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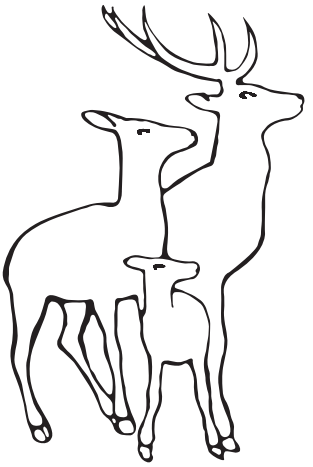

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Original Article

Elevated visfatin levels illuminate the inflammatory path in bronchopulmonary dysplasia

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ABSTRACT

Background. Bronchopulmonary dysplasia (BPD) is a chronic lung disease in premature infants caused by an imbalance between lung injury and lung repair in the developing immature lungs of the newborn. Pulmonary inflammation is an important feature in the pathogenesis of BPD. The aim of this study was to evaluate the relationship between the inflammatory microenvironment and the levels of visfatin and nesfatin-1, which are among the new adipocytokines, in BPD patients.

Methods. The groups consisted of 30 patients with BPD and 30 healthy children. Plasma levels of visfatin and nesfatin-1 and inflammation-related markers including interleukin-4 (IL-4), interleukin-10 (IL-10), nuclear factor kappa B (Nf-κB) and matrix metalloproteinase-9 (MMP-9) were determined by enzyme-linked immunosorbent assay (ELISA). RT-PCR was performed to evaluate the change in mRNA expression of visfatin and nesfatin-1 in the groups.

Results. Visfatin levels were significantly higher in the BPD group compared to the healthy control (7.05±4.07 ng/ml vs. 2.13±1.66 ng/ml, $p<0.0001$). There was a 1.36±0.12 fold increase in visfatin mRNA expression ($p<0.05$) in the BPD group. There was no significant difference in plasma levels of nesfatin-1, IL-4, and IL-10 between the groups. Although MMP-9 and Nf-κB levels were significantly higher in the BPD group ($p<0.0001$), there was no correlation between visfatin levels and MMP-9 and Nf-κB levels in BPD patients.

Conclusions. This study showed that significant changes in visfatin levels in BPD patients might be associated with the risk of developing inflammation in BPD.

Key words: adipokine, bronchopulmonary dysplasia, inflammation, nesfatin-1, visfatin.

Bronchopulmonary dysplasia (BPD) is a chronic lung disease in premature infants, resulting from an imbalance between lung injury and repair during development.[1] First described by Northway et al. in 1967, BPD was associated with infants treated for respiratory distress syndrome (RDS) using high levels of oxygen and positive pressure ventilation.[2] The disease is multifactorial, influenced by both prenatal and postnatal factors, though its molecular pathogenesis remains unclear and effective prevention methods are lacking. Despite advances in treatment, BPD continues to be a common late morbidity in preterm infants.[3]

Pulmonary inflammation plays a central role in the pathogenesis of BPD, driven by risk factors like mechanical ventilation, infections, and hyperoxia.[4] This inflammation is characterized by the presence of inflammatory cells, cytokines,

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and mediators in the lungs, which damage lung structure and induce cell death.[5]

Adipokines, proteins secreted by adipose tissue, are involved not only in energy metabolism but also in inflammatory responses in chronic diseases.[6,7] While much is known about adipokines like adiponectin and leptin in chronic obstructive pulmonary diseases, the roles of newer adipokines such as nesfatin-1 and visfatin in inflammatory lung diseases are less studied.

Nesfatin-1 is a polypeptide expressed from nucleobindin-2 (NUCB2) in the hypothalamus.[8,9] NUCB2 has also been shown to be secreted outside the central nervous system, mainly in the gastric mucosa and white adipose tissue, as well as in small amounts in the periphery, especially in adipose tissue, pancreatic endocrine beta cells and testicular tissues.[9,10] Nesfatin-1 has been linked to higher plasma levels in lung cancer patients with fat mass changes and in cystic fibrosis patients with low fat mass.[11,12] Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT), inhibits neutrophil apoptosis[13] and plays a role in nicotinamide adenine dinucleotide (NAD) biosynthesis.[14] It is synthesized not only in visceral fat but also in various tissues, including lymphocytes, hepatocytes, and pneumocytes.[13]

In the light of the existing body of knowledge, we aimed to investigate the relationship between novel adipocytokines such as nesfatin-1 and visfatin and the inflammatory microenvironment, in patients diagnosed with BPD.

Materials and Methods

Subjects

The study included thirty patients diagnosed with BPD who were being followed up in the Pediatric Pulmonary Diseases Department of our hospital. The inclusion criteria were being followed with a diagnosis of BPD and being between 0-3 years of age. Patients with infections and/or known chronic diseases were excluded from the study. We included BPD patients in the study when they visited our pediatric pulmonary diseases outpatient clinic for follow-up. The control group consisted of 30 age- and sex-matched children without infection and acute/chronic disease, who applied to our hospital's Social Pediatrics child health follow-up outpatient clinic for routine follow-up and vaccinations. The study was conducted with the approval of the Clinical Research Ethics Committee of Ankara University, and informed consent forms were obtained from each participant prior to enrollment.

Pulse oximetry saturations were measured in room air. We recorded whether they received oxygen support at home and whether they used inhaled steroids. Following the examination of the BPD patients, blood samples for a complete blood count (CBC) and C-reactive protein (CRP), and a chest X-ray were obtained. Pathological findings such as peribronchial thickness or infiltration and hyperinflation were recorded. Venous blood samples were taken from healthy controls and BPD patients for measurement of visfatin, nesfatin-1, nuclear factor kappa B (Nf-κB), matrix metalloproteinase-9 (MMP-9), interleukin-4 (IL-4), interleukin-10 (IL-10) and CRP levels, CBC and gene expression analysis.

ELISA Measurements

The plasma levels of visfatin and nesfatin-1 were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (FineTest). The analytical sensitivities of the kits were 0.188 ng/mL, and the detection range were between 0.313-20 ng/mL. The coefficients of variation (CV) within and between tests were less than 8% and 10%, respectively. The analytical sensitivity of the ELISA kit (FineTest) used for the determination of plasma levels of IL-4 was 18.75 pg/mL and the detection range was between 31.25-2000 pg/mL. The plasma levels of IL-10 were determined by the ELISA kit (FineTest), while the analytical sensitivity was 4.68 pg/mL, the detection range

was between 7.81-500 pg/mL. The intra-assay and inter-assay coefficients of variation for interleukin kits were less than 8% and 10%, respectively. The analytical sensitivity of the kit (FineTest) which was used for the detection of plasma Nuclear Factor kappa B levels was less than 0.188 ng/mL, while the detection range was between 0.313-20 ng/mL. The intra-assay and inter-assay coefficients of variation were less than 8% and 10%, respectively. The plasma levels of Matrix metalloproteinase-9 protein were also determined by a commercially available ELISA Kit (eBioscience) and the limit of detection of human MMP-9 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.05 ng/mL (mean of 6 independent assays). The calculated overall intra-assay and inter-assay coefficients of variation were 7.3% and 10.2%, respectively.

Gene expression analyses

Total RNA was isolated from whole blood samples collected into EDTA-containing tubes using the RNA isolation kit (Qiagen). cDNA samples were generated with the PCR system (Qiagen RotorGene) using cDNA synthesis kit (Qiagen). Quantitative real-time PCR (RT-PCR) was performed using the Qiagen RotorGene system. ß-actin was used as the housekeeping gene for normalization. The PCR reaction mix were prepared according to the manufacturer's recommendations. Samples were analyzed in duplicate. The forward and reverse primer sequences were as follows: 5′-GGGAGGCTAAGCAAAGAACTG-3′ forward,5′TCCATGCCTATATCTTGAAGGGA-3′ reverse for nesfatin-1; 5′-AATGTTCTCTTCACGGTGGAAA-3′ forward, 5′-ACTGTGATTGGATACCAGGACT-3′ reverse for visfatin; 5′-TGTACCGCTATGGTTACACTCG-3′ forward, 5′-GGCAGGGACAGTTGCTTCT-3′ reverse for MMP-9. The results were calculated with the $2^{-\Delta\Delta Ct}$ method and expressed as fold change in the BPD group normalized with the controls.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism (GraphPad Inc., Version 6) statistical program. Demographic data, plasma cytokine levels, oxygen saturation levels, plasma CRP levels, blood white blood cells (WBC), neutrophil and lymphocyte counts were expressed as mean±standard deviation and compared with Mann-Whitney U test. Plasma cytokine levels of BPD patients grouped according to receiving inhaled steroid treatment at home, oxygen supplementation at home and having pathological findings on chest radiography were compared with the student-t test. Real-time PCR results were analyzed using a one-way ANOVA test. Correlation analyses between plasma cytokine levels and oxygen saturation levels, plasma CRP levels, and blood WBC, neutrophil and lymphocyte counts, respectively in the BPD group; and correlation analyses between visfatin and MMP-9 and Nf-κB respectively in the BPD group were performed using the Spearman correlation test. P values <0.05 were considered statistically significant.

Results

The demographic data and clinical characteristics of patients and control subjects are presented in Table I.

Our findings showed that the plasma levels of visfatin were significantly higher in the BPD group (7.05±4.07 ng/mL) compared to the control group (2.13±1.66 ng/mL), (p<0.0001). On the other hand, nesfatin-1 levels did not differ significantly between BPD and control groups (27.09±10.92 ng/mL vs. 22.83±8.34 ng/mL, respectively, Table II). The levels of IL 4 in BPD and control groups were determined as 18.18±6.86 pg/mL and 15.73±2.68 pg/mL, respectively, while the IL-10 levels were 139.62±83.65 pg/mL and 133.32±83.16 pg/mL, respectively and no significant difference was calculated between the groups. The plasma MMP-9 levels were significantly higher in the BPD group (2.34±0.94 ng/mL) compared to the

Table I. Demographic and clinical characteristics of BPD and control groups.

Variable	BPD (n=30)	Control (n=30)
Birth week (mean±SD)	28.53± 2.9	37.85±1.97
Birth weight (gram) (mean±SD)	1188.96±489.2	3152.21±310
Current age (month) (mean±SD)	32.12±6.58	34.00±2.12
Female/Male	1/2	1.2/1.8

BPD: bronchopulmonary dysplasia.

Table II. The comparison of plasma levels of visfatin, nesfatin-1, interleukin-4, interleukin-10, matrix metalloproteinase-9 and nuclear factor kappa B in bronchopulmonary dysplasia patients and healthy controls.

	Control (n=30) mean±SD	BPD (n=30) mean±SD	p value
Visfatin (ng/mL)	2.13±1.66	7.05±4.07	<0.0001
Nesfatin-1 (ng/mL)	22.83±8.34	27.09±10.92	0.3180
IL-4 (pg/mL)	15.73±2.68	18.18±6.86	0.2527
IL-10 (pg/mL)	133.32±83.16	139.62±83.65	0.1902
MMP-9 (ng/mL)	1.46±0.64	2.34±0.94	<0.0001
Nf-κB (ng/mL)	0.81±0.58	4.51±3.86	<0.0001

BPD: bronchopulmonary dysplasia; IL-4: interleukin-4; IL-10: interleukin-10; MMP-9: matrix metalloproteinase-9; Nf-κB: nuclear factor kappa B. P < 0.05 is considered as statistically significant.

control group (1.46±0.64 ng/mL, p<0.0001). Similarly, a significant increase was observed in plasma Nf-κB levels in the BPD group (4.51±3.86 ng/mL) compared to the control group (0.81±0.58 ng/mL, p<0.0001, Table II).

According to the results of PCR experiments, mRNA expression levels of visfatin, nesfatin-1 and MMP-9 increased 1.36±0.12, 1.31±0.06 and 0.75±0.11 times in the BPD group compared to the control group, respectively (p< 0.05, Table III).

In the present study, the correlation analysis was performed between plasma visfatin levels and increased cytokines, MMP-9 and Nf-κB in the BPD group and no significant correlation was found between these cytokines (r values were 0.390 and 0.533 and p values were 0.073 and 0.074, respectively). The relationship between cytokines and various clinical variables in BPD patients was also examined by correlation analysis and no significant correlation was found (Table IV). When the relationship between cytokines and medication parameters was examined, a significant increase in MMP-9 levels was found in the group supplemented with oxygen at home compared to the group not receiving it (Table IV).

Table III. Fold change on mRNA expression levels of visfatin, nesfatin-1 and MMP-9 detected by RT-PCR in BPD group.

Age	Fold change mean±SD	p value
Visfatin	1.36±0.12	0.0121
Nesfatin-1	1.31±0.06	0.0187
MMP-9	0.75±0.11	0.0385

BPD: bronchopulmonary dysplasia; MMP-9: matrix metalloproteinase-9; RT-PCR: real-time polymerase chain reaction.
P < 0.05 is considered as statistically significant.

Discussion

Adipokines, through their involvement in inflammatory processes, may play a significant role in the pathogenesis of BPD by influencing lung inflammation and tissue damage in preterm infants. The roles of visfatin and nesfatin-1 in the pathogenesis of BPD remain largely obscure, with limited research exploring their potential contributions to lung inflammation and disease progression.

Table IV. Correlation analyses between plasma cytokine levels and oxygen saturation levels, plasma CRP levels, blood WBC, neutrophil and lymphocyte counts[a]; and comparisons of plasma cytokine levels of BPD patients grouped according to inhaled steroid treatment, oxygen supplementation and chest radiography status[b].

Parameters[a]		Visfatin		MMP-9		Nf-κB	
		r	p-value	r	p-value	r	p-value
Oxygen saturation (%)		-0.058	0.799	0.105	0.579	0.043	0.872
CRP (mg/L)		0.012	0.957	0.238	0.206	0.457	0.076
WBC (mm^3)		-0.051	0.820	-0.096	0.614	-0.021	0.937
Neutrophil (mm^3)		-0.032	0.887	0.238	0.204	0.172	0.520
Lymphocyte (mm^3)		-0.001	0.998	-0.327	0.078	-0.146	0.584
Parameters[b]		**Visfatin**		**MMP-9**		**Nf-κB**	
		Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value
Inhaled steroid treatment	+ (n=18)	7.61±1.27	0.495	0.23±0.01	0.841	0.47±0.04	0.342
	- (n=12)	6.38±1.18		0.23±0.03		0.41±0.04	
Oxygen supplement at home	+ (n=5)	8.41±1.69	0.511	0.31±0.06	0.03	0.52±0.02	0.257
	- (n=25)	6.63±1.05		0.21±0.01		0.42±0.03	
Pathological finding on chest radiography	+ (n=16)	7.51±1.48	0.634	0.24±0.02	0.479	0.45±0.04	0.910
	- (n=14)	6.66±1.04		0.22±0.02		0.44±0.05	

CRP: C-reactive protein, MMP-9: matrix metalloproteinase-9, Nf-κB: nuclear factor kappa B, WBC: white blood cells.

Visfatin is a pro-inflammatory cytokine involved in inflammatory and innate immune responses[7,15] and it was found to be associated with acute lung injury developing in the lungs.[7] In addition, inhibition of visfatin synthesis has been shown to increase inflammation and apoptosis associated with severe viral infection in the lung endothelium.[16] One study has reported that serum visfatin were elevated in stable chronic obstructive pulmonary disease and its acute exacerbations.[15] Nesfatin-1 is a recently discovered protein and it plays role in the inflammatory responses.[17,18] The studies have shown that nesfatin-1 plasma levels were found to be significantly higher in patients with lung cancer related to changes in fat mass[11,19] and in patients with cystic fibrosis with low fat mass.[20] In the present study, we reported significantly higher plasma levels of visfatin in BPD patients compared to healthy controls. Although plasma nesfatin-1 levels were higher in the BPD group compared to healthy controls, this difference was not statistically significant. Besides, mRNA expression of both visfatin and nesfatin-1 were higher in BPD patients. These results support the possible role of these novel adipocytokines on the inflammatory status of BPD patients.

Recent studies suggest that other cellular lines, such as mast cells accumulating in lung tissue and reactive T cells in peripheral blood, may influence the development of BPD.[21] The Nf-κB family of transcription factors are ubiquitously expressed, and function to regulate different cellular processes such as proliferation, differentiation, survival, and immunity.[22] There is limited data linking Nf-κB and BPD. A study on this subject, reported increased NfKB activation in tracheal lavage fluid of premature infants who develop BPD.[23] In the present study, we reported the increased plasma Nf-κB levels in BPD patients supporting the inflammatory status in BPD patients.

Matrix metalloproteinases are a family of zinc-dependent endopeptidases, and MMP-2 and -9 are gelatinases that are associated with inflammatory lung injury.[24] MMP-9 is synthesized and stored in neutrophil and eosinophil granules in the bone marrow. The production of MMP-9 is also induced by IL-1β, macrophages, Clara cells, alveolar type II cells, smooth muscle cells, fibroblasts and bronchial epithelial cells.[24] In our study, the MMP-9 plasma levels as well as mRNA expression

were found to be significantly increased in BPD patients, but no significant correlation were observed between MMP-9 and visfatin levels. It has been shown that increased MMP-9 activity in the lungs is associated with the development of BPD in newborn infants and animal models.[25] In contrast, one study reported that MMP-9 activity in the newborn lung might be a host-defense mechanism, which protects the lung against inflammatory injury, instead of playing a pathogenetic role in the development of BPD as previously suggested.[26]

In the present study, increased plasma visfatin levels were observed compared to controls, along with elevated levels of MMP-9 and NF-κB, both of which are key markers of inflammation and tissue remodeling.[27,28] Based on these findings, a correlation analysis was conducted to explore potential relationships between visfatin and other increased cytokines MMP-9 and Nf-κB, as well as some clinical variables. No significant correlation was found between visfatin and other cytokines. The lack of meaningful correlation may suggest that, while visfatin, MMP-9, and Nf-κB are individually elevated in BPD, their roles in disease progression involve different, non-interdependent pathways or mechanisms of action, rather than direct interaction. It is suggested that the complexity of the inflammatory processes in BPD involves multiple mediators contributing to lung damage through distinct but overlapping pathways. Additionally, the absence of a correlation may indicate that larger sample sizes or more targeted experimental conditions are required to better understand the interplay between these factors.

Given that receiving inhaled steroid treatment or home oxygen support may impact inflammation levels, our study compared the plasma cytokine levels of BPD patients who received these treatments with those who did not. We found that there was a significant increase in plasma MMP-9 levels only in those receiving oxygen support at home.

In conclusion, visfatin, one of the new adipocytokines, was investigated for the first time in BPD patients in the present study, and it was found that there was a significant increase in its levels. Therefore, the significant change in visfatin, MMP-9 and Nf-κB levels in BPD patients may be associated with the risk of developing inflammation in BPD. This finding is important in terms of revealing the existence of a new therapeutic target in the follow-up and treatment of the disease in question. In this context, the findings of the study will guide the development of new treatment strategies in the field of health.

Ethical approval

The study was approved by Ankara University Ethics Committee (date: 08.04.2019, number: 07-533-19).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BH, FBA; data collection: GO, NC, ST; analysis and interpretation of results: BH, NC, FBA; draft manuscript preparation: BH, NC, FBA. 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

Role of serum procalcitonin in differentiating disease flare and systemic bacterial infection among febrile children with known chronic rheumatic diseases: a cross-sectional study

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ABSTRACT

Objectives. To evaluate the role of serum procalcitonin (PCT) as a diagnostic tool to differentiate bacterial sepsis from flare-ups during febrile episodes in children with known rheumatic disorders compared to other inflammatory markers like C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR).

Methods. Previously diagnosed patients with known rheumatic disorders presenting in emergency or outpatient departments with febrile episodes were included in the study. Blood samples were collected upon admission to test for signs of infection, including serum PCT levels with routine laboratory and radiological tests. Patients with juvenile idiopathic arthritis (JIA) and systemic lupus erythematosus (SLE) were stratified using the Juvenile Arthritis Disease Activity Score (JADAS-27) and SLE Disease Activity Index (SLEDAI) respectively. Patients without bacterial focus with high disease activity were included in the flare-up group and the rest in the sepsis cohort. The diagnostic value of PCT was calculated using receiver operating characteristic (ROC) curve analysis.

Results. In the study (N=73), 41 (56.2%) patients were previously diagnosed with JIA and 28 (38.3%) had SLE. 38 patients had definite evidence of sepsis and 35 had disease flare-ups as per respective disease activity scores. There was a significant difference in PCT and CRP among the flare-up and sepsis groups. For detecting sepsis, the area under curve (0.959), sensitivity (94.7%), and specificity (74.3%) of PCT at a cut-off of 0.275 ng/mL were significantly better than those of CRP.

Conclusion. PCT is a better diagnostic test than CRP or ESR during febrile episodes in differentiating flare-ups from infection and PCT >0.275 ng/mL indicates bacterial infection with good specificity and sensitivity in children with low disease activity.

Key words: systemic lupus erythematosus, procalcitonin, rheumatic flare-ups, juvenile idiopathic arthritis, sepsis.

Children with rheumatic diseases are at an increased risk of infections[1] due to multiple reasons such as the intrinsic disturbances of immune responses[2], long-term use of immunosuppressive drugs, and associated organ complications.[3] Fever is one of the most

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common presentations of chronic rheumatic diseases, either as a flare-up in the disease course itself or an infection. Both disease flare and secondary bacterial infection follow a similar pattern of presentations, ranging from mere febrile episodes to more complex symptoms like joint pain, myalgia, shortness of breath, etc. Although huge advancements have been made in the management of sepsis and related complications, it is still a major cause of mortality across all age groups, especially in developing countries. Hence, it is of paramount importance to identify sepsis at an early stage and start the treatment before further deterioration.

Apart from sepsis, these patients typically suffer from frequent episodes of worsening of the primary disease, called disease flares. Both sepsis and flare can be life-threatening and need urgent identification. However, they fall into two opposite spectrums, having entirely different pathophysiologies and needing different lines of treatment. Early exclusion of bacterial sepsis can allow prompt administration of specific treatment of inflammatory disease with escalating immunomodulators and avoid the use of empirical antibiotics. On the other hand, timely detection and management of bacterial infections with appropriate antibiotics can prevent sepsis-related morbidity. The fact, that these two phenomena have strikingly similar presentations, makes it challenging to differentiate between them clinically.[4,5] The search for a laboratory marker has been ongoing for a long time which can distinctly point in favor of the underlying disease entity and aid in timely therapy. Conventional laboratory markers pose several limitations in assessing patients with clinical suspicion of infection. Hence in recent years, significant effort has been made to identify a novel biological marker, successfully discriminating infectious aetiologies of fever from the non-infectious ones in known rheumatic patients. The classical inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell count (WBC) are of limited use due to their lack of specificity.[6] Their diagnostic sensitivity is blunted due to the long-term use of immunosuppressive medications, especially corticosteroids, in such patients.[7] Procalcitonin (PCT) level has been studied as a reliable marker differentiating systemic bacterial and fungal infections in different trials. PCT is a 13-kDa precursor protein of calcitonin of 116 amino acids, produced by the parafollicular C cells.[8] The body produces it in the liver, lung, kidney, adipocyte, and muscle, and its serum levels increase in response to bacterial toxins and IL-1b stimulus. PCT is virtually undetectable in the plasma of healthy individuals (<0.005 ng/mL)[9] and even in case of viral infections or systemic inflammatory disorders, it is only slightly elevated. However, bacterial and fungal infections lead to a sharp increase in this value. It often ranges between 10-100 ng/mL, depending on the severity of sepsis and the inflammatory response of the body to it.[10] When the infection is controlled, its serum level is reduced promptly. This study aims to utilize this property of PCT in distinguishing bacterial infection from inflammatory flare and compare its role with other inflammatory markers in children with chronic rheumatic illnesses.

Materials and Methods

This analytical cross-sectional study was conducted in a tertiary care hospital in eastern India from January 31, 2020, to December 31, 2022.

Patients

This is a cross-sectional study where data collection was done prospectively. Purposive sampling technique was chosen to include all febrile children with diagnosed rheumatic diseases like systemic lupus erythematosus (SLE), juvenile idiopathic arthritis (JIA), and antiphospholipid syndrome (APLA), visiting the out-patient or emergency department within the data collection phase of the study fulfilling the inclusion criteria.

Scheme of the study

All children between the ages of 1 month to 12 years with previously diagnosed rheumatic disorders for at least 6 months (this excludes acute conditions like Henoch Schönlein purpura and Kawasaki disease), presenting in an emergency (ER) / outpatient department (OPD) with febrile episodes for more than 3 days (temperature > 38 °C) and being admitted, were considered for inclusion in the study. Patients with visible signs of sepsis, such as abscess, having a recent history of major trauma, known thyroid disorder, malignancies, pancreatitis, and non-bacterial infections like dengue, malaria, scrub typhus, COVID-19, etc. were planned to be excluded.

Patients with JIA and SLE were stratified using the Juvenile Arthritis Disease Activity Score (JADAS-27)[11] and the SLE Disease Activity Index (SLEDAI), respectively. The JADAS-27 score is calculated by adding the scores of 4 core set criteria and it ranges between 0-57. Worsening in any 3 out of the 4 JADAS core criteria by >30% in any JIA patient is considered a flare.[12] A lower JADAS-27 score signifies lower disease activity and is favourable for the patient's prognosis. A high JADAS-27 score indicates increased disease activity and might need escalation of therapy.[13] Children without evidence of bacterial focus with high disease activity satisfying flare criteria were placed in the "Flare-up" group while the rest were in the "Sepsis" cohort. Similarly, for SLE patients, disease activity was assessed by the SLEDAI scores, and an increase of more than three points compared to the patient's previous score was considered an SLE flare.[14,15]

Upon admission, a pre-designed and pre-tested proforma was used to record each patient's background epidemiological, clinical, and treatment information. Blood samples on the day of admission were collected from the recruited children for hemoglobin, total leukocyte count, differential count, platelet count, ESR, CRP, PCT, serum complement, anti-double-stranded DNA (dsDNA), serum ferritin, serum triglyceride, and serum fibrinogen. Culture sensitivity of blood and other body fluids was sent as mandated clinically, before starting antibiotics.

PCT values were measured using the immune-fluorescence technique on an SIEMENS ADVIA Centaur® BRAHMS machine and recorded in tabular form. Chest X-ray, abdominal ultrasound, and urine analysis were done based on the clinical scenario. Of these, three patients were excluded because of diagnostic ambiguity and the possibility of macrophage activation syndrome (MAS). The children received parenteral ceftriaxone and amikacin after collection of blood for culture and sensitivity as first-line antibiotics as per hospital policy.

Clinical presentations and findings, positive blood cultures, imaging studies, and prompt clinical response to antibiotics were considered the "gold standard" in detecting sepsis. Blood cultures are not always positive in all bacterial infections and false positives (colonization) can occur. Hence the final diagnosis was made comprehensively using all clinical findings. The children included were divided into two groups retrospectively, either disease flare or sepsis based on the final diagnosis. There were no cases in this study where sepsis and flare were clinically suspected at the same time.

Statistical analysis

All the data regarding parameters under study were maintained in a Microsoft Excel 2007 spreadsheet. Further statistical analysis was done using the Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp. 2011. Armonk, NY, USA) for Windows.

Categorical variables were presented in terms of number and percentage (%). Normally distributed continuous variables were expressed using mean ± standard deviation (SD). Serum PCT, CRP, and ESR values between the two groups were compared using an independent t-test. The diagnostic value of PCT was calculated using the Youden method

in receiver operating characteristic curve (ROC) curve analysis. The area under the curve (AUC), sensitivity, specificity, and diagnostic accuracy at the specific cutoffs were calculated to compare the different indicators. A p-value <5% was considered to be statistically significant.

Ethics statement

The Institutional Ethics Review Committee granted ethical clearance. Informed consents from parents / legal guardians in written forms were taken before including the children as study participants. All tests were performed according to relevant guidelines and indications and in adherence to the tenets of the Declaration of Helsinki.

Results

A total of 137 children, diagnosed with chronic rheumatic diseases, attended the ER/OPD of the institute during the study period. Among them, 55 children didn't present with febrile episodes, hence they were excluded. Guardians of six children declined to take part in the study and three were excluded due to a possible diagnosis of MAS. A total of 73 children were included in the study, out of which 41 (56.2%) were JIA, 28 (38.3%) were SLE patients, 2 patients had anti-phospholipid syndrome (APLA), and the remaining two children had polyarteritis nodosa (PAN) and granulomatosis with polyangiitis, respectively (Fig. 1). The background characteristics of the patients are given in Table I.

The mean±SD age of the patients was 9.66+2.3 years, with a female:male ratio of 2.7:1. The mean age of the flare group was 9.51+2.6 years and that of the sepsis group was 9.80+2.1 years (Table I). The mean period of the primary illness was 2.53+1.6 years. All the children had fever at presentation with a mean duration of 8.04 +3.4 days. Apart from fever, the most common presenting feature of the study population was arthritis (64%) followed by rash (48%) and hepatosplenomegaly (32%). Net 38 patients were identified with detectable foci of infections without worsening of disease activity and, hence were included in the sepsis group. Among them, 11 children had positive blood cultures, cerebrospinal fluid (CSF) analysis of 9 children was suggestive of bacterial meningitis, 14 had culture-positive urinary tract infection (UTI), while others had radiological evidence of bacterial infection.

The sepsis group had higher values than the flare-up group in terms of CRP (43.79+30.4 versus 17.90+11.7 mg/dL) and serum PCT (1.86+0.8 versus 0.24+0.3 ng/mL), both of which were statistically significant ($p<0.05$). However, differences in absolute neutrophil count (ANC), platelet count, and ESR between the two groups were all nonsignificant (Table II).

The AUC (0.959), sensitivity (94.7%), and specificity (74.3%) of PCT at the cutoff value of >0.275 ng/mL, with a diagnostic accuracy of 84.9% were significantly better than those of CRP with AUC (0.827), sensitivity (73.7%), and specificity (73.6%) with a diagnostic accuracy

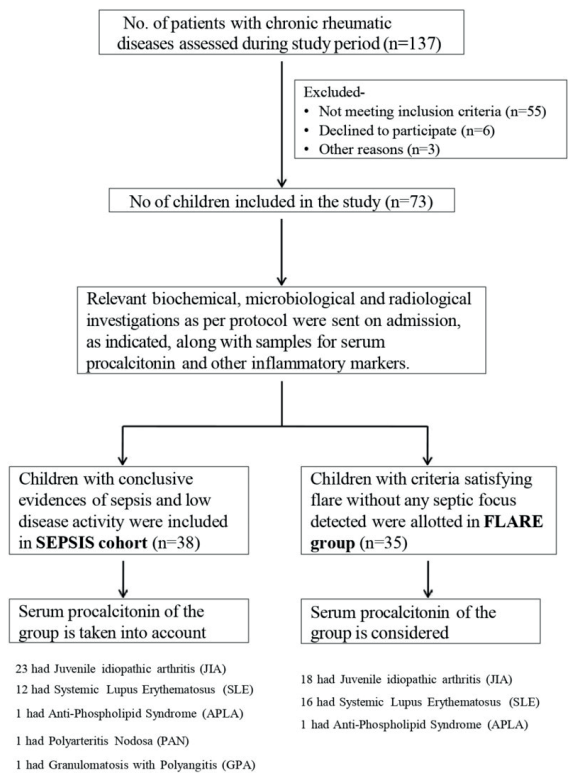

Fig. 1. Flow chart of the study population.

Table I. Demographic characteristics of the study population.

	Overall (n=73)	Flare-up (n=35)	Sepsis (n=38)
Age, yr, mean±SD	9.66 ± 2.3	9.51 ± 2.6	9.80 ± 2.1
Male gender, n (%)	20 (27.4)	9 (25.7)	11 (28.9)
Duration of fever, days, mean±SD	8.04 ± 3.4	8.54 ± 4.4	7.58 ± 2.9
Underlying condition, n (%)			
JIA	41 (56.2)	18 (51.4)	23 (60.5)
SLE	28 (38.3)	16 (45.7)	12 (31.6)
Others	4 (5.5)	1 (2.8)	3 (7.9)
Age at primary diagnosis, yr, mean±SD	7.30 ± 1.6	7.27 ± 1.8	7.31 ± 1.40
Interval since primary diagnosis, yr, mean±SD	2.53 ± 1.6	2.21 ± 1.4	2.78 ± 1.7

JIA, juvenile idiopathic arthritis; SD, standard deviation; SLE, systemic lupus erythematosus.

Table II. Comparison of inflammatory markers between subgroups of the study population (n=73).

	Flare-up (n=35)	Sepsis (n=38)	Significance*
CRP, mg/dL	17.90 ± 11.7	43.79 ± 30.4	0.001$
ESR, mm/hr	41.58 ± 15.2	49.74 ± 13.1	0.289$
Procalcitonin, ng/mL	0.24 ± 0.3	1.86 ± 0.8	<0.001$
Platelet count, x10^9/L	282.09 ± 199.1	205.83 ± 175.3	0.172$
Absolute neutrophil count, x10^9/L	5.48 ± 3.4	6.11 ± 3.0	0.180$

*p-value of independent t-test. Data presented as mean ± standard deviation.
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

of 72.60% for differentiating between flare and infection (Fig. 2). Using PCT ≥0.275 ng/mL, the sensitivity (94.7%), specificity (74.3%), positive predictive value (PPV) (80.00%), and negative predictive value (NPV) (92.8%) were higher than those of other inflammatory markers (Table III).

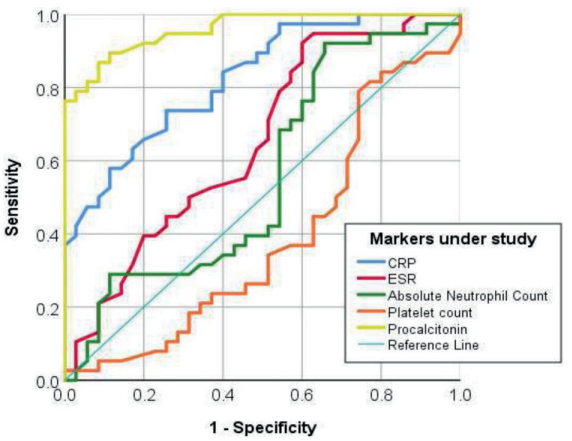

Fig. 2. Receiver operative characteristics (ROC) curve, showing different inflammatory markers under study to differentiate flare-up from bacterial sepsis. CRP, C-reactive protein, ESR, erythrocyte sedimentation rate.

Serum PCT levels on admission in all cases were compared with CRP, ESR, platelet count, and ANC using ROC curve analysis, and the optimum cut-off values for each of them were calculated by Youden's method. The cut-off values of PCT, CRP, and ESR were found to be 0.275 ng/mL, 24.1 ng/dL, and 44.5 mm/hr respectively, a value above which indicates more chance of sepsis. AUC of these parameters were 0.959, 0.827, and 0.653 respectively. Comparative ROC curve analysis reveals that serum PCT at a cut-off value of 0.275 ng/dL has the best diagnostic value among all studied parameters in differentiating flare-ups from sepsis in febrile patients with known rheumatic diseases. Serum PCT values of > 0.275 ng/dL correspond to a higher diagnostic accuracy of 84.90% with acceptable sensitivity, specificity, PPV, and NPV in predicting sepsis than the other markers, followed by CRP.

