cell-to-cell fusion without any effect on virus attachment or budding.[8] Palivizumab was approved in the USA by the Food and Drug Administration (FDA) for RSV prophylaxis in high-risk children in 1998. In Europe palivizumab was approved by the European Medicines Agency (EMA) in 1999. In the early 2000s use of palivizumab was approved in over 45 countries worldwide.[9]

Although widely used, so far there is no common guideline on the use of palivizumab. Predominately mild course of RSV infections in the majority of patients and the relatively high cost of palivizumab prophylaxis are reasons for which most of the national guidelines weigh cost-effectiveness which results in variability of conclusions and recommendations.

According to Joint Committee of Vaccination and Immunization criteria, RSV prophylaxis in developed countries is required by 0.3%-1.1% of live births.[10]

In the Republic of Montenegro, current national recommendations focus on palivizumab use in following groups: extremely preterm infants (under the 28 weeks of gestational age – 28 wGA), very preterm infants (29-32 6d w GA) with 2 or more risk factors (neurological disease, sibling in day care, etc.), preterm infants suffering from CLD/BPD, as well as infants with HSCDH less than 12 months of age at the beginning of RSV season.[11]

The effectiveness (reduced risk of RSV-related hospitalizations) and safety of palivizumab administration has been confirmed in neonates with prematurity, BPD/CLD and HSCHD in three different prospective, randomized placebo-controlled trials.[12-14] However, it is not uncommon that real-life data differ from data collected in prospective controlled randomized trials. For this reason, prospective observational studies and registries from different countries are warranted to provide valuable information related to palivizumab use in routine clinical practice.

The first RSV prophylaxis season in Montenegro was in the season 2014-2015. So far, there have been no published studies on the use of palivizumab in Montenegro. The study aimed to collect prospective data on palivizumab use, demographic data, data on neonatal hospitalization events, data on the indications

for palivizumab administration, and the frequency of RSV-related readmissions in pediatric intensive care units (PICU) (from 2014 through 2019 RSV seasons). The secondary aim was to compare the results with retrospective data of high-risk children, in the period before prophylaxis was introduced in Montenegro (from 2009 through 2013 RSV seasons), which would fulfill current criteria for palivizumab prophylaxis.

Material and Methods

The study was conducted in a prospective/retrospective single-center format in the Republic of Montenegro, in Clinical Center of Podgorica, which is the only medical institution dealing with potential candidates for palivizumab immunoprophylaxis. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Clinical Center of Podgorica (protocol code 03/01/4740/1, date of approval 14.05.2021). Informed consent was obtained from all subjects prospectively involved in the study.

The inclusion criterion was any child hospitalized for RSV infection. Data and details on the clinical presentation and course of the disease were obtained from the child's medical chart.

The statistical tests employed were Student t-test, Mann-Whitney U test, repeated measures analysis of variance (ANOVA), for continuous variables, and Fisher's exact test and Pearson chi-square test for nominal variables. A p-value of 0.05 was considered as the limit of significance. All data were examined using SPSS v. 22.0.26

Results

A total of 104 high-risk children in palivizumab prophylaxis program from 2014 through 2019 RSV seasons and 168 high-risk children without palivizumab prophylaxis from 2009 through 2013 RSV seasons were enrolled.

Demographics

Demographic data are shown in Table I. The infants enrolled in both groups were predominantly male (51.9% in the prophylaxis group; 54.2% in the control group). On average, infants had completed 30.7±4.0 wGA in the prophylaxis group and 30.1±2.4 wGA in the control group. Infants in the control group had lower birth weight and were more often twins or triplets in comparison to palivizumab group. Children with BPD who received prophylaxis were more often extremely preterm (≤28 wGA), with lower gestational age and weight average in comparison to the control group with BPD.

When comparing data for children with HSCHD, the control group was on average almost 4 weeks younger and with significantly lower birth weight than the palivizumab receiving group.

A total of 51 children (49.0%) received prophylaxis for prematurity, 33 (31.7%) for BPD, 13 (12.5%) for HSCHD, and 7 (6.8%) for "miscellaneous" indications. At the same time in control group were mostly children with prematurity (60.1%) and BPD (35.1%) ($p<0.001$). The majority of high-risk infants who received prophylaxis in the "miscellaneous" diagnostic group were diagnosed with airway congenital anomalies (3, 2.9%) in prophylaxis group; 4 (2.4%) in the control group. Other conditions in the prophylaxis group were cystic fibrosis (2, 1.9%), immunodeficiency (1, 1.0%), and neuromuscular diseases (1, 1.0%). In addition, 1 infant with HSCHD was also diagnosed with Down syndrome.

The events during hospitalization

Events during the neonatal hospitalization are shown in Table II. Most of the events were less frequent in prophylaxis group (sepsis, intraventricular haemorrhage, periventricular leukomalacia) while the duration of neonatal stay in intensive care unit was similar (64.5±31.7 days in prophylaxis group vs 66.4±23.6 days in control group).

Table I. Demographics.

		Prophylaxis with palivizumab	Control	p value
Sex	male	54 (51.9%)	91 (54.2%)	0.719
	female	50 (48.1%)	77 (45.8%)	
Multiple births – twins		19 (18.3%)	46 (27.4%)	0.020
Multiple births – triplets		1 (1%)	8 (4.8%)	
Gestational age (weeks)		30.7 ± 4.0	30.1 ± 2.4	0.188
Birth weight (g)		1620.8 ± 791.9	1448.8 ± 383.2	0.040*
Prematurity		51 (49.0%)	101 (60.1%)	
Weeks of gestation				
≤28		11 (21.6%)	17 (16.8%)	0.477
29-32		36 (70.6%)	65 (64.4%)	0.442
33-37		4 (7.8%)	19 (18.8%)	0.078
Gestational age (weeks)		30.4 ± 2.0	30.9 ± 2.2	0.348
Birth weight (g)		1452.0 ± 359.6	1515.5 ± 312.9	0.263
BPD		33 (31.7%)	59 (35.1%)	
Weeks of gestation				
≤28		23 (69.7%)	25 (42.4%)	0.012*
29-32		10 (30.3%)	31 (52.5%)	0.040*
33-37		0 (0.0%)	3 (5.1%)	0.550
Gestational age (weeks)		27.8 ± 1.4	29.4 ± 2.1	0.009*
Birth weight (g)		1100.9 ± 207.1	1263.0 ± 359.0	0.007*
HSCHD		13 (12.5%)	4 (2.4%)	
Gestational age (weeks)		37.6±3.6	33.0±0.8	0.026*
Birth weight (g)		3027.7±770.3	1740.0±240.8	0.006*
Miscallaneous		7 (6.7%)	4 (2.4%)	
Gestational age (weeks)		36.0 ± 4.7	34.0 ± 1.8	0.446
Birth weight (g)		2705.7 ± 937.8	2275.0 ± 675.4	0.444

Data are presented as n (%) or mean ± standard deviation. BPD: bronchopulmonary dysplasia; HSCHD: Hemodynamically significant heart disease/def.
* p<0.05; statistically significant

Respiratory support

Respiratory support during neonatal hospitalization was required in more than half of the subjects in both groups (71.2% in the prophylaxis group; 59.5% in control group). Duration of respiratory support averaged 13.5±16.1 days, compared to 9.6±13.1 days in the control group. Continuous positive airway pressure therapy was also more frequent in subjects in the prophylaxis group (58.7%) compared to the control group (35.7%). Oxygen therapy was required in all the subjects in the control group and in 93.3% in prophylaxis group with a duration shorter in the prophylaxis group (41.0±33.4 days) compared to the control group (46.1±19.1 days).

Preterm infants in the control group received significantly less respiratory support (mechanical and non-invasive ventilation), had longer oxygen treatment, and developed more often intraventricular haemorrhage grade 1-2 in comparison to preterm infants that received palivizumab (Supplementary Table I).

Table II. Neonatal hospitalization events.

Event	Prophylaxis with palivizumab (n=104)	Control (n=168)	p value
Days of neonatal stay (mean±SD)	64.5±31.7	66.4±23.6	0.635
Respiratory support	71.2	59.5	0.052
Duration of respiratory support in days (mean±SD)	13.5±16.1	9.6±13.1	0.022*
Surfactant	50.0	44.6	0.389
Continuous positive airway pressure	58.7	35.7	<0.001*
Oxygen therapy	93.3	100	0.001*
Duration of oxygen therapy in days, median (range)	35.5 (0.0-210.0)	42.0 (21.0-144.0)	0.001*
Proven sepsis	26.9	30.4	0.545
Retinopathy of prematurity	66.3	88.1	<0.001*
Intraventricular haemorrhage	21.2	44.6	<0.001*
Intraventricular haemorrhage grade 1-2	12.5	28.0	
Intraventricular haemorrhage grade 3-4	7.7	14.3	
Hydrocephalus	1.0	2.4	
Periventricular leukomalacia (PVL)	24.3	54.2	<0.001*

Data given as percentages (%) unless indicated otherwise.
* p<0.05; statistically significant

Similar findings were in the group of infants with BPD: children in the control group received significantly less respiratory support (mechanical and non-invasive venilation), while we found no significant difference concerning oxygen treatment, development of sepsis, or neurological complications in comparison to preterm infants that received palivizumab (Supplementary Table II).

The length of stay

When analyzing the data for infants with HSCHD, children who received prophylaxis stayed shorter in the neonatal units (21 vs. 55 days), and needed fewer days of oxygen treatment (10 vs 39 days). There were no such differences in infants with miscellaneous conditions. In both groups, we found no significant differences concerning the development of sepsis, or neurological complications (Supplementary Table III).

A total of 460 injections were administered to 104 high-risk children from 2014 through 2019 RSV seasons. Almost two-thirds of enrolled children 69 (66.3%) received five injections. The average number of injections per child was 4.4 and a median and mode of 5 injections per child.

RSV infection-related events

Children who received prophylaxis had significantly higher body weight (2752 ± 469g) compared to controls (2480 ± 457g) at discharge from the hospital (t=4,720; p<0,001). Also, there was a significant gain in body weight in every high-risk category of children that received immunoprophylaxis depending on the number of doses received (F=297.911; p=0.001) (Table III).

Readmission to the PICU due to RSV infection was significantly lower in the prophylaxis group compared to the control group (0.0 vs 16.1%, p<0.001). No lethal outcomes were observed in high-risk children with palivizumab prophylaxis compared to 2.4% in the control group during the entire study period (immunoprophylaxis - 2014 through 2019 RSV seasons; control - from 2009 through 2013 RSV seasons).

Discussion

Data presented in this paper are prospectively collected results on palivizumab usage and outcomes in 104 children from 2014 through 2019 RSV seasons, and retrospectively collected data on 168 children which would fulfill current criteria for palivizumab prophylaxis in the period before prophylaxis was introduced in Montenegro – from 2009 through 2013 RSV seasons.

Although the sample size of children receiving palivizumab in our study is much smaller compared to other studies (25,003 Canada 2005–2017[4]; 12,729 Germany; 2009–2016[15]; 3200 Russia, 2010–2014[16]; 3780 Poland, 2008–2014[17]; 589 Bosnia and Herzegovina, 2008–2014[18]) we must emphasize that this is the first study on palivizumab use in Montenegro and that only high-risk children were recruited.

In this study, palivizumab was predominately administered for primary indications. The most common indication for palivizumab administration in our study was prematurity (49%), followed by BPD/CLD (31.7%) and HSCHD (12.5%). Similar data are given in other studies although in a study by Heljic et al., there were more children with HSCHD (34.1%) compared to BPD/CLD (13.9%).[18] In data originating from the Canadian CARESS study HSCHD (10.5%) was also more frequent indication for palivizumab compared to BPD/CLD (8.4%).[4] One of the possible reasons for the lower frequency of HSCHD in our study could be the fact that all the infants with HSCHD undergo surgical treatment in institutions out of Montenegro, making the follow-up almost impossible.

The frequency of "miscellaneous" indications (6.8%) was higher compared to Bosnia and Herzegovina (2.2%)[18] and Germany (5%)[15], but lower compared to Canada (17.8%).[4] Since most guidelines on palivizumab use focus on the same three conditions for which palivizumab is most frequently prescribed in Montenegro, the only way to explain the differences in rates for the use of palivizumab in "miscellaneous" indications would be the level of strictness authorities apply in "off label" prescribing, in other words to which degree authorities are willing to accept physicians' assessment in determining priorities.[19,20]

RSV hospitalization rate in our study was 0%, which is lower compared to other studies in which RSVH ranges from 0.7% in Germany[15], over 1.6% in Canada[4] to 8.8% in France.[21] Again, we must mention that rehospitalization data in our study is related to the readmission of high-risk infants with severe RSV infections to the PICU only, both in the group with palivizumab prophylaxis and in the control group without palivizumab administration.

When comparing data to retrospective control demographic characteristics were similar with subjects being predominately male and of similar gestational age, with just a bit lower birth weight in the control group. As for medical conditions/indications for palivizumab use, the most frequent indications in both groups were prematurity and BPD/CLD, followed by HSCHD (49%, 31.7%, and 12.5% in the prophylaxis group vs 60.1%, 35.1% and 1.8% in control group). The number of subjects recruited in the prophylaxis group was approximately 35% smaller compared to the control group, which might be attributed to better health care

Table III. Body weight variation of children according to the number of palivizumab doses received.

Body weight (g)	mean	sd	med	min	max	p-value
First dose	4590.2	2216.7	4220.0	1100.0	10100.0	
Second dose	5343.9	2074.3	5050.0	1700.0	11000.0	
Third dose	5645.8	2107.1	5500.0	1700.0	11000.0	<0.001*
Fourth dose	6213.5	2013.9	6000.0	2350.0	12000.0	
Fifth dose	6727.0	1923.3	6450.0	3600.0	12000.0	

* $p<0.05$; statistically significant

(especially related to the decrease in the number of premature neonates) which has developed over time. On the other hand, the number of available doses in the study period was limited, which could potentially affect the number of subjects recruited in the prophylaxis group.

A significant number of preterm infants exhibit respiratory distress and require substantial respiratory assistance immediately after birth or upon admission to the neonatal intensive care unit (NICU) due to insufficient inspiratory effort, weak intercostal muscles, and compromised diaphragmatic function. These infants face a considerable risk of developing BPD and unfavorable neurodevelopmental outcomes, which are directly influenced by the duration of invasive mechanical ventilation (IMV) and supplemental oxygen. The strong connection between reliance on a ventilator and neurological complications, such as severe intraventricular hemorrhage and periventricular leukomalacia, highlights the severity of their condition. Also, neurodevelopmental problems have been found to be more prevalent when positive pressure support is administered for a period exceeding 60 days, irrespective of whether it is delivered through invasive or non-invasive ventilation (NIV) mode.[22] Preterm infants in the control group received significantly less respiratory support (mechanical and non-invasive ventilation), had longer oxygen treatment, and developed more often intraventricular hemorrhage grade I-II in comparison to preterm infants that received palivizumab. Most of the events during neonatal hospitalization (sepsis, intraventricular hemorrhage) were less frequent in the prophylaxis group, while the duration of neonatal stay in the intensive care unit was similar.

Less frequent events can probably be attributed to better health care provided with more equipment available and improved protocols on neonatal care (e.g. more frequent use of continuous positive airway pressure).

The biggest difference between palivizumab prophylaxis group and the control group was detected in RSV infection-related events. While there were no re-hospitalizations due to RSV and no lethal outcomes in the prophylaxis group, 16.1% of subjects in the control group were readmitted to hospital due to RSV infection, and lethal outcomes occurred in 2.4% subjects.

A prospective, multicenter, longitudinal study performed in Türkiye between 2015 and 2017[23] investigated the frequency and severity of RSV infection in infants of 29 to 35 wGA during two RSV seasons. This study showed that late preterm infants with RSV-associated lower respiratory tract infections needed significantly more hospitalization, PICU admission, and respiratory support. The duration of hospitalization was longer for RSV-positive infants. While strengthening our results, this study also emphasized the need for palivizumab prophylaxis in infants of 29-35 wGA.

Also, the children that received palivizumab prophylaxis had shown significant weight gain that was proportional to the number of doses received and neurological outcome. This positive effect of palivizumab was also recognized in the study of Orgun et al.[24] where RSV prophylaxis had positive effects on weight percentiles in infants with HSCHD. No RSV reinfection or re-hospitalization led to better and faster pulmonary function recovery, which, in combination with adequate nutrition, healthcare, and family support is a guarantee of healthy infant growth and development.

Similar results were seen in a study performed by Tavsu et al.[25], where palivizumab prophylaxis in preterm infants led to a significant reduction of RSV-related hospitalization and lower respiratory tract infections in the first and second year after prophylaxis compared to infants that had not received palivizumab prophylaxis. The Palivizumab group had shown higher bodyweight that had not reached statistical significance, and there were no significant changes in neurodevelopment between the experimental and the control group. The authors themselves recognized that this was

probably caused by inclusion criteria (preterm infants with no significant comorbidities).

Apart from being effective, palivizumab administration has also proven to be safe in our study which is similar to the results of previously published studies.[13,16,22] The use of palivizumab has also proven to be safe even in patients who are treated with palivizumab in 2 subsequent RSV seasons.[21] In our study there were no adverse effects (AE) reported which might be the result of a small sample size. The other potential reason might be the voluntary reporting of AE and serious AE by the participating physicians, unlike active surveillance ran by trained research nurses in CARESS.[26]

Limitations

Although we have provided valuable information, since data on palivizumab use in Montenegro have not been published before, there are several limitations of this study. In Montenegro, there is no electronic database on palivizumab use. For this reason, the accuracy and completeness of the primary data collected by the attending physician might limit the quality of our primary dataset. On the other hand, detection of RSV in patients hospitalized for respiratory tract infection is not mandatory during palivizumab prophylaxis in Montenegro. This fact might have resulted in underreporting of RSV-related hospitalizations. Due to the limited amount of palivizumab available during the study period the sample size was small. The sample size was also affected by recruitment of high-risk children only, who were hospitalized in the PICU.

This study report on palivizumab administered to 104 children from 2014 through 2019 RSV seasons is, best to our knowledge, the first report on palivizumab use in high-risk children in Montenegro. With the mentioned limitations kept in mind, this study demonstrates the effectiveness and safety of palivizumab prophylaxis in clinical settings and increases the pool of valuable information related to palivizumab use in routine clinical practice.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.4592

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Clinical Center of Podgorica (protocol code 03/01/4740/1, date of approval 14.05.2021).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EL, LD, LS; data collection: EL, DN; analysis and interpretation of results: EL, JL, AD, LS; draft manuscript preparation: EL, JL, AD, LS. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The role of malnutrition on outcomes of multisystem inflammatory syndrome in children (MIS-C) due to COVID-19

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ABSTRACT

Background. Malnutrition increases the complications and mortality in critically-ill children. We performed a retrospective analysis to define the impact of malnutrition on the outcomes of multisystem inflammatory syndrome in children (MIS-C) due to COVID-19.

Methods. Patients with MIS-C were evaluated for demographic features, anthropometric parameters, clinical findings and outcomes. Patients with z scores of body mass index (> 5 years) and weight-for-age (< 5 years) < -2 were considered malnourished. Sarcopenia was defined by total psoas muscle area (tPMA), calculated on abdominal computed tomography (CT) at the level of L3 and L4 vertebrae. The z scores <- 2 for tPMA were considered sarcopenia. The results of patients with and without malnutrition were compared.

Results. Twenty-seven patients were included. Forty-four percent (n=12) of patients had malnutrition. Malnutrition was classified as mild to moderate (1/3), severe (1/3) and overweight (1/3). Eighty-two % of cases had acute malnutrition. Among MIS-C symptom criteria, rash was significantly higher in children with malnutrition ($p<0.05$). Laboratory investigations showed higher ferritin levels in patients with malnutrition ($p<0.05$). The median tPMA and sarcopenia were significantly higher in patients with malnutrition when compared to patients without malnutrition (42% vs 7%, $p<0.05$). The oral feeding time, complication rates, and length of hospital stay were similar in both groups ($p>0.05$).

Conclusion. Children with MIS-C already had mild to severe malnutrition at admission. Rash and higher ferritin levels were more common in patients with malnutrition. In addition to anthropometric parameters, sarcopenia calculated using tPMA can be used to predict malnutrition in critically-ill children.

Key words: malnutrition, COVID-19, multisystem inflammatory syndrome, sarcopenia.

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The coronavirus disease 2019 (COVID-19) pandemic posed a great risk for healthcare systems and caused major morbidity and mortality. It has been reported that patients with severe COVID-19 had increased risk of malnutrition.[1] The prevalence of malnutrition is as high as 42% in COVID-19 infected patients and increases up to 66% in patients transferred from the intensive care unit.[2] Furthermore, malnutrition has detrimental effects on the prognosis of COVID-19 infection.[3] Malnourished

patients had worse outcomes and significantly higher in hospital-mortality compared to well-nourished adult COVID-19 patients.[3,4] As a result, several guidelines recommended early nutritional screening and adopted nutritional treatment for COVID-19 patients.[1,2]

Multisystem inflammatory syndrome in children (MIS-C) is a rare condition that often occurs 2 to 6 weeks after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[5] The definition of MIS-C includes evidence of severe illness, requiring hospitalization with at least two systems involvement. Acute COVID-19 has been shown to negatively affect the nutritional status of children. Longitudinal studies revealed that COVID-19 infections may cause various types of malnutrition in children.[6] Although the negative effect of COVID-19 infection on nutritional status has been well defined in adults, the impact of malnutrition on the clinical course and outcomes of MIS-C is not clear in the pediatric population.

In addition to anthropometric measures and serum protein levels, sarcopenia is also considered a component of malnutrition. It is characterized by reduced skeletal muscle mass and function.[7] Several methods have been used to defined to evaluate pediatric sarcopenia including dual energy X-ray absorptiometry and total psoas mass area (tPMA) measured on abdominal computed tomography (CT) scan.[8] The concept of sarcopenia is usually not taken into consideration in children. However, it facilitates the identification of children at risk for frailty and may help in the implementation of targeted treatments.[8] The role of malnutrition and sarcopenia has not been previously evaluated in children with MIS-C. Therefore, a retrospective study was performed to evaluate the nutritional status of children and its effect on clinical features and outcome of MIS-C.

Methods

Patients diagnosed with MIS-C between March 2019 – September 2022 were included in the study. The demographic features, anthropometric measurements, serum albumin levels, clinical findings of MIS-C, treatment options and outcomes (time to oral feeding, length of hospital stay and mortality) were retrospectively obtained from the hospital records. Sarcopenia was evaluated by tPMA on abdominal CT scans. Patients with and without malnutrition were compared for the abovementioned parameters. The study was approved by the Local Ethical Committee (GO/2022-20-22).

MIS-C definition

According to the definition of Center for Disease Control and Prevention (CDC), patients with the below criteria were diagnosed with MIS-C.[9]

- an individual aged <21 years presenting with fever (≥38.0 °C for ≥24 hours, or report of subjective fever lasting ≥24 hours),
- laboratory evidence of inflammation [including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, D-dimer, ferritin, lactate dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin]
- evidence of clinically severe illness requiring hospitalization, with multisystem (≥2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological),
- No alternative plausible diagnoses; and
- Positive for current or recent SARS-CoV-2 infection by reverse transcriptase polymerase chain reaction, serology, or antigen test; or exposure to a suspected or confirmed COVID-19 case within the 4 weeks prior to the onset of symptoms.

Inclusion criteria

1. Patients who meet the MIS-C criteria mentioned above

2. Patients with no history of chronic illness and/or cancer
3. Patients with an abdominal CT scan on admission

Exclusion criteria

1. Patients who do not meet MIS-C criteria
2. Patients with previous chronic illnesses and/or cancer
3. Patients with no abdominal CT scan.

Anthropometric measurements

The body weight, height, and body mass index (BMI) measured on admission were noted from medical records. The z scores for BMI, weight-for-age (WFA), height-for-age (HFA) and height-for-weight (HFW) were calculated according to the validated percentiles for Turkish children.[10] The definition of malnutrition was based on z scores of BMI in children > 5 years and on height-for-weight in children < 5 years. According to WHO definitions, patients were classified as normal nourished (-2 > z score < 2), minimum – mild malnutrition (z score = - 2 / -3), severe malnutrition (z score > -3) and overweight (z scores ≥ 3).[11] All children with malnutrition were also classified as having acute (BMI and/or HFW) or chronic (HFA) malnutrition (z scores < -2).

Evaluation of sarcopenia

In this study, CT scans were used to evaluate sarcopenia in children. CT scans were obtained to have a differential diagnosis of acute abdomen and GI involvement during the clinical course of COVID-19 and none of the patients underwent CT scan to assess their nutritional status. Abdominal CT scans were performed using a GE LightSpeed 16-slice CT scanner (GE Healthcare, Milwaukee, WI) and examinations were evaluated by pediatric radiologists after retrieval from the hospitals' picture archiving and communication system (PACS) (GE Medical Systems, Milwaukee, WI). The left and right psoas muscle areas were measured at L3-4 and L4-5 levels on transverse view.[12] The area was measured in mm^2 with the region of interest (ROI) measurement tool from each side (Fig. 1). The sum of both sides was obtained to have total psoas muscle areas (tPMA). The z scores of tPMA were calculated by using Pediatric Total Psoas Muscle Area (tPMA) z-score calculator' (https://ahrc-apps.shinyapps.io/sarcopenia).[13] Sarcopenia was defined as tPMA z scores < -2.[12]

Statistics

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM, USA) was used. The descriptive values were calculated as means ± standard deviations and medians (with interquartile ranges: Q1-Q3) within a 95% confidence interval. Patients with and without malnutrition were compared with chi-square test and non-parametric tests according to normality distribution, which was Mann-Whitney U test for our two groups. The p values <0.05 were considered statistically significant.

Results

Twenty-seven patients met the inclusion criteria. The mean age of the patients was 9.4 ± 4.61 years (2-16 y) and male - female ratio was 15:12. According to the above definition, 44% (n=12) of patients had malnutrition. Table I shows the demographic features, median

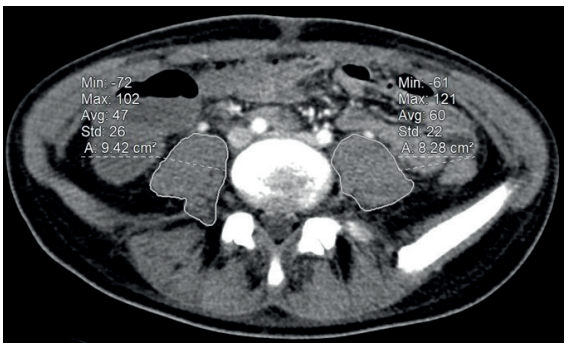

Fig. 1. Transverse contrast enhanced abdominal CT image shows bilateral psoas muscle area measurement at L4-5 level.

Table I. Demographic features, anthropometric measurements and serum protein levels in patients with and without malnutrition.

	Patients with malnutrition (n:12)	Patients without malnutrition (n:15)	P values
Age (year), median (Q1-Q3)	5.5 (4-10.25)	10 (6-13)	0.96
Gender (male: female)	6:6	9:6	0.70
Anthropometric measurements, median (Q1-Q3)			
Weight (kg)	22.5 (17,2-34.2)	45 (21-56)	0.02*
Height (cm)	122.5 (102.5-155.2)	150 (110-163)	0.27
BMI (kg/m^2)	16.5 (13.7-22.7)	19.6 (17.9-23.1)	0.14
BMI z score	-1.19 (-1.95-1.63)	0.85 (-0.13-1.72)	0.17
WFA z scores	0.25 (-1.22-0.68)	0.79 (0.22-1.33)	0.14
HFA z scores	0.99 (0.22-2.71)	0.93 (-0.47-1.21)	0.54
Acute chronic malnutrition	10:2 (83.3% : 16.7%)		
Classification of malnutrition, n (%)			
Mild- moderate	4 (33.3%)		
Severe	4 (33.3%)		
Overweight	4 (33.3%)		
Serum albumin level (mg/dl), median (Q1-Q3)	3.15 (2.59-3.39)	3.22 (2.83-3.85)	0.36

*p<0.05. BMI: body mass index, HFA: height-for-age, WFA: weight-for-age.

anthropometric measurements, and serum albumin levels in patients with and without malnutrition. Patients with and without malnutrition had similar median age and gender distributions. Patients with malnutrition were classified as mild to moderate (1/3), severe (1/3) and overweight (1/3). Eighty-two % of cases had acute malnutrition. Two patients had chronic malnutrition. When anthropometric measurements were compared, patients without malnutrition had a significantly higher median weight compared to patients with malnutrition ($p< 0.05$).

The MIS-C parameters and serum levels of inflammatory markers are listed in Table II. Among the MIS-C criteria, rash was significantly higher in patients with malnutrition ($p<0.05$). Median levels of ferritin were significantly higher in patients with malnutrition when compared to patients without malnutrition (1106.2 μg/l vs 276.1 μg/l, respectively $p < 0.05$). The comparison of inflammatory markers in both groups is summarized in Fig. 2. Ferritin levels were significantly higher in patients with malnutrition compared to patients without malnutrition ($p>0.05$). The oral feeding time, complication rates and length of hospital stay were similar in both groups. There was no mortality in both groups.

Table III shows the median levels of tPMA measurements obtained from L3-4 and L4-5 levels. The median levels of tPMA were significantly lower in patients with malnutrition (Fig. 3, $p<0.05$). Forty-two % of patients with malnutrition and 7% of patients without malnutrition had sarcopenia (z scores of tPMA <-2). However, median z scores of tPMA were not significantly different between groups (Fig. 3, $p>0.05$). When patients with and without sarcopenia were compared, there was no difference in outcomes of MIS-C in terms of time to oral feedings, length of hospitalization and mortality ($p>0.05$).

Discussion

In this study, we found that nearly half of the children with MIS-C had mild to severe malnutrition on admission and sarcopenia was observed in a significant number of these

Table II. Comparison of MIS-C findings, outcomes and median levels of serum inflammatory markers.

Parameters	Patients with malnutrition (n:12)	Patients without malnutrition (n:15)	p values
MIS-C criteria, n (%)			
- Fever	12 (100%)	15 (100%)	-
- GI symptoms	12 (100%)	15 (100%)	-
- Rash	12 (100%)	7 (47%)	0.03*
- Respiratory symptoms	1 (8%)	5 (33%)	0.18
- Neurologic findings	0	3 (20%)	0.23
- Cardiac involvement	4 (33%)	6 (40%)	0.51
- Renal involvement	1 (8.3%)	2 (13.3%)	0.58
Inflammatory markers, median (Q1-Q3)			
CRP (mg/dL)	18.2 (10.9-23.6)	13.6 (12.5-24.3)	0.77
Procalcitonin (ng/mL)	18.4 (2.5-28.9)	4.48 (0.69-13.8)	0.07
Lymphocyte count (x10^3/μL)	850 (600-1475)	700 (300-1300)	0.19
Thrombocyte count (x10^3/μL)	142 (118-183)	182 (106-290)	0.51
D-dimer (mg/L)	3.91 (1.94-7.17)	4 (2.03-7.05)	0.93
Ferritin (μg/l)	453.1 (292.8-1447.1)	215.3 (170.7-864)	0.04*
INR	1.17 (1.03-1.33)	1.25 (1.11-1.43)	0.19
Outcomes, median (Q1-Q3)			
Time to oral feeding (days)	2 (2-2.75)	2 (1-3)	0.52
Length of hospital stay (days)	7 (6-8)	9.4 (6-11)	0.42
Mortality (n)	0	0	-

*p<0.05. CRP: C-reactive protein, GI: gastrointestinal, INR: international normalized ratio.

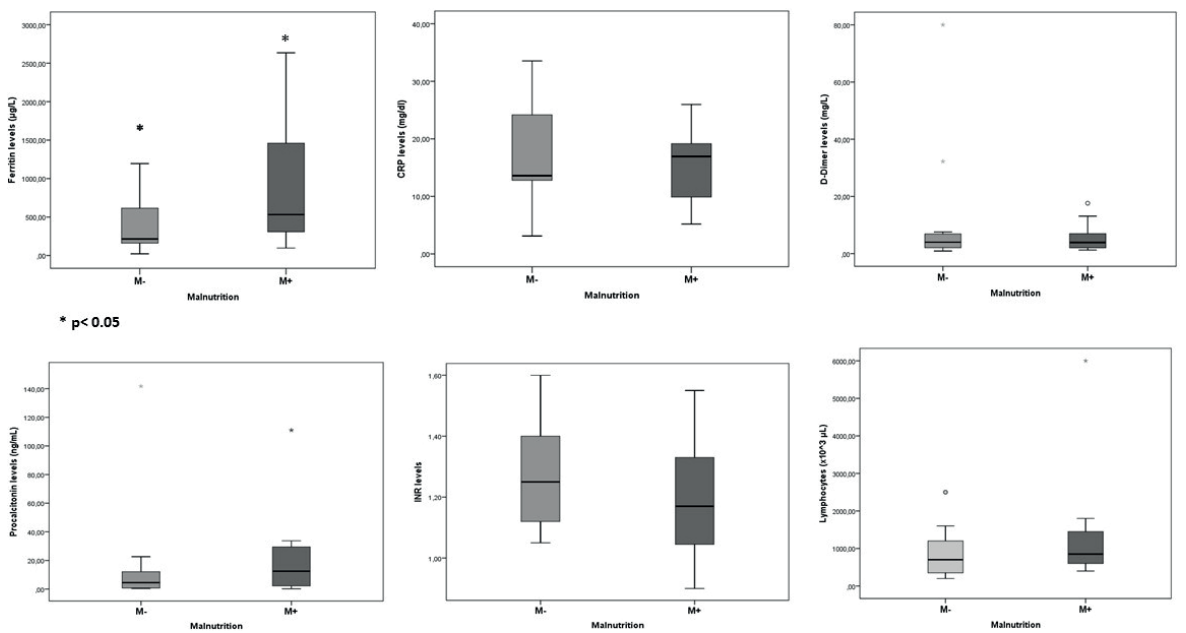

Fig. 2. Comparison of inflammatory markers in patients with and without malnutrition.