Table III. Areas under the curve of different markers as per ROC curve.

Markers	Area under curve	Cut-off value	95% confidence interval	p value	Sensitivity	Specificity
Procalcitonin, ng/mL	0.959	0.275	0.921-0.997	<0.001	94.7%	74.3%
ESR, mm/hr	0.653	44.5	0.526-0.780	0.025	63.2%	51.4%
CRP, mg/dL	0.827	24.1	0.736-0.918	0.002	73.7%	73.6%
ANC, /μL	0.561	4510	0.526-0.780	0.371	68.4%	42.9%
Platelets, /L	0.389	$170x10^9$	0.257-0.521	0.102	44.7%	37.1%

*The optimum cut-off values were calculated by Youden's method.
ANC, absolute neutrophil count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

Discussion

Sepsis and disease flare-ups are the two most important but diametrically opposite complications of any chronic rheumatic illness. Ongoing efforts are being made to establish a biomarker for the early differentiation between these two conditions in pediatric patients with chronic rheumatic conditions. However, the attempt is challenging due to scarce data among the children, especially in the Indian scenario. The current study aims to investigate the effectiveness of PCT in this regard. In addition, this study endeavors to conduct a comparative analysis between PCT and other commonly used laboratory markers, including ESR, CRP, platelet count, and ANC. Furthermore, it seeks to establish a cutoff value for diagnosis.

Bacterial infections are major causes of sepsis in SLE with the most frequent sites of infection being the respiratory tract, urinary tract, and skin. Nonetheless, a majority of SLE patients also get admitted with serious disease flares. A study in Southeast Asia on SLE patients admitted for serious complications revealed higher serum PCT levels in the bacterial infection group, a median value of 1.66 (0.04-8.45) ng/mL compared to the non–infection group, median value of 0.12 (0.02-0.81) ng/mL.[16] This study demonstrated a notable difference in serum PCT levels between the bacterial infection group and non-infection/flare groups in SLE patients. This resonates with our study, wherein only PCT and CRP were statistically significantly higher among children with sepsis. Contrary to our results, one of the studies conducted in France[17], which focused on SLE patients however concluded PCT can't reliably differentiate between flare and sepsis. However, their results were based on only 5 patients suffering from chronic infections. Supporting the relevance of our work, a systematic review and meta-analysis in 2021 illustrated that serum PCT and CRP levels were markedly elevated in SLE with bacterial infections. Notably, the authors identified PCT as a superior diagnostic marker to CRP with a high value of positive likelihood ratio.[18] Similar findings were documented in a Korean study where both CRP and PCT levels were higher in the infection group compared to the disease flare group.[19]

Research involving PCT and autoimmune diseases had already been started decades ago, but authors were unsure about a positive correlation. A study in Poland assessed PCT, CRP, and ESR in 28 children with autoimmune disease, including 9 with juvenile arthritis. They found that while ESR and CRP are sometimes elevated in children with autoimmune disease, PCT remains low in this population, but the study had its limitations.[20] Subsequent research on children with JIA suggested that serum ESR, CRP, and PCT levels could serve as viable biomarkers for distinguishing serious bacterial infection (SBI) from active JIA at presentation. Among these markers, PCT demonstrated the highest accuracy, showing minimal overlap between patients with infection and non-infectious inflammatory arthritis with a high sensitivity and specificity.[21] The current study also has similar findings where PCT at a cut-off of ≥ 0.275 ng/mL, had higher sensitivity

(94.7%) and specificity (74.3%) than the other inflammatory biomarkers in distinguishing sepsis.

Though a considerable number of studies on this topic have found serum PCT as the best diagnostic tool to differentiate the flare-up from bacterial sepsis, only a few have tried to reach an objective cut-off of PCT level. A PCT cut-off value as high as 0.5 ng/mL was observed in a study on heterogeneous rheumatic diseases.[22] A study on adult patients reported that the best cut-off value for PCT was 0.09 ng/mL.[19]

In cases of arthritis in adults, research has also shown PCT to be a useful biomarker. High PCT levels strongly suggest bacterial infection and it is better than either CRP, ESR, or WBC count in patients with RA.[23] This Japanese study used a cut-off value of 0.5 ng/mL for serum PCT with 98.2% specificity and 14.33% positive likelihood ratio. Our study had higher sensitivity and specificity at lower cut-offs, which can be explained by differences in disease activity, age, and ethnicity of the study population. One of the pediatric studies on rheumatic arthritis patients found a PCT concentration of less than 0.15 μg/mL in flare-up children[21], which is almost in congruence with our results.

A project on chronic rheumatic diseases in adults showed an area under the ROC curves (AUC; 95% CI) for CRP [0.70; (0.58-0.82)] and PCT [0.84; (0.75-0.93)] which indicated a significant difference (p<0.05). On the contrary, the authors didn't find any significant difference in predicted AUC between the CRP/ PCT levels combined and PCT levels alone (P=0.80). They determined 7.18 mg/dL to be the best cut-off value for CRP, with a sensitivity of 71.9% and a specificity of 68.1%. For PCT, the cut-off was 0.09 ng/mL, with 81.3% sensitivity and 78.7% specificity.[19]

The current study is one of the very few conducted on the pediatric population in eastern India, concerning this rather important problem with significant clinical implications. However, this study has its fair share of limitations as well. Firstly, this was a single-center study carried out in a tertiary care hospital, so hospital bias couldn't be ruled out. The sample size was small, more so, due to the COVID-19 pandemic and the consequent lockdown. Mostly JIA and SLE patients were studied in the wide spectrum of childhood rheumatic diseases as there was a lesser influx of patients with other chronic rheumatic conditions.

Secondly, there is a lack of a universally accepted uniform defining criterion for flare in JIA unlike in SLE. The core response value (CRV) criteria[9] are difficult to apply to the majority of the Indian population. Also, the authors of JADAS have themselves indicated that, although the score was devised to cover all categories of JIA, it still possesses certain limitations. A scoring system incorporating the extra-articular manifestations of systemic JIA, particularly fever and rash, would make the evaluation more robust and valid. Thirdly, PCT is elevated only in cases of bacterial and fungal infection but not non-bacterial infections like viral infections, thus making it difficult to differentiate from flare-ups. And finally, follow-up regarding long-term survival was beyond the scope of our study.

Conclusion

This study indicates that serum PCT can help differentiate between disease flare and sepsis in febrile children with chronic rheumatic diseases. PCT levels above 0.275 ng/mL in febrile patients with chronic rheumatic diseases can indicate sepsis, while a lower value may suggest non-infectious inflammation and reduce unnecessary antibiotic use.

Ethical approval

The study was approved by NRS Medical College and Hospital Institutional Ethical Committee (date: 27.01.2020, number: No/ NMC/446).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SM, MN, SM; data collection: SM, SS; analysis and interpretation of results: SM, SM, SS; draft manuscript preparation: MN, SM, SS. 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

Evaluation of metabolic syndrome components, serum uric acid levels and epicardial adipose tissue thickness in pubertal children by severity of obesity

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ABSTRACT

Background. We aimed to evaluate how the parameters used in the diagnosis of metabolic syndrome (MetS) and parameters such as epicardial adipose tissue (EAT) thickness, insulin resistance (IR), and serum uric acid (SUA) are affected according to the severity of obesity.

Methods. A total of 120 obese patients aged 10-18 years were classified as class 1-2-3 according to their body mass index (BMI) score. SUA was measured and oral glucose tolerance tests were performed on all patients. MetS components were determined according to the International Diabetes Federation 2007 criteria. IR was calculated using homeostatic model assessment for insulin resistance (HOMA-IR) and whole body insulin sensitivity index (WBISI).

Results. HOMA-IR was higher in the class 3 group than in the class 1 ($p<0.001$) and class 2 groups ($p<0.01$). WBISI was lower in the class 3 group than in the class 1 ($p=0.015$) and class 2 groups ($p<0.01$). EAT thickness was higher in the class 3 group than in the class 1 ($p<0.01$) and class 2 groups ($p<0.01$). No significant difference was found between class 1 and 2 groups for HOMA-IR, WBISI, and EAT thickness variables. The frequency of the MetS components was similar between the class of obesity groups ($p=0.702$). SUA and EAT thickness were significantly higher in the group with 2 and/or more MetS components than in the group with no MetS component. EAT thickness was positively and moderately correlated with SUA levels ($Rho=0.319$, $p<0.001$).

Conclusions. A more significant increase in cardiovascular disease risk factors, especially after class 2 obesity suggests that obese people should be followed closely and necessary interventions made for the prevention and progression of obesity. SUA and EAT thickness, an important risk factor affecting the obesity-related comorbidities, are positively correlated with each other and can be used in the follow-up of obese children.

Key words: obesity, epicardial adipose tissue, uric acid, metabolic syndrome, insulin resistance.

Obesity is one of the important childhood health problems, and its increasing prevalence can cause many serious obesity-related comorbidities such as insulin resistance (IR), type-2 diabetes mellitus (T2DM), metabolic syndrome (MetS), and cardiovascular diseases (CVD).[1,2] Although obesity is defined in terms of body mass index (BMI) determined by age and sex, BMI alone may not classify the risks of having obesity-related comorbidities. The utility of BMI in assessing obesity has been criticized for its inability to distinguish between fat, muscle, and skeletal weight. Individuals with similar BMI may have very different metabolic profiles.[3] However, Ortega et al.,[4] reported that BMI strongly predicts cardiovascular mortality

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and may be more important than total adiposity measures evaluated by complex, expensive methods.

Studies have also reported that regional, visceral, and organ-specific adiposity play an important role in the development of obesity-related comorbidities.[5] As people gain weight, fat accumulates mainly in the subcutaneous tissue. However, as weight gain continues, excess fat tends to accumulate in ectopic areas such as the liver, pancreas, kidney, muscle, and also heart.[6,7] The pathophysiological mechanisms underlying the transition from subcutaneous fat accumulation to ectopic fat accumulation have not yet been elucidated. Ectopic fat stores may contribute to obesity-related comorbidities, and this association may be relevant to clinical entities such as "metabolically healthy" obesity phenotypes.[8] Epicardial adipose tissue (EAT), which is one of the ectopic fat deposits, is a fat tissue deposit located mainly around the epicardial coronary vessels, on the right ventricle surface and on the anterior wall of the left ventricle, between myocardium and visceral pericardium.[9] Studies have shown a strong correlation between EAT thickness and anthropometric and imaging measurements of visceral adipose tissue.[10,11]

Although EAT serves important physiological functions, it has been shown in adults that excessive EAT is associated with coronary artery disease, MetS, IR, and impaired fasting glucose (IFG).[12,13] While the assessment of MetS in adults is based on criteria set by national or international organizations, assessment among children and adolescents is still unclear. Most assessments are based on adaptations based on adult criteria. Numerous biomarkers, including adipokines and inflammatory markers, have been discovered, better aiding the understanding of pathophysiology and detecting MetS early. Serum uric acid (SUA) is one of them. Studies have found that high SUA levels are associated with the risk of MetS.[14] Evaluation of EAT thickness by echocardiography has been developed as an indirect marker of CVD and metabolic changes in adults. However, studies in children are limited. Therefore, the main aim of this study is to evaluate how the parameters used in the diagnosis of MetS and parameters such as EAT thickness, IR, and SUA are affected according to the severity of obesity.

Materials and Methods

Participants

Patients aged 10-18 years with Tanner stage 3 and above who were examined and observed for obesity in Ankara Bilkent City Hospital pediatric endocrinology and pediatric cardiology clinics between September 2019 and December 2022 were included in this retrospective study. Adolescents with a BMI at or above the 95th percentile for children of the same age and sex and without chronic disease involving the endocrine, cardiac, or any other system were included in the obese group. Screening tests for T2DM in youth should be considered after the onset of puberty or > age ten years, whichever is earlier, in youth with BMI ≥ 85th percentile for age and sex with one or more of the following: family history of type 2 DM, race or ethnicity associated with higher risk, signs of insulin resistance or, low birth weight (small for gestational age) or high birth weight, maternal history of DM or gestational DM.[15] Oral glucose tolerance test (OGTT) was performed on patients who met these criteria. Patients with dyslipidemia, being investigated for high blood pressure, and a family history of early coronary artery disease were evaluated by pediatric cardiology. Obese patients who were assessed by cardiology and had an OGTT were included in the study. Previous studies reported that 1-hour OGTT glucose of > 155 mg/dL showed lower insulin sensitivity, impaired β-cell function, and worse cardiovascular risk profile and, therefore, are at greater risk of developing T2DM and cardiovascular disease.[16] OGTTs are performed at standard 0, 30, 60, 90, and 120 minutes in our department. All our patients were at Tanner stage 3 and above. The pubertal transition is a time during which rapid and dynamic changes

occur in various metabolic systems, including hormonal regulations, changes in body fat and its distribution, as well as increased insulin resistance. Insulin sensitivity changes with pubertal stages. It reaches its lowest point midway through maturation (Tanner stage 3), approaching near pre-pubertal levels at the end of maturation (Tanner stage 5).[17] To minimize differences that may arise from pubertal changes, those at Tanner stage 3 and above group were included. The control group, which is suitable for the obese group in terms of age, gender, and puberty was compared to the obese group in terms of EAT thickness. EAT thickness is routinely examined in patients undergoing echocardiography in the pediatric cardiology outpatient clinic. The healthy control group was composed of patients referred to pediatric cardiology with complaints such as chest pain and murmur, whose cardiological examination was normal, and whose BMI was below the 85th percentile for age and gender.

Patients with endocrine and syndromic causes of obesity, and using drugs that affect insulin action and secretion were excluded from the study. The study was approved by the Clinical Research Ethics Committee of Ankara Bilkent City Hospital with a decision no 23-4512 dated July 12, 2023.

Anthropometric and clinical measurements

The height and weight of all participants were measured. Weight measurement was made with electronic scales (measuring accuracy of 0.1 kg) with thin clothes without shoes. Height was measured with a Harpenden stadiometer (0.1 cm measurement accuracy), standing upright, with feet together and parallel, and with the shoulder and gluteal region touching the wall. BMI was calculated by dividing weight (kg) by height squared (m^2). Standard deviation score (SDS) of height, weight, and BMI were calculated. Obesity was diagnosed in patients with a BMI greater than the 95th percentile for age and gender.[18] Obesity is further divided into three classes according to the severity of obesity[18,19]: If BMI is ≥95th percentile to <120% of the 95th percentile according to age and gender, class 1; if BMI is ≥120% to <140% of the 95th percentile according to age and gender, class 2; if BMI is ≥140% of the 95th percentile for age and gender, class 3.

Tanner staging system was used in pubertal staging. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times after 10 minutes of rest in the supine position. The mean of the three measurements was calculated. Individuals were considered hypertensive if the mean of the measurement was 95th percentile and above or SBP ≥130 mmHg or DBP ≥85 mmHg.[20,21]

Laboratory tests

Plasma glucose (PG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), g-glutamyl transferase (GGT), SUA, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured for the obese group after 12 h of fasting by enzymatic colorimetric assays (Atellica Solution CH90, Siemens, Germany). Insulin levels were measured by chemiluminescence immunoassay (Siemens Healthineers, Erlangen, Germany).

Obese patients underwent the OGTT after 12 hours of fasting. Participants were given 1.75 g/kg glucose (maximum 75 g) in an average of 5 minutes. Venous blood samples were taken at 0, 30, 60, 90, and 120 minutes for plasma glucose and insulin. The results were evaluated according to the American Diabetes Association criteria[22]:

- Fasting plasma glucose (FPG) <100 mg/dL (5.6 mmol/L) and 2-h PG <140 mg/dL (7.8 mmol/L), normal glucose tolerance.
- FPG 100–125 mg/dL (5.6–6.9 mmol/L), IFG.
- 2-h PG 140–199 mg/dL (7.8–11.0 mmol/L), impaired glucose tolerance (IGT).

- FPG ≥126 mg/dL (7.0 mmol/L) and 2-h PG ≥200 mg/dL (11.1 mmol/L), diabetes mellitus.

IR was calculated using the homeostasis model assessment of fasting insulin resistance (HOMA-IR): FPG (mmol/L) X fasting insulin (mIU/L) /22.5.

Insulin sensitivity was calculated using whole-body insulin sensitivity index (WBISI).

$$\mathrm{WBISI} = \frac{10000}{\sqrt{G_0 \times I_0 \times G_{\mathrm{mean}} \times I_{\mathrm{mean}}}}$$

where G_0 stands for fasting glucose, I_0 for fasting insulin, G_{mean} for the mean PG concentration during the OGTT, and I_{mean} for the mean plasma insulin concentration during the OGTT.[23]

The criteria developed by the International Diabetes Federation (IDF) were used as MetS criteria[21]: Since waist circumference was not measured, other criteria of the MetS were evaluated.

- 10 to 16 years old: TG ≥150 mg/dL (1.7 mmol/L); HDL <40 mg/dL (1.03 mmol/L); SBP ≥130 mmHg or DBP ≥85 mmHg, or treatment for hypertension, or a SBP level of at least 95th percentile for sex, age and height; FPG levels ≥100 mg/dL (5.6 mmol/L) or known T2DM
- > 16 years old (adult criteria): TG ≥150 mg/dL (1.7 mmol/L); HDL <40 mg/dL (1.03 mmol/L) in males, < 50 mg/dl (1.29 mmol/L) in females; SBP ≥130 mmHg or DBP ≥85 mmHg, or treatment for hypertension; FPG levels ≥100 mg/dL (5.6 mmol/L) or known T2DM

Echocardiographic examination

All patients were evaluated by a single experienced pediatric cardiologist with the same ultrasound system (iE33, Philips, The Netherlands, Eindhoven) equipped with a broadband (1-5 MHz) X5-1 transducer. EAT, identified as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium, was measured from the left lateral decubitus position of the participants. The measurements from its thickest part were performed on each parasternal long-axis and short-axis view by directing the ultrasonic beam perpendicular to the right ventricular free wall from the reference point of the aortic annulus on the parasternal long-axis and the reference point of the interventricular septum and papillary muscle tip on the parasternal short axis section at the end of systole. The average value of three cardiac cycles from each echocardiographic view was computed. In addition, apical 4-chamber, parasternal long axis, and parasternal short axis images of all echocardiographies performed in our hospital are routinely recorded in the system.

Statistical analysis

All analyses were carried out with SPSS 25.0 (IBM, USA). The findings of the study are expressed as frequency and percentages. Normality analysis was carried out using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The variables without normal distribution are presented as median and interquartile range (IQR) with 25-75 percentiles, while variables with normal distribution are expressed as mean±standart deviation. Categorical variables were compared with the chi-square test using Yate's correction. Numerical variables with and without normal distribution were compared using the independent samples t-test and Mann-Whitney U test, respectively. One-way ANOVA and Kruskal-Wallis tests were used to compare numerical variables between more than two groups. Post-hoc multiple comparisons were made with Bonferroni or Dunnett's T3 tests according to equality of variances. Spearman correlation analysis was performed to determine the variables associated with EAT thickness and the obesity class. The correlation of SUA and EAT thickness with the scatter plot graphic is shown in Fig. 1. Multiple linear regression analysis using the backward method was performed to determine variables associated with EAT thickness. ROC analysis was

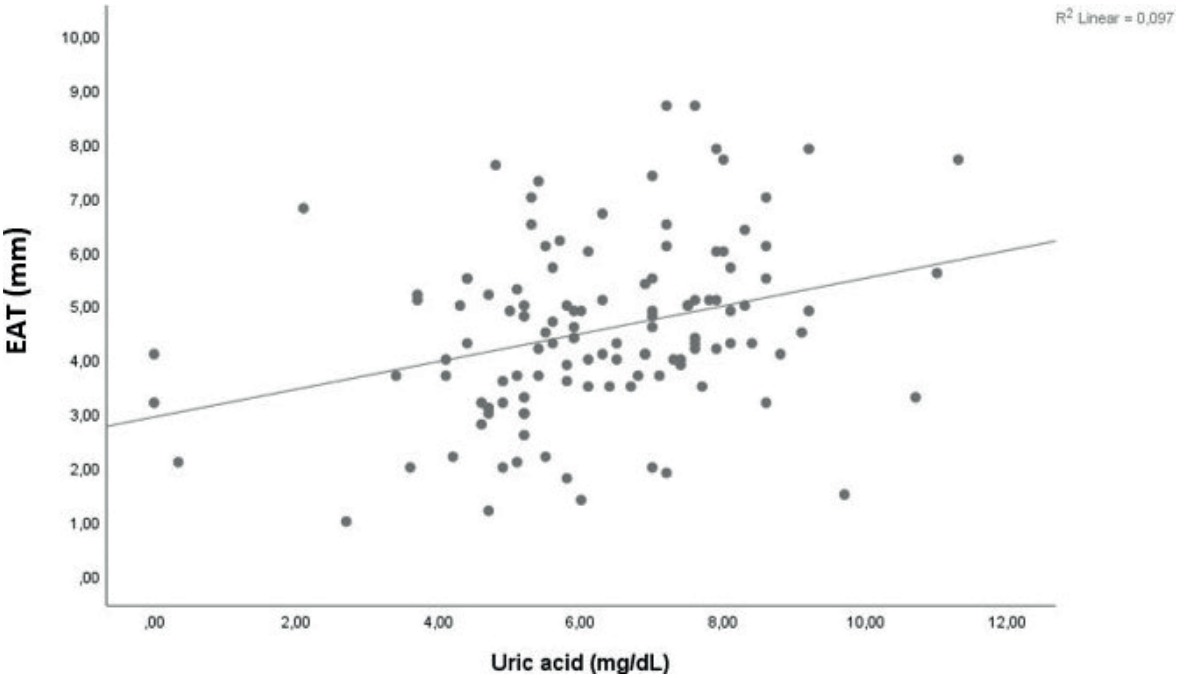

Fig. 1. Scatter plot of epicardial adipose tissue (EAT) thickness and serum uric acid level.

conducted to determine the possible association of BMI SDS and EAT thickness. The confidence interval was set at 95%, and the margin of error accepted was set to 5%. Therefore, the p <0.05 was considered significant.

Results

One hundred twenty obese patients (55 males and 65 females) with a mean age of 14.9±1.8 years were studied. Their median BMI SDS was 2.9 (Q1-Q3: 2.5-3.3). The control group included 95 normal-weight children (43 males and 52 females) with a mean age of 14.9±1.7 years. Their median BMI SDS was -0.25 (Q1-Q3: -0.73-0.44). EAT thickness was found to be statistically significantly higher in obese patients than in the healthy control group (p<0.001).

Demographic, clinical features and laboratory parameters of obese patients classified as class 1-2-3 according to the severity of obesity are presented in Table I. While the rate of males was higher in the class 1 group, the rate of females was higher in classes 2 and 3. The frequency of the female gender increased in line with the BMI category. HOMA-IR, WBISI, and EAT thickness were significantly different between classes of obesity groups. No significant difference was found between classes 1 and 2 for HOMA-IR and WBISI variables (p>0.05). Therefore, HOMA-IR was higher in the class 3 group than in the class 1 (p<0.001) and the class 2 group (p<0.01). WBISI was lower in the class 3 group than the class 1 (p=0.015) and the class 2 group (p<0.01). EAT thickness was higher in the class 3 group than in the class 1 (p<0.01) and the class 2 group (p<0.01). However, no difference was found between the classes 1 and 2 (p=0.889). A significant difference was found between groups when EAT thickness was compared between the control, class 1, 2, and 3 groups (p<0.001). Post hoc analysis showed that differences existed between the control group-class 1 group (p<0.001), control group-class 2 group (p<0.001), and control group-class 3 group (p<0.001).

Each component of MetS was compared between the class of obesity groups. The groups were similar regarding high TG levels, low HDL levels, and high blood pressure. IFG or the presence of DM could not be compared because

Table I. Comparison of demographic, clinical features and laboratory findings according to class of obesity.

	Class 1 (n=41)	Class 2 (n=47)	Class 3 (n=32)	P
Demographic and clinical features				
Female/male (n)	15/26	27/20	23/9	<0.01
Age (years)	15.0±1.9	14.9±1.9	14.9±1.7	0.941
BMI (kg/m^2)	30.7 (30.1-31.5)	33.9 (32.6-34.9)	39.6 (36.4-42.7)	<0.001
BMI SDS	2.4 (2.2-2.5)	2.9 (2.8-3.1)	3.6 (3.4-4.1)	<0.001
SBP (mmHg)	125.0 (120.0-135.0)	125.0 (120.0-135.0)	121.5 (120.0-130.5)	0.346
DBP (mmHg)	66.0 (60.0-76.3)	80.0 (70.0-90.0)	70.0 (67.3-80.0)	0.187
Laboratory parameters				
Urea (mg/dL)	23.9±5.3	22.9±4.9	21.9±3.9	0.430
Creatinine (mg/dL)	0.7±0.1	0.7±0.1	0.7±0.1	0.664
Uric acid (mg/dL)	6.1 ±2.0	6.2±1.8	6.2±1.9	0.638
ALT (U/L)	36.5 (25.0-51.5)	26.0 (21.0-39.0)	35.0 (23.3-51.0)	0.129
AST (U/L)	23.5 (16.0-30.3)	22.0 (15.0-28.0)	24.5 (17.0-31.3)	0.304
GGT (U/L)	25.0 (17.8-31.3)	19.0 (15.0-25.0)	21.0 (16.8-27.3)	0.227
Fasting plasma glucose (mg/dL)	90.7±7.3	90.9± 7.5	89.9 ±12.8	0894
Fasting insulin (mIU/L)	19.0 (8.9-44.0)	21.0 (6.0-52.0)	32.0 (15.0-107.0)	<0.001
Total cholesterol (mg/dL)	170.5± 32.9.0	164.1±29.0	166.0±34.6	0.635
HDL (mg/dL)	42.1±8.7	42.7±9.3	39.7±8.4	0.252
LDL (mg/dL)	103.1±27.2	94342.7±25.0	96.9±21.9	0.316
TG (mg/dL)	110.0 (88.0-149.5)	115.0 (88.0-158.0)	138.5 (92.5-184.3)	0.146
HOMA-IR	4.3 (3.2-6.2)	4.5 (3.5-7.0)	7.1 (5.2-9.7)	<0.001
EAT thickness (mm)	4.0±1.3	4.2±1.7	5.2±1.4	<0.01
WBISI	2.8 (1.6-2.9)	2.1 (1.4-2.9)	1.4 (1.1-1.8)	<0.01

Data presented as mean±standard deviation, or median (Q1-Q3).
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; DBP: Diastolic blood pressure; EAT: Epicardial adipose tissue; GGT: g-glutamyl transferase; HDL: High-density lipoprotein; HOMA-IR: Homeostatic model assessment for insulin resistance; LDL: Low-density lipoprotein; SBP: Systolic blood pressure; SDS: Standard deviation score; TG: Triglyceride; WBISI: Whole body insulin sensitivity index.

of the low number of cases in the groups (Table II). The frequency of metabolic syndrome components was similar between the class of obesity groups (p=0.702) (Table III). In the subgroup analysis, the proportion of patients without MetS components was significantly lower in the class 3 obesity group than in class 1 and 2 (p=0.034).

The comparisons of demographic and clinical variables regarding number of abnormal MetS components are shown in Table IV. AST level was found to be higher in the 2 and/or more MetS component group than the 1 MetS component group (p=0.013) and the group without MetS component (p<0.01). The SUA levels were different among groups. It was significantly higher in the group with 2 and/or more MetS components than in the groups with no MetS component (p<0.01) and 1 MetS component (p=0.024). WBISI value was significantly different between groups (p=0.045). WBISI was lower in the 2 and/or more MetS component group than in the groups with no MetS component (p=0.025) and 1 MetS component (p=0.039). The EAT thickness level was higher in the group with 2 and/or more MetS components than in the group without a MetS component (p=0.047); therefore, EAT thickness was statistically similar to that of the group with 1 MetS component. Also, no significant difference was found between 1 MetS component group and the group without any MetS component (p>0.05).

Table II. Prevalence of each component of metabolic syndrome according to class of obesity.

MetS Components	Class 1 (41)	Class 2 (47)	Class 3 (32)	P
Triglycerides ≥ 150 mg/dL	10 (24.3)	12 (25.5)	14 (43.7)	0.099
HDL-cholesterol < 40 mg/dL, 16+ age in females < 50 mg/dL	22 (53.6)	21 (44.6)	18 (56.2)	0.590
Hypertension	7 (17.1)	8(17.0)	8 (25)	0.619
Impaired FBG or T2DM	3 (7.3)	4 (8,5) 1(2.1)	3 (9.4) 3 (9.4)	-

Data presented as number (percentage).
FBG: Fasting blood glucose; HDL: High-density lipoprotein; MetS: Metabolic syndrome; T2DM: Type 2 diabetes mellitus.

Table III. Comparison of frequency of metabolic syndrome components between class of obesity groups.

Number and frequency of MetS components	Class 1 (41)	Class 2 (47)	Class 3 (32)	All	p
0	15 (36.5)	18 (38.3)	4 (12.5)	37 (30.8)	
1	16 (39.1)	16 (34.1)	14 (43.8)	46 (38.4)	0.702
2 and/or more	10 (24.4)	13 (27.7)	14 (43.8)	37 (30.8)	

Data presented as number (percentage).
MetS: metabolic syndrome.

Table IV. Comparison of demographic, clinical features and laboratory findings regarding number of abnormal metabolic syndrome components.

	No component (n=37)	1 component (n=46)	2 and/or more component (n=37)	p
Female/male (n)	25/12	26/20	14/23	0.157
BMI SDS	2.9±0.5	3.0±0.6	3.0±0.6	0.377
ALT (U/L)	25.5 (21.3-39.0)	30.5 (19.3-57.8)	36.0 (27.8-56.3)	0.08
AST (U/L)	19.0 (13.8-23.3)	21.0 (16.3-29.8)	26.0 (21.8-32.0)	<0.01
GGT (U/L)	19.0 (15.0-24.0)	20.0 (16.0-31.0)	24.5 (17.0-43.5)	0.085
Uric acid (mg/dL)	5.7±1.4	6.1±2.0	7.1±1.6	<0.01
HOMA-IR	5.6 (3.9-6.4)	5.3 (3.7-7.5)	6.8 (3.7-9.0)	0.060
WBISI	1.9 (1.4-2.7)	1.8 (1.3-2.5)	1.3 (1.1-1.9)	0.045
EAT thickness (mm)	4.1±1.6	4.5±1.6	5.0±1.5	0.047

Data presented as mean±standard deviation, or median (Q1-Q3).
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; EAT: Epicardial adipose tissue; GGT: g-glutamyl transferase; HOMA-IR: Homeostatic model assessment for insulin resistance; SDS: Standard deviation score; WBISI: Whole body insulin sensitivity index.

Correlation analysis of variables with the class of obesity and EAT thickness in the patient group are shown in Table V. There was a weak positive correlation between the class of obesity and EAT thickness (Rho=0.216 p=0.018), a moderate positive correlation between the class of obesity and HOMA-IR (Rho=0.342, p<0.001), and finally, a moderate negative correlation between the class of obesity and WBISI (Rho=-0.329, p<0.001). EAT thickness was weakly and negatively correlated with WBISI (Rho=-0.282, p<0.01), positively and weakly correlated with GGT (Rho=0.229, p=0.012), positively and moderately correlated with SUA (Rho=0.319, p<0.001). The simple scatter plot of EAT thickness and SUA is demonstrated in Fig. 1.

Multiple linear regression analysis revealed that male gender (p<0.001) and BMI SDS (p<0.01) were positively associated with EAT thickness, while WBISI (p=0.021) was negatively associated with it (Table VI). Age, SBP, DBP, total cholesterol, LDL, HDL, TG, HOMA-IR, and uric acid levels were not associated with

Table V. Correlation analysis of variables with class of obesity, epicardial adipose tissue thickness in patient group (n=120).

	Class of obesity		EAT thickness	
	Rho	P	Rho	P
Class of obesity	1.000	-	0.216	0.018
SBP	0.102	0.268	0.253	<0.01**
DBP	0.173	0.058	0.147	0.109
EAT thickness	0.216	0.018	1.000	-
HOMA-IR	0.342	<0.001**	0.102	0.268
WBISI	-0.329	<0.001	-0.283	<0.01**
GGT	-0.066	0.474	0.229	0.012*
Uric acid	0.034	0.710	0.319	<0.001***
Total cholesterol	-0.053	0.567	0.019	0.832
LDL	-0.101	0.268	-0.012	0.894
HDL	-0.108	0.237	-0.142	0.120
TG	0.165	0.070	0.117	0.200

DBP: Diastolic blood pressure; EAT: Epicardial adipose tissue; GGT: g-glutamyl transferase; HDL: High-density lipoprotein; HOMA-IR: Homeostatic model assessment for insulin resistance; LDL: Low-density lipoprotein; SBP: Systolic blood pressure; TG: Triglyceride; WBISI: Whole body insulin sensitivity index.
* p<0.05, **p<0.01, ***p<0.001

Table VI. Multiple linear regression analysis of factors associated with epicardial adipose tissue thickness.

Backward model final step*	Unstandardized Coefficients- B	t	p	95% Confidence Interval for B	
Variables				Lower	Upper
Constant	-0.481	-0.323	0.747	-0.3429	2.467
Gender	1.170	3.868	<0.001	0.571	1.769
BMI SDS	0.834	3.177	<0.01	0.314	1.354
SBP	0.022	1.966	0.052	0.000	0.044
WBISI	-0.343	-2.340	0.021	-0.633	-0.053

*Adjusted R^2: 0.528
BMI: Body mass index; SDS: Standard deviation score; WBISI: Whole body insulin sensitivity index.

EAT thickness (p>0.05). ROC analysis showed no significant predictive value for BMI SDS value when the EAT thickness cut-off value was 3.55 (p=0.564, sensitivity 50.0%, specificity 58.2%).

Discussion

The current study evaluated MetS components, EAT thickness, and SUA according to the severity of obesity in pubertal children. In addition, liver function tests, EAT thickness, SUA, HOMA-IR, and WBISI were examined to determine how they changed according to the MetS risk. Especially class 3 obesity in adolescent children was found to be associated with a high prevalence of abnormal levels of cardiometabolic risk factors. There was no difference between class 1 and 2 obesity in terms of both EAT thickness and cardiometabolic risk factors. As MetS components increased, higher EAT thickness, SUA, and impaired IR were detected.

Although the general statement that obese people have a higher risk of CVD and MetS than people with normal body weight is still valid, the severity of visceral adiposity is considered to be a more substantial cardio-metabolic risk factor

than body weight.[24] Therefore, neither BMI nor its derivatives alone may be very reliable for cardiometabolic risk markers. In a study by Skinner et al.[19], the risks of low HDL level, high SBP, high DBP, high TG level, and high glycated hemoglobin level were greater among children and young adults with class 3 obesity than those with class 1 obesity. Another study reported that increased BMI has a significant negative effect on IR, glycemia, lipids, and BP.[25] In our study, which is slightly different from the literature, the severity of obesity did not have a different effect on glycemia, lipids, and BP. In the class 3 group, both IRs were significantly higher, and the number of obese patients without the MetS component was significantly lower compared to the class 1 and 2 groups.