Table III. Sarcopenia, the right, left and total PMA in patients with and without malnutrition.

	Patients with malnutrition (n:12)	Patients without malnutrition (n:15)	P values
Psoas muscle area (mm^3), median (Q1-Q3)			
Right L3-4	3.66 (3.02-5.16)	6.08 (4.08-7.65)	0.01*
Left L3-4	3.73 (2.92-5.33)	6.29 (4.27-7.95)	0.03*
tPMA L3-4	7.40 (6.11-10.49)	12.32 (8.1-15.6)	0.02*
Right L4-5	4.46 (3.76-5.94)	7.44 (5.11-10.1)	0.02*
Left L4-5	4.47 (3.66-6.37)	7.75 (5.14-10.1)	0.03*
tPMA L4-5	8.93 (7.17-12.2)	15.1 (10.2-19.1)	0.02*
Z scores for sarcopenia, median (Q1-Q3)			
Z score for sarcopenia L3-4	- 1.24 (-1.89- -0.32)	-0.62 (-1.2 – 0.16)	0.22
Z score for sarcopenia L4-5	-1.21 (-1.83- -0.33)	-0.77 (-1.67- 0.7)	0.35
Presence of sarcopenia, n (%)	5 (42%)	1 (7%)	0.04*

*p<0.05. tPMA: total psoas muscle area.

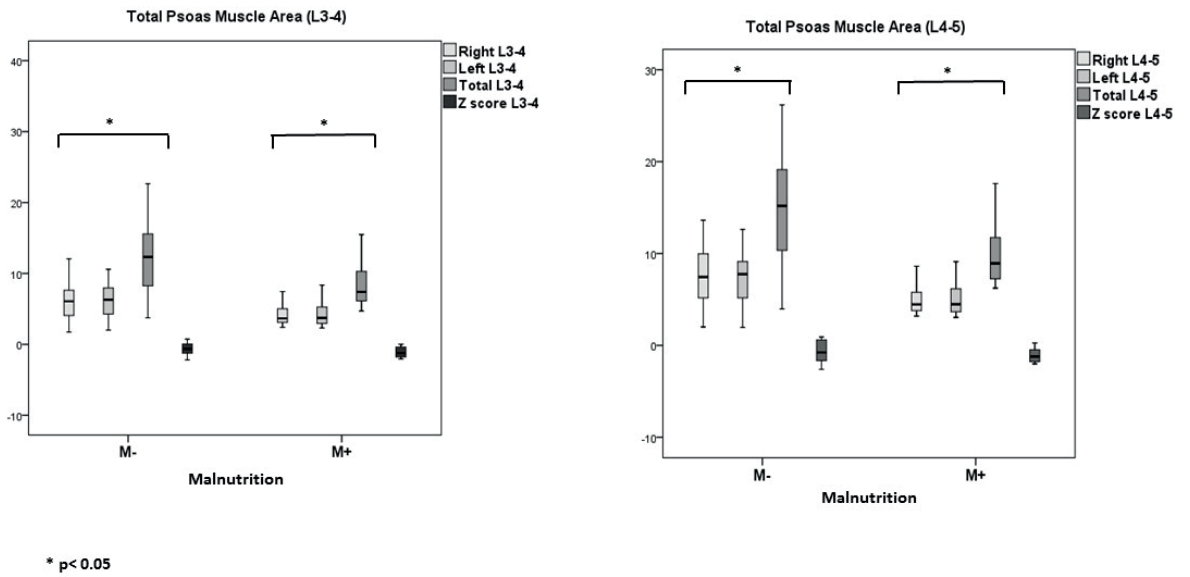

Fig. 3. The measurement of right, left and total psoas muscle area and z scores at the level of L3-4 and L4-5 vertebrae. Comparison of patients with and without malnutrition.

patients. During the COVID-19 pandemic, some of the children with SARS-CoV-2 infection developed MIS-C and faced severe morbidity, whereas most of the others neither had severe disease nor developed multisystem failure. To date, the underlying mechanisms and risk factors for developing MIS-C are not known. Malnutrition remains one of the risk factors for worse outcomes in critically-ill children.[13] Moreover, critically-ill patients with COVID-19 are at high risk of malnutrition due to the illness itself and significant metabolic alterations.[14] Although several guidelines have underlined the importance of nutritional management for COVID-19 patients in the adult population, the nutritional status and its impact on outcomes of MIS-C have not been elucidated previously. In this study, we found that 44% of children with MIS-C had mild to severe malnutrition on admission. Eighty-three percent of patients had acute malnutrition whereas only two children had chronic malnutrition. This suggests

that acute malnutrition might be a result of COVID-19, since the patients were healthy children and had no chronic diseases. The outcome of overweight patients was also similar to that of the undernourished patients. Thus, the findings of our study are not sufficient to claim that malnutrition is a risk factor for developing MIS-C, and patients with malnutrition had worse outcomes. However, screening of nutrition and adapting a nutritional supportive care should be provided for all MIS-C patients to overcome the catabolic process.

Children with MIS-C present with a wide range of clinical features and more than two systems should be involved in the diagnosis. When we compared the clinical findings with the nutritional status of children, we found that rash was seen in almost all patients with malnutrition and was significantly higher than in patients without malnutrition. Rekhtman et al.[15] reported that children with rash had less severe disease, fewer intensive care unit admissions, less shock and better outcomes when compared to children without rash. In that cohort, children with rash had less cardiac involvement. According to these contradictory results, it is not possible to conclude that the presence of rash is associated with better outcomes. Although the relation between higher incidence of rash in patients with malnutrition and outcome is not clear, the high number and heavy distribution all over the body and late improvement of rashes during the treatment period were some of our important observations in the present series. Furthermore, malnutrition may impair the immune response trigger the underlying immune mechanism of mucocutaneus findings, which may support our clinical findings.

MIS-C is characterized by a hyperinflammatory response with high levels of several inflammatory markers. Compared to COVID-19 patients, MIS-C patients had lower LDH and platelet levels and higher ESR.[16] Moreover, severe MIS-C patients had higher serum levels of white blood cell counts, absolute neutrophil count, CRP, D-dimer and ferritin levels.[16] In the present study, we observed that ferritin levels were significantly higher in patients with malnutrition when compared to patients without malnutrition. Since other inflammatory markers showed no difference with and without malnutrition in MIS-C, higher ferritin levels can be considered a sign of inflammation and malnutrition instead of a higher risk of severe MIS-C.

In addition to anthropometric features and serum protein profiles, sarcopenia is an important feature of malnutrition. The term 'sarcopenia' defines both reduced skeletal muscle mass and function.[8] However, the concept of sarcopenia is underestimated and is associated with poor outcomes in the pediatric population. Although we could not show any significant correlation between sarcopenia and the outcome of MIS-C, we suggest that sarcopenia diagnosed on tPMA can be used to predict malnutrition in critically-ill children, if an abdominal CT scan is obtained for any other reason.

Our study has some limitations. Firstly, a small number of patients were included in the analysis. A larger cohort of patients is needed to show the role of malnutrition on clinical features and outcomes of MIS-C. Therefore, it may not be possible to conclude that malnutrition is associated with a worse outcome. Secondly, to demonstrate an association between malnutrition and clinical and laboratory findings of MIS-C, additional parameters including immune and hematologic functions may be required. Also, the physical activity of the children during pandemic should be taken into consideration while evaluating sarcopenia in MIS-C patients. Despite these limitations, to the best of our knowledge this is the first study investigating the role of malnutrition including sarcopenia in MIS-C patients.

In conclusion, children with MIS-C due to COVID-19 had mild to severe malnutrition on admission. Rash and higher ferritin levels are more common in patients with malnutrition. The tPMA measurement can be used to predict sarcopenia in critically-ill children.

Ethical approval

The study was approved by Local Ethical Committee of Hacettepe University (GO/2022-20-22).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TS, KA, YÖ, KY, OA; data collection: KA, GA, YÖ, HNÖ; analysis and interpretation of the results: TS, GÖ, HNÖ, KY, OA; draft manuscript preparation: TS, KA, YÖ, HNÖ, KY, OA. All authors reviewed and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Health conditions of first-degree relatives of children with familial Mediterranean fever

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ABSTRACT

Background. Given the strong genetic background of familial Mediterranean fever (FMF), the frequently reported co-existing diseases in children with FMF should also be investigated in other family members. Therefore, we aimed to examine the medical conditions of first-degree relatives (FDRs) of our pediatric patients with FMF in the present study.

Methods. Chronic diseases of FDRs of pediatric 449 FMF, 147 juvenile idiopathic arthritis (JIA) patients and 93 healthy controls (HC) were questioned during their routine clinical visits for 9 consecutive months.

Results. A total of 1975 FDRs of 449 FMF, 690 FDRs of 147 JIA patients, and 406 FDRs of 93 HC were included into the study. The most common medical conditions were non-atopic asthma (n=71, 3.6%), type 2 DM (n=14, 2%), and tonsillectomy history (n=12, 2.95%) in the FMF, JIA, and HC groups, respectively. Atopic diseases (FMF vs. JIA: p=0.013; FMF vs. HC: p=0.014), rheumatic diseases (FMF vs. JIA: p=0.030; FMF vs. HC: p=0.017), and surgical histories (FMF vs. JIA: p<0.01; FMF vs. HC: p=0.026), including adenoidectomy, tonsillectomy, and appendectomy, were significantly more common in the FMF group than in other groups.

Conclusions. Our novel findings may contribute to understanding the hereditary burden of co-existing diseases in children with FMF and encourage further studies involving genetic screenings.

Key words: familial Mediterranean fever, arthritis, juvenile, parents.

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease, characterized by attacks of recurrent fever, abdominal pain, chest pain, arthritis, arthralgia, myalgia and/or rashes.[1] First attacks of overall 90% of patients are seen before 20 years of age.[2] Therefore, the diagnostic process of FMF mainly concerns general pediatricians and pediatric rheumatologists. Although this autosomal recessive inherited disease was firstly described in patients with certain ethnicities living around the Mediterranean region, patients from all over the world have also been reported since.[3]

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Considering the chronic pro-inflammatory process and immune-disturbed conditions caused by FMF, co-existing diseases have been widely discussed. It was recently shown that the most common comorbidities were juvenile idiopathic arthritis (JIA) and immunoglobulin A vasculitis (IgAV).[1,4] Furthermore, it was demonstrated in the studies involving all age groups that the frequencies of spondyloarthropathies, Behçet disease, Sjögren disease, polyarteritis nodosa (PAN), inflammatory bowel diseases, multiple sclerosis (MS), and psoriasis were higher in patients with FMF.[5,6]

It is well-documented that mutations in a gene called *MEFV*, which is composed of 10 exons and encodes pyrin protein are responsible

for the disease.[3] Although over 310 sequence variants in this gene have been reported and listed in an online registry named *Infevers* so far, M694V, M680I, V726A, M694I and E148Q mutations have been found to be responsible for more than 85% of the cases.[5,6] In addition, the genotype-phenotype correlation is well-established. For instance, M694V mutations were found to be significant indicators of a more severe disease course, earlier disease onset, colchicine resistance and amyloidosis.[7,8]

Although there are several studies evaluating the diseases of relatives of patients with JIA and systemic lupus erythematosus (SLE), there is no such data regarding FMF.[9-11] Given the strong genetic background of FMF, the diseases that have been previously demonstrated as co-existing in children with FMF should also be investigated in other family members. Therefore, we aimed to examine the diseases of first-degree relatives (FDRs) of our pediatric patients with FMF in the present study.

Methods

Study population

For nine consecutive months, medical conditions, and surgical histories of mothers, fathers, and siblings of healthy children, children with FMF, and children with JIA were evaluated during their regular outpatient visits. There was no disease list that may limit and divert the answers of the families. After all the participants were informed about the study in detail and informed consent was taken, they were questioned as to whether they had any chronic disease and operation history at their face-to-face appointments. The data were collected by the relevant physicians and confirmed via checking national health registries.

The FDRs of children with JIA and FMF who are being followed up by our department for at least six months were included in the study. The control group was established by the FDRs of healthy children admitted to the pediatric department for routine child-health monitoring and vaccination procedures. Among the FDRs of index cases, those with FMF were excluded from the study (n=422).

The diagnosis of FMF was established by Yalçınkaya et al. criteria[2] and the diagnosis of JIA was done according to the International League of Associations for Rheumatology Classification of Juvenile Idiopathic Arthritis.[12]

Selected disease groups

Autoimmune diseases, atopic diseases, rheumatic diseases, malignancies, and surgical histories were thought to be closely linked to FMF or its treatment for various reasons, which will be discussed in detail further. Thus, classification was performed for further analysis.

Those with at least one of the following were regarded to have the rheumatic disease: acute rheumatic fever (ARF), ankylosing spondylitis (AS), Behçet's disease (BD), gout disease, IgAV, JIA, periodic fever - aphthous stomatitis - pharyngitis - cervical adenitis (PFAPA) syndrome, rheumatoid arthritis (RA), sarcoidosis, scleroderma, Sjogren disease (SD), SLE, and uveitis.

Those with any autoimmune disease rather than the rheumatic ones, such as adrenal insufficiency, autoimmune haemolytic anemia, autoimmune hepatitis, celiac disease (CD), Graves' disease (GD), Hashimoto thyroiditis, MS, psoriasis, type 1 diabetes mellitus (DM), and vitiligo was considered to have autoimmune diseases.

Allergic urticaria, allergic rhinitis, atopic dermatitis, atopic asthma, drug allergy, and food allergy were classified as atopic diseases. While patients with acute myeloid leukemia (AML), brain tumor, breast cancer, Hodgkin lymphoma (HL), or neuroblastoma were regarded to have malignancy, those with a history of adenoidectomy, appendectomy, or

tonsillectomy were considered to have surgical history.

Ethical approval

This study was approved by the Institutional Review Board of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical School (04/04/2018-127814). All the patients or their parents gave us written informed consents, and we adhered to the guidelines of the Declaration of Helsinki throughout the research. The authors declare no conflict of interest.

Statistical analysis

All of the statistical analyses were performed by using IBM SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). Categorical variables were reported as numbers and percentages. Chi square or Fisher's exact test were used to compare the frequencies of the diseases between groups. The statistical significance was defined as p value<0.05. We employed Prism 8 software (GraphPad Software, San Diego, California) to illustrate data in graphs.

Results

Demographic findings

First-degree relatives of 449 patients with FMF (female: 255), 147 patients with JIA (female: 95), and 93 healthy children (female: 55) were included in the study. The mean age of healthy children, patients with FMF and JIA were 7.7 ± 4.6 years, 12.6 ± 4.8 years, and 11.6 ± 5.2 years, respectively. A total of 3071 participants (1975 first degree relatives of FMF patients, 690 of JIA patients and 406 of healthy children) were included in the study.

The most common medical conditions

While the most common conditions reported among the FDRs of the patients with FMF were non-atopic asthma (n=71, 3.6%), tonsillectomy history (n=66, 3.3%) and type 2 DM (n=55, 2.8%), the most common conditions detected among the relatives of patients with JIA were type 2 DM (n=14, 2%), Hashimoto thyroiditis (n=13, 1.8%), non-atopic asthma (n=13, 1.8%), and tonsillectomy history (n=13, 1.8%). Among relatives of the healthy children, tonsillectomy history (n=12, 2.95%), non-atopic asthma (n=10, 2.5%) and Hashimoto thyroiditis (n=6, 1.47%) were the most common ones (Table I).

Selected disease groups

The most common medical condition in the study group was surgical history (n=194, 6.3%). The others were as follows: atopic diseases (n=132, 4.3%), autoimmune diseases (n=121, 3.9%), rheumatic diseases (n=101, 3.3%), and malignancies (n=6, 0.2%). At least one medical condition was detected in 23.73% of the participants (n=729). Surgery history was the most common medical condition in all groups (FMF group: 149, 7.5%; JIA group: 27, 3.9%; HC group: 18, 4.4%). While autoimmune diseases were the most common disease group in both JIA (n=25, 3.6%) and HC groups (n=13, 3.2%), it was atopic diseases in FMF group (n=103, 5.2%).

Comparisons between groups

Comparing the frequencies of the medical conditions among the patients' FDRs revealed that ARF was significantly more common in the FMF group than in the JIA group (p=0.01). Besides, allergic rhinitis (p=0.008) and appendectomy history (p<0.001) were significantly more common in the FMF group than both in the JIA and HC groups (Table I).

While there was no significant difference between the JIA and HC group in terms of disease group frequencies, surgery history (FMF vs. JIA: p<0.01; FMF vs. HC: p=0.026), atopic diseases (FMF vs. JIA: p=0.013; FMF vs. HC: p=0.014), and rheumatic diseases (FMF vs. JIA: p=0.030; FMF vs. HC: p=0.017) were significantly more common in the FMF group than in JIA and HC groups (Table II).

Table I. The comparison of the disease frequencies reported among the first-degree relatives of the participants between groups, n (%).

	FMF group (n=1975)	JIA group (n=690)	HC group (n=406)	p value
Acute myeloid leukemia	0 (0%)	1 (0.14%)	0 (0%)	0.35
Acute rheumatic fever	22 (1.1%)	1 (0.14%)	1 (0.24%)	**0.01**[a]
Adenoidectomy history	34 (1.7%)	12 (1.7%)	5 (1.23%)	0.76
Adrenal insufficiency	0 (0%)	1 (0.14%)	0 (0%)	0.35
Allergic urticaria	2 (0.1%)	1 (0.14%)	0 (0%)	1.00
Allergic rhinitis	35 (1.7%)	4 (0.5%)	1 (0.24%)	**0.008**[b]
Amyloidosis	1 (0.05%)	0 (0%)	0 (0%)	1.00
Ankylosing spondylitis	5 (0.25%)	4 (0.5%)	2 (0.49%)	0.41
Appendectomy history	50 (2.5%)	3 (0.4%)	1 (0.24%)	**<0.001**[c]
Atopic dermatitis	43 (2.1%)	12 (1.7%)	3 (0.73%)	0.14
Autoimmune haemolytic anaemia	0 (0%)	1 (0.14%)	0 (0%)	0.35
Autoimmune hepatitis	0 (0%)	1 (0.14%)	0 (0%)	0.17
Atopic asthma	19 (1%)	2 (0.3%)	5 (1.2%)	0.16
Behçet's disease	11 (0.5%)	0 (0%)	2 (0.49%)	0.11
Brain tumor	1 (0.05%)	0 (0%)	0 (0%)	1.00
Breast cancer	0 (0%)	1 (0.14%)	0 (0%)	0.35
Cataract	0 (0%)	1 (0.14%)	0 (0%)	0.35
Celiac disease	1 (0.05%)	0 (0%)	1 (0.24%)	0.27
Colonic polyps	1 (0.05%)	0 (0%)	0 (0%)	1.00
Crohn disease	1 (0.05%)	0 (0%)	0 (0%)	0.75
Cystic fibrosis	1 (0.05%)	0 (0%)	0 (0%)	1.00
Drug allergy	2 (0.1%)	0 (0%)	0 (0%)	0.71
Ectodermal dysplasia	1 (0.05%)	0 (0%)	0 (0%)	0.75
Epilepsy	3 (0.15%)	1 (0.14%)	0 (0%)	0.85
Fibromyalgia	2 (0.1%)	0 (0%)	0 (0%)	0.71
Food allergy	1 (0.05%)	1 (0.14%)	0 (0%)	0.58
Gastritis	1 (0.05%)	0 (0%)	0 (0%)	1.00
Gout disease	1 (0.05%)	0 (0%)	1 (0.24%)	0.29
Graves' disease	11 (0.5%)	4 (0.5%)	1 (0.24%)	0.86
Hashimoto thyroiditis	50 (2.5%)	13 (1.8%)	6 (1.47%)	0.32
Immunoglobulin a vasculitis	1 (0.05%)	1 (0.14%)	0 (0%)	0.98
Hirschsprung disease	0 (0%)	0 (0%)	1 (0.24%)	0.13
Hodgkin lymphoma	0 (0%)	1 (0.14%)	0 (0%)	0.35
Juvenile ıdiopathic arthritis	5 (0.25%)	1 (0.14%)	0 (0%)	0.54
Lymphedema	1 (0.05%)	0 (0%)	0 (0%)	1.00
Migraine	1 (0.05%)	0 (0%)	0 (0%)	1.00
Multiple sclerosis	2 (0.1%)	1 (0.14%)	1 (0.24%)	0.59

FMF: Familial Mediterranean Fever; HC: Healthy control; JIA: Juvenile Idiopathic Arthritis; PFAPA: Periodic fever, aphthous stomatitis, pharyngitis, adenitis.

[a] Significantly more common in FMF group than in JIA group (p=0.018)

[b] Significantly more common in FMF group than in both JIA group (p=0.025) and HC group (p=0.022)

[c] Significantly more common in FMF group than in both JIA group (p=0.001) and HC group (p=0.003)

Table I. Continued.

	FMF group (n=1975)	JIA group (n=690)	HC group (n=406)	p value
Myocarditis	0 (0%)	1 (0.14%)	0 (0%)	0.35
Nephrolithiasis	0 (0%)	0 (0%)	1 (0.24%)	0.13
Neuroblastoma	0 (0%)	0 (0%)	2 (0.49%)	0.13
Non-atopic asthma	71 (3.6%)	13 (1.9%)	10 (2.5%)	0.06
Osteogenesis ımperfecta	0 (0%)	1 (0.14%)	0 (0%)	0.35
Pfapa syndrome	2 (0.1%)	0 (0%)	0 (0%)	0.57
Precocious puberty	1 (0.05%)	0 (0%)	0 (0%)	1.00
Primary hypertension	0 (0%)	0 (0%)	1 (0.24%)	0.13
Psoriasis	14 (0.7%)	1 (0.14%)	2 (0.49%)	0.22
Rheumatoid arthritis	23 (1.1%)	7 (1%)	0 (0%)	0.09
Sarcoidosis	1 (0.05%)	1 (0.14%)	0 (0%)	0.58
Scleroderma	1 (0.05%)	0 (0%)	0 (0%)	1.00
Sjogren disease	1 (0.05%)	0 (0%)	0 (0%)	1.00
Spastic paraplegia	1 (0.05%)	0 (0%)	0 (0%)	1.00
Systemic lupus erythematosus	1 (0.05%)	0 (0%)	0 (0%)	0.64
Thalassemia	2 (0.1%)	2 (0.28%)	0 (0%)	0.30
Thrombophilia	2 (0.1%)	0 (0%)	0 (0%)	1.00
Tonsillectomy history	66 (3.3%)	13 (1.8%)	12 (2.95%)	0.15
Trisomy 18	0 (0%)	1 (0.14%)	0 (0%)	0.35
Type 1 diabetes mellitus	4 (0.2%)	3 (0.4%)	1 (0.2%)	0.50
Type 2 diabetes mellitus	55 (2.8%)	14 (2%)	5 (1.2%)	0.13
Ulcerative colitis	2 (0.1%)	0 (0%)	1 (0.24%)	0.45
Uveitis	7 (0.35%)	0 (0%)	0 (0%)	0.20
Vitiligo	0 (0%)	0 (0%)	1 (0.24%)	0.132

FMF: Familial Mediterranean Fever; HC: Healthy control; JIA: Juvenile Idiopathic Arthritis; PFAPA: Periodic fever, aphthous stomatitis, pharyngitis, adenitis.
[a] Significantly more common in FMF group than in JIA group (p=0.018)
[b] Significantly more common in FMF group than in both JIA group (p=0.025) and HC group (p=0.022)
[c] Significantly more common in FMF group than in both JIA group (p=0.001) and HC group (p=0.003)

Table II. The comparison of the frequencies of certain disease groups among the first-degree relatives of the participants between groups , n (%).

	Study groups (n=3071)			p value		
	FMF group (n=1975)	JIA group (n=690)	HC group (n=406)	FMF vs. JIA	FMF vs. HC	JIA vs. HC
Any medical condition	541 (27.4%)	123 (17.8%)	65 (16%)	<0.01	<0.01	0.441
Rheumatic diseases	80 (4%)	15 (2.2%)	6 (1.5%)	0.030	0.017	0.559
Atopic diseases	103 (5.2%)	20 (2.9%)	9 (2.2%)	0.013	0.014	0.628
Autoimmune diseases	83 (4.2%)	25 (3.6%)	13 (3.2%)	0.508	0.428	0.844
Malignancies	1 (0.05%)	3 (0.4%)	2 (0.5%)	0.056	0.077	1
Surgery history	149 (7.5%)	27 (3.9%)	18 (4.4%)	<0.01	0.026	0.794

FMF: Familial Mediterranean Fever; HC: Healthy control; JIA: Juvenile Idiopathic Arthritis.

Discussion

In the present study, the diseases of the FDRs of healthy children, patients with FMF, and patients with JIA were evaluated, and compared with each other. Nearly a quarter of the participants had at least one medical condition. Non-atopic asthma, type 2 DM, and tonsillectomy history were the most common medical conditions in the FMF, JIA, and HC groups, respectively. Allergic rhinitis, ARF, and appendectomy history were significantly more common in the FMF group. While the most common group of medical conditions in all participants was surgical history, the most common group of disorders was atopic diseases. Atopic diseases, surgical history, and rheumatic diseases were significantly more common in the FMF group than in the others.

It was previously shown that while T helper-2 lymphocytes are the key elements in allergic diseases, T helper-1 lymphocyte-dependent inflammation has a pivotal role in the pathogenesis of FMF.[13,14] Therefore, a relatively low frequency of atopic diseases in patients with FMF is an expected finding. Consistently, children with FMF were shown to have a decreased frequency of atopic diseases than the general population.[15] However, more recent papers have provided contradictory findings with the previous ones. Two different studies showed a similar atopy frequency between children with FMF and their healthy peers.[16,17] Moreover, it was suggested that atopy is not only a frequent condition in FMF patients but also one of the components of the disease.[18] It was elucidated with the aid of recent increasing immunological knowledge that FMF pathogenesis is not as simple as T-helper 1 lymphocyte-driven inflammation. It also includes a large number of other immune elements, including innate immune cell-related cytokines, T-helper 17 lymphocytes, and T regulatory cells.[19,20] Besides, in addition to the T-helper-2 response, there is a significant T-helper 17 effect, mutual in both FMF and atopy, in the pathogenesis of atopic diseases.[20,21] Thus, the relationship between FMF and atopy remains unclear, considering the recent clinical and immunological findings. We found that allergic rhinitis and atopic diseases in general, were significantly more common in the FDRs of patients with FMF. Although close relatives of FMF patients probably share these complex immune mechanisms, it should be kept in mind that environmental circumstances, such as exposure to allergens rather than genetic susceptibility, play a pivotal role in allergic disorder development.

It is well-known that acute appendicitis is the most common indication of urgent abdominal surgery.[22] Abdominal signs caused by peritoneal irritation in FMF attacks are easily confused with acute appendicitis.[23] Therefore, as has been previously shown in several studies, appendectomy history might be more frequent in patients with FMF compared to the general population.[24-26] However, up to this study, FDRs of patients with FMF were not evaluated regarding their appendectomy histories. Although we excluded those diagnosed with FMF from the FDRs, appendectomy history in FDRs of FMF patients was significantly more common than in FDRs of healthy children and FDRs of patients with JIA. They might have FMF, however, *MEFV* gene mutations are not routinely performed in FDRs of patients with FMF unless they experience symptoms suggestive of FMF. Acute phase measurements have shown that healthy relatives of FMF patients tend to have subclinical inflammation, which is the main reason for several complications caused by FMF-related tissue damage.[27,28] Therefore, subclinical inflammation in these milder and undiagnosed cases may have a pivotal role for this novel finding. One of our participants in the group of FDRs of FMF patients had developed amyloidosis. This is consistent with the idea that FDRs of FMF patients may experience subclinical inflammation even if they are not diagnosed with FMF. We suggest that *MEFV* mutations should also be performed in FDRs of FMF patients who experienced acute appendicitis. On the other hand, we showed that overall surgical history was significantly

more common in the FMF group than the others, not only the appendectomy history. Adenoidectomy and tonsillectomy were the other surgical histories detected in our study population. PFAPA was previously shown to be highly related with FMF genetically and shares many common features with FMF which may cause a diagnostic challenge.[29-31] Tonsillectomy and adenoidectomy seem to be curative treatment options in most patients with PFAPA.[32] Although there were only 2 FDRs with PFAPA in the FMF group, we hypothesize that a significant amount of them were undiagnosed in the past, due to the newly growing and currently insufficient awareness of PFAPA among clinicians in our country.

In a study supported by anti-streptolysin O titters measurement, the relationship between FMF and ARF was investigated, and FMF patients were found to be more prone to develop ARF than the general population.[33] Parents of children with and without FMF were questioned regarding certain diseases previously, and it was revealed that ARF was significantly more common among family members of children with FMF.[34] Similarly, the frequency of ARF was significantly higher in FDRs of patients with FMF than FDRs of patients with JIA and healthy children in our study. Considering that arthritis is one of the cardinal signs in both ARF and FMF, the high prevalence of ARF among the FDRs of patients with FMF may be attributed to the possible misdiagnosis. On the other hand, since uncontrolled inflammatory response is a primary mechanism in both ARF and FMF, a pathogenetic similarity may be a reasonable explanation for the relationship between ARF and FMF.[35,36] It has been recently demonstrated that JIA is the most common accompanying disease in children with FMF.[1,4] Furthermore, sacroiliitis is thought to be more frequent in FMF patients.[37-39] However, in our study, the frequencies of chronic arthritis such as JIA, rheumatoid arthritis, and sacroiliitis did not differ among the FDRs of the groups. The association of vasculitis and FMF was widely investigated, and it has been showed that certain types such as IgAV, PAN and Behçet disease are more frequent in patients with FMF.[40] However, none of our participants had PAN. Besides, the frequency of vasculitis did not differ between the FDRs of FMF patients, JIA patients and healthy children in the present study. Our study revealed that not only ARF, but overall rheumatic disease was also significantly more common in the FMF group than in other groups. Although we excluded those with FMF from the FDRs of children with FMF, this novel finding which is entirely in line with the highly hereditary nature of FMF, makes us consider the possible accumulation of genetic rheumatic conditions whose inheritance pattern is yet to be unknown in this group.

Inflammatory bowel diseases (IBDs) are another investigated disease in several studies as to whether they accompany FMF, and they have been found to be more common in children with FMF.[41,42] In our study, only four participants had IBDs (ulcerative colitis:3; Crohn disease:1), and the frequency of IBDs was not found to be significantly different between groups. Similarly, neither spondyloarthropathy nor IBD were found to be significantly more common in a study comparing *MEFV* carriers and healthy controls.[34] Although the most common skin finding of FMF is erysipeloid erythema, psoriasis was also found to be associated with FMF.[35,43] Besides, a previous study reported an increased frequency of psoriasis in family members and close relatives of patients with FMF.[44] Although psoriasis was more common in FDRs of patients with FMF in the present study, there was no significant difference between the groups. While we only included the FDRs in this study, Barut et al.[44] also questioned second-and third-degree relatives in their study which found a high prevalence of psoriasis among families of FMF patients. This extended questioning may be responsible for the difference between the findings of these two studies.

Multiple sclerosis (MS), an autoimmune condition, is one of the most investigated co-morbid diseases in patients with FMF, and it was found to be significantly more frequent

compared to the general population.[45-50] Moreover, E148Q mutation has been suggested as a novel risk factor for developing MS.[50] However, only two of the FDRs of FMF patients had MS in our study, and there was no significant difference regarding the frequency between the groups. It may be related with the rarity of E148Q mutation among our index cases. However, as a limitation of this study, we did not evaluate the genetic results of our FMF patients. Beyond MS, the frequency of all detected non-rheumatic autoimmune diseases among the participants as a group of disorders was similar between FMF, JIA, and HC groups in our study. Similarly, a recent paper showed autoimmune diseases not to be increased in genetically confirmed FMF patients.[6] We evaluated this finding in accordance with the fact that the pathogenesis of FMF involves a mix of mainly innate and, to a lesser extent, adaptive immune system dysregulations.[35]

There are three main limitations to our study. Firstly, we compared the FMF, JIA, and healthy control groups regardless of the disease subtypes of the patients with JIA. Secondly, age and gender distribution of FDRs and their effects on the disease frequencies could not be assessed due to the nonavailability of data. Thirdly, we did not evaluate the genetic results either of our index FMF cases nor of their FDRs, which could be useful for a better understanding of the health condition distribution among participants. On the other hand, the main strength of the present study is that this is the first study evaluating the medical conditions with all aspects of the FDRs of patients with FMF in quite a large cohort.

In conclusion, although the co-existing diseases in children with FMF have previously been widely discussed, all medical conditions of FDRs of these patients have not been evaluated thus far. Given the strong genetic background of the disease it is necessary that fathers, mothers, and siblings of the patients with FMF are also investigated. This is a first attempt at doing this with a quite large cohort. We revealed that atopic diseases, rheumatic diseases, and surgical history were significantly more common in FDRs of children with FMF than in FDRs of children with JIA and FDRs of healthy children. This novel finding may contribute to understanding the hereditary burden of co-existing diseases in children with FMF.

Ethical approval

The study was approved by Institutional Review Board (04/04/2018-127814) of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical School.

Author contribution

Study conception and design: SY, ÖK; data collection: FH, MY, AAdrovic, AAliyeva, AG, EA, EKK, ÜG, SŞ, KB; analysis and interpretation of results: SY, FH, MY; draft manuscript preparation: SY, FH, MY. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Clinical and genetic analysis of 18 patients with *KCNQ2* mutations from South China

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ABSTRACT

Background. We aimed to delineate the genotype and phenotype of patients with *KCNQ2* mutations from South China.

Methods. Clinical manifestations and characteristics of *KCNQ2* mutations of patients from South China were analyzed. Previous patients with mutations detected in this study were reviewed.

Results. Eighteen epilepsy patients with *KCNQ2* mutations, including seven self-limited neonatal epilepsy (SeLNE), two self-limited infantile epilepsy (SeLIE) and nine developmental and epileptic encephalopathy (DEE) were enrolled. The age of onset (p=0.006), mutation types (p=0.029), hypertonia (p=0.000), and seizure offset (p=0.029) were different in self-limited epilepsy (SeLE) and DEE. *De novo* mutations were mainly detected in DEE patients (p=0.026). The mutation position, EEG or the age of onset were not predictive for the seizure or ID/DD outcome in DEE, while the development of patients free of seizures was better than that of patients with seizures (p=0.008). Sodium channel blockers were the most effective anti-seizure medication, while the age of starting sodium channel blockers did not affect the seizure or development offset. We first discovered the seizure recurrence ratio in SeLNE/SeLIE was 23.1% in South China. Four novel mutations (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G, 20q13.33del) were discovered. Each of eight mutations (c.1918delC, c.1678C>T, c.683A>G, c.833T>C, c.868G>A, c.638G>A, c.997C>T, c.830C>T) only resulted in SeLE or DEE, while heterogeneity was also found. Six patients in this study have enriched the known phenotype caused by the mutations (c.365C>T, c.1A>G, c.683A>G, c.833T>C, c.830C>T, c.1678C>T).

Conclusion. This research has expanded known phenotype and genotype of *KCNQ2*-related epilepsy, and the different clinical features of SeLE and DEE from South China.

Key words: *KCNQ2*, self-limited epilepsy, developmental and epileptic encephalopathy.

KCNQ2 (OMIM *602235), encoding a voltage-gated potassium channel, was firstly reported to be a disease-causing gene for epilepsy in 1998.[1] To date, heterozygous mutations in *KCNQ2* are considered responsible for a spectrum of disease, ranging from self-limited neonatal epilepsy (SeLNE), self-limited infantile epilepsy (SeLIE) to developmental and epileptic encephalopathy 7 (DEE, OMIM #613720).[2,3] Various types of mutations have been reported.[4,5] In the Human Gene Mutation Database, more than 400 variations in *KCNQ2* are recorded. Early onset seizures without intellectual disability/development delay (ID/DD) are common manifestations in SeLNE and SeLIE, they might spontaneously disappear or are pharmacoresponsive.[6] SeLNE/SeLIE patients also have a probability to progress into epilepsy again after late childhood.[7] DEE, in contrast, is characterized by seizures onset in the first week of life and unremitting moderate or severe ID/DD. Seizures are often resistant to regular anti-seizure medications (ASMs), and may cease between nine months and four years of age.[3] Loss of function, dominant negative effects and gain of function have been

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confirmed as the underlying mechanisms.[4,8,9] Sodium channel blockers have been found to be the most effective ASM.[10]

Recent clinical studies have described the clinical features of *KCNQ2* related diseases, including manifestations, treatment methods and prognosis.[8,11] However, there are few studies reporting patients with *KCNQ2* mutations from South China, especially from Guangdong Province, and the eletroclinical features have not yet been clarified. In this study, we aimed to explore the genetic and clinical features of eighteen epilepsy patients with *KCNQ2* mutation from South China and analyze and compare the clinical manifestations of children with the same mutation in this and previous studies.

Methods

Patients

Eighteen patients (Pt1-Pt18) with epilepsy onset in infancy and *KCNQ2* variations from 18 unrelated families were enrolled at the Department of Pediatric Neurology, Guangzhou Women and Children's Medical Center between June 2018 and August 2021. Our study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (No. 2019-40101). We have obtained informed consent from the patients' parents for the study.

Clinical analysis

Clinical data were collected, including age of onset, gender, seizure type, treatment method, developmental milestones, EEG and MRI. Before the era of genetic testing, treatment methods of 18 patients in this study were based on age, seizure type, and EEG characteristics. Since some patients had started their evaluation and treatment in other hospitals, treatment regimens were heterogeneous. Vitamin B6 was applied in some patients irregularly, and therefore was not analyzed for effectiveness in this study. Response to ASMs was considered effective if seizure frequencies decreased by more than 50% from the baseline after treatment. For the sake of simplicity in this study, developmental assessment was based on developmental milestones and ID/DD was classified as mild, moderate or severe based on the following: mild ID/DD was considered when patients acquired the developmental milestones but at a slightly worse level. Moderate ID/DD was defined as patients who were obviously delayed in milestones, but who showed progress. Patients were classified as severe or profound ID/DD if their development was nearly arrested. The descriptions of seizure and epilepsy were based on the guidelines proposed by the International League Against Epilepsy in 2017 and 2022.[12,13]

Genetic analysis

Next-generation sequencing including epilepsy gene panel sequencing, whole exome sequencing and whole genome sequencing was performed for 18 patients in genetic testing agencies. The epilepsy gene panel was designed to capture the coding exons of more than 536 genes associated with epilepsy including the *KCNQ2*. Next-generation sequencing was performed on Illumina, NovaSeq 6000 or MGI 2000 system platforms. Sequences of genes were referred to GRCh37/hg19. The average sequencing depth of epilepsy gene panels and whole exome sequencing was more than 122X. The average sequencing depth of whole genome sequencing was 28X. Variations of eleven patients (Pt2-Pt4, Pt6, Pt8, Pt10, Pt12-Pt15, Pt17) were validated with Sanger sequencing. All *KCNQ2* variants were harmonized with the ENST00000359125.7 (RefSeq NM_172107.4) of the GRCh37/hg19 human reference genome build. Variants were analyzed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG).[14,15]

Review of previous patients

Clinvar database, Human Gene Mutation Database and the literature (Supplementary table) were reviewed to analyze the clinical course, seizure types, and response to the

treatment of patients with the same mutation detected in this study.[8,11,16-40]

Statistical analysis

Data were evaluated by Fisher exact probability test, Mann Whitney U test, and Kruskal-Wallis H test for comparisons between groups using Statistical Package for the Social Sciences version 20 software. Probability values less than 5% were considered to be significant.

Results

Clinical features

Eighteen children (Pt1-Pt18) from South China, composed of eight female and ten males, were clinically evaluated in this study (Table I). Seven patients (Pt1-Pt7) were diagnosed with SeLNE, two (Pt8-Pt9) were SeLIE, and nine (Pt10-Pt18) were DEE. Fifteen patients came from the Guangdong Province, two from Guangxi (Pt2 and Pt8), and one from the Hunan (Pt3) Province. None of their parents were consanguineous. Pt17 was born prematurely at 36 weeks without asphyxia. Gestational diabetes and maternal thyroid dysfunction were diagnosed in Pt4 and Pt13, respectively. No specific abnormalities were found in blood routine test or metabolic disease screening in eighteen patients. In the brain MRI, smaller right hippocampus was found in Pt2, and diffuse atrophy was detected in Pt14 and Pt17. A positive family history of seizure was found in three patients (Pt1, Pt4 and Pt6). Seizures in three family members of two SeLNE patients (Pt4, Pt6) disappeared spontaneously without treatment in infancy. Seizures recurred to Pt1's father during adulthood.

Seizure was the initial symptom and was observed in all of them (Table I). Patients of SeLE developed seizures from 3 days to 3 months after birth, with a median of 5 days, while, in DEE patients, seizures occurred at the age ranging from several hours to six months of age, with a median of 2 days. The age of onset of DEE patients was earlier than that of self-limited epilepsy (SeLE) patients (p=0.006) (Table II). Focal onset seizure was the most common seizure type (88.9%, 16/18).

EEG often presented focal or multi-focal discharge in both SeLE and DEE patients (94.4%, 17/18), while suppression burst patterns and hypsarrhythmia were only detected in DEE patients (p=0.000) (see Table I and Table II). Initial epilepsy syndrome was Ohtahara syndrome in eight patients and West syndrome in one patient. Four patients of Ohtahara syndrome evolved to West syndrome. In eight patients with ictal amplitude-integrated electroencephalography (aEEG) in neonate, a sudden rise of the upper and lower margin followed by a marked depression in amplitude were found in eight and three, respectively.

ASMs (18/18), prednisone (3/18), and ketogenic diet (2/18) were used in this study. Generally, sodium channel blockers (SCBs), including oxcarbazepine (100%, 15/15), lamotrigine (100%, 3/3), and topiramate (66.7%, 2/3) were used and effective in 100% of patients (n=15) and its usage rate was higher in DEE patients than that in SeLE (p=0.029). In seven SeLNE patients, phenobarbital (5/7), valproic acid (5/7), topiramate (1/7), levetiracetam (1/7), nitrazepam (1/7) and oxcarbazepine (5/7) were used, in which, phenobarbital (100%, 5/5) and SCBs (100%, 6/6) were the most effective, followed by valproic acid (50%, 2/4). Lacosamide was tried in Pt3, but was too early to evaluate the effectiveness as it was only recently started during the preparation of this manuscript. They were pharmacoresponsive and became seizure-free in infancy, with a median age of 3+ months old. Oxcarbazepine, levetiracetam and valproic acid were effective in two patients with SeLIE, and both of them were free of seizures in their infancy. Nine DEE patients were responsive to SCBs, especially oxcarbazepine (100%, 9/9). In addition, nitrazepam (3/3), valproic acid (6/7), and phenobarbital (3/6) were effective in most of them. A combination of prednisone and other ASMs was found to be effective in three West syndrome patients. However, levetiracetam (0/6), vigabatrin (0/1) and ketogenic diet (0/2)

Table I. Genetic and clinical features of 18 patients with mutations from South China

Pt	Gender	Mutation/origin	Age of onset	Seizure	aEEG	EEG	Treatment		Diagnosis	Prognosis	
							Ineffective	Effective		Age at last visit	Condition
1	M	c.394G>A(p.Val132Met), Father	3d	Focal (FBTCS, TS)	/	IP: MFD →Normal	VPA	OXC	SeLNE	2y1m	SF since 3m with OXC (reduction)
2	M	c.1741C>T(p.Arg581*), *De novo*	6d	Focal (FBTCS)	/	IP: Asymmetric EW in TL→Normal	/	PB,VPA	SeLNE	2y1m	SF since 4m with VPA (reduction)
3	M	c.790T>C(p.Tyr264His), *De novo*	5d	Focal (TCS, FBTCS)	/	IP: MFD	/	TPM, OXC	SeLNE, EP	2y	SF since 5m with OXC
			3y11m	Focal (FBTCS)	/	IP: MFD	LCM(?)			4y	Occasional Sz with OXC+LCM
4	M	c.1918delC(p.Leu640Trpfs*1), Mother	7d	Focal (TCS, FBTCS)	RM	IP: MFD→Normal	/	PB,OXC	SeLNE	3y1m	SF since 5m
5	F	c.355_363delGAGAAGAGC (p.Glu119_Ser121del), *De novo*	3d	Focal (FBTCS)	RM, DA	IP: Sparse EW	/	PB,OXC	SeLNE	6m	SF since 10+d with OXC
6	F	c.296+2T>G, Mother	3d	Focal (FBTCS)	/	IP: Sparse EW →Normal	/	PB,VPA	SeLNE	1y9m	SF since 3m with VPA
7	M	20q13.33del(chr20:61974641-62324656)*1,350kb, NA	3d	Focal (TS, FBTCS)	RM	IP: MFD	VPA, LEV, NZP	PB,OXC	SeLNE	7m	SF since 3m with OXC
8	M	c.1A>G(p.Met1Val), NA	3+m		/	NA	NA	OXC	SeLIE, EP	NA	SF
			8y2m	Unknown (TCS)	/	IP: MFD	NZP	LEV		9y	Occasional Sz with NZP+LEV
9	M	c.998G>A(p.Arg333Gln), Father	2+m	Unknown (TCS)	/	IP: MFD→Normal	/	LEV,VPA	SeLIE	2y10m	SF since 5m with VPA
10	F	c.1678C>T(p.Arg560Trp), *De novo*	2d	Focal and General (FBTCS, TS, Spm) (GESELL, 2m:42)	/	IP: MFD→SB→HS, IP: MFD→IP:EW	LEV	PB, OXC, PDN	DEE, OS, WS	1y9m	SF and moderate ID/DD since 5m with OXC+PB
11	F	c.683A>G(p.His228Arg), *De novo*	2d	Focal and General (FBTCS, Spm)	RM	SB→HS, IP: MFD→ IP: MFD	PB, LEV, VGB	OXC, NZP, PDN, VPA, LTG	DEE, OS, WS	1y7m	Infrequent Sz and profound ID/DD with OXC+VPA+NZP+LTG
12	F	c.833T>C(p.Ile278Thr), *De novo*	2d	Sz: Focal and General (TS, FBTCS, Spm, CS) (GESELL 6m:10)	RM	SB, IP: MFD→HS, IP: MFD	/	PB, TPM, OXC, LTG, VPA, PDN	DEE, OS, WS	2y1m	Sz sometimes and profound ID/DD with VPA+OXC+LTG

Pt, patient; h, hours old; d, days old; w, weeks old; m, months old; y, years old; F, female; M, male; CS, clonic seizures; DA, a marked depression in amplitude; EP, epilepsy; EW, epileptic waves; FBTCS, focal to bilateral tonic-clonic seizure; HS, hypsarrhythmia; IP, interictal period; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; MFD, multifocal discharges; NA, not available; /, No; NZP, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; PDN, prednisone; RM, rise of the upper and lower margin; SB, suppression burst; SF, seizures free; Spm, spasm; Sz, seizures; TCS, tonic–clonic seizures; TL, temporal lobe; TPM, topiramate; TS, tonic seizures; VGB, vigabatrin; VPA, valproic acid; WES, whole exon sequencing; WGS, whole genome sequencing.

Table I. Continued.

Pt	Gender	Mutation/origin	Age of onset	Seizure	aEEG	EEG	Treatment		Diagnosis	Prognosis	
							Ineffective	Effective		Age at last visit	Condition
13	M	c.365C>T(p.Ser122Leu), *De novo*	8h	Focal and General (TS, FBTCS)	RM	SB→IP: MFD	PB, VPA	OXC	DEE, OS	1y2m	SF and mild ID/DD since 2m with OXC
14	F	c.1678C>T(p.Arg560Trp), *De novo*	2d	Focal and General (TS, Spm)	/	SB, IP:MFD→IP: Slow wave	LEV, KD	VPA, LTG, OXC, NZP	DEE, OS	1y1m	Frequent Sz and profound ID/DD with VPA+LTG+OXC+NZP
15	F	c.868G>A(p.Gly290Ser), NA	1d	Focal and General (TS, Spm)	/	SB→IP: Slow background, MFD	TPM, LEV	NZP, VPA,OXC	DEE, OS	1y6m	SF and moderate ID/DD since 3m with OXC
16	M	c.638G>A(p.Arg213Gln), *De novo*	2d	Focal and General (TS, TCS, Spm)	RM, DA	IP: MFD→SB,IP: MFD→HS	PB	VPA, OXC	DEE, OS, WS	3m	Frequent Sz and profound ID/DD with VPA+OXC
17	M	c.997C>T(p.Arg333Trp), *De novo*	6m	Focal and General (Spm, TS)	/	HS→IP: MFD	LEV, KD	VPA, OXC	DEE, WS	1y5m	Infrequent Sz and profound ID/DD with VPA+OXC
18	F	c.830C>T(p.Thr277Ile), *De novo*	2d	Focal and General (TCS, TS, Spm)	RM, DA	SB, IP: MFD→Slow background and sparse EW	LEV	PB, OXC	DEE, OS	6m	SF and moderate ID/DD since 3+m with OXC+PB

Pt, patient; h, hours old; d, days old; w, weeks old; m, months old; y, years old; F, female; M, male; CS, clonic seizures; DA, a marked depression in amplitude; EP, epilepsy; EW, epileptic waves; FBTCS, focal to bilateral tonic-clonic seizure; HS, hypsarrhythmia; IP, interictal period; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; MFD, multifocal discharges; NA, not available; /, No; NZP, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; PDN, prednisone; RM, rise of the upper and lower margin; SB, suppression burst; SF, seizures free; Spm, spasm; Sz, seizures; TCS, tonic–clonic seizures; TL, temporal lobe; TPM, topiramate; TS, tonic seizures; VGB, vigabatrin; VPA, valproic acid; WES, whole exon sequencing; WGS, whole genome sequencing.

Table II. Genotype and phenotype correlation.

Items		Phenotype		T	p	Seizure offset in DEE		T	p	Neudevelop. In DEE			T	p
		SeLE	DEE			Yes	No			Mild	Moderate	Profound		
Age of onset		5d (3d-3m)	2 (8h-6m)	17[1]	0.006*	1.5d (8h-2d)	2d (2d-6m)	9	0.081*	8h	2d(1d-2d)	2d(2d-6m)	9	0.099[!]
Age free of Seizures		3.5m (10d-5m)	2.5 (2m-5m)	12[1]	0.530*		NA			2m	3m (3m-5m)	/	4	0.157[!]
Mutation type	Missense mutation	4	9	13	0.029[#]	4	5	9	NA	1	3	5	9	NA
	Others	5	0	5		0	0	0		0	0	0	0	
Mutation origin	*De novo*	3	8	11[2]	0.026[#]	3	5	8[2]	NA	1	2	5	8[2]	NA
	Parental	4	0	42		0	0	0		0	0	0	0	
Mutation position of gene	From Exon1 to Exon17	8	9	17[3]	0.388[#]	4	5	9	0.714[#]	1	3	5	9	0.571[#]
Mutation position of protein	DEE-related Hot Spot	2	5	7[3]	0.335[#]		NA					NA		
	Others	6	4	10[3]										
Mutation position of protein	Regions sensitive to ASMs	6	8	14[3]	0.576[#]	4	4	8	1#	1	3	4	8	0.369[#]
	Others	2	1	3[3]		0	1	1		0	0	1	1	
EEG	HS or/and SB	0	9	9	0.000[#]	4	5	9	NA	1	3	5	9	
	no	8	0	8[1]		0	0	0		0	0	0	0	NA
SCBs	Yes	6	9	15	0.029[#]									
	No	3	0	3										
Effect of LEV	Effective	1	0	1	0.250[#]									
	Non-effective	1	6	7										
Effect of PB	Effective	5	3	8	0.182[#]									
	Non-effective	0	3	3										
Effect of VPA	Effective	3	6	9	0.523[#]									
	Non-effective	2	1	3										
Effect of NZP	Effective	0	3	3	0.250[#]									
	Non-effective	1	0	1										

T: total number, Neudevelop.: neurodevelopmental outcomes, NA: not applicable, [#]: analyzed with Fisher Exact probability test, *: analyzed with Mann Whitney U test, [!]:analyzed with Kruskal-Wallis H test, [1]: The age of onset and seizure free of Pt8 was unclear, [2]: the mutation origin of three patients was unclear, [3]: the copy number variation was not included, [4]: SCB was not used in three SeLE patients, [5]: The age of one patient with SCB was unclear, SCB:Sodium channel blockers, SeLE: self-limited epilepsy, DEE: developmental and epileptic encephalopathy, HS: hypsarrhythmia, SB: suppression burst, ASMs: anti-seizure medications, d: days old, m: months old, h: hours old.

Table II. Continued.

Items		Phenotype		T	p	Seizure offset in DEE		T	p	Neudevelop. In DEE			T	p
		SeLE	DEE			Yes	No			Mild	Moderate	Profound		
Effect of SCBs	Effective	6	9	15[4]	NA[#]	4	5	9	NA	1	3	5	9	NA
	Non-effective	0	0	0		0	0	0		0	0	0	0	
Age with SCBs		5	9	14[5]	0.242*	/	/	9	0.072*	/	/	/	9	0.188[!]
Seizure offset	Seizure Free	9	4	13	0.029[#]	NA				1	3	0	4	0.008[#]
	In Seizures	0	5	5						0	0	5	5	
Hypertonia	Yes	0	9	9	0.000[#]	NA						NA		
	No	9	0	9										
Total		9	9	18		4	5	9		1	3	5	9	

T: total number, Neudevelop.: neurodevelopmental outcomes, NA: not applicable, [#]: analyzed with Fisher Exact probability test, *: analyzed with Mann Whitney U test, [!]:analyzed with Kruskal-Wallis H test, [1]: The age of onset and seizure free of Pt8 was unclear, [2]: the mutation origin of three patients was unclear, [3]: the copy number variation was not included, [4]: SCB was not used in three SeLE patients, [5]: The age of one patient with SCB was unclear, SCB:Sodium channel blockers, SeLE: self-limited epilepsy, DEE: developmental and epileptic encephalopathy, HS: hypsarrhythmia, SB: suppression burst, ASMs: anti-seizure medications, d: days old, m: months old, h: hours old.

were ineffective in this study. At their last visit, four patients (4/9) had been in seizure-free status since two to five months following birth, with a median of 3+ months. No statistical difference in effectiveness of SCBs, phenobarbital (p=0.182), valproic acid (p=0.523), levetiracetam (p=0.250), or nitrazepam (p=0.250) was found between SeLE and DEE. In 14 patients with SCBs, the age of starting SCBs was not associated with seizure offset (p=0.242), which was also found in nine DEE patients (p=0.072). In addition, age of onset (p=0.081) did not affect the seizure prognosis in DEE patients. In the follow-up study of nine SeLE patients and four relatives, seizures recurred in three (23.1%, 3/13, Pt3, Pt8 and Pt1's father).

ID/DD in nine DEE was unremitting. We found that seizure-free patients had better cognitive and movement ability than the patients with poorly controlled seizures (p=0.008). However, age of onset (p=0.099), seizure-free age (p=0.157), age with SCBs (p=0.188), did not affect their developmental outcome. Unfortunately, we failed to perform Gesell Developmental Scale tests. In addition, hypertonia was observed in all nine DEE patients.

Genetic analysis

Genetic analysis with next-generation sequencing was performed in 18 patients, and all of them were genetically diagnosed with seventeen single-nucleotide variations (SNV) and one copy number variant (Table I). Apart from three patients without all their parents' blood samples, eleven mutations were confirmed to be *de novo*, including eight in DEE patients (100%, 8/8), and three SeLE mutations were inherited from symptomatic parents. Pt9 inherited the mutation from her asymptomatic father.

According to the ACMG recommendations on SNV[14], three variations (Pt2, Pt6, Pt8, 17.6%, 3/17) were pathogenic, eleven (64.7%, 11/17) were likely pathogenic, and the remaining three (Pt1, Pt4, Pt15, 17.6%, 3/17) were variations with uncertain significance (VUS). The three VUS

were not found in the normal control population and were reported in other research findings (Supplementary Table). The amino acids in the three cites (p.Val132Met, p.Leu640Trpfs*1 and p.Gly290Ser) were conserved in different species (http://genome.ucsc.edu/). Two of them (p.Val132Met and p.Gly290Ser) led to changes in amino acid properties. The wild-type amino acids were aliphatic and converted into sulfur-containing (p.Val132Met) or hydroxy amino acids (p.Gly290Ser). p.Leu640 was located in the last exon of *KCNQ2*, which might escape nonsense-mediated mRNA decay, however, segregation analysis revealed that the symptomatic members in the family (the proband and her mother) carried the variant whereas the healthy counterparts had the wild-type allele. Therefore, we considered those three variations to be clinically pathological, and all the patients with SNV were genetically diagnosed.

Among the SNVs, twelve missense mutations (70.6%, 12/17), one nonsense mutation (5.9%, 1/17), two small deletion mutations (11.8%, 2/17), one splice-site mutation (5.9%, 1/17), and one start-codon mutation (5.9%, 1/17) were detected, of which three (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G) were novel. SNVs were distributed in the exon 1-7, exon15 and exon 17. All nine DEE patients had missense mutations, and SeLE patients had missense mutation and other mutation types. There were differences in both mutation types (p=0.029) and mutation origin (p=0.026) between DEE and SeLE. We found that 35.3% (6/17) SNVs were located in C-terminal regions (Fig. 1), 23.5% (4/17) in the pore loop between S5 and S6, 11.8% (2/17) in S2, 11.8% (2/17) in the extracellular domain between S1 and S2, and 5.9% (1/17) in the N-terminal regions, S1, S4, and the intracellular domain between S4 and S5, respectively. Five DEE mutations and two SeLE mutations were located in S4, PD, and helices A. Fisher's precision probability test indicated no statistically significant difference in distribution between the DEE and SeLE in the *KCNQ2* gene (p=0.388), the DEE hot regions (p=0.335) or the regions sensitive to ASMs (p=0.576). Neither mutation position in the gene nor the protein determined the seizure offset (the gene, p=0.714, the protein p=1), or ID/DD outcome (the gene, p=0.571, the protein p=0.369).

A novel copy number variation of 20q13.33del (chr20:61974641-62324656) containing fourteen genes including *KCNQ2*, *CHRNA4* (OMIM*118504), *EEF1A2* (OMIM*602959) and *RTEL1* (OMIM*608833) was found in Pt7. *KCNQ2* was an established haploinsufficiency gene, and the clinical features of Pt7, such as seizure type, EEG feature and response to oxcarbazepine was consistent with the features of *KCNQ2* related disease. It was considered pathological according to ACMG guidelines in copy number variation analysis.[15]

Review of previous patients

Thirteen kinds of mutations in fourteen patients in this study were reported (Supplementary Table). Only each of eight mutations resulted in SeLN (c.394G>A, c.1918delC) or DEE (c.1678C>T, c.683A>G, c.833T>C, c.868G>A, c.638G>A, and c.830C>T). While patients with DEE mutations were diagnosed with different epileptic syndromes, six patients with c.1678C>T (75%, 6/8), two with c.833T>C (50%, 2/4), and two with c.638G>A (28.6%, 2/7) were diagnosed with Ohtahara syndrome, and two patients with c.638G>A (28.6%, 2/7) were diagnosed with West syndrome. Besides, for the first time, we diagnosed Ohtahara syndrome in patients with c.683A>G, c.868G>A and c.830C>T, and diagnosed West syndrome in patients with c.1678C>T, c.683A>G and c.833T>C. Seizure prognosis of patients with the same DEE mutation (c.1678C>T, or c.638G>A) was variable. Half of patients (3/6) with c.1678C>T and 66.7% (2/3) patients with c.638G>A became seizure-free. All the patients including Pt15 (3/3) with c.868G>A became seizure-free. Prognosis of previous patients with c.683A>G, c.833T>C and c.830C>T were not available.

Four mutations (30.8%, 4/13, c.1741C>T, c.1A>G, c.998G>A, c.365C>T) were detected in both SeLE

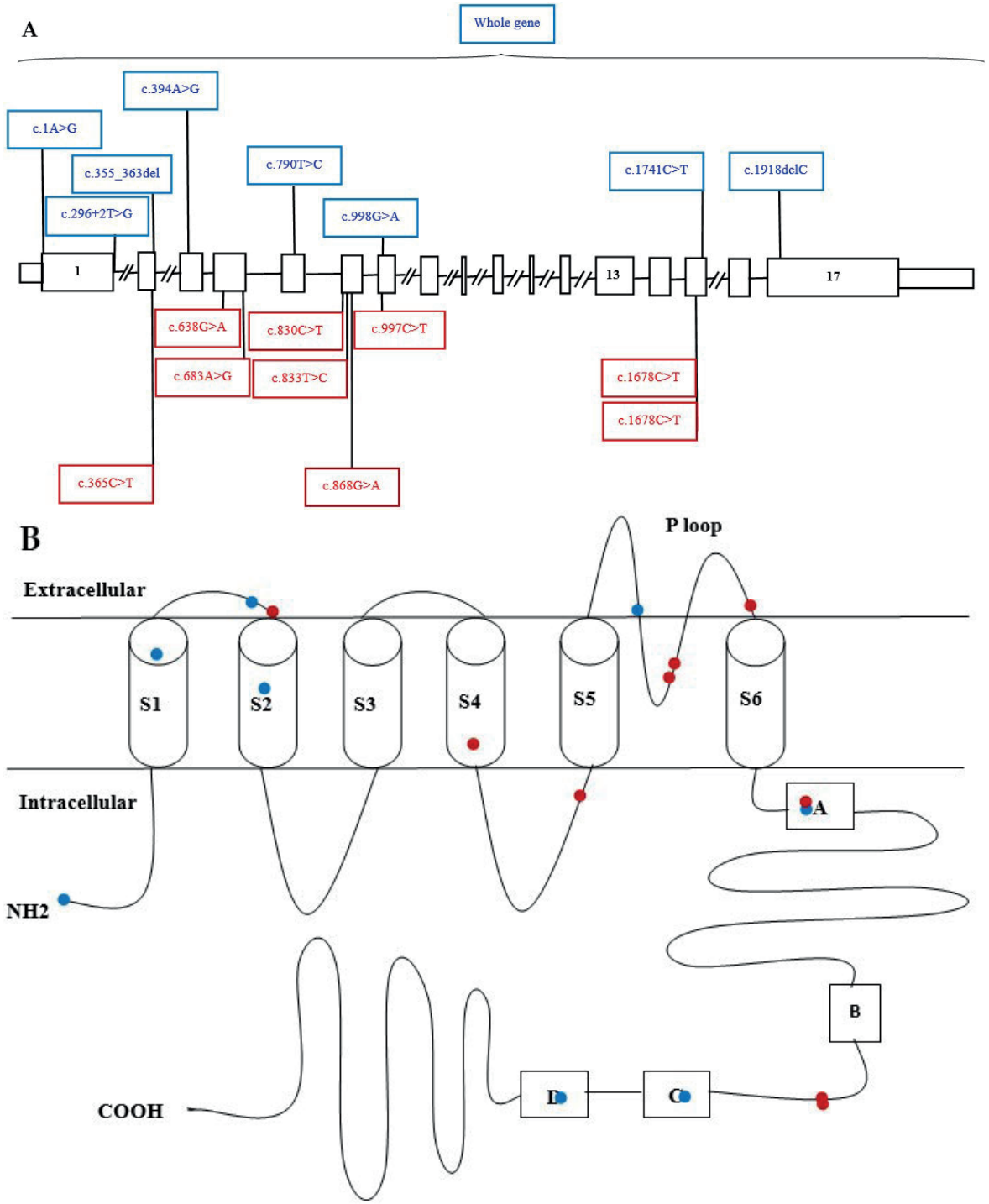

Fig. 1. (A) Mutations in *KCNQ2*. The middle of the figure is the *KCNQ2* structure. The black boxes represent exons, and the horizontal lines between the boxes are intron regions. The slightly shorter squares in exons 1 and exon 17 are non-coding sequences in exons. The size of exons and introns are based on the number their bases. Because the size of the figure is limited, we use slashes to represent the long introns. Mutations in blue were discovered in patients with self-limited epilepsy, and mutations in red were found in developmental and epileptic encephalopathy. **(B)** Mutations in *KCNQ2* protein. Mutations in blue were discovered in patients with self-limited epilepsy, and mutations in red were found in patients with developmental and epileptic encephalopathy. S: Segment.

and DEE patients. In this study, three patients (c.1741C>T, c.1A>G, c.998G>A) were SeLE, and one with c.365C>T were DEE. Pt8 in this study was the first SeLIE patient with c.1A>G, and Pt13 to be diagnosed with Ohtahara syndrome first in patients with c.365C>T. For patients in this and previous studies, we first found there was no statistical difference in seizure prognosis between DEE patients with mutations detected in both SeLE and DEE patients with mutations detected only in DEE (p=1.0). Clinical course of patients with c.997C>T (7.7%, 1/13) was not analyzed because the diagnosis of one previous patient was unclear.