Despite the importance of determining EAT thickness for its possible predictive value for CVDs and the associations of EAT thickness with indirect measures linked to excess adiposity, little research has combined the indirect (BMI, waist circumference, or blood pressure) and direct (echography) measures in pediatric populations.[26] It was reported that age, sex, and BMI could be among the most significant factors related to EAT thickness.[27] In addition, while EAT thickness has been shown to be positively correlated with BMI;[28,29] we found a positive correlation between the class of obesity and EAT thickness. We also showed that male gender and BMI SDS were positively associated with EAT thickness. For the first time it was shown in our study that EAT thickness was found to be statistically significantly higher in the class 3 group than in the class 1 and 2 group, but no significant difference was found between class 1 and 2. The fact that EAT thickness is similar in the class 1 and 2 groups and the metabolic picture is similar supports the idea that EAT also increases in the class 3 group, and the metabolic picture is more prone to deterioration. These results suggest that visceral fat distribution underlying the concept of metabolic healthy obesity is a stronger predictor of metabolic health and increased fat mass.[30] It also suggests that EAT thickness may increase more rapidly after class 2 obesity.

Studies on the relationship between EAT and obesity-related comorbidities in children are rare, and conflicting results have been found. Higher EAT thickness has been reported in adult studies in patients with MetS.[31] Mazur et al.[32] evaluated 52 obese children, and no significant difference was found in EAT thickness between obese children with and without MetS. They suggested that this discrepancy between results in adults and children may be due to the difference in metabolic activity of EAT in younger subjects and duration of exposure to obesity, which is shorter in children than in adults, hence may not be long enough to advance the chronic inflammation process.[32] Eren et al.[33] showed no statistical difference between EAT thickness in obese patients with and without MetS, and no correlation was found between EAT and ALT, TG, FPG, insulin, and HOMA-IR. In the study by Abacı et al.,[34] while EAT thickness showed a significant correlation with age, BMI, intima-media thickness, and SBP values, it was not significantly correlated with BMI SDS, glucose, insulin, HOMA-IR, TC, TG, LDL, HDL, and DBP. Despite these, Akyol et al.[35] found higher EAT thickness in the obese children with MetS than in the obese group without MetS and lean children. Although our study could not measure the waist circumference, obese patients could not be divided into MetS and non-MetS. However, they were evaluated regarding the presence of MetS components, and EAT thickness was found to be higher as the number of MetS components increased. While a correlation was found between EAT thickness and severity of obesity and SBP in our study, no correlation was found between DBP, lipid profile, HOMA-IR, and EAT thickness.

The relationship between EAT thickness and WBISI, which we have not seen before in the literature, was evaluated. Although the euglycemic hyperinsulinemic clamp is the best method for assessing insulin sensitivity, it is a complex test and rarely used in a clinical setting.[36] Therefore, different tests are used to evaluate insulin sensitivity. Homa-IR is one of

the most commonly used tests and is thought to represent mainly hepatic IR.[37] IR in peripheral organs such as skeletal muscle and adipose tissue also plays an important role in systemic IR. Yeckel et al.[38] showed that WBISI can be used to predict insulin sensitivity in obese young people and can be a good tool to assess insulin sensitivity. In our study, the severity of obesity and EAT thickness showed negative significant correlations with WBISI. In addition, WBISI was found to be significantly lower as the MetS component increased. Studies have shown that WBISI has a significant negative correlation with visceral fat, hepatic fat fraction, and pancreatic fat fraction.[39] There has been no study on its relationship with EAT thickness. While many cardiometabolic parameters do not change, the WBISI changes according to the severity of obesity and MetS risk, and its negative correlation with EAT thickness suggests that it may be a suitable parameter in the early detection and evaluation of cardiometabolic risk in obese patients.

In our study, SUA was higher in patients with increased MetS components. It is thought that high SUA levels regulate oxidative stress, inflammation, and enzymes related to glucose and lipid metabolism and constitute a mechanism for the deterioration of metabolic homeostasis.[40] In studies conducted in adults, high SUA levels have been found to be associated with MetS.[14] Similarly, in a study conducted in children, SUA levels were found to be increased in obese/overweight children, regardless of age, puberty, gender, and BMI, as well as the frequency of MetS, IR, and dyslipidemia.[41] These results suggest that the relationship between SUA and metabolic and cardiovascular risk factors begins in early childhood. In another adult study, no correlation was observed between EAT and glycemia, total serum cholesterol, HDL, or TG, while a significant positive correlation was found between EAT and SUA.[42] Studies conducted in childhood are rare, and a positive correlation has been reported between EAT thickness and SUA.[43] We also found a positive correlation between EAT thickness and SUA.

Studies have shown that liver function tests, including AST, ALT, and GGT, can be valuable parameters in evaluating the metabolic status of adults.[44] The association between MetS and liver function tests has also been demonstrated in children.[45] In our study, it was determined that as the number of MetS components increased, there was an increase in the values of liver enzymes, and a statistical difference was found between the groups in the AST levels. GGT was also investigated as a marker of MetS, and GGT levels were found to be strongly associated with cardiovascular risk factors.[46] Studies between GGT and EAT thickness are rare, and there is no study from the childhood period. A positive correlation was found between EAT thickness and GGT in adult studies.[47] A positive correlation was found between EAT and GGT also in our study.

Our study has a few limitations. Most importantly, we could not measure waist circumference or clearly distinguish patients with and without MetS. Second, the population size is small.

In conclusion, no other research has been found in the literature like our study. All parameters, such as MetS components, SUA, EAT thickness, and insulin resistance, were evaluated together according to the severity of obesity in children. Especially after class 2 obesity, the increase in EAT thickness, further decrease in insulin sensitivity, and the decrease in the number of people without the MetS component suggest that obese people should be followed closely and necessary interventions should be made to prevent obesity from progressing to further dimensions. The increase in the liver function tests, SUA and EAT thickness, and decrease in the WBISI as the number of MetS components increase show that these can be easily measurable parameters used at follow-up. Expanded case-control studies on this subject will further contribute to the literature.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Ankara Bilkent City Hospital with decision no 23-4512 dated July 12, 2023.

Author contribution

The authors confirm contribution to the paper as follows: SStudy conception and design: GB, YOS; data collection: GB, YOS; analysis and interpretation of results: GB; draft manuscript preparation: GB, YOS. 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

Impact of endocrine disorders associated with cleft lip and palate

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ABSTRACT

Background. Any impediment to the development of midline structures i.e. hypothalamus, pituitary and oral cavity may cause anatomical and functional issues. We aimed to determine the association of endocrine disorders with anatomic defects of midline structures i.e. cleft types and syndromes, as well as their impact on postoperative intensive care unit (ICU) admissions and complications.

Methods. A total of 6000 patients from the Cleft Lip and/or Palate (CLP) Treatment Center between September 2014 - February 2022 were included. Patients with physical findings or biochemistry that may indicate endocrine disorders were examined by the Division of Pediatric Endocrinology. Data concerning sex, operation age, cleft types, coexisting endocrine disorders, syndromes, echocardiography, postoperative complications as well as postoperative intensive care unit (ICU) admissions were recorded.

Results. The study group consisted of 78 patients with endocrine disorders, with a mean follow-up time of 59±7 months. One hundred and nine CLP operations were performed. The most common endocrine disorders coexisting in CLP patients were hypothyroidism (44.8%) and growth hormone (GH) deficiency (14.1%). Of the patients, 29.4% had genetic syndromes. The median age of operation in patients with endocrine disorders was 5 months (Q1-Q3: 4-8 months) for cleft lip and 15 months (Q1-Q3: 12-20 months) for cleft palate repair. Of the patients with CLP and endocrine disorders, 24% required postoperative ICU admission. Age of operation and ICU admission rates were higher compared to the general population of patients with CLP in our center ($p<0.01$).

Conclusions. Endocrine disorders, particularly hypothyroidism and GH deficiency, are frequent in CLP. Furthermore, our data suggest that endocrine disorders may complicate the postoperative course. Thus, investigation of these problems is crucial for appropriate treatment as well as adopting measures to successfully manage the postoperative course.

Key words: cleft lip, cleft palate, endocrine disorder, intensive care unit.

Cleft lip and/or palate (CLP) is the most common craniofacial anomaly in humans, with an estimated incidence of 1 in 700 live births, though this incidence may show geographical variation.[1,2] In embryonic life, there are close interactions between the hypothalamus, pituitary and the oral cavity. Any impediment to the development of these tissues may cause anatomical and functional problems. Facial clefts may indicate abnormalities in the pituitary and/or brain morphology and functions.[3,4]

In patients with CLP, intensive care unit (ICU) admission as well as postoperative

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complications may lead to serious morbidities. Furthermore, they are a cause of financial and psychological burden to the patient and their families. Moreover, failure to investigate endocrine disorders that may be associated with midline defects i.e. CLP, may cause diagnostic delay of these disorders with complications in growth and development. In this study, we aim to investigate endocrine disorders that accompany CLP subtypes, and the association of these problems on the age of operation, postoperative need for ICU admission and complications.

Materials and Methods

Medical records of 6000 patients with CLP treated in the Plastic and Reconstructive Surgery Clinic of Hacettepe University, between September 2014 - February 2022 were reviewed following approval of the ethics committee (No: GO 22/416). Patients with genetically or clinically diagnosed syndromes like Down syndrome, DiGeorge syndrome, Kabuki syndrome, Goldenhaar syndrome etc., patients with a history of hypoglycemia, growth retardation, signs of cryptoorchidism/micropenis, patients with a second midline defect like holoprosencephaly, spina bifida etc. and/or any other malformation were analyzed by pediatric endocrinologists. All diagnostic investigations and imaging had been done in the respective departments prior to applying to our clinic for cleft repair, and if available, all patients were under treatment for their conditions.

Patients diagnosed with endocrinological disorders, who provided adequate demographic and perioperative retrospective data were included in the study. Patients with insufficient demographic information and less than 6 months of follow-up data, those who had surgeries other than primary CLP repair, who lacked a definitive endocrine disorder diagnosis, or those who do had an endocrine disorder diagnosis but had been operated on in other clinics were excluded from this study. Data from a total of 6,000 patients were examined. Of these patients, 266 had been consulted with pediatric endocrinology, and 100 patients were diagnosed with endocrinological disorders. Eight of these patients were not patients who underwent primary CLP repair at our center, but were patients who had undergone surgery for reasons such as revision or alveolar cleft surgery. Fourteen patients could not be included in the study because they did not have 6-month follow-up data at our center after diagnosis (it is assumed that they were followed up at centers in their own city/country). A total of 78 patients met the inclusion criteria. Of these patients, 72 had accompanying endocrinological anomalies, while 6 patients had metabolic disorders discovered during the endocrinology consultation: Type 1 diabetes mellitus (n=1), congenital adrenal hyperplasia (n=1), and genetic metabolic diseases (n=4). The flow chart of included and excluded patients is given in Fig. 1. All surgeries were performed by the same surgical team.

Data concerning sex, age of operation, cleft types, endocrine diseases, other anomalies and syndromes accompanying CLP, echocardiography findings such as atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus (PDA), patent foramen ovale (PFO) and other cardiac anomalies, postoperative complications and ICU admission were recorded. Following examination by a genetics specialist, those who were diagnosed with a syndrome were considered as syndrome-related patients with CLP.

Cleft types were classified into 3 groups: isolated cleft lip (uni- or bilateral), isolated cleft palate (Veau 1 cleft palate or Veau 2 cleft palate), cleft lip with cleft palate (Veau 3 cleft palate or Veau 4 cleft palate).

Endocrine evaluation was performed with a detailed physical and hormonal evaluation of disorders of midline endocrine structures. Hypothyrodism was defined as low serum free thyroxine level (<12 pmol/L). Hypothyroidism was classified as primary and central according

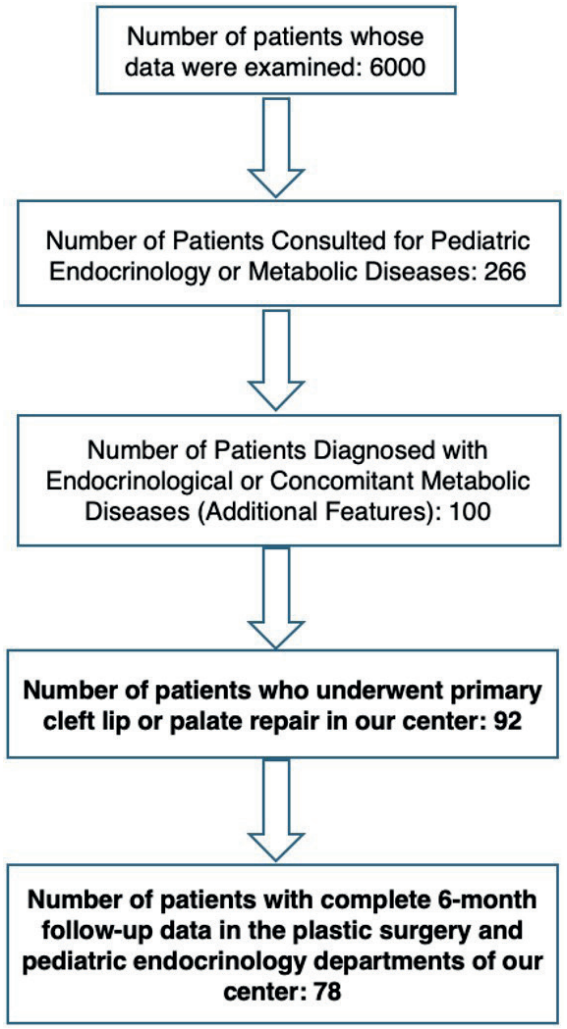

Fig. 1. Flow chart of included and excluded patients.

to TSH levels. A high TSH level accompanied by a low free thyroxine level was called primary, and a normal or low TSH level was called central hypothyroidism Some patients were on thyroid medication upon admission, and their initial pretreatment thyroid hormone levels were unknown, those were grouped into hypothyroidism, etiology unknown. Adrenocorticotropic hormone (ACTH) deficiency (secondary adrenal deficiency) was diagnosed by low morning serum cortisol (<3 μg/dL) associated with low/normal ACTH concentration or impaired peak cortisol (<19.8 μg/dL) during hypoglycemic episode or low-dose corticotropin stimulation test. Growth hormone (GH) deficiency was investigated if the patients' height was ≥2 SDS below the mean or when growth velocity was below the 25th percentile for age and sex. GH deficiency was diagnosed with low insulin-like growth factor 1 (IGF1) and insulin-like growth factor binding protein 3 (IGFBP3) and a peak GH less than 10 ng/mL in two GH stimulation tests (levodopa, clonidine). Patients with a height SDS below -2, but whose examinations for GH deficiency were not completed were defined as "short stature" only. Urinary output >2 L/m^2/day along with low urinary versus high plasma osmolality (urinary-to-plasma osmolality ratio <0.7), hypernatremia during fluid deprivation test, and elevation of urinary osmolality above 600 mOsm/kg in response to nasal 1-desamino-8-D-arginine vasopressin suggested central diabetes insipidus. Syndrome of inappropriate antidiuretic hormone secretion (SIADH) was diagnosed with clinical euvolemia without edema or dehydration and plasma osmolality <275 mOsm/kg, urinary osmolality >100 mOsm/kg and fractional sodium excretion (FENa) >1%. Male patients with micropenis and/or cryptoorchidism were grouped as patients with suspicious hypogonadism. Some patients were followed until puberty, and the data in their files were examined in terms of pubertal disorders. Delayed or absent pubertal development was defined as low serum sex steroid levels associated with inappropriately low or normal luteinizing hormone in girls older than 13 years and in boys older than 14 years.

Diagnosis of precocious puberty in girls was based on breast development (Tanner stage ≥ 2) before age 8 years and a peak LH level ≥5 IU/L during gonadotropin releasing hormone (GnRH) test. Biochemical data of the patients were also analyzed retrospectively. In the face of apparent hypocalcemia and hyperphosphatemia inappropriately low plasma parathyroid hormone (PTH) levels suggested hypoparathyroidism.

One patient presented with hyperglycemia and was diagnosed with type 1 diabetes mellitus based on International Society for Pediatric and Adolescent Diabetes (ISPAD) 2022 guidelines[5],

which was considered to be a coincidental development rather than association.

Data concerning postoperative ICU needs of patients were collected from medical files. Postoperative complications are listed as fistula formation in the repaired palate and dehiscence of the repaired lip.

Statistical analysis

For numeric variables, descriptive statistics such as mean, standard deviation, median were calculated. Normally distributed variables are presented as mean ± standard deviation and non-normally distributed variables are presented as median (interquartile range: Q1-Q3). In statistical analysis, patient-based situations were accepted as independent events and spread of numeric variables was evaluated with the Kolmogorov-Smirnov normality test.

Comparisons between two independent groups were made by Mann-Whitney U test because the assumptions of parametric tests were not met. The relationships between categorical variables and operation-based situations were analyzed with chi-square test. Comparisons of our results with a generally accepted known value were made with one-sample Wilcoxon signed rank test. All analyses were done using IBM SPSS version 23 (IBM, 2015). The statistical significance was considered to be $p<0.05$.

Results

A total of seventy-eight patients diagnosed with endocrine disorders (n=78) were included in this study, encompassing 109 surgical procedures for CLP. Among the patient cohort, 59% (n=46) were male, while 41% (n=32) were female. Of the 109 operations, 43.1% (n=47) were for cleft lip repair and 56.9% (n=62) were for cleft palate repair. The mean follow-up time was 59+/-7 months. The median age of surgery was 5 months (Q1-Q3: 4-8 months) in patients with cleft lip who had endocrine disorders. The median age of surgery was 15 months (Q1-Q3: 12-20 months) in those with cleft palate and endocrine disorders. In our clinic, the median age of surgery for the general population of patients with cleft lip is 3 months and for patients with cleft palate is 9 months (unpublished data). The age of surgery for both cleft lip and cleft palate patients with endocrine disorders were delayed significantly, compared to those who did not have an endocrine disorder ($p<0.01$). Out of these 78 patients, 7.3% (n= 6) had isolated cleft lip (uni- or bilateral), 29.4% (n= 23) isolated cleft palate (Veau 1 or Veau 2), 63.3% (n= 49) had both CLP (Veau 3 cleft palate or Veau 4 cleft palate). All endocrine abnormalities are listed in Table I. The most frequent endocrine disorder was hypothyroidism, in 44.8% (n=35) of the patients. Among these patients, 15 had central hypothyroidism and 5 had primary

Table I. Associated endocrinological and metabolic diseases (N=78).

Concomitant endocrinological and metabolic diseases	Number (%)
Hypothyroidism	35 (44.8%)
• Central hypothyroidism	15 (19.2%)
• Primary hypothyroidism	5 (6.4%)
• Etiology unknown	15 (19.2%)
Hypogonadism	4 (5.1%)
Suspicious hypogonadism	
• Micropenis	3 (3.8%)
• Cryptorchidism	14 (17.9%)
• Micropenis & cryptorchidism	2 (2.5%)
Growth hormone deficiency	11 (14.1%)
Diabetes insipidus	4 (5.1%)
Central adrenal insufficiency	5 (6.4%)
SIADH	1 (1.3%)
Short stature	6 (7.6%)
Multiple pituitary hormone deficiency	3 (3.8%)
Hypoparathyroidism	9 (11.5%)
Precocious puberty	4 (5.1%)
Type 1 diabetes mellitus*	1 (1.3%)
Congenital adrenal hyperplasia*	1 (1.3%)
Genetic metabolic diseases*	4 (5.1%)**

SIADH: Syndrome of inappropriate antidiuretic hormone secretion.
*Conditions appearing coincidentally in CLP patients
**mitochondrial disease, mucopolysaccharidosis, molybdenum cofactor deficiency, dihidrolipoamide dehydrogenase deficiency.

hypothyroidism; the etiology of hypothyroidism in the remaining 15 patients was unknown. A second endocrine disorder was present in 10/15 of the patients with central hypothyroidism. None of the 5 patients known to have primary hypothyroidism had any other accompanying endocrine disorders. Among patients with hypothyroidism whose etiology could not be determined 2/15 had a second endocrine disorder (1 cryptorchidism, 1 short stature). Of the patients in our series, %37.1 (n= 13) were detected through neonatal screening, and %6.9 (n= 22) of the patients, including patients with central hypothyroidism, were diagnosed as a result of advanced examinations performed by the pediatric endocrinology department at our center.

Of the patients, 5.1% (n= 4) had a diagnosis of hypogonadism. Nineteen patients had micropenis and/or cryptorchidism, however their files did not include the hormonal investigations required for a diagnosis of hypogonadism. The number of patients and their percentages in the study population are given in Table I. It was observed that endocrine anomalies were less prevalent in patients with isolated cleft lip (n= 6/1190, 0.5%), whereas in bilateral CLP patients (n= 21/997, %2.1) these anomalies were significantly more prevalent (p=0.0014). The accompanying endocrine abnormalities of syndromic patients are given in Table II.

Nineteen patients (24%) with clefts who had endocrine disorders required postoperative ICU admission. The rate of postoperative ICU admissions was 34% (n=2/6) in isolated cleft lip patients, and 43.5% (n=10/23) in isolated cleft palate patients. The rate of postoperative ICU admissions in cleft palate and lip patients was 14.5% (n=7/49). Postoperative ICU admission rates in patients with endocrine disorders were found to be significantly higher than in those without (p<0.01). In the case of cleft lip operations, postoperative ICU admission was

Table II. Distribution of accompanying endocrine disorders in syndromic and non-syndromic patients (N=78).

Accompanying endocrinological disorders	Syndromic patients (N=23), n (%)	Non- syndromic patients (N=55), n (%)	p-value
Hypothyroidism	6 (26.1%) - 2 with Down syndrome - 1 w/ Turner syndrome - 1 w/ Sathra-Chotzen syndrome - 1 w/ Emmanuel syndrome - 1 w/ 18q deletion	29 (52.7%)	0.03
Hypogonadism	7 (30.4%) - 1 with Down syndrome - 1 w/ Goldenhaar syndrome - 1 w/ Smith-Magenis syndrome - 1 w/ Opitz G/BBB syndrome - 1 w/ Patau syndrome - 1 w/ Carnevale syndrome - 1 w/ overgrowth syndrome	16 (29.1%)	0.90
Growth hormone deficiency	1 (4.3%) (Kabuki syndrome)	10 (18.2%)	0.16
Diabetes insipidus	1 (4.3%) (18q deletion)	0 (%0)	0.29
Central adrenal insufficiency	1 (4.3%) (Carnevale syndrome)	4 (7.3%)	1.00
Hypoparathyroidism	6 (26.1%) (DiGeorge syndrome)	3 (5.4%)	0.02
Precocious puberty	1 (4.3%) (Rubinstein-Taybi syndrome)	3 (5.4%)	1.00

Table III. Patients with endocrine diseases who are admitted to intensive care unit after cleft lip and cleft palate surgery.

Accompanying endocrinological disorders	ICU admissions after cleft lip surgery, number/total (%)	ICU admissions after cleft palate surgery, number/total (%)
Hypothyroidism	5/22 (23%)	7/24 (29%)
Hypogonadism	5/17 (29%)	5/17 (23%)
Diabetes insipidus	3/4 (75%)	0/1 (0%)
Central adrenal insufficiency	2/2 (100%)	3/3 (100%)
SIADH	1/1 (100%)	0/0 (0%)
GH deficiency	1/6 (16.7%)	2/9 (22.2%)
Hypoparathyroidism	0/2 (0%)	1/6 (16.7%)
Precocious puberty	1/2 (50%)	2/4 (50%)
Congenital adrenal hyperplasia	1/1 (100%)	0/0 (0%)

Note that a patient may have only cleft lip surgery, only cleft palate surgery, or both cleft lip and palate surgery.
GH, growth hormone; ICU, intensive care unit; SIADH, Syndrome of inappropriate antidiuretic hormone secretion.

significantly higher in patients with impaired water metabolism (diabetes insipidus, DI: n=4; SIADH: n=1) compared to other comorbidities ($p<0.05$). Of patients with DI, 75% (n=3) were admitted to the ICU after cleft lip surgery ($p=0.019$). Again, only the patient with SIADH required ICU admission after cleft lip surgery (100%). The rate of ICU admissions following cleft lip repair procedures in patients diagnosed with precocious puberty was 50% (n=1); it was 29% (n=5) for patients with hypogonadism, %16.7 (n=1) for patients with GH deficiency and %23 (n=5) for patients with hypothyroidism.

None of the patients with hypoparathyroidism had the need for postoperative ICU admission after cleft lip repair. Patients with adrenal insufficiency (central adrenal insufficiency n=5, primary adrenal insufficiency due to congenital adrenal hyperplasia n=1) also had significantly higher ICU admission rates following both cleft lip and cleft palate operations ($p<0.05$). Table III shows the patients who were admitted to ICU after CLP surgery with endocrine diseases mentioned in the results.

In addition to endocrine abnormalities, 53.9% (n=42) had variable cardiac anomalies while thirty-six patients (46.1%) had normal echocardiography findings (see Table IV). As postoperative complications, oronasal fistula formation was seen in 18% (n=13) of cleft palate patients, and dehiscence of the repaired lip was seen in 3% (n=2) of the patients in the current cohort.

Discussion

The most common congenital anomaly of the head and neck region is the CLP deformity. During the development of cranial features, there is a close interaction between the oral cavity and the pituitary gland. Diencephalon and oral ectoderm are in close relation, especially in the first two stages of development of the pituitary.[6,7] In a study performed on mice, it was shown that the anterior pituitary is dramatically affected in mice with orofacial clefts.[2] In the present study, a comprehensive endocrine evaluation was not conducted for

Table IV. Echocardiography findings of the patients.

Echocardiography finding	Number (%)
Normal	36 (46.1%)
ASD	10 (12.9%)
VSD	4 (5.1%)
PFO	7 (8.9%)
PDA	2 (2.5%)
Other cardiac anomalies	4 (5.1%)
Patients with more than one cardiac anomaly	16 (20.6%)

ASD, atrial septal defect; PDA, patent ductus arteriosus; PFO, patent foramen ovale; VSD, ventricular septal defect.

all patients; only those presenting with overt clinical symptoms underwent endocrine assessment. Among the total cohort, 78 out of 6000 patients (1.3%) were identified to have endocrine disorders associated with CLP. Some of these associations may be coincidental, such as cases with type 1 diabetes or primary adrenal insufficiency due to congenital adrenal hyperplasia, or genetic metabolic diseases. However, most are caused by common developmental pathological processes affecting midline structures. To the best of our knowledge, there is only one study with a very small number of participants investigating accompanying endocrine anomalies in neonatal patients with CLP, regardless of whether there was any symptoms or not. It was found that 70% (22/31) of CLP patients had some kind of hormonal disorder, 13 having a single endocrine abnormality, while 9 had multiple endocrine deficiencies.[2] Our study is valuable in its depiction of the prevalence of symptomatic endocrine disorders in CLP patients and the impact of those on the operation.

One of the most striking points among our findings is the low frequency of endocrine disorders in patients with isolated cleft lip (n=6, 0.5%), whereas in bilateral CLP patients (n=21, %2.1) these anomalies were significantly more prevalent (p=0.009). In accordance with our results, Rudman et al.[8] also found that bilateral CLP is the most commonly associated type with GH deficiency. These findings suggest that the association between CLP and hypothalamic-pituitary anomalies in the early stages of embryonic life should be further investigated.

In our clinic, the mean age of surgery is 3 months for cleft lip patients, and 9 months for cleft palate patients. In our study, it is evident that delaying the timing of surgical intervention for CLP patients with endocrine or genetic disorders may lead to early feeding difficulties and, subsequently, speech impairments later in life. In patients with endocrine disorders accompanying CLP, reaching the weight required for surgery may be delayed and there may be developmental delays. Additionally, delays in anesthesia administration due to abnormalities in blood laboratory values and developmental abnormalities can postpone surgical intervention. While it is essential for these patients to undergo surgery under optimal pediatric and anesthetic conditions, we believe that performing surgery beyond the routine timeline may adversely impact outcomes, such as speech development. A comprehensive study including speech parameters in these patients whose surgery time is delayed may also be beneficial.

In our practice, the postoperative ICU requirement rate for cleft surgery is overall 6.2%.[9] However, this rate was found to be 24% in our study group. This emphasizes the importance of the postoperative ICU in CLP patients with endocrine disorders and concomitant metabolic diseases. In our series, hypothyroidism was the most common endocrine disorder associated with CLP. Hypothyroidism, if not diagnosed and treated preoperatively, may danger the patient's life intra- or postoperatively leading to bradycardia and hypothermia.

Furthermore, in our study, an additional accompanying endocrine disorder was present in 12 of the 35 patients with hypothyroidism. The prevalence of congenital hypothyroidism in the general population and in Türkiye is 1 in 3000-4000 and 1 in 650 respectively. Previous studies show that hypothyroidism may be seen 18-24 times more frequently in patients with CLP.[10] In our series, the prevalence was 6/1000. Furthermore, national newborn screening with TSH levels is not able to detect all cases with central hypothyroidism[11], which was the most common type of hypothyroidism in our cases. Thus our findings suggest that it could be beneficial to consider examining CLP patients for hypothyroidism and other hormonal deficiencies prior to surgery in select cases, such as those who have suspicious physical examination findings like hypotonia, or even minor anomalies in biochemical blood tests, which may indicate a water or cortisol metabolism disorder, since they are not only common, but also easy to investigate and may

predict the presence of other more serious endocrine problems that may be life-threatening during the operation.

GH deficiency is the other common endocrine disorder in our series. There is no definitive incidence study on GH deficiency in Türkiye. Therefore, a comparison cannot be made with our series. According to the study by Feldt-Rasmussen and Klose[12], the general incidence of GH deficiency is thought to be 2-3:10,000. Other studies show that the prevalence of growth delay in the general population is 5-6%, while it is 21% in children with CLP.[13] The growth delay in these patients is thought to be associated with problems in feeding during the early infancy period. However, the close relationship between midline clefts and pituitary anomalies should also be considered as a preventable and easily treatable cause of growth delay in these infants. In a study by Rudman et al., the prevalence of GH deficiency was determined to be 40 times higher in patients with clefts.[8] Given that GH deficiency was identified by pediatric endocrinology in 11 out of the 6000 patients included in our study, we conclude that GH deficiency occurs 6 to 9 times more frequently in patients with CLP compared to the general population.

Considering the problems faced by short individuals in society, it is important to diagnose and treat GH deficiency, which is a common and treatable cause of short stature in patients with CLP. Early intervention with GH therapy can improve growth outcomes and mitigate the social prejudices associated with short stature. This would help improve psychosocial well-being of these children. We therefore recommend that children with craniofacial clefts who exhibit growth delay undergo further evaluation for GH deficiency through GH stimulation testing. Additionally, growth retardation should be recognized as an easily assessable parameter and a potential indicator of hypopituitarism. In our series, hypogonadism and findings suggestive of hypogonadism were common endocrine abnormalities accompanying CLP. The derangement in the hypothalamus – pituitary - gonad axis may lead to hypogonadotropic hypogonadism. Mini-puberty, or physiological puberty during early infancy, offers a good opportunity to objectively assess hypogonadotropic hypogonadism at a time when surgery plans for infants with CLP are typically being formed. Such evaluation is also easy, practical, and may be predictive of hypopituitarism, i.e. deficiency of other pituitary hormones. In our study population, retrospective research was conducted on patients who were diagnosed with pubertal anomalies, hypogonadism and growth retardation later in life, and it was discovered that all of these patients were admitted to the ICU at the time of their cleft operations. It may be speculated that the abnormal laboratory results caused by the hormone deficiencies and accompanying disorders and midline defects may have contributed to their admission to the ICU following anesthesia.

Another potential problem in cleft lip/palate patients is adrenal insufficiency, which, if untreated, could be fatal, especially under the stress of an operation. Poor adrenal reserve caused by secondary adrenal insufficiency may not be able to withstand the stress of surgery and may result in life-threatening intraoperative hypoglycemia and hypotension. According to our research, patients with secondary adrenal insufficiency were more likely than those without any underlying conditions or those with other hormone deficits to require postoperative ICU stay following both CLP repair procedures. For such patients, early diagnosis and treatment of adrenal insufficiency is crucial, as well as anticipatory planning for postoperative ICU conditions before the operation. Our study's key finding is that it is crucial to identify endocrine problems in infants with orofacial clefts since failure to do so could result in the demise of the patient. More extensive and prospective research is required on this topic.

Analysis of accompanying congenital anomalies in our patient population showed that 29.4% of the patients had clinically or genetically diagnosed syndromes. Our results are consistent with the study conducted by Vallino-Napoli et al. on 2022 CLP patients, which reported that one out of every 3 patients had accompanying anomalies.[14] These defects are prevalent enough in CLP patients to warrant a thorough investigation for associated syndromes and congenital malformations.

According to the literature, oronasal fistula formation occurs in 23% of patients within the general cleft population following repair procedures, while dehiscence of the repaired lip is observed in 0-7.5% of cases.[15,16] Our patient population also showed similar results: oronasal fistula formation was seen in 18% (n=13) of cleft palate patients, and while dehiscence of the repaired lip was seen in 3% (n=2) of the patients.

As for cardiac anomalies in our patient population, 42 patients (53.9%) had at least one cardiac anomaly. The most common isolated cardiac anomaly was ASD, seen in 12.9% of the patients. Of the patients, 5.1% had VSD only, 8.9% had PFO only, 2.5% had PDA only, while 20.6% of the patients had more than one type of cardiac anomaly. In a study by Çalış et al.[17] the rate of cardiac anomalies in the general cleft population was found to be 15.6%. Our findings may suggest that in CLP patients with endocrine disorders, the incidences of a cardiac anomaly or multiple cardiac anomalies are higher, therefore a preoperative screening with echocardiography may be beneficial in preventing possible postoperative complications.

The retrospective nature of the study, having a single-center patient population are the limitations of this study. It is also probable that since the institution is a major referral center of complicated pediatric cases, the rate of CLP patients with endocrine disorders are higher compared to other patient populations presented in the literature.

Conclusion

The two endocrine conditions most commonly seen in CLP patients are hypothyroidism and GH deficiency. Endocrine conditions, which also include ACTH deficiency, may delay the age at which lip and palate repair procedures are performed, and may increase the need for postoperative ICU admissions. Therefore, in select cases, especially when the patient presents with failure to thrive or with syndromic features, it could be beneficial and reasonable to screen the patients before surgery with simple blood tests such as the thyroid hormone, GH and ACTH levels, because identifying hypothyroidism or GH deficiency may help predict other significant endocrine diseases that are life-threatening if left untreated.

Ethical approval

This study was conducted following approval of the ethics committee of Hacettepe University. (No: GO 22/416).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: GÇ, EB, MÇ, FFÖ; analysis and interpretation of results: GÇ, EB, NE, MÇ, FFÖ, DCE, NG, AÖ; draft manuscript preparation: GÇ, NE, EB, AÖ. 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 outcome of functional constipation in Saudi children

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ABSTRACT

Background. Understanding the outcome of functional constipation (FC) for both patients and physicians is essential, yet it has been infrequently reported worldwide. The objective of this report was to update the outcomes of FC in Saudi children.