Discussion

This study is a genetic and clinical analysis for epilepsy patients due to *KCNQ2* mutations, including the largest sample from South China. Electroclinical features in this study are consistent with previous reports[4,7,10], but we identified four novel *KCNQ2* mutations (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G, 20q13.33del) and discovered some distinct characteristics, which might result from the special genetic background of the patients or environmental factors in South China.

In this study, self-limited epilepsy (SeLE) (9/18) and DEE (9/18) accounted for half each, and the ratio of SeLNE (7/9) to SeLIE (2/9) was 7:2. Seizure and EEG/aEEG features, response to SCBs, seizure offset, and ID/DD outcome, mutations types were consistent with previous reports.[7,11] In addition, we also found several distinct characteristics, including the difference in age of onset between SeLE and DEE, the benefit of seizure-free to ID/DD outcome. The age of onset of DEE was earlier than that of SeLE in this study. However, all the seizures started in infancy, we could not classify the patients by the age of onset alone. The age of onset, mutation location, and the age with SCBs did not determine the seizure or ID/DD offset. Opposite prognosis in Pt10 and Pt14 who had the same mutation suggested that other modifiers or environmental factors might be involved in the pathogenesis of the disease. Previous studies have found several features of mutation distributions in *KCNQ2* protein that S4, ion pore domain (PD), helices A and B were discovered as four hot spot regions related to DEE, and mutations in patients who were responsive to ASMs mainly located in S2, PD, S4, S6, C-terminal region and extracellular region.[41] However, no mutation distribution was similar between patients with DEE and SeLE or between patients with variable response to ASMs in this study. Besides, we found hypertonia in nine DEE patients, which has rarely been reported in previous reports.[35] Abnormal neuronal excitability after several gene mutations was found to be related to hypertonia[42], which suggests that hypertonia and DEE might have a common pathogenic mechanism. Hypertonia was found in none of the SeLE patients, implying it to be an important symptom to distinguish DEE from SeLE. After treatment, nine SeLE patients in this study became seizures-free, and seizures in three family members disappeared spontaneously without treatment in infancy. This underlines the importance of identifying the etiology as early as possible to make the treatment decision and correctly predict the disease prognosis. Aggressive ASMs might not be required for SeLNE without frequent seizures and we may consider reducing or even stopping the ASMs earlier for SeLE to reduce the adverse reactions. It was reported that ketogenic diet was effective in *KCNQ2* related diseases[43], however, it had little effect in seizure control in this study. Different mutations and the special genetic background of South China might contribute to this, however more studies are needed to confirm this. In this study, 23.1% SeLE patients had recurrent seizure, which was higher than the proportion of 10-15% in previous reports[7], reinforcing the idea that a long-term follow-up study is necessary for SeLE patients in South China.

In this study, we found four novel mutations (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G, 20q13.33del), expanding the

mutation spectrum of *KNCQ2*. Consistent with previous reports[4,37], *de novo* missense mutations often led to DEE. However, mutations in the three SeLE patients were *de novo*. Mosaicism in their parents might be a possibility.[4,5] Mutations in Pt9 originating from an asymptomatic father may be explained by mosaicism in the father as well as an unreliable history. Genetic analysis for multiple tissues or RainDrop™ PCR of their parents would help to confirm this.

Variable phenotypes of 20q13.3 microdeletion syndrome, including seizures, brain malformation, ID/DD and psychological abnormalities, were reported.[44] In this study, we found a mild phenotype in Pt7 with 20q13.33 deletion, involving *KCNQ2*, *CHRNA4*, *EEF1A2*, *RTEL1* and other ten genes without identified diseases. Pt7's clinical features were different from epilepsy nocturnal frontal lobe (OMIM #600513) caused by *CHRNA4* mutations, DEE33 (OMIM #616409) resulting from *EEF1A2* mutations, and acute myeloid leukemia and dyskeratosis congenita due to *RTEL1* mutations. Furthermore, *CHRNA4* haploinsufficiency does not cause a disease and mutations in it are located in or close to the M2 region of the receptor and the gain-of-function effect is responsible for the disease.[45] Therefore, *KCNQ2* gene was considered the causative gene in Pt7, and we will also conduct a long follow-up study to observe whether other genes contribute to this phenotype.

In the analysis of clinical features of patients with thirteen kinds of reported mutations in this and previous reports, we found that patients were responsive to sodium channel blockers, and heterogeneity in epileptic syndrome and seizure prognosis was found in DEE mutations. Clinical heterogeneity might be the effect of other genetic modifier, and environmental factors. It was proposed that pre- or perinatal risk factors such as neonatal hypoxia and preeclampsia in pregnancy could amplify the pathophysiological impact of *KCNQ2* mutations.[46] Additional mutations in other genes or the involvement of other genetic variants that can further regulate the reduced M-channel function may also play a role.[23] In addition, gender was once reported to be a factor in intrafamilial variability.[47] Pt13 with c.365C>T in our study suffered more serious symptoms than those in previously reported patients. We speculate that the thyroid dysfunction of his mother during pregnancy might have played a role. In addition, epilepsy syndromes of Pt8 (c.1A>G), Pt10 (c.1678C>T), Pt11 (c.683A>G), Pt12 (c.833T>C), Pt13 (c.365C>T), and Pt18 (c.830C>T) in this study has enriched the phenotype caused by the mutation they carried.

Generally, even though the mutations in *KCNQ2* were confirmed to lead to different phenotypes including SeLE and DEE, there were some overlaps in the mutation types, mutation origin, mutation distribution in the gene or the protein, and the positive response to SCBs, and different phenotypes were observed in patients with the same mutation even in the same family, illuminating that assessment of the impact of *KCNQ2* pathogenic variants is complicated, and a long-term follow-up study is necessary.

Unfortunately, the number of patients in this study was not large enough and therefore some of the results need to be confirmed with future research. We also did not conduct Gesell Developmental Scale test for most of the patients to evaluate their developmental outcome. Genetically, we failed to get the parents' DNA sample in three patients, which prevented us from determining the source of the mutation of the patients. In future studies, we will continue to conduct clinical and genetic analyses of more patients with *KCNQ2* gene mutations to make a greater contribution to the understanding of the disease.

Clinical and genetic analysis of eighteen patients from South China were conducted and this study identified four novel mutations and discovered some distinct features, which was enabled a deeper understanding of the clinical features of *KCNQ2*-related disease and the difference between SeLE and DEE in South China.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.4593

Acknowledgments

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Ethical approval

Our study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (No. 2019-40101). We have obtained informed consent from the patients' parents for the study.

Author contribution

The authors confirm contribution to the paper as follows: funding support, genetic data analysis, and drafting the first manuscript: BC; study design, data confirmation, manuscript reviewing and edition: BP; data collection and clinical analysis: YT, XL, HZ, and WC; aEEG analysis and drafting the EEG part of the manuscript: XW; statistical analysis and making tables and figures: HS. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Hyaline fibromatosis syndrome: a rare, yet recognizable syndrome

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ABSTRACT

Background. Hyaline fibromatosis syndrome is a rare autosomal recessive disorder caused by *ANTXR2* pathogenic variants. The disorder is characterized by the deposition of amorphous hyaline material in connective tissues. The hallmarks of the disease are joint contractures, generalized skin stiffness, hyperpigmented papules over extensor surfaces of joints, fleshy perianal masses, severe diarrhea, and gingival hypertrophy. The severity of the disease varies and prognosis is poor. No specific treatment is yet available. Most patients with the severe form of the condition pass away before the second year of age. In this study, we describe the clinical and molecular findings of a cohort of seven hyaline fibromatosis syndrome patients who were diagnosed and followed up at a single tertiary reference center in Turkey.

Methods. Genomic DNA was extracted by standard salting out method from peripheric blood samples of three patients. In one patient DNA extraction was performed on pathology slides since peripheric blood DNA was not available. All coding exons of the *ANTXR2* were amplified and sequenced on ABI Prism 3500 Genetic Analyser.

Results. Sanger sequencing was performed in 3 patients and homozygous c.945T>G p.(Cys315Trp), c.1073dup p.(Ala359CysfsTer13), and c.1074del p.(Ala359HisfsTer50) variants were identified in *ANTXR2*. All patients passed away before the age of five years.

Conclusions. HFS is a rare, progressive disorder with a broad phenotypic spectrum. HFS can be recognized easily with distinctive clinical features. Nevertheless, it has poor prognosis with increased mortality due to severe clinical decompensation.

Key words: Hyaline Fibromatosis Syndrome, juvenile hyaline fibromatosis, infantile systemic hyalinosis, *ANTXR2*, *CMG2*.

Hyaline fibromatosis syndrome (HFS, OMIM #228600) is an autosomal recessive, rare condition, characterized by hyaline deposits in multiple organ systems.[1] The term encompasses two disorders previously known as juvenile hyaline fibromatosis and infantile systemic hyalinosis, the latter representing the severe form of the former.[2] HFS is caused by homozygous or compound heterozygous pathogenic variants in *ANTXR2* (anthrax toxin receptor-2, OMIM *608041), also known as *CMG2* (capillary morphogenesis protein gene-2) localized on chromosome 4q21.[1]

HFS presents in early infancy and clinical features include stiff skin, subcutaneous nodules, and progressive and painful joint contractures due to continuous accumulation of proteinaceous material in the dermis, as well as pearly papules in the face, neck, and perianal region, hyperpigmented patches over bony

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prominences of joints and gingival hypertrophy as the disease progresses. Mild dysmorphic facial features including coarse face, macrocephaly, frontal bossing, ear malformations, and depressed nasal bridge may be seen in patients with HFS, some of which appear gradually as the patients advance in age.[1-5] The disease has a variable course. While some patients present in early infancy with systemic and visceral involvement eventually leading to early demise in the majority, other patients present later in life with a milder form affecting only the face and digits. In this study, the clinic-pathological and molecular features of seven HFS patients are described.

Material and Methods

The study protocol was approved by Hacettepe University Ethics Committee, Ankara, Türkiye (GO 04-30/2022). The patients clinically diagnosed with HFS between the years 2000-2022 were included in the study. The clinical and radiographic findings were reviewed retrospectively and sequence analysis of *ANTXR2* was performed when DNA sample was available. Written informed consents were taken from the parents.

Of seven patients with a clinical diagnosis of HFS, DNA sample was available in four (Patient 1, 5, 6 and 7) of them in whom Sanger sequencing of *ANTXR2* was performed. Segregation analysis was performed in two families (Patient 6 and 7). Sanger sequencing images are shown in Supplementary Fig 1. Genomic DNA was extracted by standard salting out method from peripheral blood samples. DNA was extracted from formalin-fixed paraffin-embedded skin tissue in patients whose peripheral blood samples were not available. The coding sequence of *ANTXR2* including at least 20 bp of adjacent untranslated regions or intronic sequences was amplified from genomic DNA. PCR products were evaluated by agarose gel electrophoresis. After PCR purification, The BigDye Terminator v.3.1 Cycle Sequencing Kit was used for the chain termination method, and reaction products were applied to ABI Prism 3500 genetic analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The detected variants were classified according to the ACMG (American College of Medical Genetics and Genomics) criteria.[6]

For Patient 1, Sanger sequencing of *ANTXR2* did not reveal any pathogenic variant in the exons where PCR amplicons could be formed (except exon 8 and 9). A deletion encompassing exon 8 and 9 was suspected, but MLPA probe for *ANTXR2* was not available. We used control, maternal, paternal, and patient samples and a decreased amplicon was detected in parents compared to that of the control, suggesting the presence of a heterozygous deletion of these regions. So, we designed several primers to reveal the breakpoints of the deletion between the exon 7 and 10. The list of primers is shown in Supplementary Table I. First, intron 7 was divided into six parts. While the amplicons of the first five parts were formed, the sixth part was not observed in the patient. Later, two more primer pairs were designed in part 6, and they formed amplicons. Primers were also designed for a region within intron 9, and no amplicon was formed confirming that a part of intron 9 was also located within the deletion region. Agarose gel appearances of the PCR amplicons are shown in Fig 1. A deletion between intron 7, part 6, section 2 and exon 10 was suspected, but PCR amplicon could not be formed between them.

Microarray analysis was performed in one patient (Patient 1) to investigate copy number variations. Microarray analysis using Agilent Technologies 4x180K platform (Agilent Technologies, Santa Clara, CA, USA) was performed to detect exon and whole-gene deletions or duplications. Data analysis was done using Agilent scanner and Feature Extraction software. Results were processed by Agilent Cytogenomics software. Detected CNVs were analyzed using in-house data and public databases, such as DECIPHER, DGV, and ClinVar.

Table I. Clinical, radiological and genetic features of the seven patients.

Features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Summary
Gender	Male	Male	Male	Male	Male	Male	Male	7/7 male (100%)
Consanguineous parents	+ (Same village)	+	+	+	+ (Same village)	+	+	7/7 (100%)
Onset of symptoms	2 months	1 week	3 weeks	6 months	24 months	1.5 months	2 weeks	Median 1.5 months
Survival	16 months	18 months	9 months	18 months	54 months	18 months	10 months	Median 18 months
Weight	4.7 kg (-3.5 SD)	7.1 kg (-1.7 SD)	5.1 kg (-2.5 SD)	4.8 kg (-6.9 SD)		5.6 kg (-1.4 SD)	6.1 kg (-1.9 SD)	
Length		65 cm (-2.2 SD)	64 cm (-0.6 SD)	55 cm (-7.7 SD)	NR	60 cm (-1.2 SD)	61 cm (-2.5 SD)	
(Age at evaluation)	(6 months)	(8 months)	(5 months)	(17 months)		(4 months)	(6 months)	
Coarse face	-	-	-	+	-	-	+	2/7 (28.5%)
Gingival hypertrophy	+	+	-	+	+	-	+	5/7 (71.4%)
Thickened skin	+	+	+	-	+	+	+	6/7 (85.7%)
Hyperpigmented macules	+	+	+	+	+	+	+	7/7 (100%)
Pearly papules	+	+	+	+	+	+	+	7/7 (100%)
Perianal plaques	+	+	-	+	+	-	+	5/7 (71.4%)
Cutaneous nodules	+	+	+	+	+	-		5/7 (71.4%)
Joint contractures	+	+	+	+	+	+	+	7/7 (100%)
Chronic diarrhea	-	+	+	+	NR	+	+	5/6 (83.3%)
Excessive diaphoresis	+	+	-	-	-	-	-	2/7 (28.5%)
Osteopenia	+	NR	-	+	NR	+	-	3/5 (60%)
Periosteal reaction	+	NR	-	-	NR	+	+	3/5 (60%)
Skin biopsy	Amorphous eosinophilic hyaline accumulation	-	-	-	PAS+, amorphous eosinophilic hyaline accumulation	-	-	
ANTXR2								
NM_058172.6	Probable	DNA	DNA	DNA	13	11	13	
Exon	deletion of exon 8 and 9	was not available	was not available	was not available				
Nucleotide change					c.1074del	c.945T>G	c.1073dup	
Amino acid change					p.Ala359HisfsTer50	p.Cys315Trp	p.Ala359CysfsTer13	
ACMG classification					Pathogenic	VUS	Pathogenic	

cm: centimeter, kg: kilogramme, NR: not recorded, PAS: Periodic acid-Schiff, SD: standard deviation, VUS: variant of unknown significance.

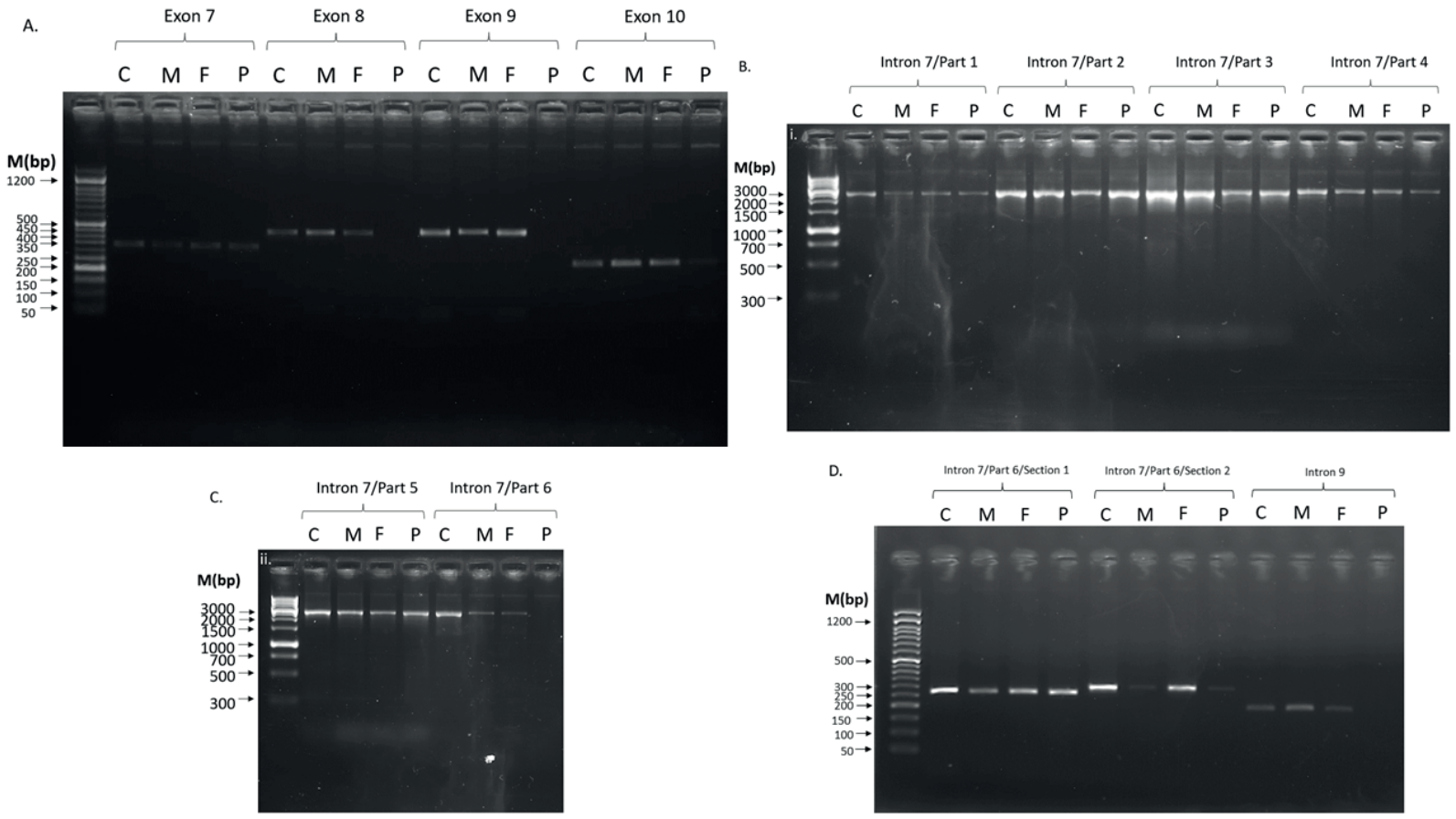

Fig. 1. For patient 1, while PCR amplicons of exon 7 and exon 10 occurred, PCR amplicons of exon 8 and exon 9 were not formed (A). Agarose gel appearance of the PCR reaction were performed with 6 pairs of primers generated from inside intron 7 to find the deletion breakpoint in both panels. PCR reaction was performed with primers designed for the first 4 parts of intron 7 using control, maternal, paternal, and patient samples. At the end of this PCR reaction, the PCR amplicon formed for all samples (B). PCR reaction was performed with primers designed for the last 2 parts of intron 7 using control, maternal, paternal, and patient samples, and PCR amplicons generated of the control, mother, and father samples for both parts (C). However, no amplicon formed in the PCR reaction was performed with the patient sample using the last primer pair. Agarose gel appearance of PCR amplicons was generated with primers designed from part 6 of intron 7 and inside intron 9 to get even closer to the deletion breakpoint. As a result of the PCR reactions performed using these 3 different primer pairs, PCR amplicons occurred in the control, maternal and paternal samples, while no PCR amplicon showed in the region covering intron 9 in the patient (D).

C: Control, M: Mother, F: Father, P: Patient

Results

In this study, all seven patients were male. The parents of the patients were either consanguineous or from the same village. The most common complaints of the patients on admission were progressive joint contractures and irritability, with symptoms manifesting as early as 1 week (median age: 1.5 month). Four patients had dysmorphic features including coarse face, wide and depressed nasal bridge, anteverted nares, long philtrum, thin lips or gingival hyperplasia. All patients exhibited joint contractures, hyperpigmented macules over extensor surfaces of joints and pearly papules in face, neck or perianal region. Thickened skin was detected in six patients and cutaneous nodules were detected in five patients. Two patients also experienced excessive diaphoresis, while chronic diarrhea was present in five patients. Radiographic findings revealed osteopenia in three patients and periosteal reaction in three patients. In Patient 3, intestinal wall thickness was detected on x-ray, which was further confirmed with ultrasound. Furthermore, skin biopsies from two patients showed Periodic acid-Schiff (PAS) positively stained amorphous eosinofilic hyaline accumulation in the papillary dermis. Unfortunately, all patients died before reaching the age of 5 (median age at death: 18 months), due to respiratory infections, septicemia or electrolyte imbalance.

Three variants in the *ANTXR2* gene were detected in three patients (Patient 5: c.1074del, p.Ala359HisfsTer50, Patient 6: c.945T>G, p.Cys315Trp and Paitent 7: c.1073dup, p.Ala359CysfsTer13). Sanger sequencing and microarray analysis did not reveal any pathogenic variant in Patient 1.

The clinical, radiographic, and genetic findings of patients with a clinical diagnosis of HFS are summarized in Table I. The patient photographs, radiographies and microscopic evaluation of the skin biopsy for patient 5 are shown in Fig 2, Fig 3, and Fig 4 respectively.

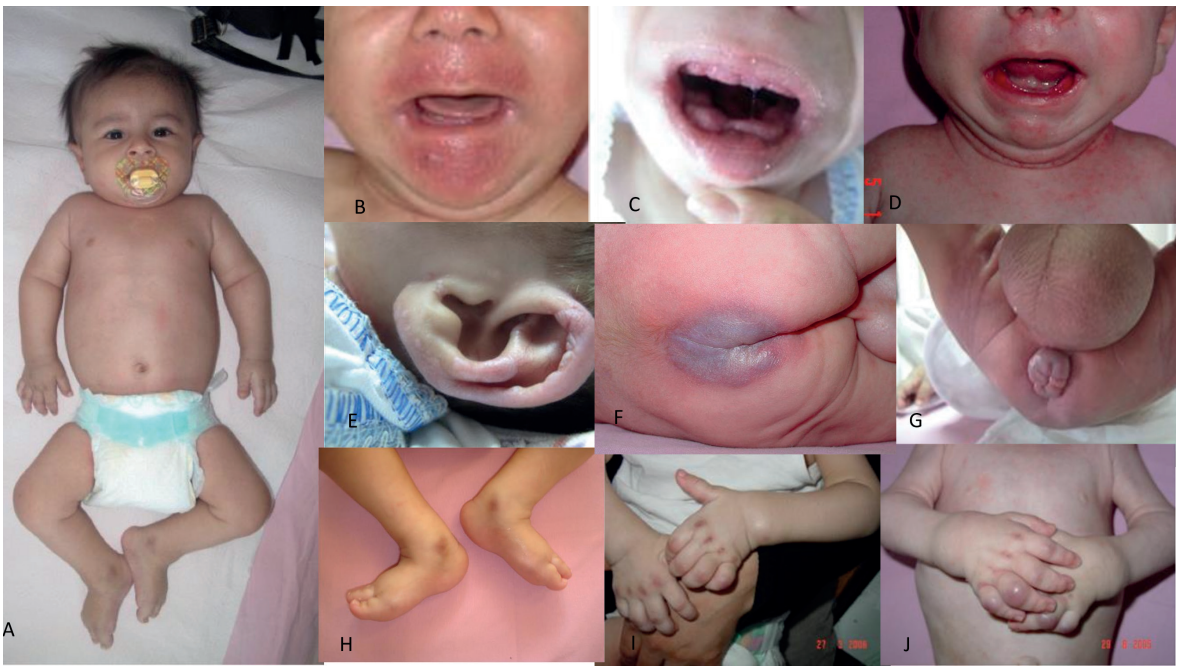

Fig. 2. Photographs of the six patients in this cohort are shown. Note the frog leg position of low extremities, patient 6 (A), perioral erythematous pearly lesions, patient 5 (B), gingival hypertrophy, patient 4 (C), pink pearly papules on perioral region and neck, patient 1 (D), the thickness of auricle, patient 4 (E), perianal purplish plaques, patient 1 (F), perianal fleshy lesion and hydrocele, patient 4 (G), purplish lesions on malleolar region, patient 3 (H), purplish lesions on dorsum of the metacarpophalangeal and interphalangeal joints, patient 2 and 1, respectively (I, J).

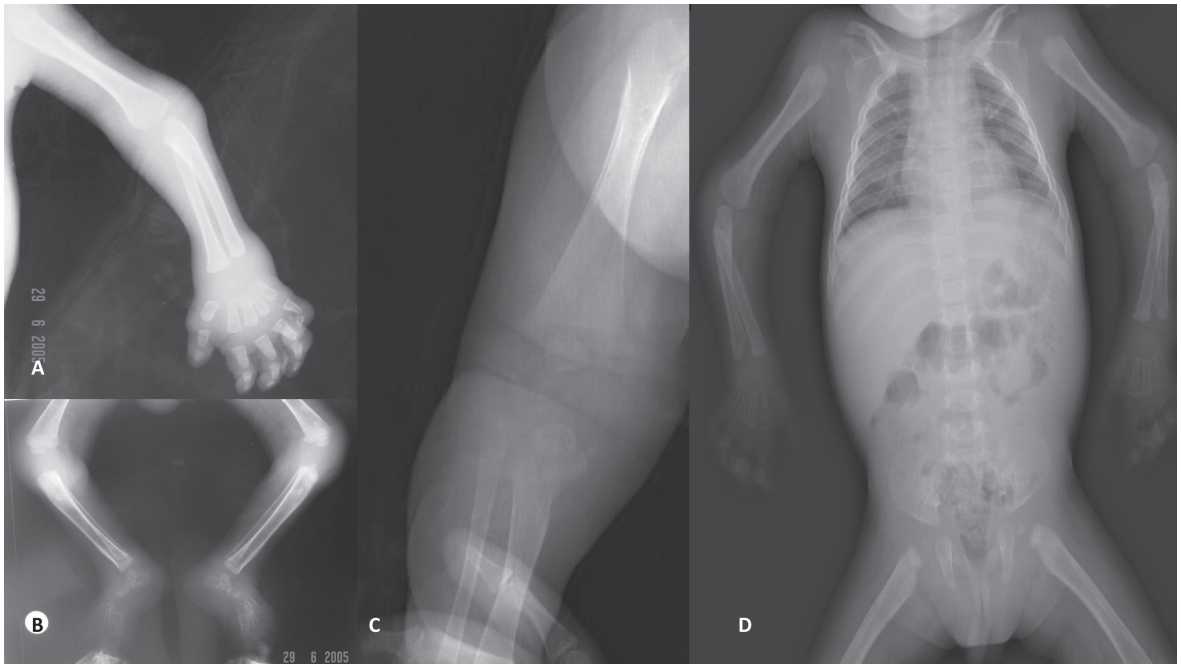

Fig. 3. X-ray images reveal osteopenia, camptodactyly, and periosteal reactions.
(A, B: patient 1, C: patient 6, D: patient 7).

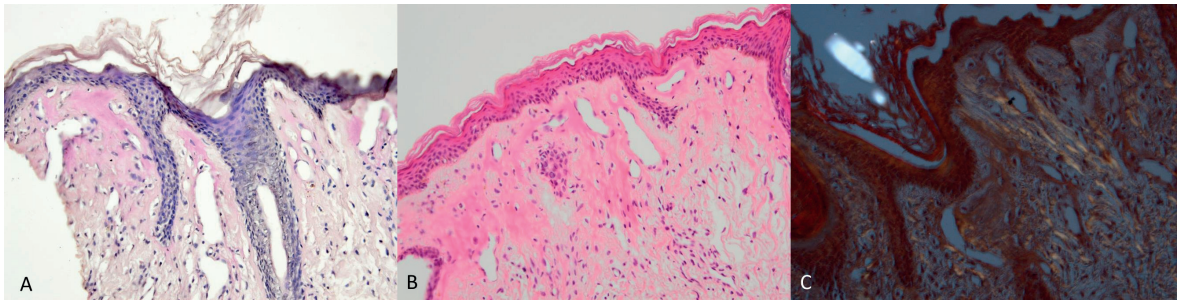

Fig. 4. Microscopic evaluation of skin biopsy for patient 5 is shown. A small amount of PAS-positive (B, 200x), hyalinized amorphous substance accumulated in the papillary dermis accompanied by few (myo) fibroblasts (A, H&E 200x). To exclude amyloid deposition, note the absence of green birefringence under polarized light in Congo red stain (C, Congo red under polarized light, 200x).

Discussion

HFS is a rare, progressive genetic disorder caused by loss-of-function mutations in *CMG2*, also known as *ANTXR2*.[4,7] The name, *ANTXR2*, stems from its well-documented role in binding and endocytosis of Bacillus antracis toxins.[8,9] To date, 116 HFS patients with a confirmed molecular diagnosis, have been reported.[10] In this report, we aimed to further expand the clinical and molecular spectrum of this rare disorder by presenting seven new patients and a new variant of *ANTXR2* gene.

The gene, *ANTXR2*, encodes a single-pass transmembrane protein and this protein contains an extracellular N terminal von Willebrand A domain, an immunoglobulin-like domain, a transmembrane domain, and a cytosolic tail.[11] von Willebrand A (vWA) domain binds to both laminin and collagen IV, suggesting that the protein interacts with

the extracellular matrix.[3,11] ANTXR2 is an important regulator of collagen VI homeostasis and mediates its intracellular degradation.[12] Accumulation of collagen VI is known to lead to fibrosis in tissues, and inactivating extracellular metalloproteases (MMP).[12] Why some body parts are particularly sensitive to the effects of loss-of-function mutations of *ANTXR2* is still unknown. However, it is suggested that in HFS patients, collagen IV accumulates in certain body parts and tissues and this accumulation leads to progressive loss of tissue integrity and function.[12,13]

HFS was first described by Murray et al.[14] in 1873 as molluscum fibrosum. Since then, many different terms have been used for this disorder. While early-onset severe forms were called 'infantile systemic hyalinosis' mild forms were called 'juvenile fibromatosis syndrome'. These two entities were suggested to represent different degrees of severity of a single clinical entity and therefore were merged under one name: "Hyaline fibromatosis syndrome".[2,15]

It is known that HFS is relatively common in the Turkish ethnicity.[2] Casas-Alba et al.[2] reported a cohort of 84 HFS patients, and Turkish ethnicity was the second most common ethnicity with a total 12 patients (%14.2), after Indians. HFS is equally seen in males and females.[3] Interestingly all seven patients in this study were male probably due to the small size of the patient cohort.

The diagnosis of HFS is based on the clinical features, biopsy findings, and genetic tests. Among the reported 116 patients, the most common clinical feature was joint contracture/stiffness with a frequency of 95%.[10] All seven patients in our cohort had joint contractures consistent with the literature.

Gingival hypertrophy is a common finding and thickened skin can also be seen.[1,3] All seven patients in this report exhibited different degrees of skin findings. Hyperpigmented macules and pearly papules were the most common cutaneous findings in our cohort and detected in all patients (100%), while they were observed in 35% and 37% of the other reported patients, respectively. The frequency of thickened skin was 85.7% and it was also higher than other reported patients (26%). Gingival hypertrophy and cutaneous nodules were detected with a similar frequency to the literature.[10]

Central nervous system involvement is not an expected finding, due to the absence of *ANTXR2* expression in the brain.[4] Intellectual disability has never been reported in HFS.[10] In patient 4, wide anterior fontanelle, preauricular skin tag, ventricular septal defect and cleft palate were detected, additionally. While wide fontanelle and skin tags are reported features in HFS, cleft palate and VSD have never been reported before.[1,16] These findings may be a rare feature of HFS or may be a part of an additional genetic disorder. Since we could not perform other comprehensive genetic tests, we cannot rule out another accompanying disorder.