Methods. Clinical data including age, sex, response to management, duration of follow up, and type of management were collected from the notes of each clinic visits and phone call follow-ups.

Results. The study included 268 children followed up for a 7 year duration. The median age of onset was 4 (0.1 to 13) years, and 123/268 (46%) were male. There was an increasing recovery rate with increasing duration of follow up with an overall recovery rate of 79%. There was no significant association between recovery and age at onset (p=0.0860) or duration of constipation (P=0.124). Management by pediatric gastroenterologists did not increase rate of recovery (81% vs. 77%, p=0.432) or being cured (47% vs. 36%, p=0.108) significantly. According to the parents of children who recovered, diet in association with polyethylene glycol (PEG) and toilet training were most helpful. Poor diet and nonadherence to medications were the most common causes of lack of recovery.

Conclusions. The higher rates of recovery in this Middle Eastern childhood population than other populations are possibly related to cultural characteristics. The parents' views support the importance of diet associated with other modalities as important parts of management. Further research is needed to identify correctable causes of nonadherence to treatment to improve recovery.

Key words: chronic constipation, prognosis, Saudi children.

Functional gastrointestinal disorders are common in infants and children worldwide. The Rome IV criteria for functional constipation (FC) issued by the Rome Foundation are currently used in clinical practice to identify children with chronic or recurrent constipation not caused by organic conditions.[1,2] In a recent review, Fedele et al.[3] reported higher prevalence rates of FC in Western compared with Asian populations, including an 18.2% prevalence in a recent review in 2023.[4] In another review, the prevalence of FC in North and South America, China, Sri Lanka and India, including infants and adolescents, ranged from 0.5 to 29.6%.[5] Similarly, recent studies from the Kingdom of Saudi Arabia (KSA) reported prevalence between 9.1% and 34.5% in infants and toddlers, respectively.[6-8]

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Despite this high prevalence and well-known clinical features worldwide[9-11], data on the outcome are relatively scarce. In a systematic review based on Western populations published in 2010, the overall 6- to 12-month follow up recovery rate was 60.6%.[12] However, the authors indicated that the studies were heterogeneous, using different definitions, populations, outcome measures, and follow-up periods, resulting in large variations in recovery rates. In addition, although children with FC account for about 3% of general pediatric (GP) clinic visits and 25% of the pediatric gastroenterology (PGI) clinics[9], data on the effect of type of clinical care on recovery rate are limited. In non-Western populations, data on the outcome of FC are even more scarce. Therefore, the objective of this study was to determine the outcome of FC in Saudi children, a Middle- Eastern pediatric population, including the factors associated with recovery.

Materials and Methods

The study was performed at King Khaled University Hospital, King Saud University in Riyadh, KSA. This hospital provides primary, secondary and tertiary medical care. The inclusion criteria were: 1) Children 13 years of age or younger, diagnosed with FC based on the Rome IV criteria during the study period from 2014 to 2023. 2) Documented follow-up clinical visit notes. 3) Response to follow-up phone calls for children who did not attend the clinic for more than one year. The study design consisted of a review of medical records and follow-up via phone calls. The information collected included the sex, age of onset, duration of constipation, management modality, duration of follow up, and whether the patient was managed by GPs or PGIs. Follow-up data at each clinic visit included the interval between visits and the outcome, including response to management. Further long-term follow-up data for children with no clinic visit for more than one year were obtained via phone calls.

The outcome, assessed either at the clinic or by phone follow-up, was classified into two categories: 1) Recovery if the child had a normal bowel habit (≥ 3 soft bowel motions per week) for at least three months. This category was divided in two groups: The improved group, if the children still needed medication; and the cured group, if the children were completely off medications. 2) No recovery if the child still had constipation despite conventional treatment for more than 3 months. Other information obtained from the parents during follow up phone calls included the most helpful treatment for those who recovered and the cause of no recovery from the parent's point of view.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 26.0 software (IBM, Inc., Armonk, NY, USA).[13] Descriptive statistics (median, range, frequency, and percentage) were used to describe the quantitative and categorical variables. Bivariate analysis with Pearson's chi-square test was used to observe associations between categorical study variables and clinical response to treatment. Nonparametric statistical tests (Mann–Whitney U test and Kruskal–Wallis test) were used to compare the means of the skewed quantitative variables in relation to follow-up outcomes (cured, improved, recovered and nonrecovered). A P value ≤ 0.05 was used to indicate statistical significance.

Ethical approval: This study was approved by the College of Medicine Institutional Review Board, King Saud University (No. 21/01096/IRB).

Results

Two hundred sixty- eight children with FC met the inclusion criteria with a duration of follow up from 0.5 to 7 years. The median and the range of age of onset of FC were 4 (0.1 to 13) years, and 123/268 (46%) patients were male. Management started with parental education (37%), including information on the chronic nature of the condition, slow response to treatment, the need for continuous medication adherence and

follow up. The dietary advice included fluids, vegetable and fruit intake. Parental education was delivered by pediatric residents or pediatric gastroenterology fellows. Treatment included disimpaction at presentation (32.3%), by sodium phosphate enema (18%) or polyethylene glycol (PEG) (8%), maintenance with PEG (60%) or lactulose (33%), and other medications including bisacodyl, sodium picosulfate – magnesium citrate, probiotics and glycerin suppositories in 7%. The outcome by duration of follow up is shown in Table I, indicating variation of rates of recovery and cure, with the duration of follow up with an overall recovery and cure rates of 79% and 41%, respectively. Table II shows the factors associated with recovery, indicating no significant association with either demographic or clinical features (P>0.05). Table III depicts a comparison of the recovery rate in children managed by GPs and PGIs, indicating a tendency of higher recovery rate. According to the parents of children who recovered, the most helpful modalities included diet, PEG, lactulose, and toilet training in 38%, 33%, 27%, and 13%, respectively. Finally, the most common causes of lack of recovery reported by the parents were poor diet and nonadherence to medication in 29/35 (83%). Other causes included unavailability of medication and unsuitable toilet facilities.

Discussion

Information on the outcome of FC in children is essential for accurate education in patients and physician. In this study, the data collected from the medical records of clinic visits were supplemented with prospective follow-up data obtained via phone calls of families of children who missed the clinic appointments for one or more years. This approach increased the duration of follow-up and allowed longer term estimation of recovery rates.

Table I. Outcome of functional constipation by duration of follow up.

Duration of follow up (years)	Number of children	Recovery, n (%)		No recovery, n (%)
		Improved	Cured	
0.5 – 1	21	13 (81)	3 (19)	5 (23.8)
1 – 2	76	32 (55)	26 (45)	18 (24)
2 – 3	59	28 (58)	20 (42)	11 (19)
3 – 4	45	22 (61)	14 (39)	9 (20)
4-5	37	20 (61)	13 (39)	4 (11)
5-7	30	11 (52)	10 (48)	9 (30)
Overall	268	126 (59)	86 (41)	56 (21)

The differences in rates of recovery in relation to duration of follow-up was not statistically significant (P=0.779).

Table II. Factors associated with favorable outcomes in children with functional constipation (N=268).

Variables	Recovery(n=211)	No Recovery(n=57)	P value
Age at onset, yr, mean± SD	5.08 (3.5)	4.62 (3.2)	0.393
Sex, n (%)			0.518
Males (n=123)	99 (80%)	24 (20%)	
Females (n=145)	112 (77%)	33 (23%)	
Duration of constipation, mo, mean±SD	11.69 (16.73)	10.74 (15.9)	0.717
Duration of follow up, mo, mean± SD	35.32 (18.0)	34.67 (19.4)	0.811
Treating physician, n (%)			0.429
General pediatrician (n=157)	121 (77%)	36 (23%)	
Pediatric gastroenterologist (n=111)	90 (81%)	21 (19%)	

SD: standard deviation.

Table III. Outcome of functional constipation by type of care.

Variables	General pediatric clinics (n=157)	Pediatric gastroenterology clinic (n=111)	P value
Male sex, n (%)	66 (42.0%)	57 (51.4%)	0.132
Age at onset, yr, median (range)	4 (0.1-13)	4 (0.1-13)	0.779
Duration of constipation, mo, median (range)	3 (0.1-84)	8 (0.1-120)	0.015
Maintenance medication, n (%)			0.099
Lactulose	55 (65.5%)	29 (34.5%)	
Polyethylene glycol	94 (54.7%)	78 (45.3%)	
Duration of follow up, yr, median (range)	2.5 (0.5- 7)	2.9 (0.5-7)	0.096
Recovery, n (%)	121 (77%)	90 (81%)	0.432
Cure among those with recovery, n/N (%)	44/121 (36%)	42/90 (47%)	0.108

The outcome of FC by duration of follow up

Comparison of our results with the Western literature is challenging because of the heterogenous nature of most reports. The finding of 75% recovery rate (improvement and cure) after 0.5 to 1 year follow up in this study is higher than the mean of 60.6% recovery regardless of the need of laxative.[14-16] After a follow up from 1 to 2 years, the 76% recovery rate in this report is higher than the reported range between 48% - 69.3% regardless of laxative use.[17,18] In addition, the 70% recovery rate in children followed 5 to 7 years is higher than the reported 56.3% regardless of laxative use.[17,19,20] Finally, the 79% overall recovery rate during the follow up from 0.5 to 7 years duration is clearly higher than previous reports. The reasons for the higher recovery rate in this study compared to most reports are not clear. Possible explanations include population differences in cultural, dietary and bowel habits (such as diets richer in fiber and non-Western types of toilets), but more recent data and extended follow up of the patients are more likely. Data from non-Western populations are scarce. In a study from Thailand, after one year of follow-up, the 78% recovery rate is comparable to the 75% in this study.[21] However, in a study from Brazil, after a mean follow up period of 2.8 years, the 50.6% recovery rate is lower.[22] It is possible that their patients who were referred from primary to tertiary care may be more difficult to treat, whereas our patients are treated in a primary, secondary, and tertiary care settings.

Factors associated with recovery

Our finding of no significant association between age at onset or duration of constipation and recovery is consistent with previous reviews reporting no significant impact of these factors on outcome.[10] Although not statistically significant, the trend of increasing recovery with the duration of follow up is consistent with a study from Brazil.[22]

The effect of the type of clinical care on the recovery rate

The finding of higher recovery rate (including rate of being cured) in children managed by PGI's than those managed by GP's is consistent with the conclusions of previous reports.[17,22] It is possible that the lack of statistical significance is related to the significantly different durations of constipation in children managed by PGIs, suggesting more severe disease (P=0.015). Accordingly, our data support the general practice that children with FC may be primarily managed by GPs and prompt referral to PGIs be reserved for cases not responding to conventional management.

Parental viewpoints on treatment modalities

Parental viewpoints on the most helpful management for recovery and causes of nonresponse are important for physicians when designing management programs. To our knowledge, this issue has rarely been reported worldwide. The parents' beliefs that

diet, associated with other modalities, was most helpful is consistent with most literature, supporting the need to include dietary advice in the management program of children with FC.[9] Finally, the finding by parents that nonadherence to medication and poor diet were the most common causes of non-recovery highlights the need for further studies to identify the causes of nonadherence to improve recovery.

Study limitations

The most important limitation is the retrospective design which includes potential recall bias of some information such as onset of constipation and incomplete documentation in the medical notes. Another limitation is the hospital-based setting with the potential of missing milder cases that did not require attending hospitals.

Conclusion

The higher rate of recovery in this Middle Eastern childhood population than other populations is possibly related to cultural characteristics. The parents' views support the importance of diet associated with other modalities as important parts of management. Further research is needed to identify correctable causes of nonadherence to treatment to improve recovery.

Ethical approval

This study was approved by the College of Medicine Institutional Review Board, King Saud University. (date: 23.01.2024. number: 24/1008/IRB).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MEM, data collection: H A, MK, NA, RA, NAH, NAZ, AAS and AA, draft manuscript preparation: MEM, SA. 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

Heat shock protein 70 levels in children with nephrotic syndrome

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ABSTRACT

Background. Idiopathic nephrotic syndrome (NS) is the most prevalent glomerular disease in children. Heat shock protein 70 (HSP70) is synthesized in response to diverse stress factors like infections and oxidative stress. We aimed to evaluate serum and urine levels of HSP70 in children with steroid-sensitive nephrotic syndrome (SSNS) and to assess changes in HSP70 levels with prednisolone treatment. Additionally, we seek to determine whether serum and urine levels of HSP70 can differentiate between frequently relapsing and infrequently relapsing cases in children with SSNS.

Methods. A total of 36 patients with SSNS and 35 healthy children were included in the study. Samples were taken from all patients at four time points; before corticosteroid treatment (day 0) and on days 15, 30, and 90 after the initiation of corticosteroid treatment. Serum and urine levels of HSP70 were measured by enzyme-linked immunosorbent assay (ELISA).

Results. In the NS group before steroid treatment (day 0), urine HSP70 (uHSP70) levels and urine HSP70/creatinine (uHSP70/Cre) ratios were significantly higher ($p<0.0001$), whereas serum HSP70 (sHSP70) levels were lower ($p=0.002$), compared to the healthy group. uHSP70 levels decreased gradually during prednisolone treatment in the patient group ($p<0.0001$). There was no difference in terms of sHSP70, uHSP70, and uHSP70/Cre ratios between patients with frequently relapsing and infrequently relapsing.

Conclusions. Our study demonstrates that uHSP70 levels are elevated in SSNS prior to treatment and decrease with prednisolone therapy, reflecting reduced renal stress and damage. uHSP70 may be a useful biomarker for monitoring renal damage and treatment response. Serum and urine levels of HSP70, as well as uHSP70/Cre ratios, did not differentiate between frequent and infrequent relapses.

Key words: pediatric, steroid-sensitive nephrotic syndrome, heat shock protein 70, HSP70, relapse, prednisolone.

Idiopathic nephrotic syndrome (NS) is the most prevalent glomerular disease in children, characterized by edema, nephrotic-range proteinuria, and hypoalbuminemia.[1] The molecular mechanisms underlying NS involve significant alterations in podocytes, including effacement of foot processes, cytoskeletal modifications, and reorganization of the slit diaphragm, leading to pronounced proteinuria.[2] Podocyte dysfunction may be idiopathic, genetic, or reactive etiologies stemming from mechanical stress, medications, toxins, viral infections, and unidentified circulating proteins.[3] Heat shock protein 70 (HSP70) is expressed in various renal cell types, including podocytes, mesangial cells, and different tubular epithelial cells.[4] As a member of the heat shock protein family, HSP70 is synthesized in response to stressors

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such as infections and oxidative stress, playing crucial roles in the structural shaping of new proteins, restoring partially denatured proteins, and degrading irreversibly damaged proteins.[5] Increased HSP70 expression is observed under stressful conditions and in diseases like chronic glomerulonephritis, tubulointerstitial nephritis, diabetic nephropathy, and chronic kidney disease, suggesting its influence on glomerular disease progression and response mechanisms.[4,6,7] Glucocorticosteroids are the primary treatment for NS, offering renoprotective effects by modulating podocyte gene expression and promoting prolonged podocyte survival, thereby reducing proteinuria.[8] Approximately 85–90% of NS patients achieve complete remission within 4–6 weeks of glucocorticoid treatment, classifying them as having steroid-sensitive nephrotic syndrome (SSNS).[9] However, around 76%-93% of these patients experience a relapse, with a substantial proportion (57%) facing frequent recurrences.[10] Relapses represent a significant clinical challenge in the long-term management of children with idiopathic NS. Firstly, children experiencing relapses are at an elevated risk of glucocorticoid-related adverse effects due to their exposure to higher cumulative steroid doses. Secondly, frequent relapses may develop, necessitating the initiation of additional immunosuppressive therapies. Identifying predictors of relapse risk could guide more personalized treatment strategies, allowing clinicians to optimize therapy choices before drug administration and mitigate the adverse effects associated with prolonged steroid use. Unfortunately, there is currently a lack of clinical or laboratory biomarkers that reliably predict the likelihood of frequent relapses. While various studies suggest potential predictors of relapse frequency[11-13], no biomarker has yet been validated to accurately forecast which patients are likely to experience recurrent relapses or require ongoing steroid and/or other immunosuppressive treatments.

This study aims to evaluate serum and urine levels of HSP70 in pediatric patients with SSNS and to assess changes in HSP70 levels in response to prednisolone treatment. Additionally, it seeks to determine whether HSP70 levels can distinguish between frequently relapsing and infrequently relapsing cases in children with SSNS.

Materials and Methods

Ethics committee approval was obtained in 2013 by the Clinical Research Ethics Committee of Istanbul University Istanbul Faculty of Medicine (2013/706), and sample collection began for our study. Serum and urine samples were collected from patients during their first episode of NS between 2014 and 2023 and then stored at -80 °C. In 2023, additional ethics committee approval was obtained to use the collected samples in the current study to investigate HSP70 as a biomarker by the Clinical Research Ethics Committee of Istanbul University Istanbul Faculty of Medicine (2023/2222), in accordance with the Declaration of Helsinki. Idiopathic NS was diagnosed in all patients in accordance with the criteria recommended by the International Study for Kidney Diseases in Children.[14] SSNS was defined as complete remission within 4 weeks of prednisolone treatment at a standard dose (60 mg/m²/day or 2 mg/kg/day, maximum 60 mg/day). Frequently relapsing was defined as having ≥2 relapses in the first 6 months following remission of the initial episode, or ≥3 relapses in any 12-month period.[15] Infrequently relapsing was defined as having <2 relapses in the 6 months following remission of the initial episode or fewer than 3 relapses in any subsequent 12-month period.[15]

Patients who experienced their initial episode of SSNS between January 2014 and December 2023 and fulfilled the inclusion criteria were enrolled in the study. Serum and urine samples were taken from all patients at four time points; prior to starting corticosteroid therapy (day 0) and on

days 15, 30, and 90 after starting corticosteroid therapy. All patients received standard oral steroids (2 mg/kg/day) for 4 weeks as induction therapy. Since proteinuria was negative in patients with NS, corticosteroid treatment was continued on alternate days for 4 weeks, then the dose was gradually tapered off, and the steroid was discontinued. Patients were divided into two groups: frequently relapsing and infrequently relapsing. Subjects in the control group were healthy children without any acute or chronic diseases.

The inclusion criteria were as follows:

- Patients with idiopathic NS
- Patients with SSNS
- Age 1-18 years
- Glomerular filtration rate (eGFR) ≥ 90 mL/min/1.73 m^2

The exclusion criteria were as follows:

- Secondary causes of NS (e.g., Henoch-Schönlein purpura, lupus nephritis)
- Patients with steroid-resistant nephrotic syndrome (SRNS)
- Presence of any acute or chronic infection or systemic disease

Written informed consent was obtained from the parents of the patients and the controls.

Measurement of serum and urine HSP70

Blood and urine samples were collected from the patient and promptly delivered to the laboratory. Blood samples were centrifuged at 2,500 g for 10 minutes to separate the serum. Following centrifugation, aliquots were stored at -80°C until analysis. On the day of analysis, samples were thawed at room temperature, and measurements were performed using the Human Heat Shock Protein 70 antibody (HSP70-Ab) ELISA Kit (Cat no: KTE62748) purchased from Abbkine (Abbkine, Inc, China) in accordance with the manufacturer's instructions. The manufacturer's guidelines for sample preparation and storage recommend storing samples at -80 °C if they are to be preserved for three years or longer. Accordingly, the samples were stored at -80 °C for long-term preservation, and repeated freeze-thaw cycles were strictly avoided. All samples were handled and stored under identical preanalytical conditions, ensuring uniform exposure and minimizing potential preanalytical effects on the results. HSP70 levels were expressed as ng/mL. The detection and quantification limits for HSP70 were set at <0.05 ng/mL. The intra-assay coefficient of variations (CV) for HSP70 were 7.9% and 8.6%; respectively. Urine creatinine levels were measured using an Architect c16000 analyzer (Abbott Laboratories, Abbott Park, IL, USA), with results reported in mg/dL. The urine HSP70/creatinine (uHSP70/Cre) ratio was expressed in ng/mg.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 22.0 for Windows (IBM Corp., Armonk, N.Y., USA). The normality of the parameters was tested using the Shapiro-Wilk test. Descriptive analyses were presented as medians and interquartile range (IQR) for non-normally distributed and ordinal variables. Mann–Whitney U test was used for between-group comparisons. Chi-square test was performed to evaluate the qualitative data. The relationships among variables were analyzed using Spearman's correlation coefficients. Friedman test was performed to evaluate the course of serum, urine HSP70 (uHSP70) and uHSP70/Cre ratio on days 0, 15, 30, and 90 following the initiation of corticosteroid treatment. For significant pairwise comparisons, Wilcoxon test was applied, with Bonferroni correction used to adjust for multiple comparisons. Post-power analysis was performed and the power was found to be 72%. p values < 0.05 were considered statistically significant.

Results

A total of 36 patients with SSNS and 35 healthy children were included in the study. Age and sex distribution were comparable between the NS and control groups (p>0.05, Table I). In the NS group prior to starting corticosteroid therapy (day 0), uHSP70 levels and uHSP70/Cre ratios were significantly higher compared to the healthy group (p=0.001 and p=0.034, respectively, Table I). Conversely, serum HSP70 (sHSP70) levels were lower in patients with NS than in the controls (p=0.002). In the patient group, there was a negative correlation between age and uHSP70/Cre ratio (r=-0.520, p=0.002), yet no correlation was detected between sHSP70 and uHSP70 (p>0.05). There was also a negative correlation between serum albumin and uHSP70 (r=-0.352, p=0.045), and a positive correlation between the spot urine protein creatinine ratio (UPCR) and uHSP70 (r=0.347 p=0.048) in NS patients prior to starting corticosteroid therapy (Table II). sHSP70, uHSP70 levels, and uHSP70/Cre ratio were independent of sex (p=0.624, p=0.381, p=0.870; respectively).

When we evaluated uHSP70 levels on days 0, 15, 30, and 90 after starting corticosteroid therapy, we observed a gradual decrease in uHSP70 levels in the patient group (p<0.0001) (Fig. 1). sHSP70 levels and uHSP70/Cre ratios showed a fluctuating course, and there was no statistically significant difference between day 0 and day 90 of the steroid treatment (p>0.05, Fig. 1).

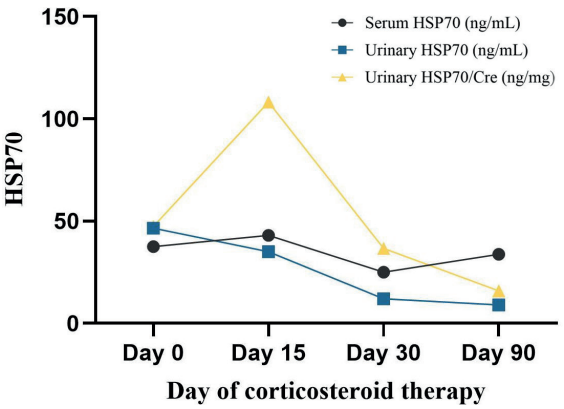

Fig. 1. Course of serum and urine levels of HSP70 with corticosteroid therapy.

GraphPad Prism version 10.4.1 (free trial) was used to produce this figure. HSP70: heat shock protein 70.

Table I. Comparison of age, sex, and HSP70 levels between the nephrotic syndrome patients prior to starting corticosteroid therapy (day 0) and controls.

Parameters	Nephrotic syndrome (n=36)	Controls (n=35)	p
Age (years)	5.3 (3.2-9.3)	7.5 (4.9-9.2)	0.067
Female/Male (%)	36/64	51/49	0.193
Serum albumin (g/dL)	1.8±0.5 (1.0-2.9)	-	
Urine protein creatinine ratio (mg/mg)	8.1±4.5 (2.2-19.5)	-	
Serum HSP70 (ng/mL)	37.5 (31.2-44.1)	49.2 (39.9-68.7)	0.002
Urine HSP70 (ng/mL)	46.5 (37.5-55.6)	33.9 (31.3-40.0)	0.001
Urine HSP70 creatinine ratio (ng/mg)	47.4 (31.3-96.2)	37.2 (21.6-60.5)	0.034

Data are given as percent, mean ± SD (min-max) or median (interquartile range) as appropriate. NS: nephrotic syndrome, HSP70: heat shock protein 70.

Table II. Correlations between HSP70 levels and various parameters in the nephrotic syndrome patients prior to starting corticosteroid therapy (day 0).

Parameters		Serum HSP70	Urine HSP70	Urine HSP70 creatinine ratio
Age	r	-0.103	-0.103	-0.520
	p	0.564	0.563	0.002
Serum albumin	r	0.112	-0.352	-0.192
	p	0.535	0.045	0.292
Urine protein creatinine ratio	r	0.149	0.347	-0.041
	p	0.407	0.048	0.824

HSP70: heat shock protein 70; r: Spearman's correlation coefficient.

The total follow-up period of the patients was 7.4±1.5 years (range: 4.3-9.4 years), and the total number of attacks was 3±2 (range: 1-11). The total number of attacks in frequently relapsing patients was 5±3 (range: 3-11), while in infrequently relapsing patients, it was 2±2 (range: 1-6). Patients with frequent and infrequent relapsing were compared in terms of HSP70 levels, and no significant differences were found between the two groups in terms of sHSP70, uHSP70, and uHSP70/Cre (p>0.05) on days 0, 15, 30, and 90 (Table III).

Discussion

This study aimed to evaluate serum and uHSP70 levels, as well as the uHSP70/Cre ratio in children with SSNS and to assess changes in these levels with prednisolone treatment. Our results revealed significantly higher uHSP70 levels and uHSP70/Cre ratios in the NS group before steroid treatment compared to healthy controls. Conversely, sHSP70 levels were lower in NS patients.

The elevated uHSP70 levels prior to treatment suggest that HSP70 might serve as a marker of renal damage and stress, reflecting glomerular dysfunction and podocyte injury. This finding aligns with previous research showing HSP70 overexpression in various renal pathologies, including chronic glomerulonephritis and diabetic nephropathy.[6,16] The increased uHSP70 levels may reflect mechanical stress and injury in the kidneys. In NS, decreased anionic charge on the glomerular filtration barrier contributes to increased permeability and proteinuria.[17] Normally, albumin, a small protein with a molecular weight of 66.5 kDa, does not pass through the glomerular barrier due to its negative charge. In conditions like minimal change disease, where the negative charge on podocytes is lost, albumin and other small proteins such as IgG and transferrin are excreted in the urine. HSP70, with a molecular

Table III. Comparison of HSP70 levels prior to starting corticosteroid therapy (day 0) between nephrotic syndrome patients with frequently relapsing disease and those with infrequently relapsing disease.

Parameters	Frequently relapsing (n=11)	Infrequently relapsing (n=25)	p
Age, years	5.9 (2.4-13.7)	4.9 (3.7-7.8)	0.396
Female/Male (n)	4/7	9/16	0.983
Day 0 of corticosteroid treatment			
Serum HSP70, ng/mL	37.4 (31.2-52.5)	37.6 (31.2-43.0)	0.818
Urine HSP70, ng/mL	51.4 (33.9-56.8)	44.1 (38.0-52.2)	0.696
Urine HSP70 creatinine ratio, ng/mg	60.6 (35.7-158.6)	40.6 (29.2-92.0)	0.414
Day 15 of corticosteroid treatment			
Serum HSP70, ng/mL	42.9 (37.2-58.6)	43.0 (37.6-49.2)	0.689
Urine HSP70, ng/mL	37.2 (33.9-48.9)	32.9 (29.9-44.7)	0.178
Urine HSP70 creatinine ratio, ng/mg	107.3 (50.9-364.5)	109.2 (49.8-174.7)	0.756
Day 30 of corticosteroid treatment			
Serum HSP70, ng/mL	35.2 (19.2-49.3)	24.3 (9.1-38.6)	0.235
Urine HSP70, ng/mL	16.3 (10.7-30.9)	10.8 (6.4-25.0)	0.305
Urine HSP70 creatinine ratio, ng/mg	33.7 (18.0-59.9)	48.9 (22.1-139.4)	0.345
Day 90 of corticosteroid treatment			
Serum HSP70, ng/mL	33.8 (24.0-82.5)	34.5 (19.2-50.2)	0.188
Urine HSP70, ng/mL	11.5 (4.8-15.3)	8.9 (7.3-18.3)	0.593
Urine HSP70 creatinine ratio, ng/mg	15.6 (4.9-24.4)	24.0 (7.7-75.0)	0.219

Data are given as median (interquartile range). HSP70: heat shock protein 70.

weight of approximately 70 kDa, is similar in size to albumin and carries a neutral or slightly positive charge. The glomerular filtration barrier selectively filters based on both molecular size and charge, which affects the passage of proteins like HSP70, similar to albumin.[18]

In our previous study on sHSP70 levels in IgA nephropathy (IgAN) and idiopathic NS, higher sHSP70 levels were observed in IgAN and IgA vasculitis nephritis (IgAVN) compared to idiopathic NS.[19] However, when comparing the NS and control groups, sHSP70 levels in patients with idiopathic NS were slightly higher than in controls.[19] These findings are contrary to the findings of the current study. This discrepancy could be attributed to differences in patient demographics, disease profiles, and treatment statuses. Notably, our NS group comprised younger patients with more cases of minimal change disease and steroid-sensitive cases, which may account for these differences. It was interpreted that HSP70 was detected at low serum levels because it was excreted from the glomerulus like albumin in NS.[18]

Our study observed a gradual decline in uHSP70 levels on the 15th, 30th, and 90th days following the initiation of corticosteroid treatment. The observation that uHSP70 levels decrease with prednisolone treatment suggests that uHSP70 might be a more sensitive marker for renal damage and treatment response compared to sHSP70. While uHSP70 levels decreased with treatment, indicating reduced renal stress, sHSP70 levels remained stable, highlighting the potential of uHSP70 as a useful biomarker for monitoring kidney damage and therapeutic efficacy.

Frequent relapsing patients require long-term immunosuppressive treatment and are at risk for complications such as osteoporosis, hypertension, cataracts, and sperm abnormalities.[20] Biomarkers that predict relapse patterns at initial presentation are crucial. Our study found no significant differences in HSP70 levels between frequently and infrequently relapsing SSNS patients, suggesting that HSP70 may not effectively differentiate relapse frequency. This highlights the need for additional biomarkers or clinical parameters to predict relapse patterns more accurately.

The most significant aspect of our study is its longitudinal design, which permitted us to monitor changes in HSP70 levels over time in children with idiopathic NS who received steroid treatment during their initial episode. One of the limitations of our study was the post-power analysis of 72%, which is suboptimal as values below 80%. Another potential limitation pertained to the long-term storage of serum and blood samples at -80 °C. As the ELISA kit manufacturer advised storage at -80 °C for periods exceeding three years, the samples were maintained at this temperature for extended durations. Repeated freeze-thaw cycles were avoided, and all samples were analyzed concurrently. Thus, it was assumed that all samples experienced uniform conditions, minimizing any pre-analytical variation in results. However, data regarding the stability of proteins like HSP70 during long-term storage at -80 °C remain limited.

In conclusion, our study shows that uHSP70 levels are elevated in SSNS prior to treatment and decrease with prednisolone therapy, reflecting reduced renal stress and damage. While HSP70 may be a useful biomarker for monitoring renal damage and treatment response, it does not differentiate between frequent and infrequent relapses. Further research is needed to confirm these findings and identify additional biomarkers for better prediction and management of SSNS.

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Ethical approval

The study was approved by the Ethical Committee of Istanbul University Istanbul Faculty of Medicine (2013/706 and 2023/2222). Written informed consent was obtained from the parents of the patients and the controls.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BA, ZNYY, AG, AY; data collection: BA, ZNYY, AY; analysis and interpretation of results: BA, ZNYY, AG, AY; draft manuscript preparation: BA, ZNYY, AG, AY. 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 importance of targeted next-generation sequencing based genomic profiling in the diagnosis of childhood acute myeloid leukemia: a single center experience

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ABSTRACT

Background. The management of pediatric acute myeloid leukemia (AML) is based on the prognostic risk classification of initial leukemia. Targeted next-generation sequencing (NGS) is a reliable method used to identify recurrently mutated genes of pediatric AML and associated prognosis.

Methods. In this study, we retrospectively evaluated the prognostic, and therapeutic utility of a targeted NGS panel covering twenty-five genes, in 21 children with de novo and 8 with relapsed or secondary AML.

Results. Variants were detected in 44.8% of patients, and 63.2% of them were in the signaling pathway genes. The number of variants per patient and diversity increased with age. The panel results affected hematopoietic stem cell transplantation decisions, especially in core binding factor AML, and allowed the categorization of diseases according to current classifications. Panel results also pointed out predisposition to germline leukemia to the extent of the panel coverage. No targeted therapy was used based on the variants, and none of the variants were used to monitor minimal residual disease.

Conclusions. Targeted NGS results, along with well-known genetic aberrations and treatment responses, can guide treatment modalities. The coverage of the routine panels should include proven mutations of childhood AML and germline leukemia predisposition genes.

Key words: acute myeloid leukemia, children, mutation, next-generation sequencing.

Acute myeloid leukemia (AML) is a molecularly and clinically heterogeneous clonal disease characterized by uncontrolled proliferation of immature myeloid cells due to complex genetic alterations that impair hematopoiesis. Pediatric AML comprises less than 20% of childhood leukemia and, nearly 40% of patients relapse after first-line therapies.[1-3]

Childhood AML development is generally enhanced by chromosomal rearrangements that create chimeric fusion genes, which often involve transcription factors. Subsequently, acquired mutations in other pathways, often tyrosine kinase or RAS, cooperate with chromosomal rearrangements.[4] The Cancer Genome Atlas network reported 23 genes that recurrently

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mutated in adult AML patients, which were grouped into nine functional categories including; transcription-factor fusions (18% of cases), nucleophosmin 1 (*NPM1*) mutations (27%), tumor suppressor genes (16%), DNA-methylation-related genes (44%), activated signaling genes (59%), chromatin-modifying genes (30%), myeloid transcription-factor genes (22%), cohesin-complex genes (13%), and spliceosome-complex genes (14%).[5]

Management of pediatric AML patients is constructed on the prognostic risk classification of leukemia. The morphologic, immunophenotypic evaluation and specific cytogenetic and molecular abnormalities are critical prognostic indicators. Most genetic methods widely used in developing countries, such as karyotype analysis, fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR) do not provide adequate information for the current prognostic classifications and targeted treatment modifications. Targeted next-generation sequencing (NGS) is a rapid, precise, and cost-effective prognostic method that is routinely used in adult AML patients.[6] Since the genetic profiles of adult and pediatric AML patients are diverse with similar phenotypes, the applicability of molecular genetic results of adult AML to children is limited. The value of NGS for pediatric AML patients has not yet been entirely determined.

This study aimed to determine the prognostic and therapeutic benefits of panel-based targeted NGS results for pediatric AML patients.

Materials and Methods

Study design and patient recruitment

Fifty children with pediatric AML were diagnosed in Ankara Bilkent City Hospital Pediatric Hematology Clinic between November 1st, 2019 and July 31st, 2023. Diagnoses were determined according to morphological, immunophenotypic, and genotypic criteria. At diagnosis, cytogenetic analysis by conventional G-banding, fluorescence in situ hybridization (FISH) analysis for t(8;21), t(15;17), t(9;22), inv16, 5q del, 7q del, 11q23, *TP53* (17p13) del and PCR analysis for *RUNX1-RUNX1T1*, *PML-RARA*, *CBFB-MYH11*, *KMT2A-AFF1*, and *BCR-ABL* were performed in all patients. The targeted NGS myeloid panel testing was performed for 21 de novo AML and 8 relapsed or secondary AML patients. None of the relapsed patients had an NGS myeloid panel study at their primary diagnosis. De novo AML patients were treated according to the Berlin-Frankfurt-Munster (BFM) AML 2019 protocol. Patients with relapsed AML received various combination therapies, such as idarubicin - fludarabine - cytarabine, gemtuzumab - ozogamicin, and venetoclax.

The results were analyzed according to the AML subgroups and age groups, as follows; infants (<3 years), children (3-14 years), and adolescents (15-18 years).

Next-generation sequencing analysis

The QIAact Myeloid DNA UMI Panel (Qiagen, Hilden Germany) was performed with GeneReader NGS System at diagnosis. The study material was fresh bone marrow in all patients. The panel included a total of 25 genes, seven of which with whole coding region (*CEBPA, DNMT3A, EZH2, MPL, RUNX1, TET2,* and *ZRSR2*); and the remaining 18 genes with specific exons (*ASXL1, CALR, CBL, CSF3R, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, NPM1, NRAS, SETBP1, SF3B1, SH2B3/LNK, SRSF2, TP53,* and *U2AF1*). This panel accurately and sensitively determines significant insertion/deletion (InDel) mutations and single nucleotide variants. Utilizing unique molecular index technology allows the detection of low-frequency variants. Variants were analyzed with Qiagen Clinical Insight Software, including population frequency databases, public variant databases, and in-silico prediction tools. The actionable variants were evaluated in the light of the 2017 recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American

Pathologists, which is a four-tiered system to categorize somatic sequence variations based on their clinical significances. These include: Tier I variants with strong clinical significance; Tier II, variants with potential clinical significance; Tier III, variants of unknown clinical significance; and Tier IV, variants deemed benign or likely benign.[7] As a principle of routine clinical practice, written informed consent was obtained from the legal guardians of patients for NGS analysis and other genetic tests, at the time of AML diagnosis. The ethics committee of Ankara Bilkent City Hospital approved the study (E2-23-3712).

Statistical analysis

Mean, standard deviation, frequency, and percentage were used for descriptive statistics. Analyses were conducted using SPSS (version 20.0).

Results

The electronic medical records of 50 children with 38 de novo AML and 12 relapsed or secondary AML were examined. Among these patients, 21 de novo and 8 relapsed/secondary AML patients were evaluated with the NGS AML panel at diagnosis and included in the study. The mean age of de novo AML patients was 8.3±6.2 years, and 38.1% (n=8) were male, whereas the mean age of relapsed/secondary AML patients was 10.8±6.1 years and 75% (n=6) were male. In 11 patients, metaphases could not be obtained for karyotype analysis. Demographic features, disease characteristics, karyotype analysis, FISH, PCR, and targeted NGS results of de novo AML patients are shown in Table I and relapsed or secondary AML patients are shown in Table II. Twenty patients (69%) had cytogenetic anomalies detected either by karyotype analysis or FISH. By NGS panel study, 19 variants were detected in 13 patients (44.8%), two of whom didn't have a cytogenetic anomaly. The 63.2% of variants (12/19) were in the signaling pathway genes.

Eight patients (27.6%) were under 3 years of age, twelve patients (41.4%) were between 3 and 15 years of age, and nine patients (31%) were 15 years or older. Patients younger than 3 years had 0.38 (3/8) variants per patient, whereas patients between 3 and 15 years had 0.50 (6/12) and patients older than 15 years old had 1.11 (10/9). The variants detected in patients younger than three years of age were in the signaling pathway (*NRAS, KRAS*) and spliceosome (*U2AF*) genes. Patients between 3 and 15 years of age had mutations in the signaling pathway (*FLT3, KIT)* and tumor suppressor genes *(TP53*). However, the variants detected in patients older than 15 years were seen in a wide variety of functional categories, such as chromatin-modifying (*ASXL1)*, signaling pathway (*FLT3, KIT, NRAS*), myeloid transcription factor (*RUNX1, CEBPA*), and DNA methylation genes (*DNMT3A*).

The NGS results were also assessed according to AML subgroups. Five patients had t(8;21) and four of them had variants detected by the NGS panel study. Two de novo AML patients had *KIT* variants (Patient #9 and Patient #11), one de novo AML (Patient #19) had copartner *RUNX1* and *DNMT3A* mutations and one relapsed patient (Patient #26) revealed an *NRAS* variant. Allogeneic hematopoietic stem cell transplantation (HSCT) was performed, considering *KIT* exon 17 mutation (N228K) in Patient #11 and copartner mutations in Patient #19. They had also experienced suboptimal (<3 log reduction in the bone marrow transcripts) *RUNX1-RUNX1T1* transcript reduction after consolidation chemotherapy.

Six patients had acute promyelocytic leukemia (APL) and the NGS panel detected aberrations in two of them. A *FLT3-ITD* mutation was detected in a de novo APL patient (Patient #14) which was not cause to change treatment protocol. The other variant was a *KIT* mutation which was detected in a 17-year-old secondary APL patient (Patient #28) whose initial diagnosis was precursor B acute lymphoblastic leukemia (pre-B ALL) followed by AML with inversion 16, five years later she relapsed as APL. This *KIT* mutation, with unknown clinical significance and 43% variant allele frequency (VAF),

Table I. The clinical and genetic characteristics of de novo AML patients.

Patient number	Age at diagnosis (years)	Gender	Disease subtype	Karyotype	FISH	Variant (NGS), VAF, classification	HSCT in CR1	Outcome	Overall survival (months)
1	0.7	M	FAB M0	43-47, XY, del(7q), del(17p)	TP53 deletion	None	Planned	Remission, alive	4
2	0.8	F	FAB M5	46, XX	*KMT2A* rearrangement	*KRAS* 35%Tier 1A *U2AF* 35% Tier 2C	No	Dead, refractory disease	10
3	1	F	FAB M7	46, XX	None	None	Yes	Remission, alive	30
4	1	F	FAB M7	51-54, XX, t(1;22) (q12;p11.2)	None	NRAS 3% Tier 1A	No	Dead (CR)	4
5	1.5	F	FAB M5	46, XX	None	None	No	Remission, alive	15
6	2	F	FAB M7 Down syndrome	Unsuccessful	None	None	No	Remission, alive	5
7	2	M	FAB M2	46, XY, t(7;9;11)	None	None	Yes	Remission, alive	11
8	3	F	FAB M4	Unsuccessful	None	None	No	Remission, alive	6
9	6.5	M	FAB M0	Unsuccessful	t(8;21)	KIT c.1254_1255delCG 26% Tier 3	Planned	Remission, alive	4
10	7	M	FAB M1	Unsuccessful	t(8;21)	None	No	Remission, alive	7
11	10	F	FAB M1	Unsuccessful	t(8;21)	KIT N822K 22% Tier 2C	Yes	Remission, alive	23
12	10	F	FAB M2	46, XX	None	None	No	Remission, alive	18
13	11	F	FAB M2	Unsuccessful	5q deletion	FLT3-ITD 13% Tier 2A	Yes	Dead (CR)	11
14	11	F	FAB M3	46, XX	t(15;17), inversion 3	FLT3-ITD 39% Tier 2A	No	Remission, alive	32
15	12	F	FAB M3	Unsuccessful	t(15;17)	None	No	Remission, alive	22
16	14	M	FAB M3	46, XY	t(15;17)	None	No	Remission, alive	23
17	15	F	FAB M3	Unsuccessful	t(15;17)	None	No	Remission, alive	24
18	15	F	FAB M0	Unsuccessful	Monosomy 7	*ASXL1* 15% Tier 2C *NRAS* 66% Tier 2C	Yes	Remission, alive	17
19	17	M	FAB M1	46, XY	t(8;21)	*RUNX1* 8,6 % Tier 1A *DNMT3A38* % Tier 1A	Yes	Dead (CR)	12
20	17	M	FAB M1	46, XY	None	*CEBPA* %39 Tier 1A *NRAS* %46 Tier 1A	Yes	Remission, alive	28
21	17.5	M	Myeloid sarcoma	46, XY	None	None	No	Remission, alive	9

CR: complete remission; F: female; FAB: French–American–British classification; FISH: fluorescent in situ hybridization; HSCT: hematopoietic stem cell transplantation; *KMT2A*: Lysine methyltransferase 2A gene; M: male; NGS: next generation sequencing; VAF: variant allele frequency.

Table II. The clinical and genetic characteristics of relapsed or secondary AML patients.

Patient number	Age at diagnosis (years)	Gender	Diagnosis	Disease subtype	Karyotype	FISH	Variant (NGS), VAF, classification	HSCT in CR2	Outcome	Overall survival (months)
22	2	M	Relapsed AML (After HSCT)	FAB M5	46XY, ins(15;2) (q21q22,q14.3q21)	None	None	No	Dead, refractory disease	2
23	5	M	Relapsed AML	FAB M7	51-52, XY	None	None	No	Dead (CR)	10
24	5	F	Relapsed AML (After HSCT)	FAB M4	Unsuccessful	None	*TP53* p.R248Q 24% Tier 1A *TP53* p.G279fs*27 21% Tier 1A (2 different mutations in TP53)	Yes	Remission, alive	26
25	10	M	Secondary AML (Previously Pre-B ALL)	FAB M3	46, XY	t(15;17)	None	No	Remission, alive	28
26	15	M	Relapsed AML	FAB M0	45, XY-19 / 45, XY-14	t(8;21)	*NRAS* 47 % Tier 2C	Yes	Dead (CR)	8
27	16	M	Therapy related AML (Previously Ewing sarcoma)	FAB M4	Unsuccessful	*KMT2A* rearrangement	*FLT3* c.2505T>G 7% Tier 1A *FLT3* c.2516A>G 5% Tier 1A	No	Dead, refractory disease	1
28	17	F	Secondary AML (Previously Pre-B ALL, AML with inv16)	FAB M3	46, XX	t(15;17), TP53 deletion	*KIT* p.R804Q 43% Tier 3	No	Remission, alive	22
29	17	M	Secondary AML (Previously T ALL)	FAB M0	46, XY	None	None	No	Relapsed, alive	23

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CR: complete remission; F: female; FAB: French–American–British classification; FISH: fluorescent in situ hybridization; HSCT: hematopoietic stem cell transplantation; *KMT2A*: Lysine methyltransferase 2A gene; M: male; NGS: next generation sequencing; VAF: variant allele frequency.

was confirmed as a germline heterozygote mutation after remission. Four patients had acute megakaryoblastic leukemia (AMKL). The myeloid NGS revealed an *NRAS* variant with a low VAF in addition to hyperdiploidy and t(1;22) in one of them (Patient #4).

De novo AML patients, one with 5q deletion (Patient #13) revealed a *FLT3-ITD* mutation and one with monosomy 7 had both *ASXL1* and *NRAS* mutations (Patient #18). They underwent HSCT based on high-risk cytogenetics. Another de novo AML patient (Patient #20) with both *CEBPA* and *NRAS* mutations did not provide any metaphases or didn't have any abnormal results with FISH or PCR techniques. However, based on 39% VAF of the *CEBPA* variant suggesting a monoallelic expression, the case was classified in the intermediate-risk group and HSCT was performed due to induction failure in the first complete remission.

Two patients had *KMT2A* rearrangements accompanied by variants detected by the NGS panel, and both of them died due to refractory disease. One (Patient #2) was an infant with de novo AML and had both *KRAS* and *U2AF* gene mutations. The other was an adolescent, who had therapy-related AML (Patient #27) with two different *FLT3* variants. A 5-year-old girl who relapsed after HSCT (Patient #24) also had two distinct variants in the same gene, *TP53*, while peripheral blood chimerism was 95%. Another secondary AML patient (Patient #29) previously diagnosed with T cell ALL revealed no mutations detected by the NGS myeloid panel. Of interest, his sister also had pre-B ALL, and whole exome sequencing (WES) revealed a pathogenic splicing *ETV6* mutation in a heterozygote state in both siblings indicating germline autosomal dominant *ETV6*-related leukemia predisposition.

No targeted therapy, even *FLT3* inhibitors, had been used based on the detected variants, and none of the variants were used for minimal residual disease (MRD) monitoring in our study group.

Discussion

Following the identification of genetic structural abnormalities and balanced chromosomal translocations in AML, the classification of leukemia evolved from a morphology-based classification to genetic-based criteria. With the advances in sequencing techniques, awareness of changes in specific genes has increased over the last decade. The Therapeutically Applicable Research to Generate Effective Treatments (TARGET) AML project revealed that somatic mutations in children are lower than in adults but increase with age. These mutations were commonly found in transcriptional regulators and signaling mediator genes.[8] Recently, European Leukemia Net (ELN) recommended screening the mutations in *FLT3, IDH1, IDH2, NPM1, CEBPA, DDX41, TP53, ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* and *ZRSR2* genes at diagnosis of adult AML to establish the diagnosis, prognosis and identify therapeutic targets.[6,9] We identified an increasing number and diversity of variants by age, mostly in signaling pathway genes (*FLT3*, *KIT*, *NRAS*, *KRAS*), almost in half of the patients by an NGS panel covering most of the ELN recommended genes. In a more comprehensive study, NGS of >150 cancer-related genes, Ishida et al.[10] evaluated 27 pediatric AML patients and similar to our results, reported the most frequent variations in *KRAS*, *NRAS*, *KIT*, and *FLT3* genes. Although we didn't use any variants to detect MRD, the detection of molecular MRD was associated with a significantly higher relapse rate; except *DNMT3A, TET2*, and *ASXL1* mutations which are often present in persons with age-related clonal hematopoiesis.[11]

Core binding factor (CBF) AML, shares a common pathogenic mechanism involving rearrangements of the CBF transcriptional complex characterized by the presence of either t(8;21) (q22;q22) or inversion 16(p13q22)/t(16;16). There is growing evidence for considerable genotypic heterogeneity in this group. Activating mutations of tyrosine kinase/RAS pathway genes such as *NRAS*, *KIT,* and *FLT3* are commonly found in CBF-AML. Exon

17 *KIT* mutations, including D816V, D816H, D816Y, and N822K, confer poor outcomes. Also, mutations in genes of the methylation group (i.e., *DNMT3A, TET2*) had a strong negative prognostic impact in CBF-AML.[12] The contribution of c*KIT* N822K mutation influenced the HSCT decision in Patient #9 and Patient #11 underwent HSCT considering the co-occurrence of *RUNX1* and *DNMT3A* mutations in addition to t(8;21). *DNMT3A* mutations have also been reported as frequent concurrent mutations in *RUNX1*-mutated AML with a negative impact on overall survival.[13] Furthermore, those CBF-AML patients did not have sufficient *RUNX1-RUNX1T1* transcript level decline, which is recommended for MRD monitoring to predict relapse and guide treatment in some reports.[14,15] *NRAS* variants are the most frequently observed in t(8;21) positive AML and are associated with improved outcomes, most likely due to sensitivity to higher doses of cytarabine.[14,16] We observed an *NRAS* variant in a relapsed patient (Patient #26).

Acute promyelocytic leukemia is a distinct type of leukemia, and *FLT3 ITD* mutation frequency is reported as 27% in de novo APL. Somatic mutations are also found in *WT1, NRAS, KRAS, ARID1B*, and *ARID1A* genes whereas, the mutations of other common AML genes, including *DNMT3A, NPM1, TET2, ASXL1, IDH1/2*, and *KIT* are usually absent.[17] In our study, *FLT3-ITD* mutation was detected in a de novo APL patient who had a high leukocyte count and inversion 3 (Patient #14). Besides being a poor prognostic factor in combination with a chromosomal abnormality like inversion 3, the high allelic burden of *FLT3 ITD* is associated with high leukocyte counts.[18,19] Interestingly, an adolescent APL patient (Patient #28) who had first experienced pre-B cell ALL and then AML with inv16 had an unusual *KIT* mutation with a high VAF (43%), which was later confirmed as a germline variant.

Acute megakaryoblastic leukemia is a genetically heterogeneous disease. Non-Down syndrome AMKL patients have several rearrangements other than t(1;22) and can be classified into risk groups according to cytogenetics.[20,21] In addition to rearrangements, recurrent mutations in *GATA1*, *JAK* kinase, *STAT*, cohesion, *CTCF*, *RAS* pathway genes, and cytokine receptor genes, mostly *MPL*, have also been reported in children with AMKL.[22] Non-Down syndrome AMKL patients harboring *GATA1* mutations may have similar biology with Down syndrome AMKL and reduced intensity chemotherapy might be efficient in these patients.[23] Besides, patients with Down syndrome can also develop AML without *GATA1* mutations with a poorer prognosis, necessitating intensified chemotherapy.[24] Bone marrow aspiration may be difficult due to extensive myelofibrosis in the course of AMKL. Next-generation sequencing is a sensitive method to detect variants in the presence of low blast percentage in fibrotic bone marrow.[25] We detected an *NRAS* variant with a very low VAF in one AMKL patient (Patient #4), which didn't affect our treatment modality.

KMT2A rearrangements are common cytogenetic abnormalities in AML and have intermediate to adverse prognosis depending on the various partner genetic aberrations. *KRAS* mutations have been reported to coexist with high-risk *KMT2A* fusions, with significantly lower event-free and overall survival.[26] A *KRAS* mutation was detected in a refractory infant patient (Patient #2) suggesting her worse prognosis. Our other patient with *KMT2A* rearrangement (Patient #27) had two distinct mutations in *FLT3* and was refractory to chemotherapy. The interaction of menin protein to *KMT2A* is critical in leukemia generation. Combined menin and *FLT3* inhibition represents a novel and promising therapeutic strategy for patients with *KMT2A* rearranged AML with concurrent *FLT3* mutation.[27] It may be possible to add *FLT3* inhibitors to menin inhibitors in the future in the management of patients like Patient #27.

Myeloid malignancies with *TP53* mutations are distinct entities associated with complex cytogenetic abnormalities, advanced age, chemoresistance, and poor outcomes. They are frequently detected in AML related to increased genomic instability, therapy-related AML, or

AML with myelodysplasia.[28] Myeloid neoplasms with mutated *TP53* (AML and myelodysplastic syndrome) are classified as a separate disease category in the 2022 International Consensus Classification (ICC) of myeloid neoplasm because of their similar aggressive behavior irrespective of the blast percentage.[29] And recently, *TP53* alterations, either mutations or deletions, were found to be associated with the most dismal prognosis in pediatric AML patients and the importance of concurrent *TP53* mutation and deletion analysis has since been underlined.[30] In addition to *TP53* deletions detected by FISH in two patients (Patient #1 and Patient #28), two different mutations in the *TP53* gene were detected in Patient #24 during the relapse after HSCT. The presence of multi-hit *TP53* mutations, as it occurred in Patient #24, can be confirmed by the presence of two or more distinct *TP53* mutations and corresponds to a highly aggressive disease.[31,32]

AML with myelodysplasia-related genetic alterations is designated by the ICC and World Health Organization (WHO) 2022 classification. According to WHO classification, AML, myelodysplasia-related (AML-MR) is defined as a neoplasm with ≥20% blasts expressing a myeloid immunophenotype with either one or more cytogenetic abnormality [complex karyotype, 5q deletion, monosomy 7, 7q deletion, 11q deletion, 12p deletion, monosomy 13, 17p deletion, isochromosome 17q or idic(X)(q13)] or somatic mutations in *ASXL1*, *BCOR EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2* genes.[33] With similar cytogenetic abnormalities and the same mutations, ICC also includes *RUNX1* mutations for this category.[29] Patient #18, who had *ASXL1* (VAF 15%) and *NRAS* (VAF 66%) mutations in addition to monosomy 7, can be classified as AML-MR according to both groups.

Our study evaluated the contribution of targeted AML NGS myeloid panel in managing childhood AML. However, the panel was created mainly for adult AML and covered the genes most recommended by the ELN. The current WHO classification system introduced "myeloid neoplasms with germline predisposition" as a new class of myeloid neoplasms.[33] In addition to detecting somatic mutations in myeloid neoplasms, panels containing germline gene variations will be helpful. Non-hematopoietic tissue or post-treatment remission samples should be tested with NGS to confirm germline pathogenic mutations associated with hematological malignancies. Our panel did not cover the most common germline mutations, such as *ANKRD26* and *ETV6*. It also did not cover the *WT1* gene. *WT1* mutations have poor prognosis and its combination with *FLT3-ITD* variants confer a poorer prognosis.[34] Another missing gene in the panel was *GATA1*, which is important for the precise diagnosis of AMKL patients, both with Down syndrome and without Down syndrome. Our study's limitations are the coverage of the used myeloid NGS panel and the small sample size.

In conjunction with well-known recurrent cytogenetic abnormalities, the NGS myeloid panel can identify prognostic risk groups and treatment responses in children with AML. Our NGS results provided information regarding the prognosis and allowed us to guide treatment individually. For some patients, HSCT decisions were strengthened. The coverage of the routinely used AML panels should be expanded with other proven mutations of childhood AML and germline leukemia predisposition mutations. The widespread use of NGS in pediatric hematology-oncology clinics may also obtain precise and reliable data for direct targeted therapies and MRD monitoring.

Ethical approval

The ethics committee of Ankara Bilkent City Hospital approved the study (date: 27/03/2023, number: E2-23-3712).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design:

DK, BÇ, NYÖ, NY; data collection: DK, BÇ, AKY, MI, FBK, FTY, TB, DGG; analysis and interpretation of results: DK, BÇ, NY; draft manuscript preparation: DK, BÇ, NYÖ, NY. All authors reviewed the manuscript 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

The Health-Related Quality of Life scores and joint health in children and young adults with hemophilia

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ABSTRACT

Background. Patients with hemophilia should be evaluated for joint health and overall health in their visits. The aims of this study were to evaluate joint health and health-related quality of life (HRQoL) in patients with mild, moderate, and severe hemophilia; determine which patient groups to focus on and whether there are any neglected patient groups.

Methods. This was a single-center, cross-sectional study. Patients were evaluated by ultrasonography (Hemophilia Early Arthropathy Detection with Ultrasound [HEAD-US]), physical examination (Hemophilia Joint Health Score version 2.1 [HJHS-2.1]), and HRQoL scales (EQ-5D/EQ-VAS and Haemo-QoL).

Results. Thirty-nine patients with regular follow-up were evaluated for a total of 234 joints. When hemophilia severity was compared with the HEAD-US and HJHS-2.1, a significant difference was found between severe and non-severe hemophilia. On the other hand, when patients' total HEAD-US scores were compared with total HJHS-2.1 scores, no statistically significant correlations were found; only a statistically significant but negligible correlation was detected when HEAD-US and HJHS-2.1 scores were examined at joint level. No significant difference was found when mild, moderate or severe hemophilia were compared with the HRQoL scores. Also, HEAD-US scores and HRQoL scores were not correlated, showing that the HRQoL score did not change whether the patient has arthropathy or not.

Conclusion. Despite recent advances in treatment options for hemophilia, arthropathy in patients with severe hemophilia remains challenging. For the follow-up of pediatric hemophilia, the HEAD-US and HJHS should be used together because their correlation was weak. Although patients with severe hemophilia are at higher risk in terms of arthropathy, patients with mild/moderate hemophilia should not be ignored because their HRQoL is not different from that of severe hemophilia.

Key words: hemophilia, hemophilic arthropathy, health-related quality of life, HJHS, HEAD-US.

Hemophilia is a congenital bleeding disorder, characterized by a deficiency of coagulation factor VIII, defined as hemophilia A or factor IX, defined as hemophilia B.[1] Although the pathophysiology, distribution of the factor levels, and pharmacokinetic characteristics of infused factors are different, there are no significant differences in expected bleeding between hemophilia A and B.[2,3] The severity of hemophilia is defined by the factor levels. Patients with factor levels below 1% are defined as severe, patients with a factor levels between

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1-5% are defined as moderate and patients with factor levels 5-40% are defined as mild hemophilia. Patients with severe hemophilia tend to experience more spontaneous bleeds, especially into their joints, and therefore develop chronic hemophilic arthropathy. When the factor level is higher, it is unlikely to experience spontaneous bleeds and develop complications. The treatment of hemophilia involves the acute treatment of bleeding and prophylactic treatment with regular factor replacement for preventing bleeding and the development of hemophilic arthropathy with regular and continuous factor replacement.[1,4,5] For optimization of follow-up, objective criteria are needed, such as bleeding frequency, physical examination, imaging studies, and health-related quality of life (HRQoL).[6]

This study was undertaken with the aim of examining the joint health and overall health of patients with hemophilia (PwH). We aimed to identify the differences between mild, moderate, and severe hemophilia and thus learn which patients to focus while on ensuring that patients with moderate and mild hemophilia are not neglected, especially in childhood and young adulthood.

Materials and Methods

This study was a single-center, cross-sectional study with 39 PwH over the age of 4 years who were regularly followed in our center (Fig. 1). As part of the clinical follow-up of PwH, joint health assessments were conducted with the Hemophilia Joint Health Score (HJHS) 2.1 and Hemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) together with simultaneous assessments of HRQoL (EQ-5D-3L, EQ-VAS, Haemo-QoL/Haem-A-QoL).

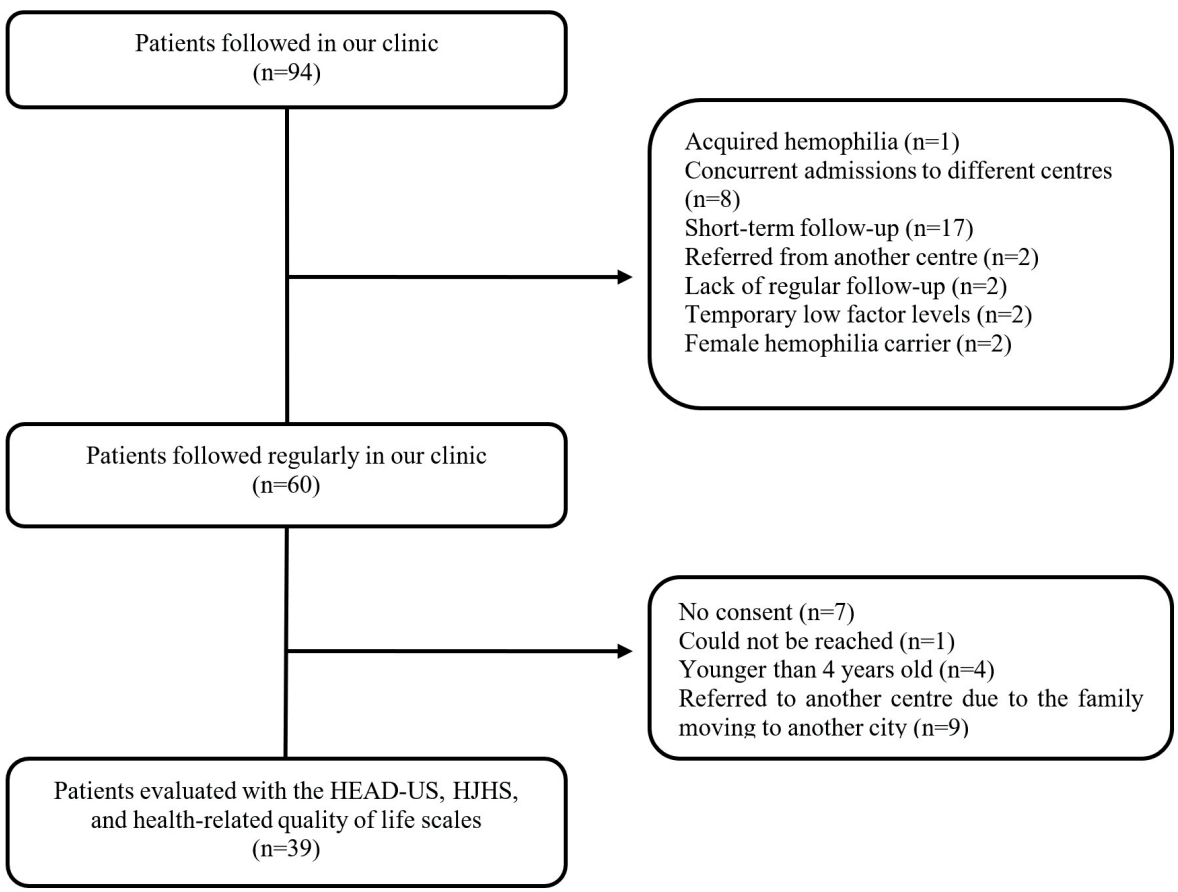

Fig. 1. Flowchart of patient selection.

All procedures were approved by the İstanbul Faculty of Medicine Ethics Committee and complied with the principles of the Declaration of Helsinki and its 2008 amendment. All participants older than 18 years of age and the legal guardians of the patients under 18 years of age were informed of the purpose and content of the research and expressed their informed consent in writing to participate in the study.

The HEAD-US is a scoring system developed by Martinoli et al. that evaluates synovitis, cartilage, and subchondral bone damage in six joints of the elbows, knees, and ankles.[7] The HJHS is a scoring system for clinical evaluation developed by the International Prophylaxis Study Group (IPSG). As part of version 2.1 of the HJHS, the same six joints are evaluated for swelling, duration of swelling, muscle atrophy, crepitus, flexion loss, extension loss, pain, and strength. Additionally, the patient's global gait is evaluated. This scoring system was originally developed for patients between the ages of 4 and 18 years, and it was later validated for use in adults.[8-10] Higher scores are associated with worse joint health for both scoring systems.[7-10]

The EQ-5D is a generic HRQoL metric developed by the EuroQol Group. Proxy versions filled out by parents are used for children between the ages of 4 and 7 years. Five dimensions are assessed: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension is divided into three levels: no problems, some problems, and extreme problems. Patients or their parents are asked to mark points on a visual analogue scale (VAS) ranging between 0 (worst imaginable health state) and 100 (best imaginable health state). If all dimensions show no problems, the individual is said to have a full state of health.[11]

The Haemo-QoL, developed by von Mackensen et al., measures the HRQoL of PwH. There are different versions for different ages, and the scale is completed by interviewing patients between the ages of 4 and 7 years. The total score and each field score can vary between 0 and 100, with higher scores indicating lower HRQoL.[12,13]

Statistical analysis

Descriptive statistics were presented as numbers and percentages for qualitative variables and as medians, minimums, and maximums for quantitative variables. In comparisons of continuous variables, the Mann-Whitney U test was used, while comparisons of more than two groups were performed with the Kruskal-Wallis test. Pairwise comparisons were examined with a post-hoc test with Bonferroni correction. Quantitative variables were examined by Spearman correlation analysis.

IBM SPSS Statistics 21 was used for these analyses, the confidence interval was kept at 95%, and the analysis results were interpreted by comparing them with the $p<0.05$ level of significance.

Results

A total of 39 patients followed at our center, 35 (89.7%) of whom were diagnosed with hemophilia A and 4 (10.3%) of whom were diagnosed with hemophilia B were evaluated. These patients were not currently or previously inhibitor-positive. For severe, moderate, and mild hemophilia; the ages were 12.77 years (4.96-24.15), 7.11 years (4.41-15.95), 15.31 years (11.90-21.89), the factor activity levels were 0.285% (0-0.90), 2.05% (1.00-3.30), 18.00% (8.70-29.30), the median annual bleeding rates (ABR) were 2.5 (0-30), 2 (0-6), 2 (0-40) and the annual joint bleeding rates (AJBR) were 1.5 (0-30), 0 (0-2), 0 (0-2); respectively. All patients received standard half-life factor treatments. The clinical characteristics of the patients are given in Table I.

Patients were evaluated for joint health based on HEAD-US and HJHS-2.1 scores and for HRQoL. The median HEAD-US score of the patients was 0 (0-19), while the median HJHS score was 1 (0-12). The median EQ-VAS score was 90 (50-100) and the median Haemo-QoL score was 22 (6.64-76.19). 43.6% (n=17) of patients reported that they were well in all areas evaluated by the EQ-5D. The median values of HEAD-US,

Table I. Clinical characteristics of the patients (n=39).

	Mild hemophilia (n=7)	Moderate hemophilia (n=8)	Severe hemophilia (n=24)
ABR	2 (0-40)	2 (0-6)	2.50 (0-30)
AJBR	0 (0-2)	0 (0-2)	1.50 (0-30)
Age at time of study, yr	15.31 (11.90-21.89)	7.11 (4.41-15.95)	12.77 (4.96-24.15)
Age at diagnosis, yr	6.70 (1.50-15.01)	1.94 (0.54-8.13)	0.69 (0.02-6.13)
Age at first treatment, yr	9.97 (1.50-14.56)	2.01 (1.18-8.87)	1.07 (0.17-6.34)
Age at prophylaxis (n=26), yr	-	4.32 (2.94-5.70)*	2.90 (0.17-13.82)
Age at first bleeding, yr	2.50 (0-11)	1.28 (0.08-8.86)	0.50 (0.02-6.02)
Factor level (%)	18.00 (8.70-29.30)	2.05 (1.00-3.30)	0.285 (0-0.90)

Data presented as median (min-max).
ABR, annual bleeding rate; AJBR, annual joint bleeding rate; yr, years.
*n=2

Table II. HEAD-US, HJHS-2.1, EQ-VAS, and Haemo-QoL scores of the patients in relation to hemophilia severity (n=39).

	Mild hemophilia (n=7)	Moderate hemophilia (n=8)	Non-severe hemophilia (mild-moderate) (n=15)	Severe hemophilia (n=24)
HEAD-US	All patients had 0 points	0 (0-5)	0 (0-5)	3.50 (0-19)
HJHS-2.1	0 (0-8)	0 (0-4)	0 (0-8)	1.50 (0-12)
EQ-VAS	85 (50-100)	85 (50-100)	85 (50-100)	95 (50-100)
Haemo-QoL	14.29 (6.64-33.44)	23.38 (16.67-76.19)	21.48 (6.64-76.19)	22.78 (6.64-69.05)

Data presented as median (min-max).
EQ-VAS, EuroQoL Visual Analogue Score; Haemo-QoL, Hemophilia Quality of Life Questionnaire; HEAD-US, Hemophilia Early Arthropathy Detection with Ultrasound; HJHS-2.1, Hemophilia Joint Health Score version 2.1.

HJHS-2.1, EQ-VAS, and Haemo-QoL scores according to hemophilia severity are provided in Table II. Haemo-QoL and EQ-5D scores and subscores are shown in Fig. 2 and Table III. The most problematic area was pain in the EQ-5D and family in the Haemo-QoL.