Failure to thrive is a common finding among HFS patients. Some of them have severe protein-losing enteropathy.[1] Chronic diarrhea was present in 52% of the reported patients[10] and in the present study it was present in five (71%) of our patients. Intestinal biopsy showed villous atrophy, edema, hyalinosis, and lymphangiectasia in these patients.[1]

Biopsy findings are mainly non-specific but worthwhile in the correct clinical setting. Skin biopsy shows a PAS-positive amorphous eosinophilic substance thought to contain glycoproteins and collagen.[1] Hyaline deposits are also seen in many other tissues including the dermis, intestines, skeletal muscles, heart, trachea, esophagus, stomach, lymph nodes, adrenals, thyroid, spleen, and thymus.[4,17] It is important that specific biopsy findings may not be seen in the early stages of HFS[1,13] as was the case in the first biopsy of Patient 5 in the present study. In Patient 5, PAS+ hyaline accumulation was demonstrated in the second skin biopsy.

In 2009, Nofal et al.[3] developed a grading system for HFS and Denadai et al.[15] modified

this system in 2012. According to this modified grading system, HFS was classified into four grades: mild, moderate, severe, and lethal.[15] While grade 1 included skin and/or gingival involvement, grade 2 included joint and/or bone involvement, additionally. Internal organ involvement is seen in grade 3. Grade 4 is the most severe form and included organ failure and/or septicemia.[15] According to this grading system, six of the seven patients in this study are classified in group 4, and all passed away before the age of 2 years, due to organ failure or septicemia. Only one patient (Patient 5) survived until 56 months.

HFS has a poor prognosis and most of the patients with severe/lethal form die before the age of two, due to respiratory tract infections, septicemia, organ failure, or intractable protein-losing diarrhea.[3] Today, no specific treatment is available and the treatment is mainly palliative. Pain management and nutritional support are important for these patients. Physiotherapy may not be tolerated due to severe intractable pain. Surgical excision for gingival hypertrophy and large, ulcerated subcutaneous nodules may be performed. Genetic counseling is quite important and it should be explained to parents that this rare disorder has a 25% possibility of recurrence and preimplantation genetic diagnosis may be an option, particularly in countries with a high consanguineous marriage rate like Turkey.

In this study, we demonstrated four variants in the *ANTXR2* gene in four patients whose DNA samples were available. The two frameshift variants in exon13 (c.1073dup and c.1074del) were hotspots for HFS.[2,13] The other missense variant (c.945T>G) has also been previously reported.[13] We described the deletion of exons 8 and exon 9 in the *ANTXR2* gene. Although large deletion and entire exon deletion have previously been reported in HFS patients in the literature[10,15,18], a deletion involving exon 8 and exon 9 has never been reported. When removing the minimum deleted region according to the reference genome, approximately 2000 base amplicon would be obtained in PCR using intron 7 part 6 section 2 forward and exon 10 reverse primers, but we were unable to amplify this region that would show the deletion breakpoint. We suggest that this may be due to an additional structural variant (inversion, repeat sequence insertion, etc.). We could not delineate the breakpoints of a probable deletion in Patient 1 with the aid of other genetic tests (such as MLPA, qPCR, optical genome mapping or whole genome sequencing). In addition, we could not perform Sanger sequencing to three patients since their DNA samples were not available. These are the limitations of the present study.

In conclusion, HFS presents a significant challenge in clinical management, as there is currently no cure for this rare genetic disorder. The focus of treatment is primarily on providing supportive care and addressing the various medical complications that may arise. A multidisciplinary approach involving healthcare professionals from different specialties such as dermatology, orthopedics, algology and genetics is essential in order to effectively manage the diverse symptoms and complications associated with the condition. Symptomatic treatment may include surgical intervention for contractures, physical therapy to improve joint mobility, and management of skin lesions. Additionally, close monitoring and management of potential complications such as growth retardation, joint contractures, and respiratory issues are crucial in providing comprehensive care for individuals affected by HFS. While there is currently no definitive cure, ongoing research and advancements in medical knowledge offer hope for improved management and quality of life for patients with this condition.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.4511

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Ethical approval

The study protocol was approved by Hacettepe University Ethics Committee, Ankara, Türkiye (GO 04-30/2022).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TD, PÖŞK, GEU, KB; data collection: TD, HNG; analysis and interpretation of results: HNG, KK, ÖT, PÖŞK, GEU, KB; draft manuscript preparation: TD, HNG, KK, ÖT, PÖŞK, GEU, KB. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Congenital heart defects and postoperative follow-up of patients with Williams syndrome as a single center experience and review of the cases from Türkiye

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ABSTRACT

Background. Cardiovascular system involvement is quite common and the leading cause of morbidity and mortality in patients with Williams syndrome (WS), most of whom need surgery. The present study aimed to provide a detailed evaluation of the features of surgical procedures and outcomes of patients with WS given as single-center experience, and additionally to make a detailed review from Türkiye.

Materials and Methods. Thirty-five children with WS diagnosed between the years 1992 and 2021 were evaluated retrospectively including cardiovascular data, surgical treatment features, and outcomes. A total of six articles from Türkiye were evaluated.

Results. A total of 35 patients with Williams Syndrome (24 male) with a median age of cardiologic diagnosis of 6 months (range, 2 days-6 years) were evaluated. The cardiac defects of the patients with WS were found as supravalvular aortic stenosis (SVAS) (n=30, 85%) and peripheral pulmonary stenosis (PPS) (n=21, 65%). Additional cardiac anomalies were seen in 71% patients. The rate of SVAS and PPS surgery in all patients with WS was 77.1%. The median surgical age of the patients was 2.5 years (range, 7 months-15.5 years). No patients died due to surgery. But one patient died because of ventricular tachycardia due to anesthesia at the beginning of angiography. A total of 138 (63% male) patients with WS were evaluated from the articles published in Türkiye. Of 138 patients, 64.4% had SVAS, 52.1% had PPS, and 39.8% had additional cardiac anomaly. The median follow-up period ranged from 17 months to 18 years, and six (4.3%) patients died in the early postoperative period.

Conclusion. Cardiovascular system involvement is extremely common and is the leading cause of morbidity and mortality in patients with WS, often requiring surgical intervention. As seen in our study including 35 patients with WS and in publications from Türkiye, SVAS in patients with WS generally requires surgery, especially in the first year of life. PPS, on the other hand, requires surgery less frequently than SVAS, and pulmonary stenosis appears to decrease over time.

Key words: Williams syndrome, supravalvular aortic stenosis, peripheral pulmonary stenosis, congenital heart defects.

Williams syndrome (WS), also known as Williams-Beuren syndrome, is a rare genetic disease characterized by typical faces, growth delays, mild intellectual disability, extroverted personality, hypercalcemia, and congenital heart defects (CHD). Although the exact frequency of WS is unknown, it is estimated to be approximately 1 in 7500-15000.[1,2]

The diagnosis of WS is genetically confirmed through fluorescence in situ hybridization. Approximately 90% of patients with WS have a microdeletion of chromosome 7, which includes the elastin gene. Disruption of the elastin gene

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as a result of microdeletion on chromosome 7q11.23 causes a deficiency or abnormal accumulation of elastin during cardiovascular development during the intrauterine period. As a result of the abnormal accumulation of elastin, various CHDs occur in patients with WS.

The most common CHDs in WS are supravalvular aortic stenosis (SVAS), peripheral pulmonary stenosis (PPS), mitral valve prolapse (MVP), and coarctation of the aorta (CoA).[3] Surgery is usually required for these CHDs. In the literature, different surgical techniques such as single patch, Doty, and Brom techniques are used in patients when WS are reported; the type of surgical techniques to be chosen varies from patient to patient and from center to center. Although there are reports including large case series about the long-term follow-up of patients with WS who underwent surgery from different countries in the literature, reports including large series about surgery of patients with WS reported from our country are scant. Our clinic is a tertiary-level congenital heart surgery center, accordingly, we have many patients with WS who have undergone surgery and followed up in our clinic.

This study including 35 patients with WS, most of whom have had surgery, aimed to give detailed data including clinical features, cardiac defects, follow-up periods, and the surgical techniques performed as well as their outcomes as a single-center experience. Additionally, we conducted a literature review of patients with WS reported from Türkiye to date to give more detailed information.

Materials and Methods

Study population

We retrospectively reviewed the current records of 35 patients who were followed up with a diagnosis of WS at our hospital between the years 1992 and 2021. The study was approved by Başkent University Institutional Review Board (Project No: 63 KA20/231). The study protocol was conducted in accordance with the ethical guidelines of the 1975 Helsinki Declaration, as revised in 2008.

Data collection

The diagnosis of WS was confirmed through fluorescence in situ hybridization or clinical features by a medical geneticist. Cardiovascular data, patient histories, and physical characteristics were reviewed on the medical records of patients identified with WS. Congenital heart disease/abnormalities were diagnosed through echocardiography and cardiac catheterization. We analyzed the variables including age, gender, age at time of diagnosis, and degrees and types of cardiac abnormalities.

Echocardiographic evaluations were performed in accordance with the guidelines of the American Society of Echocardiography. In echocardiographic evaluations, a peak velocity (Vmax) ≥ 1.6 m/s or a peak pressure gradient ≥ 10 mm Hg between pre- and post-stenotic segments was considered vascular stenosis. SVAS and PPS were divided into three categories based on the peak systolic pressure gradient at the echocardiography: mild: Vmax 2 – 2.9m/s, moderate: Vmax 3.0 – 3.9 m/s, and severe: Vmax ≥4 m/s.[4]

Mitral valve prolapse was diagnosed as systolic displacement of one of the mitral leaflet from the plane of the mitral annulus to the left atrium over 2 mm in a parasternal long-axis view on echocardiography.

Surgical records were reviewed to determine anatomic measurements and interventions performed, time and method of surgery, treatment, and follow-up. During the follow-up period, postoperative complications, the development of restenosis, the need for reoperation, mortality rates in surgical patients, and the final status of SVAS and PPS values in non-surgical patients were evaluated.

Literature search

Williams syndrome research in cardiology and cardiovascular surgery from Türkiye was included in this study. The study was conducted by evaluating articles published online. PubMed, Google Academic, TR-Index, and Researchgate databases were searched between the years 1990 and 2022. The latest search was conducted on September 31st, 2022. The searches were performed using the keywords *Williams syndrome*, *Williams-Beuren syndrome*, *supravalvular aortic stenosis*, and *pulmonary arterial stenosis*, which were in either the title, abstract or keywords published in Turkish and English from Türkiye. Articles with incomplete and/or limited information about patients with WS and case reports were not included in the study.

Statistical analyses

Statistical analyses were performed using SPSS version 23.0 software package (SPSS, Inc., Chicago, IL, USA). Qualitative variables are shown as the number of cases (n) with percentages (%), and quantitative variables as mean ± standard deviation (SD). A normality distribution test (Shapiro-Wilk test) was performed for continuous data.

Results

A total of 35 patients with WS who were followed at our clinic were evaluated. The clinical and demographic features of the patients with WS are given in Table I. Twenty-four (68.6%) patients were male. The age of cardiologic diagnosis ranged between 2 days and 6 years (median; 6 months). The presenting symptoms of the patients were murmur (n=18, 51%), cyanosis (n=4,11.4%), and wheezing (n=1, 2.8%). The cardiac defects of the patients with WS were SVAS (n=30, 85%) (isolated SVAS in 12 patients) and PPS (n=21, 65%) (isolated PPS in three patients). Eighteen (51%) patients had both SVAS and PPS. Additional cardiac anomalies were seen in 25 (71%) patients. The most frequently seen additional cardiac abnormalities were coarctation of the aorta (CoA), which was seen in six patients, bicuspid aortic valve (BAV) in five patients, ventricular septal defect (VSD) in four patients, and MVP in three patients (Table I). Hypertension was seen in four (11.4%) patients and no renal pathology was found in these patients.

The rate of SVAS and PPS surgery in all patients with WS was 77.1% (27/35). The surgical age of patients with WS ranged from seven months to 15.5 years (median 2.5 years). The surgical features of patients with WS are given in Table I.

Supravalvular aortic stenosis (SVAS)

SVAS was detected in 30 (85%) of 35 patients with WS, 21 (70%) of whom had severe SVAS. All patients with severe SVAS underwent surgery. The median SVAS surgery age of our patients with WS was 42 months. Sixteen (76.1%) of 21 surgical patients with WS due to SVAS were aged younger than 5 years. Of the 21 patients, 10 patients received a single patch, and 11 received double patches (Doty technique) and isthmus expansion. One patient who received a single patch underwent surgery due to stenosis distal of the patch after SVAS surgery (Table I). According to the postoperative SVAS gradient at the last visit, 10 patients showed a decreasing trend in SVAS gradient (mean: 11 mm Hg) and eight patients showed an increasing trend (mean: 8 mm Hg). Nine patients with WS had moderate or mild SVAS, none of whom underwent surgery. The SVAS gradients of patients with mild or moderate SVAS showed a decreasing trend during the follow-up. At the last visit, there were no patients with severe SVAS in the surgical or non-surgical groups (Table I).

Peripheral pulmonary stenosis (PPS)

PPS was detected in 21 of 35 patients with WS. Of 21 patients, surgery was performed for PPS in six (26%) patients (Table I). The mean initial gradients were 53.1 ± 23.7 mm Hg. Pulmonary artery gradients were measured at an average of 13.4 ± 14 mmHg at the last follow-up visits.

Table I. Clinical, demographic characteristics, observed cardiac anomalies, outcome of cardiac surgery, and doppler changes of patients with Williams syndrome.

Clinical features	Number of patients (n)
Cardiologic diagnosis ages	Median: 6 months (2 days-6 years)
Gender	Male: 24(68 %), female: 11(32 %)
Cardiac anomalies	
SVAS	Total: 30 (85.7%) (isolated: 12 (34.2%))
PPS	Total: 21 (60%) (isolated: 3 (8.5%))
SVAS and PPS	18 (51.4%)
Additional cardiac anomalies	25 (71.4%) [CoA: 6 (17.1%), BAV: 5 (14.2%), VSD: 4 (11.4%), MVP: 3 (8.5%), PDA: 2 (5.6%), Hypoplasia of the aorta : 1 (2.8%), Left coronary hypoplasia: 1 (2.8%), Aortic insufficiency (moderate and severe): 3 (8.5%)]
Surgical treatment	
SVAS surgery (total)	21(60%)
Surgery technique of SVAS	Single-patch: 10 (28.5%), Doty technique (Y patch): 11 (31.4%)
Reoperation of SVAS	1 (2.8%)
PPS Surgery	6 (17.1%)
Additional surgery and angioplasty	13 (37.1%) (CoA repair: 5, VSD repair: 2, MVR: 2, AVR: 1, CoA balloon angioplasty: 3)
SVAS Gradient	
Initial	66.1±36 mmHg, mild and modere: 9 (25.7%), severe: 21 (60%)
Last visit	19±13 mmHg, mild and modere: 30 (85.7%), severe: 0
PPS Gradient	
Initial	53.1±23.7 mmHg, mild and modere: 10 (28.5%), severe: 11 (31.4%)
Last visit	13.4±14 mmHg, mild and modere: 20 (57.1%), severe: 1 (2.8%)
Follow-up	
Follow-up time (years)	Median: 5.6y (1.1y-15.6y)
Postoperative follow-up period (years)	Median: 3.6y (1y-14.4 y)
Exitus	1 (2.8%) during anesthesia

AVR: Aortic valve replacement, BAV: Bicuspit aortic valve, CoA: Coarctation of the aorta, MVP: Mitral valve prolapse, MVR: Mitral valve replacement, PDA: Patent ductus arteriosus, PPS: Peripheral pulmonary stenosis, SVAS: Supravalvular aortic stenosis, VSD: Ventricular septal defect

Pulmonary artery gradients were shown to decrease over time during follow-up in most of our patients with PPS. Since the PAP values of our 6 patients remained high even after the age of one year (PAP: 47-115 mmHg), surgery was performed on these patients, whose ages ranged from 12 months to 53 months, after the age of 1 year. Surgical intervention to the pulmonary artery was performed in 4 of 6 patients due to severe pulmonary artery branch stenosis with SVAS, 1 patient due to PPS and CoA, and 1 patient due to right pulmonary arterial stenosis with VSD closure. Surgical intervention in the pulmonary artery was in the form of widening the pulmonary artery with a patch.

Balloon pulmonary angioplasty was performed in one patient due to restenosis after surgery. Balloon angioplasty was performed in this patient at the age of 1 year due to supravalvular pulmonary stenosis, and the gradient, which was 63 mmHg before balloon angioplasty and regressed to 36 mmHg after balloon angioplasty. The pulmonary gradient of this patient, who had no additional problems during follow-up, was measured as 8 mmHg at the last follow-up.

Surgery of other cardiac anomalies

Surgery was performed for CoA in 5/35 (15.6%) patients. Balloon angioplasty for CoA was performed in one patient. During the follow-up, CoA balloon angioplasty was performed in three patients due to recoarctation of CoA. Mitral valve replacement was performed in two patients with mitral valve insufficiency due to MVP. Aortic valve replacement was performed in one patient with aortic valve insufficiency (Table I).

Follow-up period

The follow-up of the patients ranged from 1.1 to 15.6 (median: 5.6) years and postoperative follow-up periods were between 1 and 14.4 (median: 3.6) years. Forty-five percent of the patients with WS had a follow-up longer than 5 years. One patient died because of ventricular tachycardia due to anesthesia at the beginning of angiography but no additional complications during catheterization or surgery were seen.

Published articles from Türkiye

In the review of the literature, published articles related to WS from Türkiye were identified. After excluding case reports, 15 studies were evaluated. Seven SVAS articles were excluded due to incomplete and/or limited information on patients with WS. Two WS studies were excluded due to including the same patients from the same centers in previous years however the last publication of theirs was included in the study. Hence, a total of six articles were included in the study (Fig. 1).

A total of 138 patients (87 (63%) male) with WS from six articles were evaluated. Age at WS diagnosis ranged from 1 month to 14.5 years. Isolated SVAS was reported in 45 (32.6%) patients, isolated PPS in 27 (19.5%) patients, and 44 (31.8%) patients had both SVAS and PPS (Table II).

Supravalvular aortic stenosis (SVAS)

SVAS was seen in 89 (64.4%) patients with WS and 20 (22.4%) underwent surgery. In the 20 surgical patients, the Doty technique was performed in 11 patients, a single patch was used for two patients, and the Brom technique was used in one patient. There was insufficient information about the surgical procedures of SVAS performed on six patients. According to these reports, reoperation was not needed for any patient (Table II).

Peripheral pulmonary stenosis (PPS)

PPS was seen in 72 (52.1%) patients with WS, seven (9.7%) underwent surgery and nine (12.5%) patients underwent balloon angioplasty. Severe PPS was seen in 20 patients with WS at the initial visit and one patient with WS at the last visit. No patients needed reoperation.

The number of patients with additional cardiac anomalies was 55 (39.8%) and the most common additional cardiac anomalies were MVP (n=18, 13%), VSD (n=12, 8%), and CoA (n=6, 4.3%).

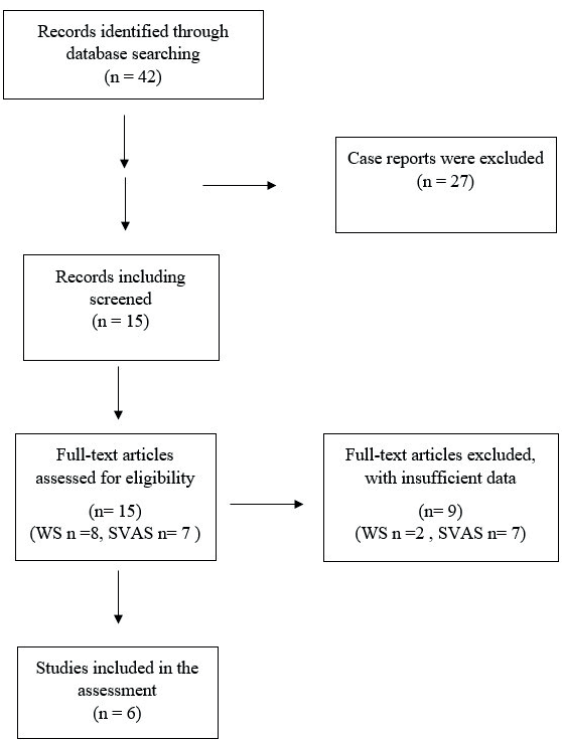

Fig. 1. Diagram of the strategy used for study inclusion.

SVAS: supravalvular aortic stenosis, WS: Williams syndrome.

Eight patients underwent surgery for additional cardiac anomalies.

The median follow-up times of articles ranged between 17 months and 18 years. During the follow-up, six (4.3%) patients died in the early postoperative period (Table II).

Discussion

In the present study, to provide a detailed evaluation of the surgery of patients with WS, we presented our single-center experiences and additionally conducted a detailed review from Türkiye. In our study, 35 patients with WS with follow-up periods between 1.1 and 15.6 years and postoperative follow-up periods between 1 and 14.4 years were evaluated. SVAS was detected in 85%, PPS in 65%, and additional cardiac anomalies were detected in 71% of WS patients. Surgery was performed in 77.1% of these patients. To our knowledge, the present study is the largest study to date regarding the surgical procedures of patients with WS from Türkiye.

Table II. Features of clinical, cardiac abnormalities, and surgery of patients with Williams syndrome in articles reported from Türkiye.

Author		Samanlı et al.[21]	Baykan et al.[15]	Ergul et al.[23]	Sarısoy et al.[18]	Gürses et al.[7]	Gürsoy et al.[19]	Total	Our study
Year of study		1997	2009	2012	2013	2018	2021		2023
Patient (n)		14	31	45	9	12	27	138	35
Sex (male/female)		8 (57%) / 6 (43%)	20 (64%) / 11 (36%)	27 (60%) / 18 (40%)	6 (66%) / 3 (34%)	8 (66%) / 4 (34%)	18 (66%) / 9 (34%)	87 (63%) / 51 (37%)	24 (68%) / 11 (32%)
Age at diagnosis		8y (2m-12y)	1m-13y	4.6y (3m-13y)	19m (3m-9y)	33m (1m-14.5y)	4y (1-8y)	1m-14.5y	6m (2d-6y)
Follow up (year)		3.7y±2.4y	41m ±26m	6.9y (6m-18y)	17m (2-74m)	33m±14m	5y (5-8y)	17m-18y	5.6y (1.1-15.6y)
Exitus		1 (during catheterization)	2 (early period)	2 (early period)	1 (early period)	-	-	6 (4.3%)	-
SVAS	Isolated	3 (21,4%)	12 (38%)	16 (35%)	2 (22%)	2 (16%)	10 (37%)	45 (32.6%)	12 (34.2%)
	Total	8 (57%)	20 (64%)	33 (73%)	7 (78%)	5 (41%)	16 (59.2)	89 (64.4%)	30 (85.7%)
SVAS surgery		1 (7.1%)	3 (9.6%)	8 (17.7%)	6 (66.6%)	-	2 (7.4%)	20 (14.4%)	21 (60%)
PPS	Isolated	6 (42%)	11 (35%)	2 (4.4%)	2 (22%)	3 (25%)	4 (14.8%)	27 (19.5%)	3 (8.5%)
	Total	11 (78%)	19 (61%)	19 (42%)	7 (78%)	6 (50%)	10 (37%)	72 (52.1%)	21 (60%)
PPS surgery			-	1 (2.2%)	6 (66.6%)	-		7 (5%)	6 (17.1%)
SVAS+PPS		5 (35.7%)	8 (38%)	17 (37%)	5 (55%)	3 (25%)	6 (22.2%)	44 (31.8%)	18 (51.4%)
Additional cardiac anomalies (Total)		2 (14%)	8 (38%)	28 (62%)	4 (44%)	5 (41%)	8 (29%)	55 (39.8%)	25 (71.4%)
MVP			4 (13%)	10 (22%)		1 (8%)	3 (11.1%)	18 (13%)	3 (8.5%)
VSD			2 (6.5%)	5 (11%)	1 (11%)	3 (25%)	1 (3.7%)	12 (8%)	4 (11.4%)
CoA		1 (7%)	1 (3.2%)	2 (4.4%)		1 (8%)		6 (4.3%)	6 (17.1%)
BAV			1 (3.2%)	1 (2.2%)			1 (3.7%)	3 (2.1%)	5 (14.2%)
Coronary artery anomaly		1 (7%)						2 (0.7%)	1 (2.8%)
Hypoplasia of the aorta		1 (7%)		4 (8,8%)				5 (3.6%)	1 (2.8%)
Others		2 (14%)		7 (15,5%)	3 (33%)	3 (25%)	3 (11.1%)	18 (13%)	5 (14.2%)

BAV: Bicuspit aortic valve, CoA: Coarctation of the aorta, MVP: Mitral valve prolapse, PDA: Patent ductus arteriosus, PPS: Peripheral pulmonary stenosis, SVAS: Supravalvular aortic stenosis, VSD: Ventricular septal defect.

In the literature, the rate of cardiovascular system involvement in patients with WS was reported as 60-80% and was the leading cause of morbidity and mortality.[5,6] From Türkiye, Dolunay et al. reported the frequency of cardiovascular system involvement in patients with WS as 83%.[7] The most common cardiac abnormalities in patients with WS are reported as SVAS, PPS, and CoA.[3] In addition, other congenital heart diseases such as BAV, MVP, VSD, atrial septal defect, and atrioventricular septal defect have been reported in patients with WS.[2] A detailed cardiac examination should be performed on every patient suspected of having WS because it is associated with high rates of CHDs and cardiac involvement. In addition, it should be recommended to evaluate the possibility of WS in children with anomalies such as SVAS and PPS, which were frequently seen in patients with WS.

Supravalvular aortic stenosis is the most common cardiac anomaly in patients with WS, reported with a frequency of 37-75%. Patients with severe SVAS often require surgery in the first years of their lives.[5] Patients with SVAS with moderate and mild stenosis are generally diagnosed later, and it has been reported that stenosis gradients tend to decrease over time, and the need for surgery decreases in advanced ages. When we evaluated the articles in Türkiye, 72.4% of patients with WS were diagnosed as having SVAS, and surgery was performed on 22.4% of them. In the present study, SVAS was found in 85% of patients with WS, of which 70% underwent surgery due to severe stenosis. The high SVAS incidence in patients with WS and high surgical rate due to severe SVAS in our study is related to our center being a tertiary pediatric cardiovascular surgery center. The median SVAS surgical age of our patients with WS was 42 months. We had only five patients who underwent surgery for SVAS aged over 5 years. The stenosis gradients of our patients with moderate or mild SVAS also showed a tendency to decrease over time, in line with the literature.

The first technique used in SVAS surgery is the single-patch aortoplasty technique described by McGoon et al.[8] Later, Doty et al. used an inverted Y-shaped patch that extended towards the non-coronary and right coronary sinus.[9] It has been reported that the Doty technique is more successful than McGoon et al.'s method and the frequency of reoperation is less. Brom et al. improved the technique by patching all three sinuses.[10] Myers sliding aortoplasty is an autologous technique where the aorta is reconstructed without the need for prosthetic material.[11] The Doty technique and Brom technique are seen as more preferred in SVAS surgery throughout recently published articles from Türkiye.[12] Koçyildirim et al. found no significant difference between the two techniques in an SVAS article, in which single-patch and three-patch techniques were compared.[13] Bostan et al. reported that reoperation was required for a patient who underwent single-patch surgery during the follow-up period.[14] We used the single-patch aortoplasty technique in 10 patients with WS in our clinic in the first years, but the Doty technique was preferred in 11 WS patients in procedures performed after the year 2010. The SVAS type of most patients in our study (71%) was the hourglass-type. In this cohort, only one patient required reoperation. This patient was our patient with hourglass-type SVAS who was operated on with a single patch and developed stenosis distal to the patch. We observed no restenosis in our patients who underwent SVAS surgery performed with the Doty technique.

Pulmonary artery stenosis is the second most common cardiac anomaly, reported with a frequency of 37-75% in patients with WS. It is more common in the first year of life. Many studies have reported that pulmonary arterial stenosis improves over its natural course in time and PPS needs less surgery than SVAS in patients with WS.[6] In the articles reported in Türkiye, PPS is the second most common cardiac anomaly in patients with WS, and surgery was performed in 12.5% of patients with PPS. Baykan et al. performed balloon

valvuloplasty on seven of 10 WS patients with PPS and observed no restenosis.[15] In our study, PPS was the second most common cardiac anomaly, shown in 60% of patients with WS, and surgery was required in only 28.5% of patients. Similar to the literature, it has been reported in articles reported from Türkiye that PPS gradients tend to decrease at follow-up, which we also observed in our study. The upper limits of the incidence of pulmonary arterial stenosis in patients with WS in the present study compared to the literature in general might be due to the fact that our clinic is known as a tertiary cardiac surgery center and most of our patients are referred to our clinic from different centers from Türkiye due to their severe clinical findings.

In the literature, the most common cardiac anomalies other than SVAS and PPS in patients with WS have been reported as CoA, MVP, and VSD.[3,5,16] In the articles reported from Türkiye, the rate of other cardiac anomalies is 39.8%, and MVP, VSD, and CoA are the most common, respectively. In our study, CoA, bicuspid aortic valve, and VSD were the most common cardiac anomalies in patients with WS, respectively. In our study, CoA was the third most common cardiac anomaly, which also required surgery in patients with WS.

MVP in patients with WS was reported at different rates in previous studies. In the study of Collins et al., which included 270 patients with WS, MVP was reported as third among the cardiac anomalies at a rate of 15%.[6] Cha et al. reported the frequency of MVP in patients with WS as 22.5% (18/80) in their study, six of whom underwent mitral surgery.[17] When the studies from Türkiye are evaluated, the frequency of MVP is seen as 12% but no patient who was operated on for MVP was reported.[18,19] In our study, unlike the literature, MVP was shown only in three (8.5%) patients, two of whom required mitral valve replacement due to severe mitral insufficiency.

Coronary artery anomalies in WS are usually seen as coronary artery stenosis, especially ostial stenosis in patients with WS.[3] Coronary ostial narrowing may be present, leading to myocardial ischemia and a higher risk for sudden cardiac death. Coronary artery anomalies have been reported in approximately 5% of patients with WS.[5] Collins et al. reported 6% coronary artery anomalies in their study. By contrast, Cha et al. observed no coronary artery anomalies in their study of around 80 patients.[6,17] In studies from Türkiye, Akkaya et al. reported a coronary anomaly in one patient in their study and Samanlı et al. reported a single coronary root with tetralogy of Fallot, pulmonary artery hypoplasia, and PPS.[20,21] Ergul et al. detected coronary artery anomalies in 26% (10/38) of patients with WS using computed tomography angiography studies in patients with WS.[22] In our study, we described left coronary hypoplasia in a 5-year-old patient with WS who was diagnosed as having SVAS and PPS and underwent surgery for SVAS. No coronary artery intervention was performed in this patient and we observed no additional problems at follow-up.

Elastin haploinsufficiency results in systemic arteriopathy in Williams syndrome. Hypertension is a clinical condition reported in 3-30% of patients with WS and increases with older age. Although the cause is mostly unknown, diffuse aortic stenosis, CoA, and renal artery stenosis have been reported as causes of hypertension.[3,5] Two of the studies conducted in Türkiye mentioned the hypertension rate in patients with WS as 22% (Ergul et al.) and 12.9% (Baykan et al.).[15,23] The incidence of hypertension in our cohort was found as 11.4% (4 patients). However, renal or thoracic artery stenosis or any renal pathology was not detected in any of these four patients. While, among our other patients in whom hypertension was not observed, we had four patients in whom we detected anomalies on urinary USG. (Right renal agenesis in two patients, double ureter in one patient, nephrocalcinosis in one patient). Due to the high incidence of renal and urinary abnormalities in Williams syndrome, performing a urinary and renal analysis and sonographic evaluation of the patients is recommended.

Cardiovascular abnormalities are the leading cause of morbidity and mortality in WS patients. In the literature, it was reported that anesthesia-related rhythm problems, sudden cardiac arrest, and postoperative complications were seen as causes of early mortality in patients with WS. The rate of early mortality in patients with WS has been reported as 2-11% in the literature. In the cohort study of Wessel et al., the incidence of sudden death in patients with WS was reported as 1/1000 patient-years.[24] In the articles reported from Türkiye, a total of 6(4.3%) patients died, five were in the early postoperative period, one was during catheterization. We saw no early mortality postoperatively in our series. One of our patients with WS died of cardiac arrest after ventricular tachycardia during anesthesia before catheterization. Careful preoperative preparation of patients with WS should be undertaken before every elective procedure and physicians should be aware of anesthesia-related rhythm problems.