When patients were divided into two groups as severe and non-severe (moderate and mild) hemophilia and compared in terms of the HEAD-US, HJHS-2.1, EQ-VAS, and Haemo-QoL, a significant difference was found only for HEAD-US scores (p=0.001, p=0.598, p=0.309, and p=0.721, respectively). When the patient group was divided as severe, moderate and mild hemophilia; there was a statistically significant difference between the HEAD-US scores of patients diagnosed with mild and severe hemophilia (p=0.006), but no significant difference was detected between mild and moderate (p=1.000) or moderate and severe hemophilia (p=0.052). When the analysis was repeated according to the factor levels of the patients; there was a statistically significant moderate negative correlation between the patients' factor levels and the HEAS-US scores (p=0.001, r=-0.530), and no significant correlation was found between patients' factor levels and the HJHS, EQ-VAS, and Haemo-QoL scores (p=0.874, p=0.431, p=0.451, respectively).

All patients with severe hemophilia (24/24) and two patients with moderate hemophilia (2/8) were on prophylactic treatment, whereas the remaining patients with moderate hemophilia (6/8) and all patients with mild hemophilia (7/7) were managed with on-demand therapy. Two of the patients with moderate hemophilia were receiving prophylaxis due to frequent bleeding (The first patient's factor activity level was 1.60%, aged 12.52, diagnosed at 2.88, started prophylaxis at 2.94 years old. The second patient's factor activity level was 1.80%, aged 8.64, diagnosed at 0.63, started prophylaxis at

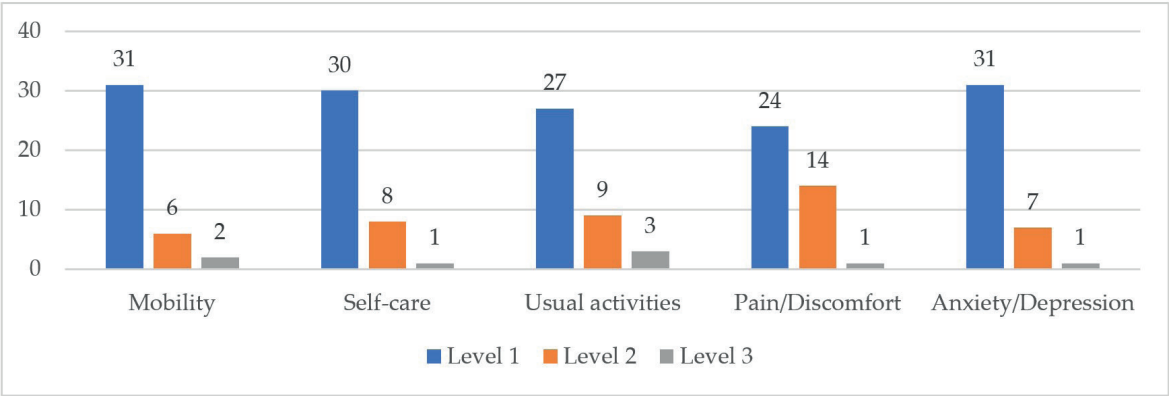

Fig. 2. Distribution of the patients according to the dimensions of the EQ-5D scale.

Table III. EQ-VAS and Haemo-QoL scores and Haemo-QoL subscores.

	n	Median (Range) (%)	Number of patients with the highest score
EQ-VAS	39	90 (50-100)	
Haemo-QoL	39	22 (6.64-76.19)	
Physical health	39	12.5 (0-100)	4
Feelings	39	6.25 (0-83.33)	0
View of yourself	39	16.67 (0-100)	4
Sports	39	27.78 (0-81.25)	6
Treatment	39	25 (0-100)	5
Family	33	45 (12.50-100)	11
Friends	33	25 (0-100)	3
Other people	33	8.33 (0-100)	1
Dealing with hemophilia	32	19.64 (0-100)	2
Perceived support	25	31.25 (0-100)	7
Future	20	22.50 (0-75)	1
Relationships	13	0 (0-62.50)	1
Work	5	6.25 (0-37.50)	0
Family planning	5	0 (0-6.25)	0
Sexuality	6	0 (0-75)	1

EQ-VAS: EuroQoL Visual Analogue Score; Haemo-QoL: Hemophilia Quality of Life Questionnaire

5.70 years old). Because the treatment modality changes according to clinical phenotype; patients were divided into two groups: patients who are receiving prophylaxis and on-demand therapy. Between these two groups, a significant difference was found only for HEAD-US scores (p=0.000). There was no significant difference between HJHS-2.1, EQ-VAS, and Haemo-QoL scores (p=0.546, p=0.489, p=0.872, respectively). When the patients' total HEAD-US scores were compared with their total HJHS-2.1 scores, EQ-VAS scores, and Haemo-QoL scores, no significant correlations were found (p=0.074, p=0.862, and p=0.210, respectively). When the patients who described a state of complete well-being in the EQ-5D and the patients who reported having any problems were compared, no statistically significant difference was detected in HEAD-US, HJHS-2.1, or EQ-VAS scores between the two groups (p=0.082, p=0.564, and p=0.053, respectively), but a statistically significant difference was detected for their Haemo-QoL scores (p=0.001).

When the correlation of the HEAD-US and HJHS-2.1 was examined at the joint level, a statistically significant but negligible correlation was detected (p<0.001, r=0.244) (Fig. 3). When we looked at the 234 joints to understand why the correlation was negligible, 165 joints with HEAD-US and HJHS-2.1 scores of 0 (165/234, 70.51%), 34 joints (34/199, 17.09%) with a score of 1 or more from the HJHS-2.1 when the HEAD-US was 0, and 21 joints (21/186, 11.29%) with a score of 1 or more from the HEAD-US when the HJHS-2.1 was 0 were observed. Thus, the rate of joints with scores that were incompatible with each other was 23.5% (55/234) among all evaluated joints. When the HJHS-2.1 score was 0, arthropathy was identified by the HEAD-US for 21 joints (synovitis in 18 joints, cartilage damage in 17 joints, bone damage in 6 joints). When the HEAD-US score was 0, among the joints that had scores of >0 from the HJHS-2.1, 25 joints had a non-zero score for crepitation, 1 joint had a non-zero score for loss of extension, and 8 joints had a non-zero score for pain.

Discussion

In the study by Jiménez-Yuste et al., in which the severity, treatment types, and HEAD-US scores of patients with hemophilia B were compared, it was shown that there was a difference between hemophilia severity and HEAD-US scores in most joints when the results were explored at the joint level.[14] In the study by Fang et al. examining knee joints, differences were found between moderate and mild and between severe and mild hemophilia in terms of HEAD-US and HJHS scores, whereas no difference was found between severe and moderate hemophilia. The reason for this was explained as spontaneous bleeding being unusual in cases of mild hemophilia.[15] In this study, we have shown that HEAD-US scores differ statistically between mild and severe hemophilia.

In a study conducted by Xu et al., where the HRQoL of 875 patients was examined with various scales, the Haem-A-QoL was found to be positively correlated with the EQ-5D and negatively correlated with the EQ-VAS.[16] Since there is no threshold value set for the EQ-5D in Türkiye, a relevant comparison could not be made, but when the patients who described themselves as having a state of complete well-being according to the EQ-5D and the patients who reported any problems were examined, there was no statistical difference in EQ-VAS

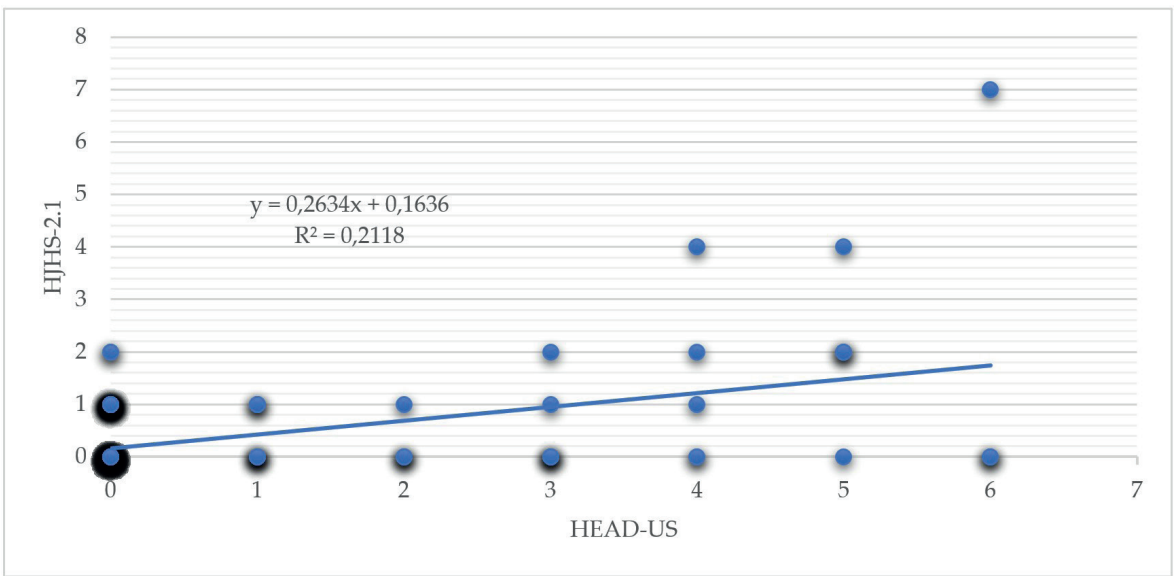

Fig. 3. Scatter plot of HEAD-US and HJHS-2.1 scores at joint level.
HEAD-US, Hemophilia Early Arthropathy Detection with Ultrasound; HJHS-2.1, Hemophilia Joint Health Score version 2.1

scores between the two groups but a statistically significant difference was detected for the Haemo-QoL. In our study, it was seen that the most problematic area of the EQ-5D was pain, while it was family for the Haemo-QoL. Baek et al. showed that the most affected areas for children and adolescents were support, friends, and coping with the disease among patients in Korea with moderate and severe hemophilia.[17] In the study by Oldenburg et al. including patients with severe hemophilia A aged >12 years, the patients received the highest scores in the areas related to sports and the future.[18] In a study in China, patients had the worst scores for the anxiety/depression domain of the EQ-5D-5L, followed by the pain domain; it was observed that the areas where the most problems were reported were pain and then mobility.[16] We believe that the problematic domains may change from culture to culture.

In the PROBE study, the HRQoL scores of patients with moderate and mild hemophilia were found to be significantly lower compared to those of the healthy population.[19] Similar to our study, Cheung et al.[20] showed that HRQoL scores did not change with disease severity. This finding contradicts the results of a study conducted by Daffunchio et al.[21] including patients with mild hemophilia and an average age of 35.9 years, in which it was emphasized that HRQoL was lower among patients with arthropathy. The reason for this difference may be that the median age of the patients in our study was 13 years or that we evaluated patients with all severity levels of hemophilia in our study.

In a study conducted by Foppen et al.[22] that included patients who were diagnosed with severe and moderate hemophilia and receiving prophylaxis, a statistically significant correlation ($p<0.01$, $r=0.700$) was found between HEAD-US and HJHS scores. In our study, no correlation was detected at the patient level ($p=0.074$), while a negligible correlation was detected at the joint level ($p<0.001$, $r=0.244$). The difference in findings on the correlation between the HEAD-US and HJHS-2.1 may be due to the fact that our study was conducted with all hemophilia patients or that more joints were evaluated. In the MoHem study, where HEAD-US and HJHS scores and arthropathy were examined in patients with moderate hemophilia, a statistically significant correlation was found between the HEAD-US and HJHS ($r=0.70$ for elbows, $r=0.60$ for knees, $r=0.65$ for ankles). At the same time, 24% incompatible results were detected. It was observed that when the HJHS score was 0 for 5% of the joints, the HEAD-US scores were 1 or above, and when the HEAD-US score was 0, 26% of the joints received HJHS scores of 1 or above. Crepitus was found to be present in 31% of knees with normal HEAD-US results.[23] In our study, when 234 joints were evaluated, there were 165 joints with 0 points from both the HEAD-US and HJHS-2.1 (165/234, 70.51%), 34 joints (34/199, 17.09%) with a score of ≥1 from the HJHS-2.1 when the HEAD-US score was 0, and 21 joints (21/186, 11.29%) with a score of ≥1 from the HEAD-US when the HJHS-2.1 score was 0. The percentage of joints with scores that were incompatible with each other was 23.5% (55/234) among all joints. Although this is similar to the rate reported by the MoHem study considering discordance among all joints, in our study, the rate of joints with abnormalities detected by the HJHS-2.1 when the HEAD-US score was 0 was found to be lower while the rate of abnormalities detected by the HEAD-US when the HJHS-2.1 score was 0 was found to be higher. The most significant difference between our study and the literature is that the correlation of the HEAD-US and HJHS-2.1 at the joint level varies, but they were found to be less correlated in this study than in the MoHem study.

Since the correlation between the HEAD-US and HJHS-2.1 was found to be lower than previously reported in the literature, an attempt was made to determine which data disrupted that correlation. According to Hilliard et al., when the intraclass correlation of the HJHS was examined, pain and crepitation were found to be the least reliable variables and swelling, muscle atrophy, and walking were found to be

the most reliable.[8] When the subscores of the HJHS-2.1 and HEAD-US were examined in our study, it was observed that the patients scored highest for crepitus and second highest for pain when the HEAD-US score was 0. In this regard, our data are similar to the findings reported in the literature.

The present study is valuable because it emphasizes that HRQoL is not necessarily correlated with patients' arthropathies, since the ways in which children perceive the world differ from those of adults.

Study limitations

The limitations of this study are that it was a cross-sectional design and a limited number of patients were included. More accurate results could be achieved with regular prospective follow-up of patients, the course of their arthropathies, and their HRQoL scores, as well as with studies including larger numbers of patients.

Conclusions

In our study, it was concluded that pediatric patients with hemophilia should be followed with both the HEAD-US and HJHS in terms of arthropathy in childhood because the correlation between them is weak. Additionally, more attention should be paid to patients with severe hemophilia. However, the fact that a patient does not have arthropathy or that a patient has moderate or mild hemophilia does not necessarily mean that the patient's HRQoL scores will be better. In terms of holistic health care, it is necessary to also pay proper attention to patients with moderate and mild hemophilia.

Ethical approval

All procedures were approved by the İstanbul Faculty of Medicine Ethics Committee on 25.11.2022 (meeting no: 21, file no: 2022/1947) and complied with the principles of the Declaration of Helsinki and its 2008 amendment.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AÜ, BG; data collection: BG, MB, GB, AÜ; analysis and interpretation of results: BG, AÜ; draft manuscript preparation: BG, AÜ. All authors reviewed the results and approved the final version of the article.

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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

Risk factors of disease severity and mechanical ventilation requirement in childhood Guillain-Barré Syndrome

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ABSTRACT

Background. This study aimed to investigate the risk factors associated with the severity of the disease, the need for mechanical ventilation (MV) and poor prognosis in the early stages of Guillain-Barré Syndrome (GBS).

Methods. Data of children who met GBS diagnostic criteria were evaluated retrospectively. The sample was divided into three binary subgroups according to severe GBS (Hughes Functional Grading Scale [HFGS] ≥ 4 at admission), mechanical ventilation (MV) requirement, and poor prognosis (inability to walk independently, HFGS ≥ 3 after six months). Various clinical, laboratory and electrophysiological parameters were compared between these subgroups.

Results. The mean age of 63 children with GBS was 91.55±49.09 months. 13 (20.6%) patients required MV and 4 (6.3%) patients died. Associated risk factors for the need for MV in severe GBS were found to be autonomic dysfunction, bulbar palsy, sensory impairment, lowest total Medical Research Council (MRC) scale for muscle strength score at admission, high modified Erasmus GBS respiratory failure score (mEGRIS), high neutrophil-lymphocyte ratios (NLR) and high systemic immune-inflammation index (SII) values (p<0.001, p=0.003, p=0.033, p<0.001, p<0.001, p=0.037 and p=0.042, respectively). The lowest total MRC scale for muscle strength score at admission was a significant indicator of poor prognosis (p<0.001).

Conclusions. Autonomic dysfunction, bulbar palsy, sensory impairment, lowest total MRC scale for muscle strength score at admission, high mEGRIS score, high NLR and SII values are potential risk factors for the need for MV in children with severe GBS. The lowest total MRC scale for muscle strength score at admission was associated with poor prognosis.

Key words: Guillain-Barré Syndrome, disease severity, mechanical ventilation, prognosis, risk factors, pediatric.

Guillain-Barré Syndrome (GBS) is an acute immune-mediated peripheral polyradiculoneuropathy and is the leading cause of acute flaccid paralysis in children. The incidence of GBS is 0.62 cases per 100,000 person-years in children aged 0 to 9 years and 0.75 cases per 100,000 person-years in children and adolescents aged 10 to 19 years.[1]

Respiratory failure requiring mechanical ventilation (MV) affects 20–30% of patients with GBS and is the most important prognostic factor for severe GBS.[2] Therefore, early recognition of respiratory failure in patients with GBS is of great importance.[3] Accurately predicting GBS patients who will need MV in the early stages of the disease may improve disease outcomes by allowing clinicians to determine personalized treatments in a timely manner. Previous studies on the predictors for respiratory failure have been reported as shorter time from onset to admission, bulbar

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involvement, total Medical Research Council (MRC) scores for muscle strength score at admission <20/60, higher GBS disability score, lower vital capacity, hypoalbuminemia, neck muscle weakness, inability to raise elbows, inability to stand, inability to cough, dysautonomia, low single breath rate, increased liver enzymes, lower proximal/distal compound muscle action potential ratio, nerve conduction block, longer phrenic nerve latency and acute inflammatory demyelinating polyneuropathy (AIDP) versus acute motor axonal neuropathy (AMAN) or acute motor and sensory axonal neuropathy (AMSAN) in GBS subtypes.[4-16] Modified Erasmus GBS respiratory failure scores (mEGRIS) accurately predict the risk of respiratory failure in the early course of GBS.[3,16] Most of these studies have been conducted in adults with GBS, and significant heterogeneity has been observed between studies. There are however a limited number of studies in the literature on MV risk factors in children with GBS.[17-24]

In this study, we aimed to investigate risk factors associated with the severity of GBS and poor prognostic risk factors and predictors of the need for mechanical ventilation in severe cases of GBS.

Materials and Methods

Study design

The study population consisted of patients younger than 18 years who met the diagnostic criteria for GBS and received sequential treatment during their hospitalization at the İnönü University Faculty of Medicine Hospital Pediatric Neurology Unit between December 2003 and January 2023. Medical records of the patients included in the study were recorded on a predesigned questionnaire. GBS diagnostic classification accuracy levels were defined for each patient.[3] The functional status of the patients was evaluated according to the Guillain-Barré Syndrome Disability Scale (Hughes Functional Rating Scale [HFGS]) at the time of hospital admission and approximately six months after discharge.[25]

Ethics approval

The study was approved by İnönü University Ethics Committee (date: 02.05.2023, number: 2023-4580).

Study inclusion and exclusion criteria

All patients who presented with acute flaccid paralysis and met the diagnostic criteria for GBS, were younger than 18 years of age, and complete medical files were included in the study. Patients diagnosed with acute flaccid paralysis due to other causes, patients diagnosed with diseases such as polio, botulism, toxic neuropathy or diphtheria-related neuropathy, Bickerstaff encephalitis, Miller Fisher syndrome, critical illness polyneuropathy or myopathy, and chronic inflammatory demyelinating polyradiculoneuropathy were excluded from the study.

Data collection

Patients' demographic and clinical characteristics, including age, gender, season of onset, infection history, time from onset of GBS symptoms to hospitalization, length of hospital stay, deep tendon reflex, cranial nerve involvement (facial, glossopharyngeal and vagus nerves), sensory impairment, need for MV, autonomic nerve dysfunction, and therapeutic methods used were recorded and analyzed retrospectively. GBS severity was assessed at admission using HFGS and the lowest total MRC scale for muscle strength score, whereas respiratory failure was predicted with mEGRIS. Patients with HFGS score of ≥ 4 at admission were considered to have severe GBS.[26] Patients with an HFGS score of ≥ 3 (inability to walk independently) within six months after discharge were considered to have poor prognosis.[27] Cerebrospinal fluid (CSF) protein, neutrophil (N), lymphocyte (L), platelet (P), neutrophil-to-lymphocyte ratio (NLR=N/L), platelet-to-lymphocyte ratio (PLR=P/L),

systemic immune-inflammation index (SII = P x [N/L]) values and nerve conduction study (NCS) results were recorded.

Assessment of GBS severity and functional neurological deficit

All patients were evaluated in terms of disease severity and functional neurological deficit using HFGS and MRC. HFGS grades and corresponding functions were as follows: '0': no symptoms; '1': minor symptoms and the ability to run; '2': able to walk 10 meters or more without assistance but unable to run; '3': able to walk 10 meters in an open area with assistance; 'unable to walk unaided' '4': bedridden or wheelchair-bound; '5': requiring ventilation support for at least part of the day; and '6': dead.[23] MRC scores used to evaluate muscle strength were calculated according to the strength of six bilateral muscles in four extremities and ranged between 0 and 60. Accordingly, an MRC score of 0 meant quadriplegic, whereas an MRC score of 60 indicated normal muscle strength.[28] The lowest total MRC and highest HFGS scores were considered to indicate the worst status of GBS.

Grouping of patients with GBS

Patients (n=63) were divided into two subgroups according to disease severity. Accordingly, severe GBS (HFGS score ≥ 4, n=27) and non-severe (HFGS score< 4, n= 36) GBS were included in the subgroups.

Patients with severe GBS were further divided into two subgroups according to whether patients were in need of MV, such that: patients requiring MV (MV subgroup) and patients with normal ventilation (NV subgroup). Intensive care unit (ICU) physicians decided the indication for starting MV based on vital signs and laboratory data (pediatric protocols or standard criteria).[29]

In general, the patients whose conditions had improved or were stable in our neurology service were discharged from the hospital. Patients who could walk independently approximately six months after discharge were considered to have good prognosis (HFGS<3, n=50) and patients who could not walk independently were considered to have poor prognosis (HFGS ≥ 3, n=13).

Respiratory failure prediction

Respiratory failure was predicted based on mEGRIS scores. Accordingly, first, the patients whom the time from the onset of first symptoms to admission was > 7 days, between 4 days and 7 days, and ≤ 3 days were assigned 0 points, 1 point, and 2 points, respectively. Secondly, the patients with and without facial palsy and/or bulbar palsy at admission were assigned 1 point and 0 points, respectively. Thirdly, the patients with a lowest total MRC scale for muscle strength score at admission between 60 and 51, 50 and 41, 40 and 31, 30 and 21, and ≤20 were assigned 0 points, 1 point, 2, 3, and 4 points, respectively. By adding up the three points mentioned above, a total mEGRIS score of 0 to 7 points was obtained. Patients with mEGRIS scores of 0 to 2, 3 to 4, and 5 to 7 were considered to be at low, moderate, and high risk of respiratory failure, respectively.[4]

Statistical analysis

The descriptive statistics obtained from the collected data were expressed as mean ± standard deviation values or median with minimum and maximum values in the case of continuous variables determined to conform and not to conform to the normal distribution, respectively, and as frequency (n) and percentage (%) values in the case of categorical variables. Shapiro- Wilk test was used to analyze the normal distribution characteristics of continuous (numerical) variables. Independent samples t-test and Mann-Whitney U test was used to compare quantitative variables. Yates' chi-square with continuity correction and Fisher's exact tests were used to compare qualitative variables. Probability (p) statistics of ≤ 0.05 were deemed to indicate statistical significance. IBM SPSS Statistics 27.0 (Statistical Product and Service Solutions for Windows,

Version 27.0, IBM Corp., Armonk, NY, U.S., 2020) software package was used to conduct the statistical analyses.

Results

Demographic characteristics of pediatric patients with GBS

The mean age of the 63 children with GBS included in the study sample was 91.55 ± 49.09 (range: 17-180) months at admission. Of these patients, 33 (52.4%) were male. In terms of infection history, 36.5% of the patients had upper respiratory tract infections, 25.4% acute gastroenteritis, and one patient each had hepatitis A, chickenpox and brucellosis. Cranial nerve involvement was present in 31 (49.2%) patients. Of these patients, 15 (23.8%) had bulbar palsy, 13 (20.6%) facial palsy, and 3 (4.8%) both bulbar and facial involvement. Sensory impairment and autonomic dysfunction was present in 28 (44.4%) and 16 (25.4%) patients, respectively. The mean time from the onset of symptoms to hospitalization was 4.31± 2.56 days. The mean length of hospital stay was 9.76±5.67 (range: 4-37) days. There were 27 (42.9%) patients with severe GBS. The lowest total MRC scale for muscle strength in patients with poor prognosis was 36.44 ± 4.10 (median: 38, range: 30 to 42). The mean mEGRIS score of the overall study group was 3.88 ±1.58 (range: 2-7). There were 20 (31.7%) patients in the high-risk group according to the mEGRIS scores. The most common electrophysiological GBS subtype in the overall study group was AMAN, seen in 31 (49.2%) patients, followed by AMSAN, seen in 18 (28.6%) patients, and AIDP, seen in 14 (22.2%) patients. In terms of treatment methods used, the first preferred method was intravenous immunoglobulin (IVIg) administration to 43 (68.3%) patients within 24 hours after admission to the hospital, and plasmapheresis was applied after IVIg to 20 (31.7%) patients who did not show a significant improvement in muscle strength.

During clinical follow-up, 13 (20.6%) patients required MV, 4 (6.3%) died, and 59 (93.7%) were discharged. The common causes of death of the 4 patients monitored on MV were respiratory failure, autonomic dysfunction and cardiac arrest. Penicillin-sensitive *Streptococcus pneumoniae* was detected in the respiratory tract secretion culture of the first patient. Urosepsis (100000 cfu/ml *Escherichia coli* was found in the urine culture) was detected in the second patient. The third patient died due to aspiration pneumonia, pleural effusion, gastrointestinal bleeding and multiorgan failure. The fourth patient had bronchopneumonia (with widespread infiltration on chest radiography) at the time of admission. Echocardiography results of 4 patients were evaluated as normal.

The risk factors for severe GBS

No statistically significant difference was observed between severe and non-severe GBS subgroups in terms of age and gender ($p > 0.05$). In the severe GBS subgroup, the time from onset of first symptoms to hospitalization was shorter, albeit not significantly, ($p > 0.05$). There was a statistically significant difference in seasonal morbidity between GBS subgroups. Admission in summer season was significantly higher in the severe GBS subgroup than in the non-severe GBS subgroup ($p = 0.018$). There was a statistically significant difference between GBS subgroups in terms of electrophysiological GBS subtype. In the severe GBS subgroup while AMSAN was most common, AIDP was significantly higher in the non-severe GBS subgroup ($p=0.013$). Cranial nerve involvement, autonomic dysfunction and sensory impairment were observed in significantly more patients in the severe GBS subgroup than in the non-severe GBS subgroup ($p <0.001$ for all cases) (Table I). There was no statistically significant difference between the severe and non-severe GBS subgroups regarding CSF protein, N, L, P, NLR, PLR, and SII values ($p >0.05$) (Table II).

The risk factors for MV need

The mean age of pediatric GBS patients was younger in the MV subgroup, albeit not significantly, than in the NV subgroup

Table I. Comparison of clinical characeristics and presentation of GBS between severe GBS and non-severe GBS groups.

Variables*	Categories	GBS classification		Total	Chi-square statistics	*p*
		Non-severe GBS group (n=36) (HFGS 0,1,2,3)	Severe GBS group (n=27) (HFGS 4,5,6)			
Gender	Girl	16[a] (53.3%)	14[a] (46.7%)	30 (100.0%)	0.107	0.743[2]
	Boy	20[a] (60.6%)	13[a] (39.4%)	33 (100.0%)		
Cranial Nerve	No	26[a] (81.3%)	6[b] (18.8%)	32 (100.0%)	23.452	<0.001[1]
Damage	Facial paralysis	8[a] (61.5%)	5[a] (38.5%)	13 (100.0%)		
	Bulbar palsy	2[a] (13.3%)	13[b] (86.7%)	15 (100.0%)		
	Facial and bulbar palsy	0[a] (0.0%)	3[b] (100.0%)	3 (100.0%)		
Sensory	No	27[a] (77.1%)	8[b] (22.9%)	35 (100.0%)	11.091	<0.001[2]
Disorder	Yes	9[a] (32.1%)	19[b] (67.9%)	28 (100.0%)		
Autonomous	No	36[a] (76.60%)	11[b] (23.4%)	47 (100.0%)	25.554	<0.001[2]
changes	Yes	0[a] (0.00%)	16[b] (100.0%)	16 (100.0%)		
Pain	No	3[a] (100.0%)	0[a] (0.00%)	3 (100.0%)	-	0.253[3]
symptoms	Yes	33[a] (55.0%)	27[a] (45.00%)	60 (100.0%)		
Season at	Spring	7[a] (58.3%)	5[a] (41.7%)	12 (100.0%)	9.821	0.018[1]
admission	Autumn	17[a] (68.0%)	8[a] (32.0%)	25 (100.0%)		
	Summer	7[a] (33.3%)	14[b] (66.7%)	21 (100.0%)		
	Winter	5[a] (100.0%)	0[b] (0.0%)	5 (100.0%)		
EMG	AIDP (myelin)	12[a] (85.7%)	2[b] (14.3%)	14 (100.0%)	8.884	0.013[1]
	AMAN (axonal)	18[a] (58.1%)	13[a] (41.9%)	31 (100.0%)		
	AMSAN (myelin and axonal)	6[a] (33.3%)	12[b] (66.7%)	18 (100.0%)		
Total		36 (57.1%)	27 (42.9%)	63 (100.0%)		

*: Variables are expressed as frequency (percent).
[1]: Pearson chi-square, [2]: Continuity correction [3]: Fisher's exact test.
Each superscript letter denotes a subset of GBS categories whose column proportions do not differ significantly from each other at the 0.05 level. The statistically significant difference is expressed in bold.
AIDP, Acute inflammatory demyelinating polyneuropathy; AMAN, Acute motor axonal neuropathy; AMSAN, Acute motor and sensory axonal neuropathy; EMG, Electromyelography; GBS, Guillain-Barré syndrome; HFGS, Hughes Functional Grading Scale.

Table II. Comparison of laboratory parameters and presentation of GBS between severe GBS and non-severe GBS groups.

Variables*	GBS classification		*p*
	Non-Severe GBS group (n=36) (HFGS 0,1,2,3)	Severe GBS group (n=27) (HFGS 4,5,6)	
Age	95.14 ± 51.01 ǀ 73.5 (25 - 180)	86.78 ± 46.94 ǀ 82 (20 - 180)	0.667
MRC at admission	43.06 ± 3.72 ǀ 44 (34 - 50)	29.19 ± 6.91 ǀ 30 (16 - 42)	<0.001[1]
CSF protein	98.19 ± 49.76 ǀ 81.05 (46 - 257)	98.04 ± 47.32 ǀ 87.4 (45.9 - 230.3)	0.978[2]
Neutrophil	5.1 ± 1.99 ǀ 4.4 (2.6 – 12.3)	5.64 ± 2.62 ǀ 5 (1.79 – 12.2)	0.437[2]
Lymphocyte	2.83 ± 1.13 ǀ 2.54 (1.36 – 6.9)	2.79 ± 1.28 ǀ 2.75 (0.9 – 6.1)	0.802[2]
Platelet	347.75 ± 100.3 ǀ 309 (214 - 681)	343.37 ± 97 ǀ 342 (147 - 556)	0.776[2]
NLR	2.1 ± 1.23 ǀ 1.93 (0.65 – 5.13)	2.68 ± 2.51 ǀ 1.69 (0.57 – 10.17)	0.708[2]
PLR	141.4 ± 74.04 ǀ 126.43 (35.22 – 412.33)	150.73 ± 93.05 ǀ 114.71 (62.18 – 463.33)	0.945[2]
SII	771.52 ± 632.49 ǀ 559.78 (158.48 - 3051.23)	998.62 ± 1220.88 ǀ 560.91 (111.3 – 5652.67)	0.771[2]

*: Variables are expressed as mean ± std. deviation ǀ median (minimum-maximum).
[1]: Independent samples t-test, [2]: Mann-Whitney U test.
The statistically significant difference is expressed in bold.
CSF, Cerebrospinal fluid; GBS, Guillain-Barré syndrome; HFGS, Hughes Functional Grading Scale; MRC, Medical Research Council; NLR, Neutrophil to lymphocyte ratio; PLR, Platelet to lymphocyte ratio; SII, Systemic immune inflammation index.

($p > 0.05$). The length of hospital stay was significantly longer in the MV subgroup than in the NV subgroup (15 ± 9.8 days vs. 8.4 ± 2.83 days, $p = 0.047$). Of the 13 children who needed invasive MV, 4 died and 9 were discharged with poor functional recovery (negative outcome). The presence of bulbar paralysis, autonomic dysfunction and sensory impairment in children with GBS were identified as significant clinical risk factors for a need for MV ($p < 0.001$) (Table III). The mean mEGRIS score of the pediatric GBS patients was statistically significantly higher in the MV subgroup than in the NV subgroup (6.38 ± 0.51 vs. 3.24 ± 1.02; $p < 0.001$). In parallel, the lowest mean total MRC scale for muscle strength score at admission was statistically significantly lower in the MV subgroup than in the NV subgroup ($p < 0.001$) (Table IV). No statistically significant difference was found between the MV and NV subgroups regarding CSF protein, NLR, PLR and SII values ($p > 0.05$) (Table IV).

The risk factors for MV need in children with severe GBS

The mean age of the 27 pediatric patients with severe GBS at the onset of first symptoms of GBS was 86.08 ± 47.63 months. Of these patients, 13 (48.1%) were male ($p > 0.05$), and 13 needed MV. Comparison of the pediatric severe GBS patients with and without the need for MV is shown in Table V. There was no significant difference between the pediatric severe GBS patients with and without MV need in terms of the season of admission ($p > 0.05$). mEGRIS score was statistically significantly higher in pediatric severe GBS patients with a need for MV than in those without a need for MV ($p < 0.001$). In parallel, the lowest mean total MRC scale for muscle strength score at admission was statistically significantly lower in pediatric severe GBS patients with a need for MV than in those without MV need (23.38 ± 4.72 vs. 34.57 ± 3.18, $p<0.001$). There were significantly more patients with clinical risk factors, like autonomic dysfunction, bulbar palsy, and sensory impairment among pediatric severe GBS patients with a need for MV compared to those without MV need ($p < 0.001$, $p=0.003$, and $p=0.033$, respectively). In terms of anti-inflammatory markers, NLR and SII values calculated at admission were statistically significantly higher among pediatric severe GBS patients with a need for MV compared to those without MV need ($p=0.037$ and $p=0.042$, respectively) (Table VI).

The risk factors for GBS prognosis

No statistically significant difference was observed between GBS subgroups with poor and good prognosis in terms of age and gender ($p > 0.05$). The lowest total MRC scale for muscle strength score at approximately six months after discharge was statistically significantly lower in the GBS subgroup with poor prognosis than in the GBS subgroup with good prognosis [49.84 ± 4.22 (median:50) versus 36.44 ± 4.10 (median:38); $p < 0.001$]

Discussion

We investigated potential risk factors regarding the severity of GBS, the need for MV in the early stages of the disease, and prognosis, using a cohort of children diagnosed with GBS. In 42.8% (27) of the patients with GBS, the disease was severe, 20.6% (13) required MV, and 6.3% (4) died. The potential predictors for severe GBS were found to be summer admission, cranial nerve involvement, autonomic dysfunction, sensory impairment, lowest total MRC scale for muscle strength score at admission, and AMSAN electrophysiological subtype. The predictors of MV requirement in severe GBS patients were found to be bulbar palsy, autonomic dysfunction, sensory impairment, lowest total MRC scale score for muscle strength at admission, high mEGRIS score, high NLR and high SII values. Lowest total MRC scale for muscle strength score at admission was the factor associated with poor prognosis. These results may assist clinicians in accurately predicting the development of respiratory failure in children with GBS using clinical

Table III. Comparison of clinical features and presentation of GBS between MV and NV groups.

Variables*	Categories	Ventilation type		Total	Chi-square statistics	*p*
		Normal ventilation group (n=50)	Mechanical ventilation group (n=13)			
Gender	Girl	24[a] (80.0%)	6[a] (20.0%)	30 (100.0%)	0.000	0.999[2]
	Boy	26[a] (78.8%)	7[a] (21.2%)	33 (100.0%)		
Cranial Nerve Damage	No	32[a] (100.0%)	0[b] (0.0%)	32 (100.0%)	32.939	<0.001[1]
	Facial paralysis	12a (92.3%)	1[a] (7.7%)	13 (100.0%)		
	Bulbar palsy	5[a] (33.3%)	10[b] (66.7%)	15 (100.0%)		
	Facial and bulbar palsy	1[a] (33.3%)	2[b] (66.7%)	3 (100.0%)		
Sensory Disorder	No	34[a] (97.1%)	1[b] (2.9%)	35 (100.0%)	12.853	<0.001[2]
	Yes	16[a] (57.1%)	12[b] (42.9%)	28 (100.0%)		
Autonomous changes	No	47[a] (100.0%)	0[b] (0.0%)	47 (100.0%)	-	<0.001[3]
	Yes	3[a] (18.8%)	13[b] (81.3%)	16 (100.0%)		
Season at admission	Spring	9[a] (75.0%)	3[a] (25.0%)	12 (100.0%)	2.575	0.447[1]
	Autumn	21[a] (84.0%)	4[a] (16.0%)	25 (100.0%)		
	Summer	15[a] (71.4%)	6[a] (28.6%)	21 (100.0%)		
	Winter	5a (100.0%)	0[a] (0.0%)	5 (100.0%)		
Total		50 (79.4%)	13 (20.6%)	63 (100.0%)		

*: Variables are expressed as frequency (percent).
[1]: Pearson chi-square, [2]: Continuity correction [3]: Fisher's exact test.
Each superscript letter denotes a subset of GBS categories whose column proportions do not differ significantly from each other at the 0.05 level. The statistically significant difference is expressed in bold.
GBS, Guillain-Barré syndrome; MV, mechanical ventilation; NV, Normal ventilation.

Table IV. Comparison of laboratory parameters and presentation of GBS between MV and NV groups.

Variables*	Ventilation type		*p***
	Normal ventilation Group NV (n=50)	Mechanical ventilation Group MV (n=13)	
Age	92.98 ± 49.85 ǀ 74.5 (25 - 180)	86.08 ± 47.63 ǀ 96 (20 - 180)	0.760
MRC at admission	40.68 ± 5.23 ǀ 42 (30 - 50)	23.38 ± 4.72 ǀ 24 (16 - 28)	<0.001
mEGRIS	3.24 ± 1.02 ǀ 3 (2 - 5)	6.38 ± 0.51 ǀ 6 (6 - 7)	<0.001
CSF protein	99.51 ± 49.35 ǀ 81.05 (45.9 - 257)	92.81 ± 45.7 ǀ 92 (53.1 – 230.3)	0.852
Neutrophil	4.99 ± 1.88 ǀ 4.45 (2.4 – 12.3)	6.64 ± 3.16 ǀ 5.4 (1.79 – 12.2)	0.051
Lymphocyte	2.91 ± 1.22 ǀ 2.61 (1.36 – 6.9)	2.43 ± 1 ǀ 2.2 (0.9 – 4.2)	0.262
Platelet	343.22 ± 95.16 ǀ 327 (147 - 681)	356.08 ± 112.4 ǀ 338 (194 - 556)	0.734
NLR	1.99 ± 1.14 ǀ 1.63 (0.65 – 5.13)	3.72 ± 3.27 ǀ 2.17 (0.57 – 10.17)	0.055
PLR	135.72 ± 68.55 ǀ 120.49 (35.22 – 412.33)	182.63 ± 117.38 ǀ 169.09 (62.18 – 463.33)	0.255
SII	707.14 ± 559.27 ǀ 558.48 (158.48 – 3051.23)	1490.83 ± 1630.15 ǀ 762.86 (111.3 - 5652.67)	0.083

*: Variables are expressed as mean ± std. deviation ǀ median (minimum-maximum).
**: Mann-Whitney U test.
The statistically significant difference is expressed in bold.
CSF, Cerebrospinal fluid; GBS, Guillain-Barré syndrome; mEGRIS, Modified Erasmus GBS Respiratory Insufficiency Score; MRC, Medical Research Council; MV, Mechanical ventilation; NLR, Neutrophil to lymphocyte ratio; NV, Normal ventilation; PLR, Platelet to lymphocyte ratio; SII, Systemic immune inflammation index.

Table V. Comparison of clinical characteristics between MV and NV groups with severe GBS.

Variables*	Categories	Severe GBS patients (n=27)		Total	Chi-square statistics	*p*
		Normal ventilation (n=14)	Mechanical ventilation (n=13)			
Gender	Girl	8[a] (57.1%)	6[a] (42.9%)	14 (100.0%)	0.034	0.853[2]
	Boy	6[a] (46.2%)	7[a] (53.8%)	13 (100.0%)		
Cranial Nerve Damage	No	6[a] (100.0%)	0[b] (0.0%)	6 (100.0%)	11.882	0.003[1]
	Facial paralysis	4[a] (80.0%)	1[a] (20.0%)	5 (100.0%)		
	Bulbar palsy	3[a] (23.1%)	10[b] (76.9%)	13 (100.0%)		
	Facial and bulbar palsy	1[a] (33.3%)	2[a] (66.7%)	3 (100.0%)		
Sensory Disorder	No	7[a] (87.5%)	1[b] (12.5%)	8 (100.0%)	-	0.033[3]
	Yes	7[a] (36.8%)	12[b] (63.2%)	19 (100.0%)		
Autonomous changes	No	11[a] (100.0%)	0[b] (0.0%)	11 (100.0%)	14.136	<0.001[2]
	Yes	3[a] (18.8%)	13[b] (81.3%)	16 (100.0%)		
Season at admission	Spring	2[a] (40.0%)	3[a] (60.0%)	5 (100.0%)	0.449	0.8801
	Autumn	4[a] (50.0%)	4[a] (50.0%)	8 (100.0%)		
	Summer	8[a] (57.1%)	6[a] (42.9%)	14 (100.0%)		
	Winter	0[a] (0.0%)	0[a] (0.0%)	0 (0.00%)		
Total		14 (52.0%)	13 (48.0%)	27 (100.0%)		

*: Variables are expressed as frequency (percent).
[1]: Pearson chi-square, [2]: Continuity correction [3]: Fisher's exact test.
Each superscript letter denotes a subset of GBS categories whose column proportions do not differ significantly from each other at the 0.05 level. The statistically significant difference is expressed in bold.
GBS, Guillain-Barré syndrome; MV, Mechanical ventilation; NV, Normal ventilation.

Table VI. Comparison of laboratory parameters and presentation of GBS between MV and NV groups with severe GBS.

Variables*	Severe GBS patients (n=27)		*p***
	Normal ventilation (n=14)	Mechanical ventilation (n=13)	
Age	87.43 ± 48.09 ǀ 76 (27 - 170)	86.08 ± 47.63 ǀ 96 (20 - 180)	0.884
MRC at admission	34.57 ± 3.18 ǀ 34 (30 - 42)	23.38 ± 4.72 ǀ 24 (16 - 28)	<0.001
mEGRIS	4.14 ± 0.86 ǀ 4 (3 - 5)	6.38 ± 0.51 ǀ 6 (6 - 7)	<0.001
CSF protein	102.9 ± 49.97 ǀ 81.6 (45.9 – 192.7)	92.81 ± 45.7 ǀ 92 (53.1 – 230.3)	0.771
Neutrophil	4.7 ± 1.61 ǀ 4.63 (2.4 – 7.9)	6.64 ± 3.16 ǀ 5.4 (1.79 – 12.2)	0.109
Lymphocyte	3.13 ± 1.45 ǀ 2.83 (1.6 – 6.1)	2.43 ± 1 ǀ 2.2 (0.9 – 4.2)	0.254
Platelet	331.57 ± 82.74 ǀ 342 (147 - 459)	356.08 ± 112.4 ǀ 338 (194 - 556)	0.698
NLR	1.72 ± 0.84 ǀ 1.52 (0.79 – 3.81)	3.72 ± 3.27 ǀ 2.17 (0.57 – 10.17)	0.037
PLR	121.11 ± 51.32 ǀ 97.38 (72.79 – 221.25)	182.63 ± 117.38 ǀ 169.09 (62.18 – 463.33)	0.159
SII	541.58 ± 246.79 ǀ 496.73 (268.71 – 1265.75)	1490.83 ± 1630.15 ǀ 762.86 (111.3 – 5652.67)	0.042

*: Variables are expressed as mean ± std. deviation ǀ median (minimum-maximum).
**: Mann-Whitney U test.
The statistically significant difference is expressed in bold.
CSF, Cerebrospinal fluid; GBS, Guillain-Barré syndrome; mEGRIS, modified Erasmus GBS Respiratory Insufficiency Score; MRC, Medical Research Council; NLR, Neutrophil to lymphocyte ratio; PLR, Platelet to lymphocyte ratio; SII, Systemic immune inflammation index.

features available at the time of admission, making clinical decisions regarding patient transfer to the ICU, and providing counseling for prognosis.

Early detection of patients with severe GBS may reduce residual sequelae and mortality. However, few studies have evaluated the severity of GBS.[27] In the current study, summer admission was among the factors predicting severe GBS. This finding may be associated with the increase in gastrointestinal infections in spring and summer.[1] Additionally, AMSAN variant, cranial nerve involvement, autonomic dysfunction, and the lowest total MRC scale score for muscle strength at admission were found to be associated with GBS severity. These results were consistent with previous studies.[3,19,27] In the current study, the time from symptom onset to hospital admission was not associated with disease severity. This may be attributed to differences between studies on whether it is the time from the onset of symptoms to hospitalization or the time of peak functional neurological deficit, and the difficulties in determining the exact time of peak functional neurological deficit in children with GBS.[4,23,24]

In the current study, 20.6% (13) of the patients required MV. Our MV rate was consistent with previous studies (20–30%).[2,3] The potential predictors of MV requirement in patients with severe GBS were found to be autonomic dysfunction, presence of bulbar palsy, lowest total MRC scale for muscle strength score at admission, and high mEGRIS scores.[3,9,10,16,27,30-32] It is of great importance to predict the need for MV early, as 60% of these patients may experience many complications that increase the risk of mortality, therefore early recognition and intervention can improve the prognosis.[33,34] It has been reported that severe muscle weakness (MRC <20) is more likely to progress to MV.[32] It has been shown that the NSB score model (Neck muscle weakness, Single breath count, Bulbar palsy) developed in patients with GBS can accurately predict MV requirement.[8] Single breath count (SBC) < 20 (inability to count 1 to 20 out loud in a single breath) is a useful bedside tool that can assess the need for MV, but may be an indicator rather than a predictor.[3] The current study showed that high NLR and SII values may be potential predictors of MV requirement in children with severe GBS. Inflammatory markers may reflect an underlying proinflammatory state and immunological dysfunction in patients with GBS.[35] A relative decrease in adaptive immunity, reflected by an elevated NLR value, may lead to dysregulated proinflammatory responses that contribute to the development of GBS.[36] High NLR may be useful in the evaluation of diagnosis, prognosis and treatment response in patients with GBS and may also predict the need for MV.[36] SII can predict disease severity and short-term prognosis, and is even more valuable than NLR in predicting the need for MV.[37]

In the current study, 4 patients (6.3%) died. A large series of 527 adult patients with GBS reported a mortality rate of 2.8%.[38] Mortality rates reported in the pediatric age group vary between 6.5-12.7%.[19,39,40] Two of the patients who died were the youngest patients in our cohort. The presence of bulbar palsy, respiratory failure and autonomic dysfunction were common features of all of them. The time from onset of muscle weakness to admission was between 1 and 5 days, and the disease appeared to progress rapidly. Three patients required MV on the first day of admission. Duration of stay in the ICU was between 3-10 days. In the literature, the most frequently identified causes of death in GBS are respiratory failure, pneumonia, cardiovascular complications and autonomic dysfunction.[38,39] Risk factors for mortality have been reported to be associated with older age, more severe weakness at admission, need for ventilation and pre-existing comorbidity, and a longer delay between the onset of weakness and presentation.[38] The mortality rate in GBS can be reduced by more intensive management of respiratory failure and dysautonomia, early treatment of infections, and greater attention to patients with cardiovascular risk factors.[38]

GBS has a variable clinical course and outcome, but patients are treated with a standard approach. Patients with a poor prognosis may benefit from treatment as long as nerve degeneration can be detected early when it is potentially reversible and treatment is most effective. GBS guidelines recommend that the risk of poor prognosis be assessed at the early stage of the disease.[3] Predicting both short-term prognosis (likelihood of needing MV) and long-term prognosis (likelihood of being able to walk unaided after six months) is important for treatment goals and counseling. The predictors for poor prognosis in GBS (with a large amount of evidence) have been reported to be older age, prior history of gastroenteritis, higher GBS disability score at presentation, lower MRC total scores at admission, and reduced compound muscle action potential amplitude on NCS.[3] The current study found that a lower MRC scale score at admission may predict poor prognosis for the patient. A study conducted in Turkey has shown that the duration of weakness, length of hospital stay and need for ventilation may negatively affect prognosis.[41]

The research has some limitations. First, the single-center retrospective design of the study allowed only a limited number of clinical features to be analyzed. Therefore, data such as functional vital capacity, which are quantitative indicators of the likelihood of need for ventilation, could not be collected. Second, the relatively small number of our participants (given the rarity of GBS in children) limited our ability to identify underlying risk factors for GBS (such as associated sources of infection, electrophysiological subtypes, or autoantibodies) as prognostic predictors.

In conclusion, the potential predictors of MV requirement in severe GBS patients were found to be bulbar palsy, dysautonomia, sensory impairment, lowest total MRC scale for muscle strength score at admission, high mEGRIS score, high NLR and high SII values. The lowest total MRC scale for muscle strength score at admission was shown to be associated with poor prognosis. Multicenter prospective studies for early prediction of outcome in GBS are needed to develop clinical prognostic prediction models valid for clinical practice and future therapeutic trials.

Ethical approval

The study was approved by İnönü University Ethics Committee (date: 02.05.2023, number: 2023-4580).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: GY, AKA; data collection: GY; analysis and interpretation of results: GY, AKA, BÖ, SG; draft manuscript preparation: GY, AKA. 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

Comparison of ocular posterior segment parameters in the pediatric population with migraine without aura and tension-type headache

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ABSTRACT

Background. This study aims to compare the posterior ocular structure parameters in children with migraine without aura (MWA), tension-type headache (TTH), and a healthy control group.

Methods. The study included 31 patients with MWA, 29 patients with TTH, and 38 healthy controls between 6 and 18 years of age. For all participants, the detailed eye examination and measurements including peripapillary retinal nerve fiber layer (pRNFL) thickness, central macular thickness (CMT), subfoveal choroidal thickness (SCT), macular vessel densities and foveal avascular zone (FAZ) parameters measured by optical coherence tomography (OCT) and OCT-angiography (OCTA), were obtained from the patient files.

Results. The mean age was 12.1±3.3 years in MWA patients, 12.4±2.8 years in TTH patients, and 11.9±3.8 years in the healthy controls (p=0.844). Among the groups, the mean pRNFL thickness, CMT, and SCT values were lowest in the MWA group. However, this difference was not statistically significant (p=0.621, p=0.854 and p=0.201, respectively). The mean and four-quadrant (superior, inferior, temporal, nasal) pRNFL thicknesses, the CMT, and the SCT were not statistically significant between the groups (p=0.621, p=0.500, p=0.186, p=0.565, p=0.744, p=0.854 and p=0.201, respectively). The macular vascular densities were lower in MWA patients than in the other two groups, and there was a statistically significant difference between the groups only in the nasal quadrant of the deep retinal capillary plexus (p = 0.014). There were also no statistically significant differences between the groups in the superficial and deep FAZ area parameters (p=0.652 and p=0.985).

Conclusion. This study suggested that differential diagnosis between MWA and TTH can be difficult in childhood, as these conditions, which can present with ocular symptoms, may also be characterized by changes in posterior segment parameters. Long-term studies incorporating OCT-A in larger patient populations may provide valuable insights into retinal changes associated with these two distinct headache spectrums.

Key words: pediatric migraine, pediatric tension-type headache, optical coherence tomography-angiography, macular vessel density, foveal avascular zone.

Headache is a common symptom, affecting nearly 52.0% of the population.[1] Headache is also common in the pediatric age group, with studies showing that various types of headaches can develop in 57-82% of children up to 15 years of age.[2] The average age of headache onset is 7.5 years.[3,4] The failure to diagnose and manage headache has a negative impact on the quality of life in childhood. The disability caused by chronic headaches in childhood has

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been shown to be similar to the loss in quality of life caused by cancer and arthritis.[5]

The most common types of primary headaches in the pediatric age group are migraine and tension-type headaches (TTH). The differential diagnosis depends on clinical symptoms, according to the International Classification of Headache Disorders, third edition (ICHD-III) of the International Headache Society (IHS).[6] The prevalence of migraine is 20 % in the 15-25 age group, however, the prevalance of TTH in young subjects varies between 0.9-72.3%.[7,8] It can be difficult to distinguish between these two spectra in children. Two theories explain the development of migraine: vascular and neurogenic. According to the vascular theory, migraine is triggered by activation of the trigeminal vascular system (TGVS), which maintains vascular tone and innervates blood vessels in the brainstem, extracranial tissues, and eyes.[9] The pathophysiology of TTH remains insufficiently understood. One theory suggests increased sensitivity of pain pathways in the central and peripheral nervous systems, and another hypothesis involves hyperactivation of myofascial nociceptors.[10,11]

Some ocular symptoms, such as photophobia, visual field loss, and periocular pain may develop in migraine and TTH patients.[12-14] However, the reason for these ocular conditions is not known. Some studies have reported changes in neuronal and vascular structures in the posterior segment on optic coherence tomography (OCT) and OCT-angiography (OCTA) imaging in migraine patients, and these studies demonstrated that migraine may cause impairment of retinal microcirculation, retinal nerve fiber layer (RNFL) thinning and ganglion cell loss in the retina.[15-17] However, there have been no studies of TTH using OCTA.

It is known that the cause of the ocular symptoms seen in both migraine without aura (MWA) and TTH cannot be fully explained. In addition, the difficulty of clinically differentiating these two types of primary headaches in children, increases the value of objective diagnostic tools. Therefore, this study aimed to comprehensively evaluate the posterior segment parameters concerning neural and vascular changes in MWA and TTH.

Materials and Methods

This retrospective study was conducted by the Pediatric Neurology and Ophthalmology Departments of Dr. Lütfi Kırdar Kartal City Hospital between May and June 2022. The study was approved by the Ethics Committee of Dr. Lütfi Kırdar Kartal City Hospital and conducted according to the tenets of the Declaration of Helsinki (Protocol Number: 2022/514/224/11). Written informed consent was not obtained from participants due to the retrospective nature of this study.

Thirty-one patients diagnosed with MWA and 29 patients diagnosed with TTH according to ICHD-III edition of the IHS, were included in the study, and 38 age- and gender-matched patients presenting to the Ophthalmology Department with minor symptoms such as refractive error were included as the healthy control group. The patients in this study consisted of those who were followed for at least 6 months due to headache before the differential diagnosis. The clinical and demographic data were collected from the medical records of all patients, including age, sex, diagnosis, age of onset, pain characteristics (including triggers and alleviating factors), associated symptoms (e.g., headache prodromes or a history of bruxism), disease duration following differential diagnosis, attack frequency and duration, treatments used, family history, and neurological and general physical examinations. Detailed eye examinations were also obtained for all patients including best corrected visual acuity (BCVA) with Snellen charts, slit-lamp biomicroscopy, fundus examination with a 78-diopter lens, and intraocular pressure (IOP) with Goldman applanation tonometry. For all participants, only the measurements of the right eye were examined due to the similarity of right and left eye analyses. In addition, all data such

as examinations and measurements were taken during the period when the patients were free of attacks.

Patients with previous ocular diseases (such as corneal and lens diseases, uveitis, and retinal diseases), a history of intraocular surgery, ocular or head trauma, ocular hypertension or glaucoma, drug or radiation treatment, and any systemic and neurological diseases except migraine and TTH were excluded from the study. Patients with BCVA ±4 D were also excluded from the study. In the healthy controls, patients were excluded if they had been diagnosed with any type of headache or reported having headaches for any reason in the previous six months. Patients were also excluded if they were under the age of four or over the age of eighteen, or if they were unable to cooperate with the measurements.

OCT images of all patients were obtained using a swept-source OCT device (DRI OCT Triton plus-Topcon, Japan) with a light source of 1050 nm wavelength and a scanning speed of 100,000 axial per second. Macular and optic nerve head parameters were assessed in a single scan with deep penetration wide-angle (12x9 mm) imaging at the posterior pole in OCT images (Fig. 1a, b). The central subfoveal choroidal thickness (SCT) was obtained by drawing a perpendicular line between the posterior edge of the retinal pigment epithelium and the choroid-sclera junction in the fovea region, using cross-sectional OCT-B scans by the same experienced technician. All SCT measurements were performed at the same time of the day (from 09.00 to 12.00 in the morning) to avoid daily variations.

OCTA images were measured by scanning a 3x3 mm^2 area on the fovea of the patients. The "en-face images" were taken from the superficial capillary plexus (SCP) and deep capillary plexus (DCP). The SCP is located from 2.6 μm below the internal limiting membrane (ILM) to 15.6 μm below the inner plexiform layer (IPL) while DCP is located between 15.6 μm and 70.2 μm below IPL. The quantitative analysis, including FAZ parameters and capillary density, was performed using the

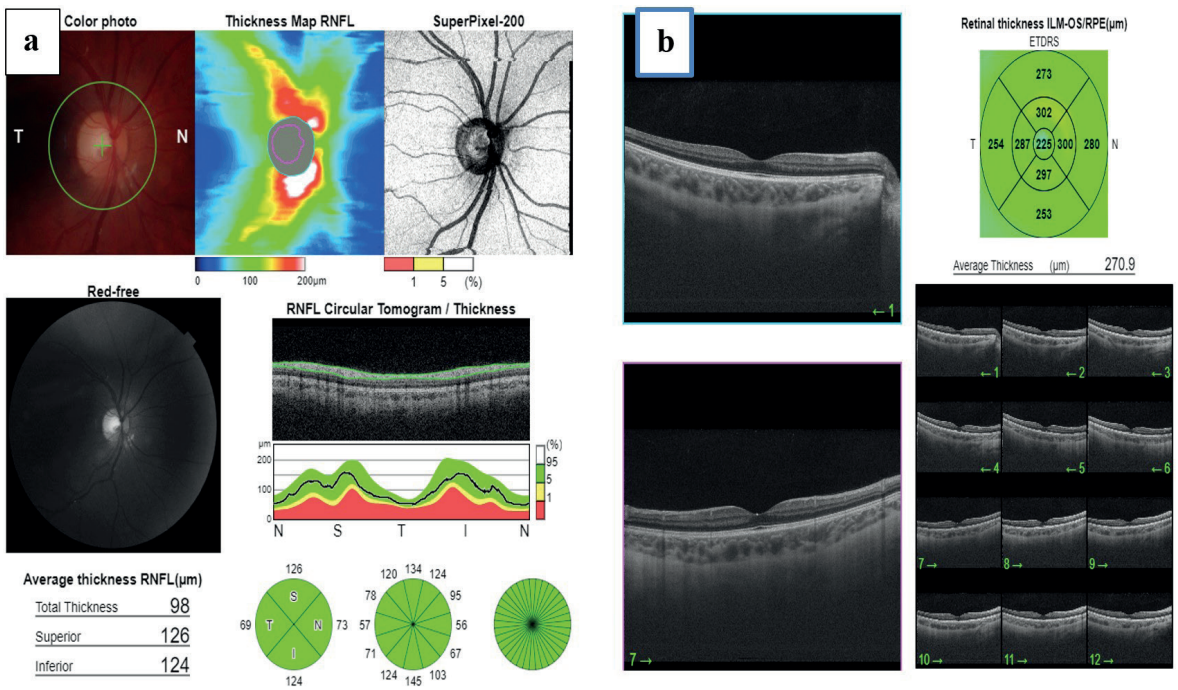

Fig. 1a. Optical coherence tomography images of the optic disc, **1b.** Optical coherence tomography images of the macula.

IMAGEnet program developed by Topcon (Tokyo, Japan). In addition, the FAZ area was measured manually (by the same experienced technician) in all study participants. The device automatically measured SCP and DCP, and the vessel density was determined as the percentage of the area occupied by the vessels. The device focused on two concentric circles with radii of 1 and 3 mm in the center of the fovea, dividing the area between them into four sections: superior, inferior, temporal, and nasal. The device also calculated the capillary densities in the superficial and deep plexus in 5 regions (Fig. 2).

Statistical analysis

The continuous variables were presented as mean+/-standard deviation or median (interquartile range) based on their distribution characteristics. The normality and homogeneity assumptions were assessed using the Shapiro-Wilk and Levene tests, respectively. The one-way ANOVA test was used to compare normally distributed and homogeneous data and the Kruskal-Wallis test was used to compare non-normally distributed continuous data. The Student's t-test was used to evaluate two groups with normally distributed continuous variables, the Mann-Whitney U test for non-normally distributed continuous variables, and the chi-square test for categorical variables. The Jamovi 2.2.5 statistical package program was used for statistical tests and $p<0.05$ was considered statistically significant.

Results

The study included 31 patients with MWA, 29 patients with TTH, and 38 healthy controls. The mean age was 12.1±3.3 years in MWA patients, 12.4±2.8 years in TTH, and 11.9±3.8 years in the healthy controls (p=0.844). 54.8% (n=17) of the patients in the MWA group, 72.4% (n=21) in the TTH group, and 60.5% (n=23) in the healthy controls were female (p=0.359). There was no statistically significant difference between the groups for age and sex. Also, there were no statistically significant differences between disease duration after differential diagnosis, age of onset, and frequency of attacks. The demographic and clinical characteristics of the groups are shown in Table I.

Biomicroscopic and dilated fundus examinations revealed no pathology in any of

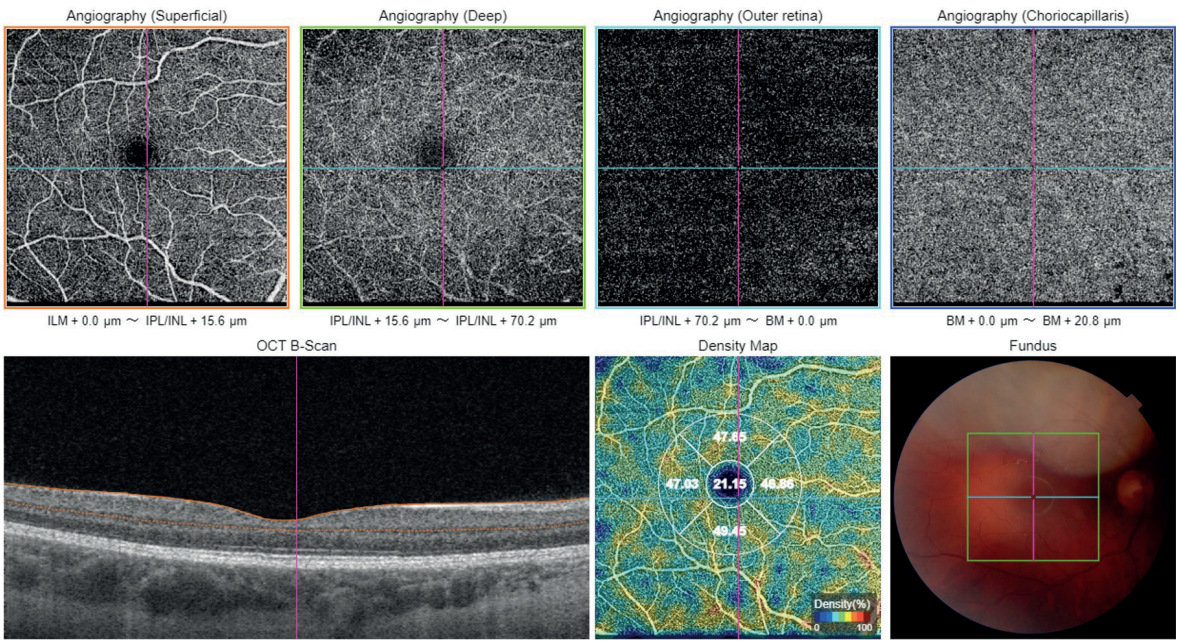

Fig. 2. Optical coherence tomography angiography images of the macula.

Table I. Clinical and demographic features of the groups.

	MWA	TTH	Healthy Controls	p
Age (years)	12.1 ± 3.3	12.4 ± 2.8	11.9 ± 3.8	0.844[K]
Gender (Female/Male)	17/14	21/8	23/15	0.359[C]
Refraction Error, SE, D	-0.82 ± 1.22	-1.41 ± 4.67	-1.26 ± 1.67	0.437[K]
Family history	61.3% (n=19)	37.9% (n=11)	-	0.071[C]
Disease duration (months)	19.2 ± 21.8	18.4 ± 10.5	-	0.336[U]
Attack frequency (per month) (range)	4.0 (1.5-6.0)	4.0 (2.0-6.5)	-	0.596[U]
Age of onset (months)	127.0 ± 40.0	134.0 ± 37.2	-	0.519[T]

C: Chi-square test, D: Diopter, K: Kruskal-Wallis test, MWA: Migraine without aura, SE: Spherical equivalent, TTH: Tension-type headache, T: student's t-test, U: Mann-Whitney u test.

the participants. Among the groups, the mean pRNFL thickness, central macular thickness (CMT), and SCT values were lowest in the MWA group. However, mean pRNFL thickness and the four-quadrant (superior, inferior, temporal, nasal) RNFL thicknesses were not statistically significant between groups (p=0.621, p=0.500, p=0.186, p=0.565, and p=0.744, respectively). The CMT and four-quadrant (superior, inferior, temporal, nasal) macular thicknesses were not statistically significant between MWA patients, TTH patients, and healthy controls (p=0.854, p=0.230, p=0.174, p=0.434, and p=0.333, respectively). SCT was also not statistically significant between groups (p=0.201). All parameters are listed in Table II.

According to macular vessel density parameters, there was no statistically significant difference between the groups in SCP and DCP measurements, except for the nasal quadrant of the DCP, although the vessel densities in both SCP and DCP were lower in MWA patients than in TTH patients and healthy controls (Table III). There was a statistically significant difference in the nasal quadrant of the DCP between the MWA patients, TTH patients, and the healthy controls (46.2±5.9, 48.8±3.2, and 49.5±3.1, respectively, p=0,014). It was observed that this difference was statistically significantly lower in the MWA patients than in the healthy controls. In addition, both the superficial and deep FAZ areas were larger in MWA patients than in both TTH patients and the healthy controls. However, there were no statistically significant differences between the superficial and deep FAZ area parameters between the groups (p=0.652 and p=0.985) (Table III).

Table II. Comparison of peripapillary retinal nerve fiber layer thickness, macular thickness, and subfoveal choroidal thickness values between the groups.

	MWA	TTH	Healthy Controls	p
Average RNFL (μm)	107 ± 8.3	109 ± 9.0	109 ± 7.1	0.621[A]
Superior quadrant RNFL (μm)	132.0 ± 11.7	135.0 +/- 11.5	134.0 ± 13.9	0.500[A]
Inferior quadrant RNFL (μm)	133.0 ± 13.5	141.0 ± 20.4	137.0 ± 12.9	0.186[A]
Temporal quadrant RNFL (μm)	78.4 ± 12.7	76.2 ± 9.0	79.6 ± 9.8	0.565[K]
Nasal quadrant RNFL (μm)	85.4 ± 12.2	83.3 ± 8.8	85.1 ± 12.2	0.744[A]
Central MT (μm)	236 ± 18.7	239 ± 23.8	238 ± 16.5	0.854[A]
Superior quadrant MT (μm)	310 ± 13.5	311 ± 19.0	317 ± 13.1	0.230[K]
Inferior quadrant MT (μm)	305 ± 12.8	304 ± 25.3	313 ± 14.1	0.174[K]
Temporal quadrant MT (μm)	294 ± 15.0	293 ± 38.2	295 ± 28.0	0.434[K]
Nasal quadrant MT (μm)	309 ± 18.6	313 ± 14.8	314 ± 16.7	0.333[K]
SCT (μm)	360 ± 72.3	395 ± 74.2	370 ± 80.2	0.201[A]

A: ANOVA test, K: Kruskal-Wallis test, MWA: Migraine without aura, MT: Macular thickness, RNFL: Retinal nerve fiber layer, SCT: Subfoveal choroidal thickness, TTH: Tension-type headache.

Table III. Comparison of macular vessel density of both superficial and deep plexus and foveal avascular zone area among the groups.

		MWA (n=31)	TTH (n=29)	Healthy Controls (n=38)	p
CVD superficial (%)	Parafoveal	20.3 ± 3.7	22.0 ± 4.1	20.6 ± 4.0	0.200
	Superior	45.2 ± 4.2	45.4 ± 4.3	47.3 ± 2.9	0.051
	Inferior	42.4 ± 4.6	43.1 ± 5.2	44.1 ± 4.2	0.252
	Temporal	45.6 ± 3.5	46.4 ± 3.5	46.6 ± 2.8	0.408
	Nasal	44.2 ± 4.0	45.3 ± 2.8	45.7 ± 2.9	0.169
CVD deep (%)	Parafoveal	18.3 ± 3.6	19.9 ± 3.9	19.4 ± 4.5	0.297
	Superior	48.7 ± 5.4	49.4 ± 4.5	51.1 ± 3.3	0.095
	Inferior	45.0 ± 5.8	46.4 ± 5.1	46.8 ± 4.6	0.346
	Temporal	47.8 ± 4.96	49.4 ± 4.0	50.0 ± 3.2	0.085
	Nasal	46.2 ± 5.9S	48.8 ± 3.2	49.5 ± 3.1S	0.014*
FAZ μm^2	Superficial	303.7 ± 97.1	281.8 ± 101.9	291.2 ± 78.9	0.652
	Deep	333.5 ± 83.7	333.2 ± 116.9	329.6 ± 105.0	0.985

*ANOVA test, CVD: Capillary vessel density, FAZ: foveal avascular zone, MWA: Migraine without aura, TTH: Tension-type headache.