The follow-up period is important for WS. The present study included 29 years of experience with a median of 5.6 years of follow-up; 45% of our patients were followed for more than 5 years after surgery. Our patients with WS with mild and moderate SVAS did not require surgery during follow-up. Similarly, in the articles from Türkiye, it was reported that there was a tendency for SVAS and PPS gradients to decrease in their mean follow-up period ranging from 17 months to 6.9 years. According to literature about follow-up of WS patients, it is recommended that the cardiology evaluation for elastin arteriopathy occurs at least annually until age five years and every two to three years thereafter; and renal and bladder ultrasound examination every ten years.[25] In patients with WS, surgery may be required for supravalvar aortic or pulmonary artery stenosis, mitral valve insufficiency, and/or renal artery stenosis.[25] Anesthesia consultation and electrocardiogram are recommended prior to sedation and surgical procedures.[25] At our clinic, follow-up periods of WS patients, and outpatient clinic controls with echocardiography and electrocardiography are performed, usually at between 6-month and one-year postoperative intervals, however neonatal period and patients with serious cardiac findings may be evaluated more frequently.

Limitations

Our study has some important limitations due to its retrospective design and long study period. Since Williams syndrome is a very rare syndrome, we conducted a scan of the first patient diagnosed with Williams syndrome in our clinic, and this period consisted of 29 years. First of all, some changes were observed in the diagnosis, surgical method, and treatment applied to patients with WS during the study period. Second, we could not make a clear assessment of the frequency of cardiac anomalies and the incidence of WS in our patients with WS. Thirdly, the follow-up period we stated in our study is the period from the diagnosis of the cardiac disease of the patients to the last check-up at our clinic. Due to our clinic being a reference tertiary center in the diagnosis and surgical treatment of congenital heart diseases, we have patients from many parts of Türkiye, and some of these patients applied only for evaluation of surgery. For this reason, unfortunately, the follow-up period of some of our patients in our clinic was very short. This caused the average follow-up period of the current study to be short, and the rate of surgical intervention in our patients to be higher compared to the literature. Additionally, since we are a pediatric cardiology clinic, our patients over the age of 18 are transferred to adult cardiology clinics. Lastly, in this study, we only evaluated original articles about patients with WS from Türkiye to provide homogeneity; however, because WS is a rare syndrome, publications from Türkiye have usually been reported as case reports in the literature. Larger series and longer follow-up times are needed to give more detailed information about children with WS.

Conclusion

Cardiovascular system involvement is quite common and the leading cause of morbidity and mortality in patients with WS, frequently requiring surgery. The present study provides a detailed evaluation of the features of surgical procedures and outcomes of patients with WS through our single-center experiences and provides a detailed review from Türkiye. SVAS usually requires surgery, especially in the first year of life, PPS requires less surgery than SVAS, and pulmonary stenosis is seen to decrease over time. Aortic hypoplasia causes stenosis distal to the patch after SVAS surgery and increases the frequency of reoperations. The Doty technique and isthmus dilation process decrease the frequency of this stenosis. To the best of our knowledge, the present study is the largest reported to date about surgical procedures of patients with WS from Türkiye.

Ethical approval

The study was approved by Başkent University Institutional Review Board (Project No: KA20/231).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AO, NKT; data collection: AO, İE, MÖ; analysis and interpretation of results: AO, İE, MÖ, BV; draft manuscript preparation: AO, İE, BV, SA, NKT. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Exercise capacity and muscle strength in patients who have undergone the Fontan procedure: a retrospective follow-up study

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ABSTRACT

Background. Due to their relationship with clinical progression, follow-up of exercise capacity and muscle strength is important for optimal disease management in patients who have undergone the Fontan procedure. We aimed to retrospectively analyze exercise capacity and muscle strength trajectory over approximately 2 years.

Methods. Exercise capacity was assessed using an exercise stress test with the modified Bruce protocol on a treadmill, hand grip and knee extensor strength using a hand dynamometer, and body composition using a bioelectrical impedance device. Exercise capacity, muscle strength, and body composition follow-up data recorded between 2020 and 2022 were compared.

Results. Fifteen patients [median age from 17 (first assessment) to 18 years (last assessment), 5 females)] with a 20-month median follow-up time were analyzed retrospectively. There was an increase in weight, height, body mass index, and body fat weight ($p<0.05$). There was a tendency for increased handgrip strength (%) ($p=0.069$), but no significant difference was observed in the knee extensor strength of patients during the follow-up period ($p>0.05$). The changes in heart rate (HR) and oxygen saturation were higher in the last test than in the first test ($p<0.05$). Maximum HR (HRmax), % predicted HRmax and HR reserve recorded during the test and HR 1 minute after the test were similar between the first and last tests ($p>0.05$).

Conclusions. After 20 months of follow-up, exercise capacity and muscle strength did not decline; instead, the body mass index and fat weight increased. Patients who have undergone the Fontan procedure may not be experiencing a decline in exercise capacity and muscle strength over relatively short time periods during childhood, adolescence, and early adulthood.

Key words: Fontan procedure, Fontan circulation, exercise capacity, muscle strength, follow-up, exercise testing.

The importance of rehabilitative management in patients who have undergone the Fontan procedure (hereafter referred to as "Fontan patients" for brevity) has increased due to their longer life expectancy thanks to medical advancements. Exercise is crucial for providing better disease management as a diagnostic and therapeutic tool in the Fontan population.[1] Decreased exercise capacity is common in Fontan patients due to a variety of potential factors such as primary diagnosis, Fontan physiology, and pre-and postoperative clinical characteristics. The four main factors implicated in decreased exercise capacity in the Fontan circulation are preload failure, chronotropic incompetency, restrictive lung problems, and underlying and residual lesions

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such as no subpulmonary ventricle, valve disease, and systemic ventricular dysfunction. These are usually caused by the absence of a subpulmonary pump and high systemic venous pressure, which are specific to the physiology of the Fontan circulation.[2]

In addition, peripheral muscle strength is an extracardiac factor that can affect exercise capacity in Fontan patients.[1-3] It is accepted that the pump effect created by peripheral muscles is important in increasing preload and therefore stroke volume during exercise in Fontan patients without a subpulmonary ventricle.[4] Fontan-associated myopenia and increased adiposity are common in Fontan patients. Furthermore, increased adiposity is linked to an increased risk of Fontan failure or moderate-to-severe ventricular dysfunction.[5] Secondary sarcopenia is an adjunctive predictor of hospitalization and so may lead to a negative prognosis in this population.[6] Turquetto et al.[7] reported that reduced skeletal muscle strength is associated with suboptimal peripheral blood supply and diminished exercise capacity in Fontan patients.

The relationship between exercise capacity and clinical status has previously been reported in Fontan patients. The positive exercise capacity trajectory during childhood was linked to improved clinical status in adulthood and a significant decrease in exercise performance was associated with worse functional status in these patients.[8,9] Therefore, follow-up data obtained via periodic assessments may be of prognostic importance for clinical status and medical and rehabilitative treatment in Fontan patients. The present study aimed to retrospectively analyze the exercise capacity and muscle strength follow-up data of Fontan patients obtained between 2020 and 2022.

Materıals and Methods

Study design

This observational follow-up study was carried out retrospectively using the data of Fontan patients who were followed up in Hacettepe University's Faculty of Medicine, Department of Pediatric Cardiology and who were referred to Hacettepe University's Faculty of Physical Therapy and Rehabilitation, Cardiopulmonary Rehabilitation Unit in 2020-2022. The Hacettepe University Non-interventional Clinical Research Ethics Board approved the study (06.09.2022, GO22/786). The parental written informed consent form and the child informed assent form for patients aged 8-17 years as well as the written informed consent form for patients aged 18 years and older were waived.

Procedure and participants

The medical information of each patient was obtained from clinical records. Echocardiographic measurements were performed by a pediatric cardiologist and the exercise stress test, peripheral muscle strength test, and body composition test by a pediatric cardiologist and specialist physiotherapist during routine controls in the Department of Pediatric Cardiology and Cardiopulmonary Rehabilitation Unit. All assessments were performed by the same pediatric cardiologist and specialist physiotherapist during the follow-up.

After a detailed clinical examination of each patient, consisting of a physical examination and imaging and laboratory tests, a pediatric cardiologist confirmed their eligibility to participate in the tests. The patients meeting the following criteria were included: those between the ages of 8 and 50 years, who had undergone the Fontan operation, who were clinically stable, and had no change in ongoing medication therapy adversely affecting clinical stability in the last one year, and had at least one year follow-up after the operation. Patients unable to cooperate in the assessments and/or who had a neurological, musculoskeletal, or cognitive disorder that would affect the tests were excluded.

Assessments

Clinical characteristics

The medical and clinical information including age, sex, weight, height, age at Fontan operation, initial diagnosis, technique of Fontan surgery, functional ventricle, and fenestration was obtained from a physical examination and clinical records. The hemoglobin (Hb) and brain natriuretic peptide (BNP) values of the patients at the last outpatient visits were also recorded. Echocardiographic measurements were performed using a GE Vivid E9 with XDclear (GE Healthcare, Horten, Norway) or EPIQ CVx (Philips Medical Systems, Andover, MA, USA) according to the standards of the American Society of Echocardiography.[10] The main diagnoses of the Fontan patients and other echocardiographic findings (functional ventricular structure, Fontan pulsatility, and presence of fenestration) were noted from the echocardiography reports.

Exercise capacity

Exercise capacity was assessed with an exercise stress test using the modified Bruce protocol on a treadmill ergometer (GE T2100-ST2, GE HealthCare, WI, USA).[11-13] Heart rate (HR) and electrocardiography (ECG) changes were monitored closely during the rest, test, and recovery periods. Every 3 min during the test and in the recovery period, perceived dyspnea, leg fatigue, and fatigue were assessed using the modified Borg scale; systolic (SBP) and diastolic blood pressure (DBP) and oxygen saturation (SpO_2) were measured with a sphygmomanometer and pulse oximeter, respectively. Additionally, maximum HR (HRmax), heart rate reserve (HRR), and HR 1 min immediately after cessation of exercise (HRR_1_min) were recorded. The exercise stress test was terminated with the exhaustion of the participant, indicated by symptoms such as dyspnea, leg fatigue, fatigue, chest pain, or HR reaching 90% of predicted HRmax.[11]

Peripheral muscle strength

Knee extensor muscle strength was measured using a hand dynamometer (Lafayette Instrument Inc., Lafayette, IN, USA). The physiotherapist held the dynamometer while the patient applied maximum force to it for 5 s and the muscle force was gradually overcome; the test ended at the moment the extremity gave way. The highest value of the dominant leg achieved in three trials was used as the strength measure and recorded in kilograms.[14] Predicted percentages of normal values for children and adults were calculated by age and sex and were used in the interpretation of the measurements.[15,16]

Hand grip strength was measured using a hand dynamometer (Jamar, Sammons Preston, Rolyon, Bolingbrook, IL, USA). Measurements were obtained using standard procedures on the right and left sides, with the arms at the side of the trunk, the elbow in a 90-degree flexion position, and the forearm and wrist in a neutral position.[17] The highest value of three measurements of the dominant hand was accepted as hand grip strength and recorded in kilograms.[18] Predicted percentages of normal values for children and adults were calculated by age and sex and were used in the interpretation of the measurements.[19,20]

Body composition

Body composition was evaluated by bioelectrical impedance analysis. A Tanita Body Fat Analyzer (model TBF 300, Tokyo, Japan) was used to analyze body mass index (BMI), body fat, and lean body weight.[21] The fat-free mass index (FFMI) was recorded. It was calculated by dividing the lean body mass by the height in meters squared (kg/m^2).[22]

Statistical analysis

The statistical analyses were performed using SPSS v. 26.0 (IBM, Chicago, IL, USA). Descriptive data were presented as median (interquartile range) or number (percent) as appropriate.

Wilcoxon's test or Fisher's exact test was used to compare the follow-up data between the first and last assessment. A p-value of less than 0.05 was considered statistically significant.

Results

Follow-up data on exercise capacity, muscle strength, and body composition recorded between 2020 (first assessment) and 2022 (last assessment) from 15 Fontan patients (5 women) with a median follow-up period of 20 months were retrospectively analyzed. The clinical characteristics are presented in Table I. Although there was a difference in the age of the patients at the first and last assessment (p<0.001), there was no change in the age groups (<12 years, 12-17 years, ≥18 years) (p=0.805).

There was an increase in the weight, height, BMI, and body fat weight of the patients during the follow-up (p<0.05, Table II). Although it was not statistically significant, there was an increase in other body composition parameters as well (p>0.05, Table II). Furthermore, the median BMI z-score for pediatric Fontan patients (<18 years) was -0.59 (1.26) at the first assessment and -0.52 (1.50) at the last assessment (p=0.499). The BMI z-scores of pediatric Fontan patients ranged between -2SD and 2SD. Only one patient had a

Table I. Clinical characteristics (n=15).

Characteristics			
Age at Fontan operation (years)	7.00 (7.00)		NA
Follow-up time (months)	20 (7)		NA
Sex (female/male)	5 (34) / 10 (66)		NA
Main diagnosis			
Tricuspid atresia	6 (40)		NA
Double inlet right ventricle	2 (14)		NA
Double outlet right ventricle	2 (14)		NA
Ventricular septal defect	4 (26)		NA
Hypoplastic left heart syndrome	1 (6)		NA
Functional ventricular structure			
Left ventricle	9 (60)		NA
Right ventricle	5 (33)		NA
Undetermined	1 (7)		NA
Technique of Fontan surgery			
Lateral tunnel	1 (7)		NA
Extracardiac conduit	5 (33)		NA
Intra-/extracardiac conduit	9 (60)		NA
Pulsatility (yes/no)	5 (33) / 10 (67)		NA
Fenestrated Fontan	9 (60)		NA
	First assessment	Last assessment	p*
Age (years)	17.00 (9)	18.00 (9)	<0.001*[a]
<12 years	4 (26.70)	3 (20)	
12-17 years	5 (33.30)	4 (26.70)	<0.001*[b]
≥18 years	6 (40.00)	8 (53.30)	
Hemoglobin (g/dl)	14.75 (2.72)	14.95 (2.05)	0.551[a]
BNP (ng/L)	10.50 (12.75)	10.50 (7.15)	0.859[a]

BNP: brain natriuretic peptide, Data are presented as n (%) or median (interquartile range).
[a]Wilcoxon's test was performed. [b]Fisher's exact test was performed.
*Statistical significance is set at p<0.05.

Table II. Body composition and peripheral skeletal muscle strength (n=15).

Characteristics	First assessment (<18 years, n=9)	Last assessment (<18 years, n=7)	p*,[a]
Body composition			
Weight (kg)	51.30 (32.30)	53.40 (35.00)	0.001*
	(24.00 - 78.50)	(28.00 - 86.00)	
<18 years	38.70 (26.13)	46.00 (29.00)	0.018*
	(24.00 - 57.50)	(28.00 - 64.00)	
z-score	-0.63 (1.40)	-.56 (1.62)	0.091
	(-1.42 - 0.37)	(-1.57 - 0.44)	
Height (cm)	159.00 (31.00)	165.00 (22.00)	0.005*
	(122.00 - 181.00)	(130.00 - 182.00)	
<18 years	150.00 (24.50)	153.00 (28.00)	0.027*
	(122.00 - 165.00)	(130.00 - 170.00)	
z-score	-0.38 (1.47)	-0.56 (1.94)	0.499
	(-2.07 - 0.51)	(-2.15 - 0.61)	
Body mass index (kg/m^2)	19.70 (5.60)	19.85 (7.41)	0.008*
	(13.50 - 26.00)	(15.48 - 26.40)	
<18 years	17.40 (4.76)	19.11 (5.58)	0.066
	(13.50 - 20.30)	(15.48 - 22.77)	
z-score	-0.59 (1.26)	-0.52 (1.50)	0.499
	(-1.62 - 0.18)	(-1.50 - 0.46)	
Body fat weight (kg)	8.02 (8.09)	9.85 (16.39)	0.008*
	(1.20 - 18.80)	(1.40 - 21.58)	
Lean body weight (kg)	41.10 (28.18)	46.02 (21.56)	0.112
	(22.32 - 65.40)	(26.60 - 64.42)	
Fat free mass index (kg/m^2)	15.57 (5.36)	16.80 (3.74)	0.733
	(7.07 - 19.96)	(13.79 - 19.75)	
Peripheral skeletal muscle strength			
Handgrip strength (kg)	30.00 (22.00)	32.00 (18.00)	0.003*
	(12.00 - 50.00)	(16.00 - 52.00)	
Handgrip strength (% predicted)	83.91 (28.88)	96.47 (10.30)	0.069
	(60.04 - 146.34)	(71.29 - 145.45)	
Knee extensors strength (kg)	27.60 (11.60)	27.15 (11.45)	0.256
	(12.00 - 39.30)	(16.00 - 41.20)	
Knee extensors strength (% predicted)	65.49 (34.89)	58.78 (40.91)	0.372
	(45.43 - 136.44)	(47.64 - 115.31)	

Data are presented as median (interquartile range) (min-max).
[a]Wilcoxon's test was performed. *Statistical significance is set at p<0.05.

height z-score below -2SD at both the first and last assessments (Table II).

The data for follow-up peripheral muscle strength are presented in Table II. At the end of follow-up, there was an increase in handgrip strength in kilograms (p<0.05) and a tendency for increased handgrip strength as a percentage (p=0.069). However, no significant difference was observed in knee extensor muscle strength (p>0.05).

The changes in vital signs including HR, SpO_2, SBP/DBP, dyspnea, leg fatigue, and fatigue

Table III. Exercise stress test findings (n=15).

Characteristics	First assessment	Last assessment	p*,a
Test protocol			
Time (s)	822.00 (183.25)	806.50 (148.00)	0.570
	(586.00 - 918.00)	(636.00 - 895.00)	
Speed (km/h)	5.50 (1.50)	5.50 (0)	0.680
	(4.00 - 6.80)	(4.00 - 5.50)	
Grade (%)	14.00 (2.00)	14.00 (0)	0.655
	(12.00 - 16.00)	(12.00 - 14.00)	
Vital signs (Δ)			
HR (bpm)	33.50 (24.25)	42.00 (19.00)	0.008*
	(1.00 - 81.00)	(22.00 - 68.00)	
SpO_2 (%)	-1.00 (5.00)	-3.00 (4.00)	0.020*
	(-6.00 - 2.00)	(-7.00 - 0.00)	
SBP (mmHg)	17.50 (16.50)	26.00 (8.50)	0.065
	(-8.00 - 50.00)	(10.00 - 35.00)	
DBP (mmHg)	10.00 (15.50)	15.00 (16.50)	0.069
	(-9.00 - 30.00)	(2.00 - 30.00)	
Dyspnea (MBS)	2.00 (3.00)	3.25 (3.25)	0.345
	(0.00 - 7.00)	(-0.50 - 5.00)	
Leg fatigue (MBS)	2.00 (2.25)	3.00 (3.38)	0.138
	(0.50 - 5.00)	(0.00 - 7.00)	
Fatigue (MBS)	2.00 (3.25)	1.75 (3.75)	0.550
	(0.00 - 7.00)	(0.00 - 7.00)	
Other cardiac parameters			
HRmax (bpm)	178.50 (16.50)	181.00 (14.50)	0.432
	(141.00 - 200.00)	(123.00 - 196.00)	
HRmax (% predicted)	89.50 (8.50)	89.50 (7.00)	0.775
	(67.00 - 96.00)	(60.00 - 95.00)	
HRR (bpm)	21.00 (20.00)	23.00 (21.00)	0.198
	(7.00 - 70.00)	(15.00 - 90.00)	
HRR_1_min (bpm)	26.50 (16.25)	29.50 (13.50)	0.167
	(3.00 - 45.00)	(6.00 - 39.00)	

DBP: diastolic blood pressure, MBS: modified Borg scale, HR: heart rate, HRR: heart rate reserve, HRR_1_min: heart rate recovery 1 minute after test, SBP: systolic blood pressure, SpO_2: oxygen saturation, .
Data are presented as median (interquartile range) (min-max).
[a]Wilcoxon's test was performed. *Statistical significance is set at p<0.05.

recorded immediately before the test and during active recovery are presented in Table III. The changes in vital signs were similar except for HR and SpO_2 between the first and last assessments (p>0.05, Table III). The changes in HR and SpO_2 were higher in the last test than in the first test (p<0.05). Desaturation (a reduction in SpO_2≥4%) was recorded in four (26%) and six patients (40%) in the first test and last test, respectively (p=0.700). Additionally, during both tests, the patients reached a median of 89% and 90% predicted HRmax, respectively. HRmax, % predicted HRmax, HRR, and HRR_1_min were similar between the first and last tests (p>0.05).

All patients performed and completed the first and last exercise stress test with no adverse events in median times of 13 min 52 s and 13 min 29 s, respectively (p>0.05, Table III). The median peak speed was 5.50 km per hour and the grade was 14% in both exercise stress tests (p>0.05, Table III).

Discussion

After 20 months of follow-up, exercise capacity was similar, but HR increase and oxygen desaturation were higher compared to a similar exercise test workload in the last assessment in Fontan patients. In addition to the increase in body composition parameters including height, body weight, body fat weight, and BMI, there was also a tendency for higher handgrip strength in the Fontan patients. Exercise capacity and muscle strength may not decline in these patients over relatively short time periods during childhood, adolescence, and early adulthood.

A serial evaluation of exercise capacity may be important and predictive in terms of determining the current clinical status, prognosis, and adverse cardiovascular events in Fontan patients. In follow-up studies, decreased exercise capacity over time was generally reported in Fontan patients. In a previous study, the 5-year risk of adverse cardiovascular events was 30%, and a decrease of ≥3% in predicted peak oxygen consumption (VO_2) points/year was associated with an increase in this risk.[23] Ohuchi et al.[8] reported that a positive exercise capacity trajectory in childhood can lead to better exercise capacity, hemodynamics, pulmonary function, hepatorenal function, and body composition in adulthood in Fontan patients. In their well-designed 12-year follow-up study, Atz et al.[9] reported that a significant decrease in exercise performance was associated with worse functional status.

In contrast to the studies mentioned, there was no decrease in the exercise capacity of the patients during the follow-up period in our study. In general, exercise capacity increases during physical growth and reaches its peak in early adulthood.[24,25] Therefore, this growth may lead to confusion in the interpretation of exercise capacity. In previous studies, a cardiopulmonary exercise test (CPET) was used in the assessment of exercise capacity and longer-term follow-up was performed.[8,9,23] In our study, a CPET could not be conducted and follow-up was shorter than that in other studies. In addition, due to the heterogeneity in the test protocol and ergometer, the exercise test workloads achieved during the follow-up tests could not be compared with our results.[8,9,23] However, changes in HR increase and oxygen saturation were higher at the same workload compared to the first test. Desaturation is considered present when the SpO_2 ≥4% decreases from baseline to peak exercise.[26] In the current study, four patients had desaturation in the first assessment and six patients in the last assessment. These results show that the cardiorespiratory responses demanded by the test workload should also be greater in the last assessment. This may indicate a regression in terms of cardiorespiratory fitness in Fontan patients.

According to some studies, serum BNP levels may affect exercise capacity in patients with congenital heart disease. In their study, which excluded Fontan patients, Gavotto et al.[27] reported that BNP levels were related to impaired exercise capacity in patients with systemic right ventricles. In another study that included Fontan patients, serum BNP concentrations did not correlate with CPET parameters in adult patients with single or systemic right ventricles.[28] However, the baseline age and serum BNP concentration of the patients in these studies were higher than those in our study. As previously reported, BNP concentrations were higher in patients in more advanced New York Heart Association (NYHA) functional classes[29]; however, data on the patients' NYHA classes were not collected in our study. During the follow-up, the patients' serum BNP concentration and exercise test

parameters were mostly similar, but correlation analysis between these parameters was not performed. For this reason, the relationship between BNP concentration and exercise capacity could not be interpreted based on our results.

In the literature, peripheral muscle strength follow-up data are rarely collected in Fontan patients. Despite a tendency for higher hand grip strength in patients during follow-up, there was no significant change in lower extremity peripheral muscle strength in our study. This result may cause a positive impression, although, considering the age of the patients at the first and last assessments, it is highly likely that preserved hang grip strength was due to physical growth. In a previous study, significant myopenia, especially in the lower extremities, and increased adiposity were reported in young Fontan patients.[30] Therefore, myopenia, especially in the legs, may be associated with the absence of any increase in knee extensor muscle strength, while hand grip strength tends to increase during the physical growth process in our Fontan population. Sandberg et al.[31] reported that isometric knee extension muscle strength was impaired in adolescents (13-18 years) but not in younger children (6-12 years) who had undergone the Fontan procedure compared to the controls. In another study involving 6- to 12-year-old Fontan patients, it was reported that hand grip strength was similar to that in healthy individuals.[32]

In the current study, more than half of the population (60%) was under 18 years old; of these, 4 patients (26.70%) were below 12 years old and 5 (33.30%) were 12-17 years old. The remaining 40% of the population were 18 years old or older. As a result, in our study, both knee extension muscle strength and hand grip strength did not show a decline over approximately 2 years; in fact, there was a tendency towards increased hand grip strength. Possible explanations for this observation include the significant proportion of our patients being children and adolescents, the somatic growth process, and the promoting physical activity from the beginning of follow-up for patients. Additionally, the approximately 2-year follow-up period may be considered relatively short to observe the onset of extracardiac factors deteriorating in Fontan patients in this age group. Furthermore, in our study, subgroup analysis based on age could not be conducted due to the small sample size; if it could have been done, it could have provided more detailed clinical information about the deterioration period of muscle strength and exercise capacity in patients. Indeed, studies suggest that the decrease in muscle strength may be slower in childhood and more pronounced towards adulthood in Fontan patients.[31,32] Therefore, in future studies, longer-term analyses incorporating subgroup analyses based on age are crucial for a more comprehensive understanding of critical turnover points in terms of muscle strength and exercise capacity and determining the appropriate timing and guidance for rehabilitation in Fontan patients.

Bioelectrical impedance research reveals significant myopenia and increased adiposity in Fontan patients.[30] Greater muscle mass was correlated with better exercise capacity in this population. Lower skeletal muscle mass and a higher body fat percentage were seen in patients who experienced a late complication after their Fontan procedure.[30] Tran et al.[33] reported that Fontan patients had an unfavorable body composition profile, marked by impairments in skeletal muscle mass and a tendency for adiposity, and reduced exercise capacity and oxygen pulse (a proxy for stroke volume) were related to low skeletal muscle mass.

In the present study, the BMI of the Fontan patients were within the normal range during follow-up. Tran et al.[33] reported that BMI is a poor indicator of adiposity, and dual-energy X-ray absorptiometry often reveals excessive adiposity in Fontan patients with normal BMI. However, the Fontan patients had a significant increase in body fat weight over time in the present study. This increase was not related

to any decrease in muscle strength or exercise capacity. Previously, Longmuir et al.[32] found that Fontan patients aged 6-12 years had strength and a good body composition similar to those of healthy controls. Since the same age group constituted a significant part of our sample size, body composition results may have been better preserved in Fontan patients. Therefore, the possible worsening of body composition and consequent reduced exercise capacity and muscle strength outcomes during follow-up in patients with Fontan could not be discussed in the present study.

The most important limitation of our study was the absence of a CPET in the assessment of exercise capacity. There was no change in cardiac parameters such as HR peak, HRR, or HRR at 1 min, but a CPET can provide information about changes in metabolic, ventilatory, or gas exchange parameters during follow-up, as well as cardiovascular parameters. Another significant limitation was the small sample size. With a larger sample size, subgroup analyses based on age, surgical type, or other key clinical characteristics could be performed. This would enable more detailed clinical information to be obtained regarding muscle strength and exercise capacity prognosis in the follow-up of Fontan patients. Furthermore, the presence of follow-up data from a control group would be helpful to understand the potential impacts of physical growth on the exercise capacity and muscle strength of Fontan patients in comparison with control subjects.

At the end of 20 months of follow-up, the exercise capacity and muscle strength did not regress; rather, an increase in body fat weight was observed in the Fontan patients. There may not be a decline in exercise capacity and muscle strength in these patients over relatively short periods during childhood, adolescence, and early adulthood. Long-term follow-up studies with subgroups are crucial for gaining a clearer understanding of critical turnover points for extracardiac factors. Consequently, they can aid in determining the optimal timing of cardiac rehabilitation and guiding it in Fontan patients.

Ethical approval

The Hacettepe University Non-interventional Clinical Research Ethics Board approved this study (06.09.2022, GO22/786). The parental written informed consent form and the child informed assent form for patients aged 8-17 years, as well as the written informed consent form for patients aged 18 years and older were waived.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HT, MS, HHA, SNS; data collection: HT, SNS; analysis and interpretation of results: HT, MS, HHA, SNS; draft manuscript preparation: HT, MS, HHA, Dİİ, NVY, ECK, TK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The effect of 3D modeling on family quality of life, surgical success, and patient outcomes in congenital heart diseases: objectives and design of a randomized controlled trial

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ABSTRACT

Background. Understanding the severity of the disease from the parents' perspective can lead to better patient outcomes, improving both the child's health-related quality of life and the family's quality of life. The implementation of 3-dimensional (3D) modeling technology in care is critical from a translational science perspective.

Aim. The purpose of this study is to determine the effect of 3D modeling on family quality of life, surgical success, and patient outcomes in congenital heart diseases. Additionally, we aim to identify challenges and potential solutions related to this innovative technology.

Methods. The study is a two-group pretest-posttest randomized controlled trial protocol. The sample size is 15 in the experimental group and 15 in the control group. The experimental group's heart models will be made from their own computed tomography (CT) images and printed using a 3D printer. The experimental group will receive surgical simulation and preoperative parent education with their 3D heart model. The control group will receive the same parent education using the standard anatomical model. Both groups will complete the Sociodemographic Information Form, the Surgical Simulation Evaluation Form - Part I-II, and the Pediatric Quality of Life Inventory (PedsQL) Family Impacts Module. The primary outcome of the research is the average PedsQL Family Impacts Module score. Secondary outcome measurement includes surgical success and patient outcomes. Separate analyses will be conducted for each outcome and compared between the intervention and control groups.

Conclusions. Anomalies that can be clearly understood by parents according to the actual size and dimensions of the child's heart will affect the preoperative preparation of the surgical procedure and the recovery rate in the postoperative period.

Key words: congenital heart diseases, 3D printing, heart modeling, family quality of life, surgical simulation.

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Congenital heart disease (CHD) is a group of disorders characterized by a series of individual structural cardiac pathologies that are usually rare and involve a wide range of anatomical defects and complexity.[1] The degree to which the defect deviates from normal anatomy determines the severity of symptoms.[1-3] Globally, between 0.8% and 1.2% of all live births are affected by CHD.[4-6] Approximately 25% of children born with CHD require open-heart surgery due to defects.[7] Reliable diagnostic methods provide much better treatment options, leading to a significant reduction in mortality. According to Sachdeva et al.[8], in recent years, 3D modeling and printing technologies have been added to imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), and echocardiography (ECHO). There are differences and benefits among the different imaging methods.[9] The most recent and rapidly developing method among them is 3D printing technologies.

It is stated that beneficial results can be obtained for multiple purposes, from planning and simulation before the definitive surgical procedure to patient-specific preoperative education.[10-12] There are several techniques for modeling organs using 3D printing technology, which has developed rapidly in recent years. For the heart, two types of cardiac modeling are performed. These are filled solid models (blood pool) and hollow models. The hollow models are obtained from signals sent in a way that limits the perimeter of the area where the blood pool is located. These models are printed as a cross-section and show the intracardiac structure.[10] However, technically, the peak heart rate of children is higher than that of adults, so the images may lose clarity, require more time and effort, and may not be as useful. Solid models have filled models of the atria and ventricles. They are typically modeled and printed from contrast-enhanced CT or MR images. Extracardiac structures are very guiding in surgical simulation with easier and faster modeling than intracardiac structures.[13] Recurrent pulmonary artery stenosis and aortic coarctation can be successfully treated, and positive outcomes can be achieved with fast and patient-specific models.[10,14] The operating time of surgically simulated patients is reduced, and procedures can be completed with less cost and fewer complications.[15]

Targeted patient outcomes can be achieved by managing a multidisciplinary team that includes the patient and family and by using surgical simulation.[10,16,17] In life-threatening diseases such as CHD, diagnosis, treatment, and surgical planning are long-term processes. This process causes serious psychological distress in parents, such as post-traumatic stress disorder.[18] Parental/caregiver stress increases, and the family's quality of life deteriorates, especially when the surgical procedure and interventions are not clearly understood.[19] This situation negatively affects the postoperative recovery process of patients.[20,21] A surgical procedure performed with good technique followed by poor postoperative management renders many interventions ineffective.[10,22-24] Understanding the severity of the disease from the perspective of the parents can improve both the health-related quality of life of the child[25] and the quality of life of the family, leading to more positive patient outcomes.[26] Patient-specific modeling using 3D printing technology with images obtained through traditional methods is believed to eliminate all of these issues.

Aims

The aims of this study are to:

Aim 1: Compare the effect of education with a patient-specific 3D heart model on family quality of life with standard education/care.

Aim 2: Plan and simulate the surgical procedure with a patient-specific 3D heart model to obtain the surgeon's opinions about this method and to evaluate patient outcomes.

Hypotheses

In this study, the control group will receive only standard education/care, while the experimental

group will receive 3D model-based education in addition to standard education/care. The hypotheses are as follows:

H1: The family quality of life of the experimental group receiving preoperative education with a patient-specific 3D heart model will be higher than that of the control group.

H2: Surgical simulation using a patient-specific 3D heart model will positively affect surgical success and patient outcomes (operation time, hospital stay, intensive care unit stay, and complications) compared to standard care.

Methods

Study design

The study is a two-group pretest-posttest randomized controlled trial. The design and all phases of the randomized controlled trial (RCT) were based on the Consolidated Standards of Reporting Trials (CONSORT) 2017 recommendations and guidelines.[27] In addition, the recommendations for Standard Protocol Items Recommendations for Interventional Trials (SPIRIT 2013) checklist were followed.[28] This trial study was registered to clinicaltrials.gov in May 2023 (NCT05852106).

Study setting and population

This study will take place in two hospitals affiliated with a foundation in Istanbul, Turkey. The imaging used in this study will be reviewed by a specialist radiology doctor at another hospital belonging to the same foundation after patients have been examined and diagnosed by a pediatric cardiology specialist. The study will recruit patients whose images meet the inclusion and exclusion criteria. The study will be explained to the legal guardian/parent of the identified volunteer patients, and those who wish to participate and give written consent will be included. Recruitment will continue until the target sample size is reached.

Sample and recruitment

The study population consists of pediatric patients (0-18 years old) admitted to a foundation hospital in Istanbul within the last year. In the sample calculation of the study, the effect factor value reported by Ladak et al. was used.[29] In this study, in which they compared the health-related quality of life of children and adolescents with CHD by revealing the difference between siblings, they stated that the most significant difference was in the total quality of life score (effect size: -1.35). The sample of this study was analyzed using the G*Power (v3.1.7) statistical program with an effect size of -1.35 and 95% power (1- β), and alpha was set at 0.05. The sample size was calculated as 13 experimental, 13 control, and 26 children in total. Considering there may be a 20% loss in the study, it was decided that the total sample size should be 30 to reach the target sample size.

Randomization and blinding

For participants who agree to participate in the trial, a randomization list generated by a computerized random number generator (https://www.randomizer.org/) will be used to determine which group of patients will be enrolled. This process will be carried out by a person independent of the investigators. Due to the feasibility of this trial, it is not possible to blind the investigator and patients to group allocation. However, an independent statistician will evaluate the data. In this way, there will be no bias due to the coding of the experimental and control groups as A and B.

Eligibility criteria

The inclusion criteria are as follows: The participant has a CHD between the ages of 0-18 years, the congenital defect has extracardiac structure malformations (this is because the modeling to be done before the operation is done in a shorter time, and it is desired to be trained for preoperative education). Hollow modeling requires more detailed technique and time.[13]

In addition, the difficulty of 3D printing the hollow model made in the pilot study was also effective in this decision being a candidate for elective surgery, having a contrast-enhanced CT image taken during and before the patient's routine diagnostic procedure outside the scope of the study, having at least 15 days between the imaging and the surgical procedure plan, and having the parents/legal guardian give permission to participate in the study were the inclusion criteria of the study.

The exclusion criteria for the study are as follows: Patients who do not require CT for diagnosis or treatment (no patient will undergo CT imaging within the scope of the study unless necessary for this study only), emergency surgical procedures, heart defects involving intracardiac structures (atrial septal defect, ventricular septal defect, tetralogy of Fallot), additional anomalies/syndromes, chronic diseases (such as neurodevelopmental disorders, bleeding disorders, asthma or Down syndrome), history of cardiac arrest, contrast agent reflection in the images, image quality preventing modeling.

Data collection

This study aims to determine the eligibility of children scheduled for pediatric cardiac surgery to participate in the research. Inclusion criteria will be assessed through personal interviews, where informed consent and socio-demographic data will be collected. Following this, participants will be randomly assigned to either the experimental or control group in a 1:1 ratio using a computer-generated list created through randomization. An independent researcher, who is unaware of the group allocation, will evaluate the quality of life of the family (PedsQL) and the surgical simulation (Surgical Simulation Evaluation Form - Part I) one week before the operation. The surgical simulation and parental education will be completed on the same day. Both groups will complete the Surgical Simulation Evaluation Form - Part II on postoperative day 0. On postoperative day 15, both groups will complete the Surgical Simulation Evaluation Form - Part II and the Pediatric Quality of Life Inventory (PedsQL) Family Impacts Module (Table 1).

Table I. Participant timeline.

	Study period									
	Intervention group					Control group				
Time of evolution	Pre-op	One week before admission	Post Operative Day 0 to Discharge		Post Operative Day 15	Pre-op	One week before admission	Post Operative Day 0 to Discharge		Post Operative Day 15
Determination of patients with inclusion and exclusion criteria	x					**x**				
Written informed consent and randomization	x					**x**				
Sociodemographic Information Form		x					**x**			
Surgical simulation		x								
Surgical Simulation Evaluation Form- Part I		x								
Education with 3D heart modelling		x								
Standard education with booklet		x					**x**			
Surgical Simulation Evaluation Form- Part II				x	x				**x**	**x**
PedsQL	x				x	**x**				**x**

PedsQL: Pediatric Quality of Life Inventory, Post Op. Post Operative , Pre Op. Pre Operative

Data collection tools

Socio-demographic information form

This form was prepared in light of the literature and includes descriptive data about the child and family (child's age, height, weight, mother's age and educational status, etc.) and consists of 11 questions in total.[22-24,29]

Surgical Simulation Evaluation Form Part I and Part II

Part I includes eight questions that the surgeon should answer, such as the surgeon's age, professional experience, the effect of the 3D model on defining pathologic findings, the effect of the 3D model on surgical technique, and the strengths and weaknesses of the 3D model. Part II consists of seven questions about surgical success (the effect of the 3D model on surgical operation time and complications) and patient outcomes (operation time, hospital stay, intensive care unit stay, need for repeat operation, unusual complications) of 3D model-based interventions. The surgeon's opinions about the model will also be obtained through this form.[30-33]

Pediatric Quality of Life Inventory (PedsQL) Family Impacts Module

The Turkish validity and reliability study of this scale, first developed by Varni et al.[34], was conducted and published by Gürkan et al.[35] In this methodological study, 201 parents were included. As a result of the study, the internal consistency coefficient was 0.926. The data on the sub-dimensions and Cronbach alpha values of this scale, which has eight sub-dimensions in total, are as follows: physical (0.85), emotional (0.83), social (0.82), cognitive (0.86), communication (0.51), anxiety (0.79) activities of daily living (0.89), family relationships (0.95). A total score of 0.92 was reported. In addition, Cronbach alpha values for all subscales were also included in the original study. As a result of this study, Cronbach alpha values for all subdimensions will be reported when the measurements are completed. The scale consists of 36 questions in total and is a 5-point Likert type. Scale items are scored as never (0), rarely (1), sometimes (2), often (3), and always (4). Items are reverse scored when converted into scores (0= 100; 1 = 75; 2 = 50; 3 = 25; 4 = 0). The scale does not have a cut-off point. A high score indicates a good family quality of life functioning, while a low score indicates a negative family quality of life. Within the scope of this study, a comparison between mean scores will be made.

Data management

The researchers will ensure that all data are accurately and completely coded and consistently entered into the statistical analysis software. The researchers will keep all original documents, including medical records, questionnaires, informed consent forms, and other relevant records obtained during the study, confidential. Data will be kept for five years after the end of the study.

Preliminary study

For the study design, a preliminary study was conducted using retrospective data from the pediatric cardiologist's previously diagnosed and treated patients. Available CT images were analyzed, and images without contrast enhancement were selected and used for the pilot study. Four patient datasets were analyzed, and model testing was performed, representing approximately 10% of the total sample size of 30 patients. One intracardiac abnormality was detected in the patient data acquired for the first model. It was decided to exclude patients with intracardiac malformations from the study as the image of the heart filling with blood was perceived as a solid structure. This could not be excluded from the model until the time of the operation. In the second set of images, a coarctation of the aorta was examined and it was found that there was a stent in the vessel as a result of the completed procedure that there was a contrast reflection, and that the reflections were reflected in the modeling as a vascular heart structure, and it was not successful.

Therefore, it was decided to examine the CT scans of the patients beforehand and to include patients with appropriate CT scans in the study. Finally, the aortic coarctation of the two patients were modeled quite clearly, and the printing was completed. One of the two aortic coarctations was printed piece by piece and painted with different colors to provide more effective education. However, the compatibility and integration of the structures with each other were problematic due to the material used (Fig. 1). Therefore, the second successful model, aortic coarctation, was printed as a whole-heart model (Fig. 2).

Reconstruction of a 3D heart model

The modeling work for models to be obtained with 3D printing technology involved three steps. The first step was to model the CT images in a virtual simulation environment using computer-assisted programs.[36] Since the image resolution increases as slice thickness decreases, virtual modeling should be done on the CT images with a slice thickness of less than 1mm. In this study, the CT scans were obtained using standard techniques at 100 kVp and 256 mAs, with a slice thickness of 0.5 mm and a resolution of 512 × 512 pixels (voxels approximately 0.7 × 0.7 × 0.5 mm3).

Materialize Mimics software program was used to model the patient's heart and major mediastinal vessels. The modeling was done semi-automatically. The patient's CT images were transferred to the modeling software. The first step in the modeling process is masking. Masking with the most accurate Hounsefield unit (HU) is one of the most important factors for successful modeling. For different body tissues, different HU units are defined. For soft tissues, an average of +100 to +300 has been specified, while for harder tissues such as bone, up to +1900 HU has been reported.[37-39] For this study, the average minimum value for masking ventricles and large vessels was set between

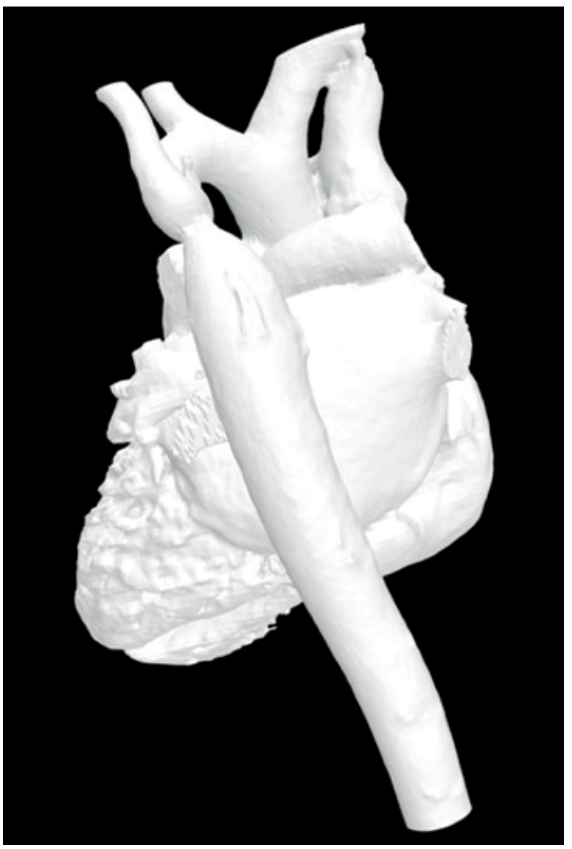

Fig. 1. A two year old female patient.

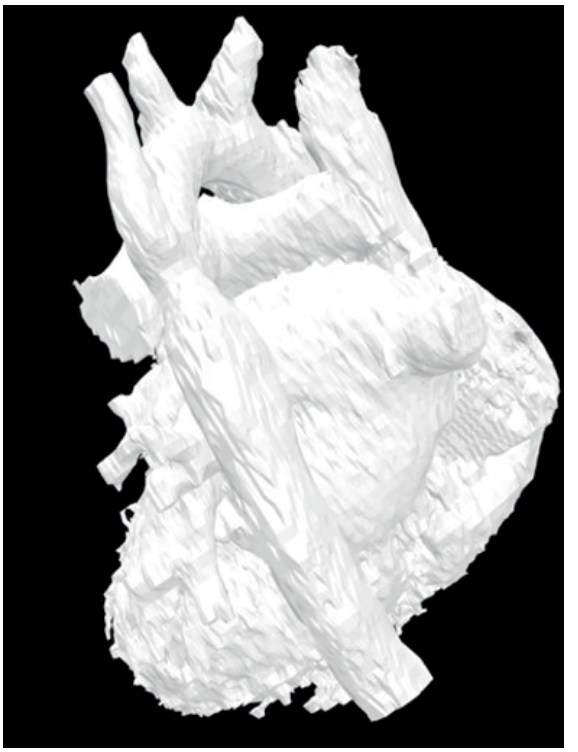

Fig. 2. A 17 year old male patient.

80 and 200 HU[40] Threshold values of min 216 HU - max 1502 HU were used. At these HU values, the blood in the heart and great vessels was masked, and the outline of the heart was revealed. As Hounsfield decreases, hollow structures (such as lungs) appear darker, while filled structures (such as bones) appear brighter and whiter.[41] Lowering the minimum HU value is necessary to make the heart walls more visible. However, this results in masking unwanted soft tissues other than the heart, such as muscle and fat. In this case, the unwanted tissues can be deleted from the mask by manual selection. The masked, unnecessary surrounding tissues other than the heart, such as muscle, bone, and fat) were removed first with the cropping mask and then manually by marking along the contours of the heart and great vessels. Thus, a model containing only the heart and the desired large vessels was created and cleaned from the surrounding tissues. With this mask, 3D reconstruction could be performed, and the model was ready for printing.

The virtual model obtained in the second step was transferred to the 3D printing machine in STL (Stereolithography or Standard Tessellation Language) format to be translated into physical conditions. Using the program's Blender, a series of surface correction operations were performed on the model. The purpose of this smoothing process was to prevent breaks and visual distortions that can occur during the printing process. However, in case where distortion occurs during this smoothing process, the validity of the model was re-checked by the radiology specialist, and the model was corrected if necessary. In the final stage, the model was checked, and the printer settings that best reflect the defect were determined (temperature 205-210 C°, layer height 0.14-0.3 mm, printing speed 50-55 mm/s). The images were printed with 1.75 mm white PLA filaments by converting the model to gcode type, the file format the printer can understand, on Ultimaker Cura, which is a free software. Although the printing time varies depending on the model size and defect type, the total printing time for a complete model was approximately 15 hours.

Re-evaluation of the model

The compatibility of the 3D heart model with the patient's radiological images were checked by a radiologist experienced in cardiac radiology and the patient's primary cardiologist surgeon. Both doctors reviewed the model and made their final decision together. If an error was detected in the model, the necessary corrections were made to the 3D model, and the printing process was repeated. This process must be repeated until a patient model that is fully compatible with the radiological images is created.

Interventions

Intervention Group:

Preoperative period: Once the surgery date is set, appointments will be made with the surgeon for surgical simulation and with the family for education one week prior to surgery. The meetings are held in separate rooms. After completion of the surgical simulation with model demonstration and radiological images, the surgeon will be asked to complete the Surgical Simulation Evaluation Form-Part I. At the same time, another researcher will complete the family sociodemographic information form and PedsQL questions in the examination room. After completion of the pre-test and the surgical simulation, the families are given a 30-minute preoperative education with the "Congenital Heart Disease Parent Education Booklet" prepared in light of the literature, together with a life-size 3D heart model obtained from their child's own heart, and drawings on paper where they are not understood. The education is followed by an average of 15 minutes of questions and answers, and the education is completed in 45 minutes. The education booklet is given to the parents after the intervention.

Postoperative Period: After surgery, the patient will be followed until discharge, and only Part II of the Surgical Simulation Evaluation Form will be completed. On the 15th postoperative day, the Surgical Simulation Evaluation Form Part II and the PedsQL will be given again as a post-test.

Control Group:

Preoperative Period: When the operation date is determined, one week before the operation, the patients included in the study's control group will be asked the Sociodemographic Information Form and Pediatric Quality of Life Inventory Family Module (PedQL) questions in the examination room. After the pre-test, standardized education will be given to the families by the same researcher. In the first half hour of this education, the disease process will be explained to the patients with the same 'Congenital Heart Diseases Parent Education Booklet' prepared in light of the literature, and the disease process will be presented with the heart model used in standard medical faculty anatomy courses, and the ununderstood parts will be detailed by drawing on paper. The remaining 15 minutes of the education will be conducted as a question and answer with the parents. In addition, a parent education booklet on CHD will be provided to the parents at the end of the education.

Postoperative Period: After the operation, the Surgical Simulation Evaluation Form Part II and PedsQL will be filled out again as post-tests for this group.

Criteria for discontinuing the recruitment

Researchers will stop the intervention if the patient has any harm during the research process. The most significant potential harm is the 3D modeling period. If the patient has emergency surgery before the intervention, prioritization will be done. One of the researchers, who is the responsible cardiologist MD, will make this decision. This patient's variables, such as the pretest, will be excluded.

To improve the patient's post-test adherence, both groups will be called and asked if they have any questions by the primary investigator after the post-op 7th day. Researchers will conduct an intention to treat test (ITT) when the research data is completed in order to protect the actual data results. In addition, two researchers will revise the data independently.

Outcomes

The primary outcome of this study is the quality of life score obtained from the Family Impacts Module of the Pediatric Quality of Life Inventory (PedsQL). In contrast, the secondary outcome is the patient outcomes of the patients who underwent surgical simulation (duration of operation, duration of hospital stay, duration of intensive care unit stay, need for repeat operation, other unusual complications other than pain, cardiopulmonary resuscitation, need for ECMO (Extracorporeal Membrane Oxygenation), seizures, rhythm changes, etc.).

Statistical analysis

Descriptive analyses will be presented as percentage and frequency values, and scale score averages will be presented as mean and standard deviation. In addition to descriptive statistical analyses, a normality test will be applied before comparative analyses. First, skewness and kurtosis values will be evaluated. If these values are between +1.5 and -1.5, parametric tests will be used.[42] In the first comparative analyses, the intergroup analysis will be performed, and no difference will be sought between the experimental and control groups. The chi-square test will be used to compare nominal variables between the two groups, and the student t-test will be applied between the nominal variables and the intragroup quality of life scale. Ordinal variables will be evaluated among themselves again with the chi-square test. At the same time, a one-way ANOVA method will be used in comparison with the average scale score within the group. In order to reveal the differences between the scale scores in the follow-ups, the evaluation will be made with the Pearson correlation test. When the study data do not fit the normal distribution, nonparametric evaluations of these tests will be performed. When necessary, expert statistician support will be obtained for further statistical analysis. The significance level of the study data will be accepted as 95%, and when $p<0.05$ in the analyses, it will be considered statistically significant.

Harms

In the trial, the researchers will record all adverse events reported by participants or unexpected and unwanted events. These possible events will be categorized as intervention-related and unrelated. The biggest risk that may be encountered within the scope of the study may be some technical problems that may occur in the model that emerges after 3D modeling, and the defect size or shape may be incorrect. In order to prevent this, a double control method will be applied. The CT report written by the specialist radiology doctor independent of the researchers in the hospital, where the patients were examined and their images were taken, will be compared with the evaluation of the specialist radiology doctor among the researchers, and the comparison process will be ensured again with the images after the model is printed.

Auditing

Auditing by the researchers will take place at every stage of the research. This means that each stage (3D modeling, data analysis, etc.) will be audited by all researchers during the process and after its completion.

Ethics approval and consent to participate

The study will adhere to the ethical principles declared by the Declaration of Helsinki. This study protocol was approved by the Acibadem University Medical Research Evaluation Board (date: 11.11.2022; approval no/number: 2022-17/50), and written institutional approval (date: 09.02.2023) was obtained for the institutions where the research will be conducted. Any protocol changes will be informed to the same ethical board and institution that gave the research permission at the beginning. The study was registered on clinicaltrials.gov in May 2023 (NCT05852106). All authors confirm that all methods will be carried out in accordance with ethical guidelines. In addition, parents of patients who meet the inclusion criteria will be informed about the study's purpose, procedure, benefits, and possible risks. Verbal consent from the children and informed written consent from their parents/legal guardians will be obtained by the researcher in a face-to-face interview. Participation will be voluntary; they will be informed that they can refuse to participate in the study or leave the study at any stage without any penalty.

Discussion

The study is expected to significantly improve the quality of life of children with CHD and their parents. Anomalies that parents can clearly understand according to the actual size and dimensions of the children's hearts will affect the preoperative preparation for surgery and the recovery rate in the postoperative period. Parents with less anxiety will be able to provide better parental care for their children during the recovery process. This will result in fewer complications.[22,23]

The recovery process after heart surgery involves a range of care needs. Many of these include care activities that involve parents, especially in the pediatric setting. Examples include painful procedures such as turning in bed, coughing and deep breathing exercises, mobilization, and wound care can be examples.[43] At this point, studies show that parents who understand the disease cooperate better.[44-46] Marella et al.[47] conducted a pilot study that showed that using 3D modeling for preoperative parental education is feasible. The study found that this method produces results that are equal to or better than the current standard of care in terms of parental understanding and knowledge. Onyekachukwu et al.[48] conducted a prospective study at a single center. The study used 3D-printed heart models to educate and counsel families. The researchers enrolled 75 participants and found that using 3D-printed heart models was highly effective in helping families understand their child's disease. The study also assessed, similar to this plnned study, caregiver satisfaction and found a significant improvement after the educational intervention

was provided Another RCT implemented by Karsenty et al.[49] with 76 patients showed that 3D heart models improve parental knowledge and reduce their anxiety level.

Good care received by patients in the postoperative period reduces complications and directly affects the recovery process. In a study conducted in 2016, 22 physicians, 38 nurses, and 10 ancillary care providers who were caring for patients with heart models created through 3D printing reported that nurses were better able to manage their patients when they received brief information about the procedure performed in the postoperative period along with the model.[50] In addition, children will be prevented from being traumatized with reduced surgical complications such as less pain and infection, and biopsychosocial healthier children will be raised.[43] Moreover, surgeons who can perform surgical simulations can perform their procedures, which will be completed in a shorter time with this method, with less risk of complications. A multicenter international study made in 2017 revealed that modeling before complex cardiac surgeries has significant effects. Surgeons stated that 19 out of 40 patients changed their surgical procedure after modeling and completed the treatment with a different surgical technique than the initial one.[15] Models obtained with 3D printers contribute significantly to preparing surgeons for complex procedures, reducing costs, and improving outcomes. According to a study by Chaudhuri et al.[51], surgical simulations with anatomical models reduced procedures by 1.5-2.5 hours. Although it is stated in the literature that cardiac surgery methods performed with 3D modeling are not very common, this study states that the cost of the procedure is significantly reduced. Similarly, Tack et al.[52] completed a research in 2021 and they mentioned that the savings varied according to the surgical procedure, ranging from 366 to 1485 euros. The most savings were achieved with the Norwood operation in atrial septal defects with 1485 euros.[52] When the operation time is reduced, more patients can be admitted, patients can be treated with fewer complications, and with the education provided, there will be fewer repeated hospitalizations and fewer outpatient visits. This also means a significant financial gain. 3D printing is a new technology and it should not be considered expensive. With proper expertise, it can potentially provide useful and low-cost 3D models.[53] In a seven-year prospective study conducted by Gomez Ciriza et al.[54] in 2021, it was discovered that affordable facilities can produce 3D-printed heart models that meet high technical and clinical standards.

Limitations

This study has some limitations. The first and most important is that patients were selected according to the quality of CT images taken during their diagnosis and treatment procedures. As a result of this practice, to protect children and avoid repeated imaging, a bias in patient selection may be identified. The second limitation is that intracardiac structures take longer to be modeled than extracardiac structures, and therefore, very common diseases such as ASD and VSD must be excluded. To conduct more feasible research, we highly recommend using imaging, modeling, and surgical procedures, which require physical proximity. One of the most significant limitations of this study is the need to obtain CT images from one hospital, carry out modeling in different laboratories, and complete printing in various locations. The most challenging and time-consuming parts were going back to the hospital, obtaining the surgeon's opinions, and revising the model. Although using this novel technology in treatment and care can be highly beneficial, future research needs to be conducted to address these limitations.

Conclusions and Recommendations

3D modeling and printing technologies are rapidly increasing today. With good technological equipment and knowledge, the use of these technologies can lead to higher family quality of life and better surgical simulation and operation preparations. The strength of

this study is that it reveals the 3D modeling and practical implementation procedures. It is important to inform the scientific world of our difficulties, challenges, and solutions for further development. To our knowledge, there has been no randomized controlled trial that uses 3D heart modeling to examine the effectiveness of family quality of life. Our study aims to fill this gap in research. In order to expand the use of these technologies and demonstrate their clinical effects, it is recommended that more studies with a larger sample size in different populations and a better technical team be included in the literature.

Declarations

Consent for publication

All authors and patients who have provided written consent for participation have agreed to the publication. As we have not yet started recruitment, we do not possess any written consent at this stage. However, the retrospective patient information provided to us for the preliminary study contained no personal identifiers. It solely consisted of medical images intended for practice and the determination of inclusion and exclusion criteria. Consequently, we did not obtain informed consent from their parents. However, in anticipation of the recruitment phase, we have prepared a written informed consent form for parents and children aged 16 years and older. Furthermore, all authors have given their final approval to the manuscript.

Availability of data and materials

All researchers will have access to the research dataset throughout the research process. The data will never be shared with any third party other than the researchers and will be kept in encrypted files for five years after the study is completed. At the end of five years, they will be destroyed.

Ethical approval

The study will adhere to the ethical principles declared by the Declaration of Helsinki. This experimental protocol was approved by the Acıbadem Mehmet Ali Aydınlar University Medical Research Evaluation Board (ATADEK) (date: 11.11.2022; approval no/number: 2022-17/50) and written institutional approval (date: 09.02.2023) obtained for the institutions where the research will be conducted. The study was registered on clinicaltrials.gov in May 2023 (NCT Number is: NCT05852106). All authors confirm that all methods will be carried out in accordance with ethical guidelines.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AAS, AVI, PI Author; data collection: AAS, AVI, EP, AC Author; analysis and interpretation of results: AAS, AVI, PI, DN, TG, AC, DOS, GNC Author; draft manuscript preparation: AAS, DOS, AVI, PI, TG, AC, CZE, GNC, DN, EP Author. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A pediatric case with hemolytic uremic syndrome associated with COVID-19, which progressed to end-stage kidney disease

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ABSTRACT

Background. Hemolytic uremic syndrome (HUS) is a serious cause of acute kidney injury in children. There is a suggestion that coronavirus disease 2019 (COVID-19) may be a trigger for HUS. In this study, we present a pediatric case diagnosed with HUS associated with COVID-19, which progressed to end-stage kidney disease.

Case. A previously healthy 13-year-old girl with fever and vomiting was referred to our hospital. Laboratory investigations revealed direct Coombs-negative hemolytic anemia, thrombocytopenia and renal impairment accompanied by COVID-19 infection. Although anemia and thrombocytopenia showed improvement on the seventh day after admission, the renal impairment persisted. The histopathological findings of a renal biopsy were compatible with both HUS and COVID-19. One month later, the patient had a recurrence of HUS, again testing positive for COVID-19. Kidney function improved with plasma exchange therapy. Eculizumab treatment was recommenced after COVID-19 PCR became negative. Anemia and thrombocytopenia did not recur with eculizumab, while renal impairment persisted. Eculizumab was discontinued after three months when genetic analysis for HUS was negative. Subsequently, the patient was diagnosed with end-stage kidney disease.

Conclusions. COVID-19 can be associated with HUS relapses, leading to chronic kidney disease. Further studies should investigate the mechanism of HUS associated with COVID-19.

Key words: COVID-19, chronic kidney disease, hemolytic uremic syndrome, SARS-CoV-2.

Coronavirus disease 2019 (COVID-19) emerged as a public health problem in 2019. Although COVID-19 primarily affects the lower respiratory tract, it has also been associated with a prothrombotic state that increases the risk of thrombotic microangiopathies such as hemolytic uremic syndrome (HUS).[1] The exact mechanism behind COVID-19 triggering HUS is unknown, although some theories have been proposed.[2] COVID-19 may cause endothelial injury and endotheliitis, leading to HUS. Accordingly, the effect may not be associated only with the direct cytopathic effect of the virus, as the subsequent inflammatory response may also contribute to the development of HUS.[3] Complement activation by COVID-19 is another potential underlying mechanism leading to HUS. The spike protein can directly induce an alternative complement pathway.[4] These findings are supported by the effectiveness of eculizumab (anti-C5 monoclonal antibody) treatment in patients with HUS triggered by COVID-19.[2,5,6]

In the current study, we present a pediatric case diagnosed with HUS associated with COVID-19, which subsequently progressed to end-stage kidney disease.

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Case Report

A previously healthy 13-year-old girl was admitted to the pediatric emergency service of another center with complaints of vomiting and fever for a week. She had pretibial edema and tachycardia. Her anthropometric measurements were within the normal range. Her laboratory results revealed renal failure (urea: 103 mg/dl, creatinine: 6.5 mg/dl) and proteinuria (3.4 g/m^2/d) on admission and mild anemia (Hgb: 10 gr/dl) with a normal platelet count. She underwent hemodialysis and received antibiotic treatment (tazobactam and ceftriaxone).

On the seventh day of hospitalization, she was referred to our center to investigate the etiology of renal failure. On admission, she had fever (38.2°C), tachycardia (124/min) and hypertension (159/100 mmHg). There were no symptoms of diarrhea, respiratory distress, oliguria or hematuria. The patient had periorbital and pretibial edema. Her laboratory results showed Coombs-negative hemolytic anemia and thrombocytopenia accompanied by increased lactate dehydrogenase (LDH) and low haptoglobin levels (Table I). She had renal failure, proteinuria (3+) and hemoglobinuria. Myoglobin and creatine kinase levels were within the normal range. Renal ultrasonography revealed normal kidney length with increased echogenicity. SARS-CoV-2 PCR was positive, so she was hospitalized in the COVID-19 unit. Cyanocobalamin was initiated for vitamin B12 deficiency [Vit B12: 158 (182-820) ng/ml]. Due to hyperparathyroidism [PTH: 700 (12-88) pg/ml] and vitamin D deficiency [Vit D: 12 (>30 μg/L)], 25-OH-D_3 and calcitriol were initiated. Serum homocysteine and urine methylmalonic acid levels were within the normal range. Complement levels, pANCA, cANCA, and anti-glomerular basement membrane antibody levels were within the reference range. Antinuclear antibody was negative. ADAMTS13 activity was normal (94%). Urine output was normal (1.3 ml/kg/hr). She continued hemodialysis due to edema and hypertension. Enalapril, doxazosin, amlodipine and furosemide treatments were initiated gradually for hypertension. On the seventh day of hospitalization, thrombocytopenia and hemolysis improved. Enoxaparin sodium was initiated due to an increased level of D-dimer and COVID-19 positivity. Methylprednisolone was started due to nephrotic syndrome. On the 12th day, COVID-19 PCR was found to be negative, and on the 18th day, the patient underwent renal biopsy, which revealed glomerular capillary obliteration, fibrin thrombi, and glomerular basement membrane duplication on light microscopy, consistent with the histopathological findings of HUS. In addition, tubular epithelial nucleus hypertrophy and an atypical appearance were detected, consistent with COVID-19 histopathological findings (Fig. 1). Thickening of arteriolar walls was observed, but there was no interstitial fibrosis. Immunofluorescence microscopy revealed mild staining with IgM and C3, leading to the diagnosis of HUS associated with COVID-19. Electron microscopy could not be evaluated due to degeneration in the glomeruli. Anti-complement factor H antibody levels were

Table I. Laboratory parameters of the patient.

Parameters (reference values)	On admission	At discharge (28th day)	2 weeks after discharge	Latest control values (six months after transplantation)
Urea (10-38 mg/dl)	103	156	122	19
Creatinine (0.5-0.9 mg/dl)	6.5	6.6	6.4	0.67
Albumin (32-45 g/L)	24	28	32	43
Hemoglobin (11.7-15.3 g/dl)	7.1	10.2	7.2	14.0
Platelet count (150-400X10^3/μL)	98	197	138	281
LDH (120-300 U/L)	495	216	498	177
Haptoglobin (0.3-2 g/L)	0.17	0.96	0.04	0.47

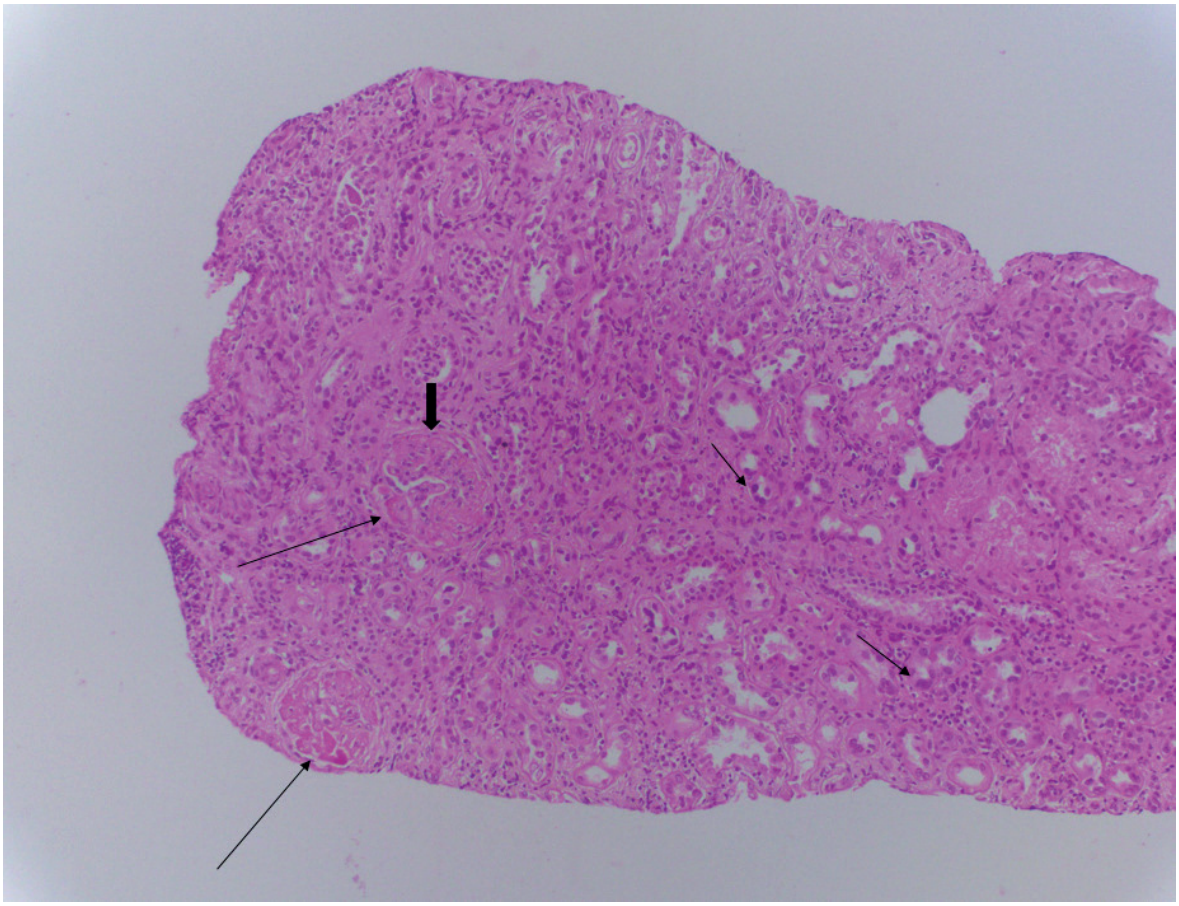

Fig. 1. Fibrin thrombus in the glomerulus (long thin arrows), capillary obliteration (short thick arrow), and nuclear atypia (short thin arrows) in tubulus epithelial cells (H&E).

within the normal range and the genetic panel for HUS (*CD46, CFH, CFHR5, CFI, C3, DGKE, THBD*) was negative. The steroid dose was tapered and the patient was discharged on the 28th day for treatment with hemodialysis three days a week.

One month after discharge, she was admitted to the pediatric nephrology clinic with no complaints. The periorbital and pretibial edema continued and she had hypertension. Laboratory results revealed hemolytic anemia with the presence of schistocytes, thrombocytopenia and renal failure consistent with HUS relapse (Table I). Eculizumab (anti-C5a monoclonal antibody therapy, 900 mg/4 weeks) was initiated. While there was no significant decrease in hemoglobin levels, the platelet counts decreased despite eculizumab therapy. In the search for other causes of thrombocytopenia, her COVID-19 PCR was positive again after four weeks, although she had no complaints. Therefore, eculizumab treatment was abandoned. Viral genome sequencing tests to distinguish between reactivation or reinfection with COVID-19 could not be performed due to the unavailability of this test. Plasma exchange was performed five times with one-day intervals, leading to an increase in the platelet count to 124×10^3/μL. Eculizumab treatment was recommenced two weeks later. Although the platelet counts remained within the normal range, the renal functions did not improve within three months of eculizumab therapy. The patient underwent renal transplantation from her mother due to end-stage kidney disease. Her renal functions and hematologic parameters were within the normal range at her last visit six months after

transplantation (Table I). An informed consent was obtained from the family for the publication of this case report.

Discussion

The case presented suffered two HUS relapses associated with the COVID-19 infection. Despite eculizumab and plasma therapy, her renal functions did not improve and she developed chronic kidney disease. This led us to consider the possibility that COVID-19 was a contributing factor to chronic kidney disease in addition to the HUS relapses.

At the time of admission to our center for further investigation, she had undergone hemodialysis in another center and was being treated with antibiotics (tazobactam and ceftriaxone). The initial clinical presentation was acute, her growth was within the normal range and renal ultrasonography revealed normal renal lengths. This led us to consider the development of acute kidney injury. We attributed hyperparathyroidism in this patient to vitamin D deficiency rather than chronic kidney disease. Our presumed diagnoses for the etiology of acute kidney injury included HUS, crescentic glomerulonephritis and acute tubulointerstitial nephritis (ATIN). There was a strong suspicion of HUS based on the clinical and laboratory findings, including direct Coombs-negative hemolytic anemia, thrombocytopenia and renal failure. Although the rapid deterioration in renal function was initially considered related to crescentic glomerulonephritis, acute tubulointerstitial nephritis was also a possible diagnosis. This consideration was based on her urine microscopy findings being inconsistent with glomerulonephritis, the absence of oliguria and the presence of COVID-19 infection. The renal biopsy findings were consistent with HUS and featured a tubular atypia appearance associated with COVID-19. We could not show the presence of COVID-19 due to the absence of specific staining. However, acute kidney injury was evidenced by the absence of interstitial fibrosis.

SARS-CoV-2 infection was reported to be a trigger for atypical HUS in children in a review.[7] Additional evidence has shown that COVID-19 may activate an alternative complement pathway, proven by the deposition of mannose-binding lectin (MBL), mannose-associated serine protease 2 (MASP2) and C3b, C4b and C5b-9 on the endothelial cells of patients with COVID-19-associated thrombosis.[8] Eculizumab treatment can thus be considered an effective treatment option in patients with HUS triggered by COVID-19. Numerous adult cases are extensively detailed in the literature[2,5,9,10] but only five pediatric HUS cases are associated with COVID-19 infection.[6,11-13] One of the pediatric cases was a 14-year-old female patient presenting with fever, abdominal pain, diarrhea and SARS CoV-2 PCR positivity.[6] She developed myocarditis, coronary artery ectasia, Coombs (-) hemolytic anemia, thrombocytopenia and acute kidney injury. The patient was initiated on continuous renal replacement therapy (CRRT) on the 10th day, and on eculizumab on the 14th day. Following the first dose of eculizumab, there was no need for CRRT and renal functions showed improvement at three weeks. Another case was a 16-month-old male with a history of intrauterine growth retardation, microcephalia, and corpus callosum agenesis.[13] He was admitted to the hospital presenting with fever, vomiting and respiratory distress, testing positive for SARS-CoV-2 PCR. He developed diabetic ketoacidosis, nephrotic range proteinuria and persistent hypertension. On the 11th day of hospitalization, he developed HUS. Following the first dose of eculizumab, anemia and thrombocytopenia showed improvement. His renal functions improved at 21 days. Dalkıran et al.[11] reported a 3-year-old pediatric case presenting with respiratory distress, fever and COVID-19 PCR positivity. On the 10th day, the patient developed HUS and was initiated on hemodialysis and plasmapheresis. Hemolysis and renal functions were improved on the 6th and 28th days of plasmapheresis, respectively. Nomura et al.[12] reported five pediatric patients with COVID-19 and kidney dysfunction, of whom two presented with HUS

and diarrhea (6- and 9-year-old males). One of them received multiple therapies, including steroids, plasmapheresis, and IVIG. However, he became dialysis dependent at seven months. The other patient received only supportive care and developed chronic kidney disease at three months.[12] Eculizumab (anti-C5 monoclonal antibody) therapy was a successful treatment option in the reported cases.[6,11,13] Therefore, we opted to initiate eculizumab therapy. After four weeks of eculizumab treatment, the platelet counts decreased, but the patient tested positive for COVID-19 again. Platelet count showed improvement under plasma exchange therapy. Platelet counts remained within the normal range and hemolytic anemia did not develop under eculizumab therapy. However, renal functions did not show improvement.

Vrečko et al.[10] reported 28 adult cases with HUS, 15 of whom received therapeutic plasma exchange (TPE) with or without steroids as initial therapy, resulting in improvement in six patients. Sixteen patients received eculizumab therapy (as monotherapy or after the failure of TPE ± steroids to improve the condition). The renal functions of 10 patients who received eculizumab therapy improved, while six did not, similar to those of our patient. She had nephrotic range proteinuria, which could lead to insufficient plasma levels of eculizumab. C5 polymorphism was associated with nonresponsiveness to eculizumab therapy in patients with atypical HUS in Asian and Japanese people.[14] However, we were unable to evaluate these genetic changes.

When the patient's genetic analysis for HUS was negative, as reported, eculizumab therapy was discontinued. After her kidney impairment remained for six months, she was diagnosed with chronic kidney disease.

The majority of patients reported in the literature with HUS triggered by COVID-19 have severe diseases[11-13] and multisystemic diseases such as respiratory distress or cardiac involvement requiring intensive care unit (ICU) admission. In contrast to previous studies, our patient had a mild disease without systemic involvement or ICU requirement. Other differences from the reported cases included the absence of oliguria and active urine sediment.

In conclusion, COVID-19 can be associated with HUS, leading to end-stage renal disease. However, further studies are needed to assess the mechanisms leading to HUS to support the treatment of patients with COVID-19.

Ethical approval

We obtained informed consent from the patient's family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SSD, EDV, YYK, FD, AD, BÖÖ; data collection: EDV, BÖÖ, FD; analysis and interpretation of results: SSD, YYK, AD; draft manuscript preparation: SSD, EDV, BÖÖ, AD, FD, YYK. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare that there is no conflict of interest.

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Case Report

Extracorporeal carbon dioxide removal for acute hypercapnic respiratory failure in a child with cystic fibrosis

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ABSTRACT

Background. Acute respiratory failure is a prevalent condition in childhood with a high rate of mortality. Invasive mechanical ventilation support may be required for the management of these patients. Extracorporeal membrane oxygenation (ECMO) is a method used when ventilation support is insufficient. However, the less invasive extracorporeal carbon dioxide removal method can be used as an alternative in cases of hypercapnic respiratory failure.

Case. A 9-year-old patient with cystic fibrosis presented to the hospital with acute respiratory failure due to pneumonia. Bilateral patchy areas of consolidation were evident in the chest x-ray. Invasive mechanical ventilation support was consequently provided to treat severe hypercapnia. Although peak and plateau pressure levels exceeded 32 cmH_2O (49 cmH_2O) and 28 cmH_2O (35 cmH_2O), respectively, the patient continued to have severe respiratory acidosis. Therefore extracorporeal carbon dioxide removal support was initiated to provide lung-protective ventilation. By Day 10, venovenous ECMO support was initiated due to deteriorating oxygenation.

Conclusion. In cases where conventional invasive mechanical ventilation support is insufficient due to acute hypercapnic respiratory failure, extracorporeal carbon dioxide removal support, which is less invasive compared to ECMO, should be considered as an effective and reliable alternative method. However, it should be noted that extracorporeal carbon dioxide removal support does not affect oxygenation; it functions solely as a carbon dioxide removal system.

Key words: extracorporeal carbon dioxide removal, extracorporeal membrane oxygenation (ECMO), hypercapnic respiratory failure, pediatric intensive care unit.

Acute respiratory failure (ARF) is the ineffective oxygenation, ventilation, or both in the respiratory system. The extracorporeal carbon dioxide removal ($ECCO_2R$) technique assists in the improvement of hypercapnia and respiratory acidosis by the removing of CO_2 from the blood.[1] This method is similar to extracorporeal membrane oxygenation (ECMO); however, it uses lower blood flow rates. Therefore, $ECCO_2R$ can be administered more readily and requires smaller venous access. In contrast, it has little or no effect on blood oxygenation.[2]

$ECCO_2R$ can be used to implement a lung-protective strategy in acute respiratory distress syndrome (ARDS), during weaning off mechanical ventilation in chronic obstructive pulmonary disease (COPD), and reduce the need for invasive mechanical ventilation (IMV) in hypercapnic respiratory failure.[3,4] This technique is more commonly used in adults, and there is no available literature on the use of $ECCO_2R$ in the pediatric age group in our country. The purpose of the present study is

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to share the experience of using $ECCO_2R$ in a pediatric patient with cystic fibrosis, who had acute hypercapnic respiratory failure.

Case

A 9-year-old girl with cystic fibrosis was admitted to the hospital due to the sudden onset of respiratory distress. She had a history of neonatal surgery for ileal atresia and had been admitted to the pediatric intensive care unit (PICU) multiple times for recurrent pneumonia. It was established that she had not received chronic respiratory support prior to this, but had not presented to the hospital for regular check-ups for over a year. Therefore, her baseline condition was not clearly known.

According to the physical examination, her general condition was poor, but she remained conscious. Her heart rate was 160/minute, blood pressure was 122/83 mmHg, respiratory rate was 52/minute, oxygen saturation was 74% at room temperature and 96% with a reservoir mask. Capillary refill time was 2 seconds. She weighed 18 kg. She had subcostal and intercostal retractions, and bilateral ronchi and crepitant rales were audible on auscultation. A chest x-ray revealed bilateral patchy areas of consolidation (Fig. 1).

Additional physiological tests yielded the following results: blood gas pH 7.27, pCO_2 79.4 mmHg, HCO_3 30 mmol/L, lactate 3.1 mmol/L, base deficit 8.9, hemoglobin (measured via complete blood count) 10 g/dL, hematocrit 31.7%, platelets 608000/mm^3, leukocyte count 20900/mm^3, blood glucose (biochemical examination) 158 mg/dL, aspartate aminotransferase 91 U/L, alanine aminotransferase 43 U/L, C-reactive protein 232.4 mg/L, procalcitonin 36.8 ng/mL, and albumin 2.3 g/L. Other blood parameters were within normal ranges.

She received oxygen support via a high-flow nasal cannula. Treatments included dornase alfa, salbutamol, and 3% NaCl nebule, along with an intravenous dose of 50 mg/kg of magnesium sulfate. Blood and urine cultures were collected, and the patient was started on teicoplanin (10 mg/kg/day), meropenem (120 mg/kg/day), and amikacin (30 mg/kg/day). Due to severe hypercapnic respiratory failure (pH: 7.06, pCO_2: 120 mmHg, HCO_3: 23.5 mmol/L, lactate: 1.3 mmol/L, base deficit: 3), the patient was intubated using a 5.0 cuffed tube with a rapid sequential intubation protocol and connected to a mechanical ventilator. She was monitored in adaptive pressure ventilation synchronised intermittent mandatory ventilation mode with a tidal volume target (V_T) of 140 mL (7.8 mL/kg), positive end-expiratory pressure (PEEP) of 6 cmH_2O, fraction of inspired oxygen (FiO_2) of 60%, frequency of 25/min, inspiratory time of 0.6 seconds, and peak inspiratory pressure (P_{peak}) of 46 cmH_2O. Right femoral central venous catheter, right radial artery catheter, and urinary catheter were inserted. Midazolam (0.2 mg/kg/h) and fentanyl (1 mcg/kg/h) infusions were started for sedation and analgesia, with rocuronium (0.4 mg/kg/h) used for paralysis. A tracheal aspirate culture was sent for further examination. Caspofungin treatment was initiated for allergic bronchopulmonary aspergillosis. Total immunoglobulin E and immunoglobulin E levels specific to *Aspergillus fumigatus* were normal.

During follow-up, persistent severe respiratory acidosis was observed (pH: 7.11, pCO_2: 102.3 mmHg, HCO_3: 23.9 mmol/L, lactate: 2.7 mmol/L, base deficit: 3.2). $ECCO_2R$ was planned due to peak and plateau pressure levels exceeding 32 cmH_2O (49 cmH_2O) and 28 cmH_2O (35 cmH_2O), respectively (Fig. 2). Additionally, a 12F hemodialysis catheter was inserted through the right internal jugular vein, and $ECCO_2R$ treatment was initiated (PrismaLung, Baxter Healthcare/ Gambro, Lund, Sweden, allowing for a blood flow rate ranging from 80 to 450 mL/min in a patient weighing 18 kg, with a surface area of 0.35 m^2). Initial settings included a gas flow rate of 2 L/min, blood flow rate of 100 mL/min, and heparin infusion rate of 10 U/kg/h. Gas flow rate and blood flow rate were adjusted according to the treatment target, while heparinization was regulated based on

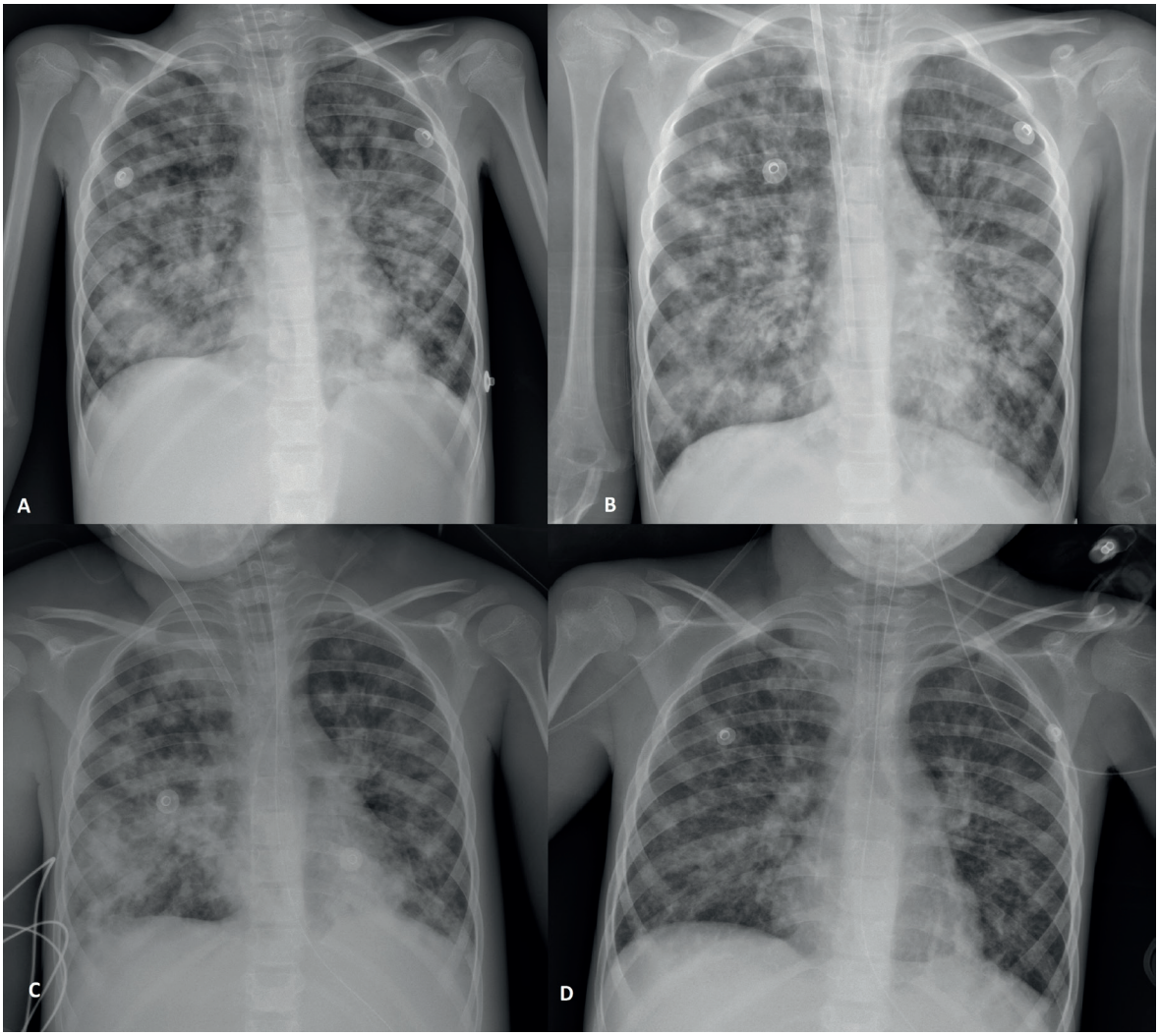

Fig. 1. Chest X-ray images of the patient.

A, Patchy-like bilateral infiltrative consolidation areas at the patient's admission; **B**, Similar ongoing consolidation areas while the patient is being placed on $ECCO_2R$ support, **C**, Increased consolidation areas on the patient's chest radiograph taken when ECMO support was started, and **D**, Chest radiograph showing the patient's recovery trend when weaned from ECMO support. $ECCO_2R$: extracorporeal carbon dioxide removal, ECMO: extracorporeal membrane oxygenation.

the activated clotting time (ACT) and activated partial thromboplastin time (aPTT) target. In $ECCO_2R$ support, we applied the following treatment targets: respiratory rate < 25/min, $V_T \leq 6$ mL/kg for ventilatory settings, and pH > 7.30, and PaCO2 < 55 mmHg for blood gas values. We maintained the ACT and aPTT targets within the ranges of 180–220 seconds and 60–80 seconds, respectively, according to the heparinization protocols. Lung-protective ventilation was performed using a mechanical ventilator with the following settings: V_T: 100 mL (5.5 mL/kg), frequency: 20/min, inspiratory time: 1 s, PEEP: 8 cmH_2O, FiO_2: 60%, P_{peak}: 30 cmH_2O. One hour after initiating the $ECCO_2R$ procedure, blood gas values were as follows: pH 7.22, pCO_2 72.3 mmHg, HCO_3 24.9 mmol/L, lactate 2.2 mmol/L, PaO_2 88 mmHg, and SpO_2 96%. The targeted blood gas values (pH>7.30, pCO_2<55 mmHg) were ultimately achieved at 10 hours (Table I). $ECCO_2R$ settings were gas flow rate of 6 L/min and blood flow rate of 160 mL/min, when the target blood gas levels were reached. During the follow-up, gas flow was

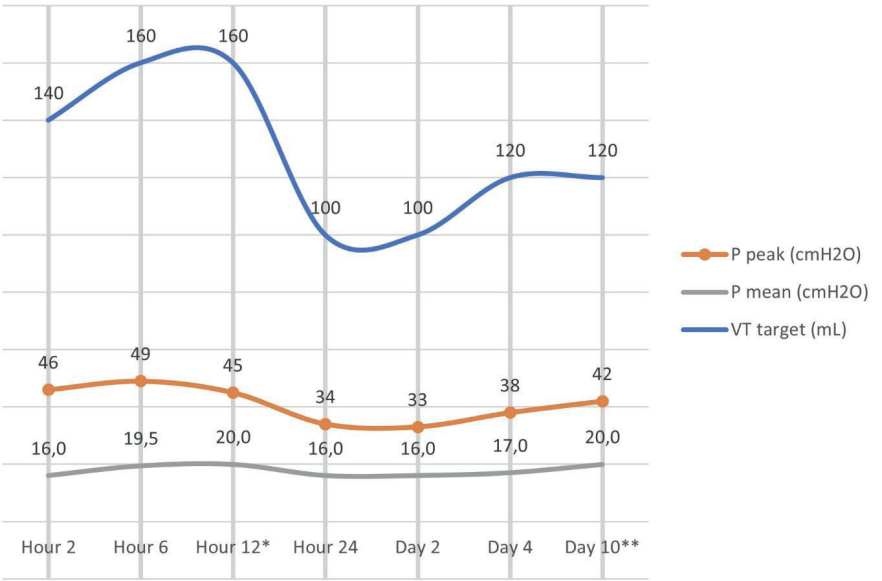

Fig. 2. Ventilator settings during follow-up.
* ECCO2R support was commenced here.
** Switch to ECMO support here, VT: tidal volume.

Table I. Blood gas parameters, invasive mechanical ventilation and $ECCO_2R$ settings, and oxygenation parameters of the patient.

	Parameters	Hour 0*	Hour 2*	Hour 6*	Hour 12*[a]	Hour 24	Day 2	Day 4	Day 10[b]
Blood gas parameters	pH	7.06	7.15	7.13	7.11	7.30	7.27	7.34	7.43
	pCO_2, mmHg	120	85	97.4	102.3	55	58.2	65.3	54.4
	HCO_3, mmol/L	23.5	23.4	24	23.9	24.1	23.7	32.1	34.8
	SpO_2, %	43.6	68	60	52.5	92	88	93.3	90.7
	PaO_2, mmHg	40.2	50	44	36.7	86	70	75.7	60.4
	Lactate, mmol/L	2.8	3.3	2.9	2.7	1.5	1.5	1.3	1.1
Mechanical ventilation settings	Frequency/min	X	25	25	25	20	20	20	22
	VT_{target}, mL	X	140	160	160	100	100	120	120
	P_{peak}, cmH_2O	X	46	49	45	34	33	38	42
	P_{mean}, cmH_2O	X	16	19.5	20	16	16	17	20
	PEEP, cmH_2O	X	6	7	8	8	8	8	10
	IT, seconds	X	0.6	0.7	0.8	1	1	0.9	0.9
	FiO_2, %	X	60	60	60	70	70	65	70
$ECCO_2R$ settings	Blood flow velocity, mL/min	X	X	X	100	160	160	220	180
	Gas flow rate, L/min	X	X	X	2	4	6	10	2
Oxygenation index		X	10.1	12.7	15.6	15	16	14.5	23.2

* Oxygen saturation index was used because blood gas was venous.
[a] $ECCO_2R$ support was commenced here, [b] Switch to ECMO support here, $ECCO_2R$: extracorporeal carbon dioxide removal, FiO_2: fraction of inspired oxygen, IT: inspiratory time, PaO_2: partial arterial pressure of oxygen, PEEP: positive end-expiratory pressure, Pmean: mean alveolar pressure, Ppeak: peak inspiratory pressure, SpO_2: pulse oxygen saturation, VT: tidal volume.

increased by 10 L/min, and blood flow rate was increased by 220 mL/min to maintain the blood gas target. No $ECCO_2R$-related complications were noted in the targeted CO_2 levels. A total of 4 filters were used. The first membrane change occurred within 24 hours due to membrane thrombosis.

An increase in oxygenation index, FiO_2, and PEEP requirement was observed during follow-up. Due to problems related to oxygenation, the patient was switched from $ECCO_2R$ support to venovenous ECMO support by Day 10. *Pseudomonas aeruginosa* growth was identified in a tracheal aspirate culture. She responded positively to appropriate antibiotic therapy, based on the culture antibiogram, for the infection. This led to improvements in ventilation and oxygenation, and she was weaned off ECMO support on Day 29, but could not be weaned off IMV support during the follow-up; hence, tracheostomy was opened as a result of chronic respiratory failure and chronic ventilation support was continued.

The authors declare that they have obtained written consent from the family for the publication of this case report.

Discussion

The annual incidence of PICU admission for ARF is 2.3%, with a mortality rate ranging from 24 to 34%. Although advancements in technology and IMV strategies have reduced mortality and morbidity in these patients, ARF remains a serious condition.[5-8] Currently, lung protective ventilation strategies are recommended in cases of respiratory failure requiring IMV support. Previous studies in the relevant literature have recommended the use of $ECCO_2R$ during acute and severe decompensation of COPD or to implement a lung-protective ventilation strategy in IMV for ARDS, especially in cases of hypercapnic respiratory failure.[4,9] The current patient was also diagnosed with cystic fibrosis and was admitted to the PICU with clinical manifestations of pneumonia, presenting a more severe acute hypercapnic respiratory failure. Despite the patient's peak and plateau pressure levels exceeding 32 cmH_2O and 28 cmH_2O, respectively, $ECCO_2R$ support was initiated due to uncontrollable hypercapnia.

In our patient (age, 9 years; body weight, 18 kg), a 12 Fr temporary hemodialysis catheter was percutaneously inserted through the right internal jugular vein. Although typically not recommended for patients weighing less than 30 kg, the effective application of $ECCO_2R$ support in this patient was made possible by achieving appropriate and adequate venous access, ensuring sufficient blood flow rate. At the time $ECCO_2R$ was first introduced, arteriovenous 15 Fr and venovenous 18–19 Fr cannula sizes were required; however, in recent years, the system can be effectively used with smaller cannula sizes.[10] Data in the relevant literature regarding venovenous $ECCO_2R$ with percutaneously placed hemodialysis catheters in pediatric patients are limited. The most prevalent $ECCO_2R$ complication is bleeding and vascular injury, which can occur during the venous cannulation procedure.[4] Nevertheless, no complications related to venous access were observed in our patient.

A previous study reported a complete recovery from hypercapnia within 21–24 hours with $ECCO_2R$ support. However, this study focused on adult patients who received $ECCO_2R$ support via separate arterial and venous routes.[11] No specific studies have been conducted in the pediatric age group regarding this matter, and there is a lack of available data in the literature comparing the rate of CO_2 removal between single venous route and separate arterial and venous access. In our case, we utilized a single venous route for $ECCO_2R$ support, successfully achieving the targeted CO_2 levels within 10 hours.

A consensus study recommended treatment targets in $ECCO_2R$ support, including P_{plato}<25 cmH_2O, respiratory rate <25/min, V_T ≤6 mL/kg, pH>7.30, and $PaCO_2$<55 mmHg.[12] In our patient, $ECCO_2R$ support settings were gradually

increased to achieve these treatment goals. The desired targets were reached with a gas flow rate of 6 L/min and a blood flow rate of 160 mL/min. However, during the follow-up, the gas and blood flow rates were increased to 10 L/min and 220 mL/min, respectively, to maintain these targets.

Heparin is required for anticoagulation during $ECCO_2R$ administration, but clear targets for anticoagulation in pediatric patients are not well-defined.[13] In a case series study, heparin infusion doses ranged from 3 to 19 IU/minute, maintaining the ACT target between 150 and 200 seconds.[14] The consensus study recommended maintaining an aPTT target of 45–70 seconds or an anti Xa activity between 0.3 and 0.5 IU/mL, using an initial heparin bolus of 40–80 IU/kg, followed by infusion.[12] In our case, the heparinization protocol used in continuous renal replacement therapy was selected. After a 20 IU/kg heparin bolus dose, an infusion of 10 IU/kg/hour was initiated and adjusted to maintain the ACT and aPTT targets between 180–220 seconds and 60–80 seconds, respectively. Thrombocytopenia gradually developed from the patient's initial admission and persisted during the $ECCO_2R$ support. It is well-established that thrombocytopenia can result from the interaction of extracorporeal support systems with blood components and the transient effect of heparin infusion.[13,15,16] Hemorrhagic events are the most common complications during $ECCO_2R$ support, often requiring blood transfusions.[4,11,17] Despite the presence of thrombocytopenia in our patient, no bleeding complications occurred, and the heparinization procedure was sustained with blood product support.

Despite the use of anticoagulation protocols during $ECCO_2R$ support, clot formation in the circuit can occur. This situation can lead to a rapid increase in $PaCO_2$ especially in the patient. Membrane thrombosis should be considered a life-threatening condition, and circuit replacement should be promptly implemented.[15,18] In a retrospective study, thrombosis was observed in 2 out of 3 patients receiving $ECCO_2R$ support with low blood flow rates in the circuit, while there was no thrombosis in 6 patients with high blood flow rates.[19] Another study reported that half of the patients with low blood flow experienced circuit thrombosis despite heparin anticoagulation.[9] In our case, $ECCO_2R$ support was initially provided at lower blood flow rates, yet circuit replacement was required during the early period (first 24 hours) due to membrane thrombosis. Subsequently, the recommended 72-hour membrane use period at higher blood flow rates was completed without any thrombosis and no thrombotic complications were observed during that period. Furthermore, two case series studies reported intravascular hemolysis.[17,18] However, we did not observe significant hemolysis in our case.

In conclusion, $ECCO_2R$ support, as a less invasive method compared to ECMO, proves to be both an effective and reliable alternative when conventional IMV support is insufficient in cases of acute hypercapnic respiratory failure in the pediatric population. Yet, it should be underscored that ECCOR support is specifically designed for carbon dioxide removal and does not exert any influence on oxygenation. It has been observed that $ECCO_2R$ can also be utilized in patients with body weights below the recommended range when adequate venous access is available. However, future studies focusing on the pediatric population and the development of standardized guidelines are necessary to better comprehend the clinical significance of the $ECCO_2R$ procedure.

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Ethical approval

Authors declare that they have written consent from the family.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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