Discussion

This study found a statistically significant difference in the nasal quadrants of the DCP between groups in the pediatric population, with MWA patients showing lower values compared to healthy controls. In addition, both the SCP and DCP parameters were lower in MWA patients than in both TTH patients and the healthy controls, but these changes were not statistically significant. The superficial and deep FAZ areas were larger in MWA patients, but not statistically significantly different between groups.

The symptoms of MWA and TTH frequently overlap in childhood. The diagnosis may also change over the years due to the development of complications and changing symptoms of primary headaches.[18,19] Kienbacher et al.[20] followed 227 patients with headache complaints in the pediatric and adolescent group for 6.6±1.6 years from the date of diagnosis. They showed that the headache complaint was resolved in 30% of the patients. In addition, the diagnosis changed in 20-25% of patients, from migraine to TTH or from TTH to migraine. The very similar epidemiological, clinical, and pharmacological features between migraine and TTH, make the differential diagnosis in children challenging. Some studies have aimed to evaluate the differences between migraine and TTH using quantitative sensory tests, brainstem excitability, laser-evoked potentials, temporal discrimination thresholds, and magnetic resonance imaging.[21-25]

There have been a few studies in the literature investigating the effect of migraine on retinal structures.[15,26,27] Kanar et al.[28] reported a statistically significant thinning in the mean pRNFL, superior and inferior pRNFL in adult patients with migraine compared to controls, only nasal quadrant of pRNFL was significantly thinner in patients with MWA than other groups. They proposed that these changes are associated with dysregulation of ocular blood flow, resulting from impaired autoregulation in individuals with migraine. Cankaya and Tecellioglu[29] reported that the retinal thickness of the fovea was thinner in adult patients in the migraine group compared with healthy controls, and they suggested that this was due to decreased blood flow in adult patients with migraine. Iyigundogdu et al.[15] showed no statistically significant difference in pRNFL and ganglion cell layer thickness in adult migraine patients compared to control

subjects. They attributed the difference between their results and other studies to differences in measurement techniques and participant characteristics. Nalcacioglu et al.[30] found no statistically significant difference between the migraine patient group and healthy controls in the pediatric group for pRNFL and macular thickness in the attack-free period. They related this situation to age-related changes in adult migraine patients, and the insufficiency of retinal and choroidal circulation due to the chronic nature of the disease in contrast to children. Rego-Lorca et al.[31] reported statistically significant reductions in RNFL thickness in the temporal and inferior-temporal in children with migraine with aura compared to patients with MWA. In addition, they detected negative correlations between the number of episodes per month and RNFL thickness.[31] On the other hand, there are few studies in the literature comparing adults with migraine and TTH. In one of them, Yener and Korucu[13] evaluated the visual field defects in adult patients with migraine and TTH and showed that adult patients with TTH have similar visual field defects as patients with migraine. The retinal changes such as pRNFL, macular thickness, and SCT were compared between adult patients with TTH and migraine in the study by Yener and Korucu, and they found no statistically significant difference.[32] In our study, no difference in mean pRNFL, four-quadrant RNFL, CMT, and four-quadrant macular thickness was observed between the groups during the attack-free period. We attributed this to the fact that the disease did not become chronic in children, considering the duration and number of attacks.

The choroidal thickness in patients with migraine has been evaluated in a few studies.[33,34] In the meta-analysis by Gouravani and colleagues, SCT was found to be reduced in individuals with migraine, particularly in those with aura compared to those without. This finding has been linked to the pathophysiology of migraine-related neovascularization.[33] Unlu et al.[34] demonstrated that SCT was thinner in adult patients with ≥5 migraine attacks per month than in healthy controls during the attack-free period and that the SCT was similar between migraine patients with ≤2 migraine attacks per month and the control group. They suggested that increasing disease frequency may contribute to decreased choroidal thickness through choroidal attenuation and vascular dysregulation. Nalcacioglu et al.[30] reported no statistically significant difference between the migraine patient group and healthy controls in the pediatric group for SCT in the attack-free period. Similarly, in this study, we observed that there was no statistically significant difference between the groups during the attack-free period. We attributed this to the fact that our study group was children and it was not yet a chronic process in terms of both MWA and TTH. Also, all measurements were taken during the attack-free period.

Additionally, some studies have demonstrated that migraine affects the vascular structures of the retina and optic nerve head. Kara et al.[35] reported an arterial resistance in the central retinal and posterior ciliary arteries using color-Doppler sonography in migraine patients compared with healthy controls. One study also implicated migraine as a risk factor for retinal vascular occlusion, ischemic optic neuropathy, and normotensive glaucoma.[36] Hamamci et al.[37] reported that in the macular OCTA measurements, SCP and DCP were significantly decreased, and FAZ area increased in the migraine with aura group compared to the control group in adult patients. There was also no statistically significant difference between the MWA group and the healthy control group in these measurements. Karahan et al.[26] also reported decreased macular DCP and increased deep FAZ area in patients with migraine with aura, which they attributed to the relationship between migraine pathophysiology and ischemia. Dereli et al.[38] reported no statistically significant changes in the macular and peripapillary microvascular densities measured by OCTA between the migraine patient and

healthy subjects during the nonattack period. They also showed that there was a negative correlation between the pediatric migraine disability assessment rating and optic disc OCTA parameters, and stated that there may be changes in the retina and optic disc vascular structures depending on the frequency, severity, and duration of attacks. Kurtul et al.[39] observed significantly lower values of all quadrants of SCP in pediatric MWA patients during the attack-free period compared with healthy controls, and no differences in FAZ area between MWA patients and healthy controls. They hypothesized that the low vascular density of the SCP is associated with hypoperfusion and ischemia. Our study observed a statistically significant reduction in the nasal quadrants of the DCP among groups in the pediatric population, with MWA patients showing particularly lower values compared to healthy controls. Furthermore, the MWA patients had lower mean SCP and DCP parameters than the TTH patients and the healthy controls, although these differences were not statistically significant. The superficial and deep FAZ areas were larger in MWA patients, but there was no statistically significant difference between the groups. We attributed this to the fact that the pathophysiology of MWA is different from that of TTH. In addition, vascular changes in MWA may originate from the nasal region.

The limitations of our study include its retrospective design, the lack of long-term outcome data for the patients, and the fact that the results cannot be generalized to all migraine patients, as the patients were selected from the MWA group during the attack-free period. Additionally, the inclusion of patients without visual symptoms and the inability to clearly specify the type of headache in the family history represent other limitations of the study. Although TTH can be classified into subtypes based on the ICHD-III edition of the IHS for adult patients, the challenges in applying these criteria to the pediatric population hindered the classification of TTH patients into subtypes, which constitutes a limitation of our study.

In summary, there was a statistically significant difference between the groups in terms of the DCP nasal quadrant, especially in patients with MWA, which was found to be lower than in healthy children. To the best of our knowledge, this is the first study to evaluate these parameters in pediatric patients with MWA, TTH, and healthy controls. We conclude that long-term follow-up studies in large patient populations using OCTA, a non-invasive, reproducible, and reliable imaging technique, may be extremely useful in understanding the pathogenesis of MWA and TTH. Additionally, OCTA could offer a unique advantage in providing objective, personalized measurements, which may be especially beneficial for pediatric patients who struggle to fully articulate their symptoms. This imaging technique could thereby enhance diagnostic accuracy and facilitate earlier identification, moving beyond reliance on subjective symptomatology. Furthermore, the findings of this study may contribute to a novel understanding of the differences between these two headache spectra, providing potential implications for future research and clinical practice.

Ethical approval

The study was approved by the Ethical Committee of İstanbul Kartal Dr. Lütfi Kırdar City Hospital (Protocol number: 2022/514/224/11).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: UK, MTK; data collection: UK, MTK, İK, İK; analysis and interpretation of results: İK; draft manuscript preparation: UK, MTK, İK. 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

Pneumatosis intestinalis: Does it always indicate necrotizing enterocolitis?

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ABSTRACT

Background. Pneumatosis intestinalis (PI) is a rare radiological finding that may be associated with various diseases. In the neonatal period, it is considered pathognomonic for necrotizing enterocolitis (NEC). Cow's milk protein allergy (CMA) is the main cause of allergy especially in term infants appearing following breastfeeding or consumption of milk-based formulas.

Case Report. We report three neonates presenting with PI and diagnosed with CMA and/or NEC. Case 1 was a 44-day-old preterm infant admitted to the hospital for nutritional deficiency and jaundice, who later developed PI and a NEC-like appearance (NEC-LA). Case 2 was born at 28 weeks' gestation and developed PI and NEC-LA five times. Case 3 was a 24-day-old term neonate who was admitted to the hospital due to acute gastroenteritis and developed PI and NEC-LA. Only case three required a surgical intervention. After feeding the infants an amino acid-based formula, clinical manifestations improved quickly, and the disease did not relapse. In our opinion, CMA was the correct diagnosis for cases 1 and 3. However, case 2 developed two NEC episodes and three NEC-LA episodes, which were thought to be related to CMA.

Conclusions. In addition to NEC, CMA should be considered in every PI, and recurrent NEC feeding should begin in accordance with a CMA management protocol.

Key words: cow's milk protein allergy, necrotizing enterocolitis, neonate, pneumatosis intestinalis.

Pneumatosis intestinalis (PI), is a rare form of air leak into the gastric and intestinal wall and often is used as the radiological finding in preterm infants to diagnose necrotizing enterocolitis (NEC).[1-3] The pathogenesis of PI is still unknown. It may occur due to inflammation, infection, ischemia, trauma, or autoimmune processes.[2] Although the etiology and clinical course are various, physicians tend to treat all PI similarly. Bowel rest, broad-spectrum antibiotics, and hemodynamic support are the basis of the treatment.[4,5] If PI is not related to NEC, enteral nutrition can be initiated early, and a prolonged course of broad-spectrum antibiotics is not recommended.[1]

The most common food allergy of infancy is cow's milk protein allergy (CMA) which is an immune-mediated hypersensitivity response to some proteins in cow's milk.[6] The immunological response can be IgE-mediated, non-IgE-mediated, or mixed and the clinical presentation depends on the type of the immunological response including cutaneous, gastrointestinal, respiratory, and systemic symptoms.[6,7] The diagnosis is based on history and physical

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examination. In exclusively breastfed infants, the mothers should be put on a cow's milk-free diet. In non-exclusively breastfed infants, a formula with reduced allergenicity for cow milk protein is recommended.[7]

Herein, we report three neonates presenting with PI with different etiologies mimicking NEC. Informed consent were obtained from the families.

Case presentations

Patient 1

A 44-day-old male baby was referred to our hospital due to nutritional deficiency and jaundice. His gestational age (GA) and birth weight (BW) were 32 weeks and 1810 g, respectively. According to the medical reports of the previous hospital, he was hospitalized in the neonatal intensive care unit (NICU) for 35 days where he received total parenteral nutrition (TPN) for three weeks as he could not tolerate breast milk or formula and was diagnosed with sepsis caused by *Serratia marcescens*. On presentation to our hospital, his weight was 2120 g (<3rd percentile), and height was 47.5 cm (10-50th percentile). Physical examination revealed umbilical hernia, and icterus. Laboratory data on admission showed conjugated hyperbilirubinemia. The infant was hospitalized in our pediatric ward for the regulation of nutrition and further investigations. Upon receiving preterm formula, he developed feeding intolerance, abdominal distention and emesis. On day of life (DOL) 55 and 56, abdominal X-ray (AXR) showed gas distension and PI (Fig. 1 A for DOL 55 and B for DOL 56). Abdominal ultrasound (AUS) demonstrated air in the intestinal wall and portal system. He was diagnosed with NEC and transferred to the NICU. Feeding was stopped, and intravenous antibiotics were initiated after the sepsis workup. C-reactive protein (CRP) was 49.9 mg/dL and fecal occult blood test was positive. He was electively intubated and placed on mechanical ventilation (MV) to reduce intraluminal pressure in the intestines and was put on gastric decompression, which relieved his abdominal distension, and the next day he was extubated. The complete blood count showed 28.9% eosinophilia and cow's milk specific IgE was positive (0.83 kU/L). Blood culture was negative. He received antibiotics for 14 days and enteral feeding was resumed with an amino acid-based formula (AABF) on DOL 70. After feeding with formula, his clinical condition remained well. The infant was discharged on AABF on DOL 84 with the diagnosis of NEC and CMA.

Patient 2

A male preterm baby with a BW of 765 g was born at 28 GA via Cesarean section due to preeclampsia. The baby was put on noninvasive MV, received prophylactic antibiotics and TPN plus trophic feeds were started with breast milk. In the first DOL the baby developed abdominal distention and an AXR was remarkable for gas distension in the bowel loops. Considering NEC, enteral feeding was discontinued. Abdominal distention regressed on DOL 5, the infant was extubated, and enteral feeding was started and increased gradually. On DOL 9, 31, 60 and 85 the infant developed vomiting and abdominal distention. Laboratory findings were within the normal range apart from elevated CRP. Fecal occult blood test was positive in every episode. AXR demonstrated PI (Fig. 1C for DOL 31-Fig. 1D for DOL 60-Fig. 1E for DOL 85). AUS confirmed NEC (Fig. 2). We additionally detected a left femoral fracture on DOL 68 (Fig. 1F) caused by metabolic bone disease of prematurity. In every episode, enteral feeding was discontinued, fluid and broad-spectrum antibiotic treatments were initiated after cultures were obtained and he was intubated to alleviate abdominal distention. Blood cultures revealed no growth, so antibiotics were given for 7-10 days, and we suspected the patient had CMA and began feeding him exclusively with AABF. The mother was put on a milk-free diet and after three days breastfeeding was resumed. However, due to the mother's non-

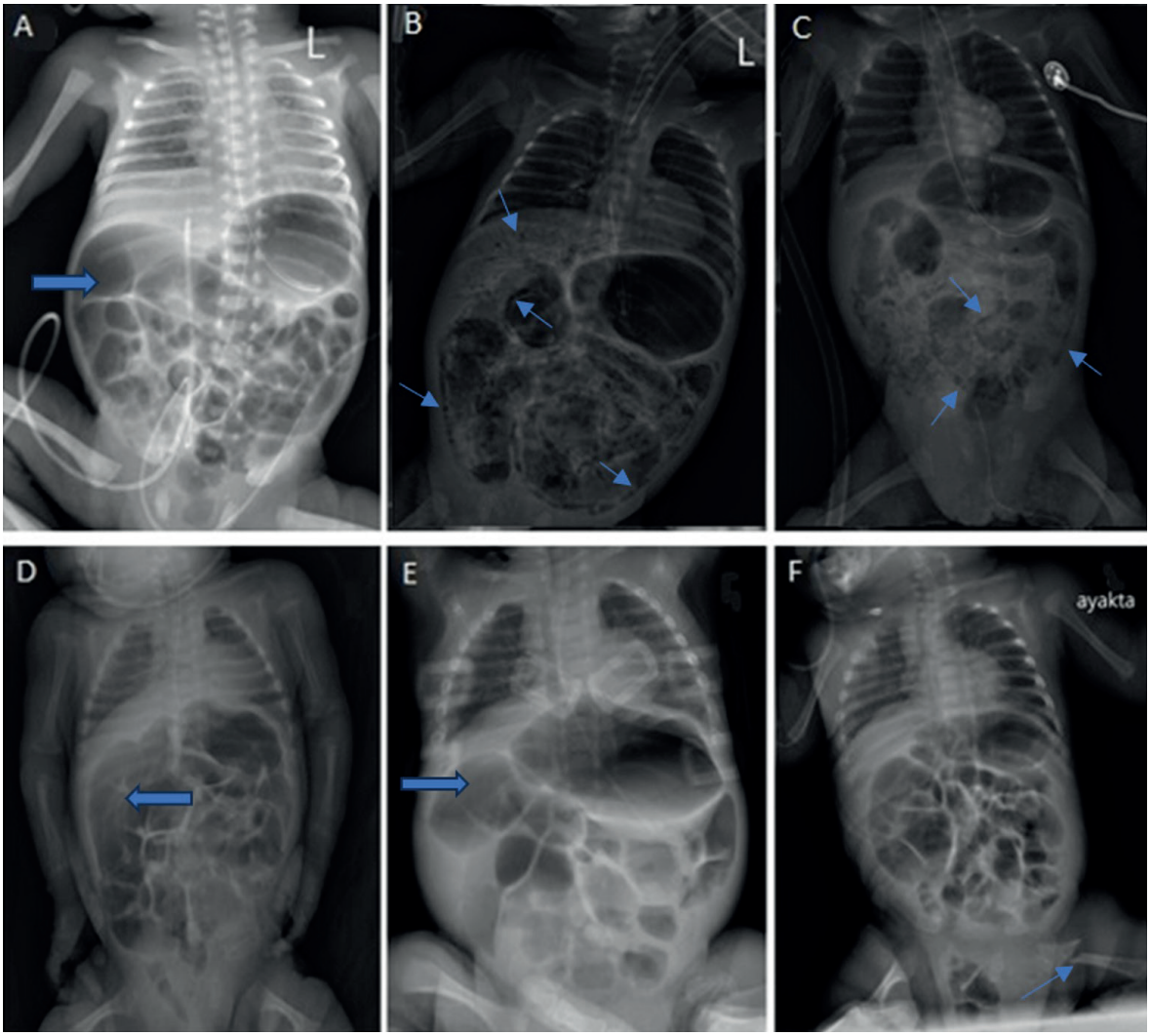

Fig. 1. A, B: Gas distension in bowel loops (thick arrow), pneumatosis intestinalis including the entire intestines and in the portal vein (thin arrows). C, D, E: Distension with gas was observed in the bowel loops (thick arrows). There were also linear and circular air lucencies in the bowel wall (thin arrows). F: Fracture in the left femur (thin arrow).

compliance with the diet, the baby experienced two additional episodes. We insisted that the mother follow a strict elimination diet. There were no further attacks after the fifth, but on DOL 152, an upper gastrointestinal tract endoscopy was performed due to a refusal of breastfeeding, which revealed esophagitis, esophagus, and gastric motility disorders. He was diagnosed with both NEC and CMA. On DOL 154, the infant was discharged on AABF and breast milk.

Patient 3

A 24-day-old baby boy was admitted to our NICU after two days of diarrhea with mucoid stools, vomiting, and fever. The baby, born at full term (39 weeks of gestation), was the first child of the 24-year-old mother and was delivered via Cesarean section. His parents reported that due to maternal lactation insufficiency, formula feeding began on DOL 10. His physical examination revealed mild

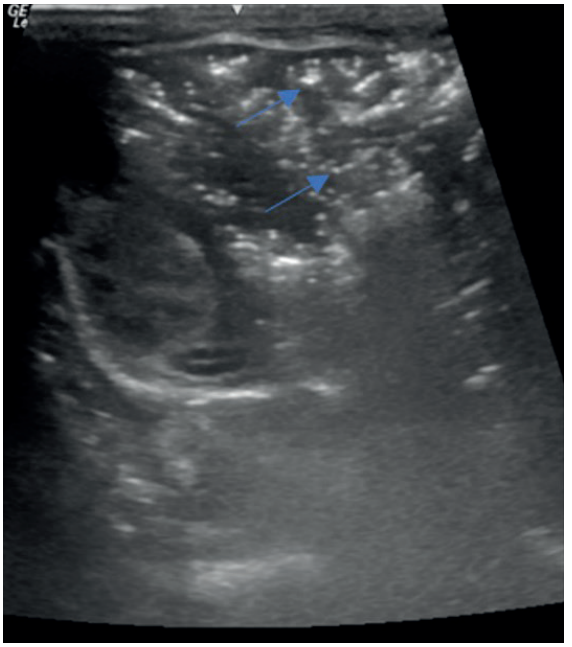

Fig. 2. Ultrasonography revealed intense air echoes in the intestinal wall and portal system (blue arrows).

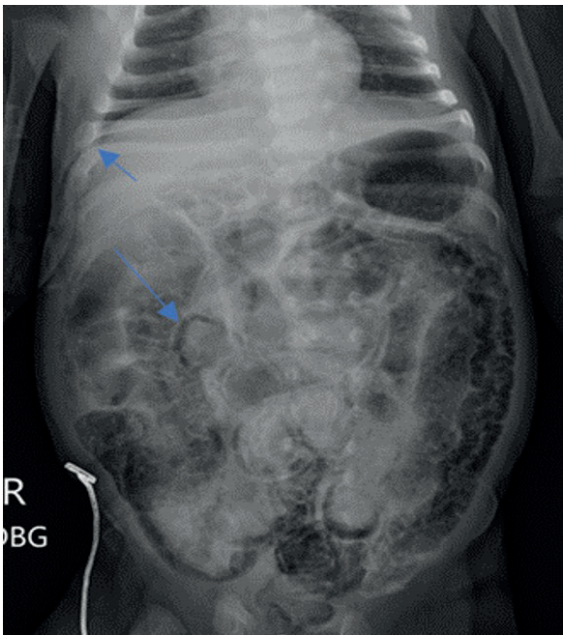

Fig. 3. Pneumatosis intestinalis (long arrow) and suspicious air lucency superposed on the lateral section of the liver (short arrow).

abdominal distention and cutis marmorata. Blood tests revealed elevated CRP levels (44 mg/dL), while stool tests for viral antigens and stool analysis were negative. Ampicillin sulbactam and gentamicin were initially given. Fluid therapy and enteral feeding were initiated. On DOL 27, the infant experienced severe abdominal distention and bloody stools. CRP was 187 mg/dL, and procalcitonin was above 100 ng/mL. AXR revealed suspicious air lucency superimposed on the lateral section of the liver and PI (Fig. 3), which was confirmed by AUS. Due to the possibility of perforation, exploration was performed, but no bowel perforation was found (Fig. 4). Enteral feeding was stopped, and TPN was initiated. The blood, peritoneal, and intraoperative abdominal free fluid cultures remained sterile, and antibiotic treatment was discontinued on DOL 37. The infant was diagnosed with CMA after suffering from acute gastroenteritis, and feeding was initiated with AABF and on DOL 45, the infant was discharged solely on AABF.

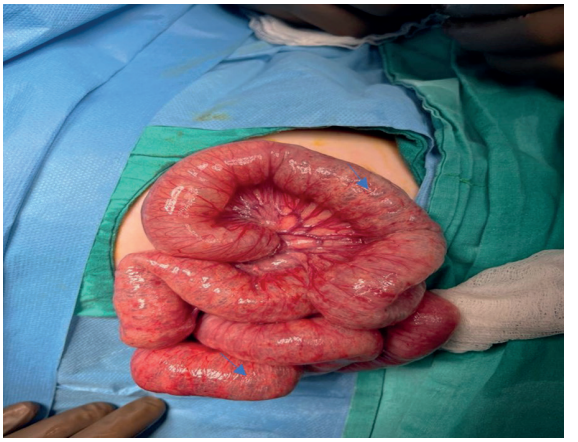

Fig. 4. During exploration, pneumatosis intestinalis (blue arrows) was observed throughout the entire small intestine, starting 75 cm distal to the Treitz ligament and extending to 5 cm from the cecum, involving all intestinal walls. The circulation, wall color and structure of the stomach appeared satisfactory. The liver and gallbladder were normal in appearance.

Discussion

In this paper, we described the clinical, laboratory, and radiological courses of three neonates with PI detected on AXR.[5,8] PI can be idiopathic or due to a benign condition (primary - 15%) or due to an unclear etiology or NEC (secondary - 85%).[8] It is often associated with NEC in the neonatal period. However, other rare conditions can also cause PI. Lenfestey et al.[9] reviewed 56 infants diagnosed with NEC. Of the 56 infants, five had an atypical course and were diagnosed with food protein-induced enterocolitis. Kalra et al.[10] reported a preterm infant who developed PI due to isolated ruptured appendicitis on DOL 13. Deutsch et al.[11] reported a 20-day-old neonate who was a victim of child abuse and presented with PI. A 30-day-old preterm infant presented with acute abdominal distension and PI, which was later diagnosed with meconium inspissation.[12] Capitanio et al.[13] reported PI in two infants with rotavirus gastroenteritis. Despite the wide range of conditions that can cause PI, it is critical to distinguish NEC from non-NEC causes because treatments differ. As suggested in the literature, upon detecting abdominal distension and PI, regardless of the cause, we stopped enteral feeding, intubated the babies to relieve abdominal distension, and initiated broad-spectrum antibiotics.[1]

NEC is a multifactorial acute inflammatory bowel necrosis affecting 10% of very low birth weight infants, resulting in death and morbidity.[3,14] As GA decreases, the onset of NEC occurs later.[15] Despite the nonspecific clinical, laboratory and radiographic findings, the mainstay of the diagnosis is AXR. AXR and AUS can show portal venous gas (PVG), PI, or pneumoperitoneum.[3,16] The initial management is supportive including bowel rest, hemodynamic support, and broad-spectrum antibiotics. In addition, to reduce gastrointestinal luminal pressure, elective intubation and ventilation, switching from noninvasive to invasive ventilation mode and gastric decompression are recommended.[1] In infants with bowel perforation, surgical resection of the necrotic bowel may be necessary.[2,17]

Cow's milk protein allergy is a benign condition that typically arises after the introduction of breast milk or milk-based formula. The exact mechanism is not clear. Most of the neonates diagnosed with CMA are term and it is mostly non-IgE-mediated. The infants may have cutaneous, gastrointestinal, respiratory, and systemic symptoms including atopic eczema, poor feeding, irritability, failure to thrive, abdominal pain, colic, vomiting, rectal bleeding and bloody or mucoid stools.[18] Infants with skin manifestations are more likely to have specific IgE positivity than infants with gastrointestinal manifestations.[7] The diagnosis of CMA and NEC in the early stage is a challenge due to similar radiologic findings including intestinal dilatation, PI and PVG. Guo et al.[19] reported that during the follow-up, intestinal motility outside the pathological area was different, and the duration of PVG was shorter in CMA, which could help distinguish CMA from NEC. To confirm the diagnosis of CMA, the CM protein must be eliminated for 1-4 weeks and then challenged or reintroduced orally.[7,18] Mothers who are exclusively breastfeeding should refrain from consuming milk and milk products while continuing to breastfeed. Non-breastfed infants should receive AABF.[6,7,18]

It is worth noting that, preterm CMA may mimic NEC and may be misdiagnosed as NEC. Furthermore, CMA was reported as a pre-existing condition that increases the risk of NEC or vice versa.[18] Despite the unknown association between NEC and CMA, it was postulated that after intestinal inflammation and mucosal damage plus atrophy, the infant becomes more vulnerable to CMA due to increased intestinal permeability.[20] As suggested in the literature, before making a definitive diagnosis, we primarily diagnosed our patients with NEC. Although case 1 was initially diagnosed as NEC, we changed the final diagnosis to CMA due to the late onset of symptoms, eosinophilia, and cow's milk specific IgE positivity. Case 2

developed five clinical episodes of enterocolitis, the first and second of which were caused by NEC, but the others were believed to be caused by CMA. Previous studies have shown that NEC causes intestinal damage and facilitates the development of CMA; additionally, CMA may predispose to the development of NEC.[18,20] Case 3 was exclusively fed CM protein-based formula, developed gastroenteritis on DOL 24, and was the only infant who required surgery for a suspected perforation. However, no surgical pathology was detected, and he was diagnosed with CMA. It was reported that PI may be associated with gastroenteritis, so we believed that the infant had CMA following gastroenteritis.[13]

In conclusion, the presence of PI on AXR is frequently regarded as a diagnostic marker for NEC. However, other conditions, such as CMA or gastroenteritis, may produce the same radiological findings. CMA is rare in neonates, and the findings may coincide with NEC. The correlation of history, physical examination, laboratory, and radiological findings is critical for accurate diagnosis and management. The significance of these three cases stems from the recognition that NEC and CMA can be developed concurrently, sequentially, or in similar ways. As a result, CMA should be considered in every PI, and if an infant develops recurrent NEC, feeding should begin in accordance with a CMA management protocol.

Ethical approval

Since this is a retrospective case report, we only obtained consent from the families. Written informed consent was obtained from parents of the patients.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: YC,TG,ÇA; data collection and literature review: YC, MAÖ; interpretation of results: YC, KK, draft manuscript preparation;MAÖ. Critically review of the manuscript: TG, ÇA. 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

Metoclopramide-induced rapid-onset psychosis in a child with methylphenidate use: a case report

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ABSTRACT

Background. Metoclopramide, a dopamine antagonist employed for its antiemetic effects, can precipitate neuropsychiatric adverse effects, including extrapyramidal symptoms and, in a few instances, acute psychosis. Although there have been reports of metoclopramide-induced psychosis in elderly individuals, there is no documentation of such incidents in children as far as we are aware.

Case presentation. This case report describes an 11-year-old girl with a history of mild intellectual disability and attention deficit hyperactivity disorder, managed with 10 mg of methylphenidate daily. She presented to the emergency department with acute gastrointestinal symptoms and was administered two tablets of metoclopramide alongside her regular dose of methylphenidate. Subsequently, she developed psychotic symptoms, disorganized behavior, and agitation. An extensive medical evaluation ruled out other organic pathologies, leading to a diagnosis of rapid-onset psychosis induced by metoclopramide. The psychotic episode, which lasted approximately two weeks, resolved with low-dose antipsychotic treatment.

Conclusions. Children, especially those with neurodevelopmental disorders, are more susceptible to a wide range of side effects. Therefore, this report highlights the necessity for careful pharmacological management. Additionally, this case represents a significant contribution to the scientific literature by being the first to document metoclopramide-induced acute psychosis in children to the best of our knowledge.

Key words: metoclopramide, acute psychosis, children, adverse drug reactions.

Children with comorbid conditions such as attention deficit hyperactivity disorder (ADHD) and mild intellectual disability often require multidrug regimens to manage their symptoms.[1] Methylphenidate, a central nervous system stimulant, is a common pharmacological treatment for ADHD[2], and is used effectively for both children with intellectual impairment and typically developing children.[2,3] Although the side effects of methylphenidate are typically transient, dose-dependent, and generally regarded as minor from a clinical standpoint[4], there is a risk of developing psychosis, albeit rarely.[5]

Metoclopramide is a dopamine receptor (D2) antagonist frequently prescribed for its antiemetic properties.[6] Although effective, metoclopramide is associated with a range of neuropsychiatric side effects, including extrapyramidal symptoms (EPS)[7,8], mood dysregulation, increased anxiety[9,10], and, in rare cases, psychotic reactions.[11]

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Methylphenidate functions by inhibiting the dopamine transporter, consequently increase the extracellular levels of dopamine.[12,13] In contrast, antipsychotic agents (including dopamine receptor blockers such as metoclopramide) exert an opposing influence on the dopaminergic system. This divergence in their mechanisms of action can lead to potential pharmacodynamic antagonism.[14] The stimulant properties of methylphenidate may counterbalance[15] or interfere with the effects of dopamine antagonists, potentially exacerbating psychiatric symptoms or precipitating new ones in certain individuals.[16]

Acute-onset psychosis, also known as supersensitivity psychosis, is a condition caused by the sensitization of dopamine receptors due to prolonged blockade by long-term antipsychotic use.[17] Past research has shown that psychosis following metoclopramide use has been documented in two elderly patients with no prior psychiatric history.[11] However, to our knowledge, metoclopramide-induced rapid-onset psychosis has not been previously reported in the pediatric population.

We present the case of an 11-year-old girl with a background of mild intellectual disability and ADHD managed with methylphenidate, who developed rapid-onset psychosis following the administration of metoclopramide for acute gastrointestinal symptoms.

Case Presentation

The case in an 11-year-old girl followed-up at another center for two years prior, with diagnoses of ADHD and mild intellectual disability. She was also on a medical regimen of 10 mg/day of methylphenidate. The patient presented to the emergency department with complaints of high fever, nausea, and vomiting. Due to her nausea and vomiting, she was prescribed 10 mg of metoclopramide tablets. On the same day, she took two metoclopramide tablets along with 10 mg of methylphenidate. By the evening, she started exhibiting restlessness and agitation. She was talking to herself nonsensically. Upon returning to the emergency department, she was given intravenous hydration. Although her symptoms eased somewhat on the following day, her nonsensical speech and behavioral problems persisted. She refused to eat, and her sleep patterns became irregular. On the fourth day of persistent symptoms, the patient was brought to our clinic and admitted to the general pediatric ward.

The patient's birth history was unremarkable, and she had no previous history of seizures. There was consanguinity between the parents, who were second-degree cousins, but had no known neurological diseases in the family.

Upon initial psychiatric examination, she was conscious, oriented and cooperative. However, her affect was blunted, and she gave nonsensical answers to questions. Her associations were disorganized. Echolalia was observed during the interview, along with auditory and visual hallucinations. Her psychomotor activity was within normal limits. For the psychotic symptoms, haloperidol was initiated at a dose of 0.6 mg/day.

Given the sudden onset of her psychotic symptoms and her young age, consultations with the neurology and infectious diseases departments were requested to explore potential underlying organic causes. The primary differential diagnoses included encephalitis, delirium, and psychosis. The infectious diseases department initiated ceftriaxone and acyclovir treatments due to the possibility of encephalitis.

The initial blood test results for the patient indicated a glucose level of 88 mg/dL. Electrolytes were within the normal range: Na 138 mEq/L, K 4.1 mEq/L, Cl 108 mEq/L, Ca 9.2 mg/dL, Mg 2.1 mg/dL, and P 4.4 mg/dL. Renal function tests revealed normal results with uric acid at 4.3 mg/dL, urea at 28 mg/dL, and creatinine at 0.45 mg/dL. Her liver function tests were also all within the normal range. Specifically, total protein was measured at 71 g/L, albumin at 45

g/L, with aspartate aminotransferase (AST) at 18 U/L, alanine aminotransferase (ALT) at 13 U/L, alkaline phosphatase (ALP) at 179 U/L, and gamma-glutamyl transferase (GGT) at 16 U/L. Additionally, lactate dehydrogenase (LDH) was 269 U/L, total bilirubin 0.55 mg/dL, and direct bilirubin 0.2 mg/dL. The patient's C-reactive protein (CRP) level was below 0.5 mg/L.

The following day, she displayed increased restlessness and excessive activity, engaging in self-harming behaviors. Additionally, her previous symptoms persisted. Cranial and diffusion magnetic resonance imaging (MRI) and electroencephalogram (EEG) results were normal, and cerebrospinal fluid (CSF) analysis showed normal results. The haloperidol dose was revised to 1 mg/day.

On the third day of hospitalization, the patient was re-evaluated and found to have a decrease in visual and auditory hallucinations, improved sleep and appetite, but increased agitation. She had a blank look and avoided eye contact, exhibiting disorganized behaviors such as urinating on the side of the bed. The haloperidol dose was reduced to 0.6 mg/day.

On the fourth day, she had bilateral rigidity in the extremities and upward eye movement, defined as a haloperidol-induced EPS side effect. Haloperidol was discontinued, and biperiden 2.5 mg was administered intramuscularly, resulting in relief of joint stiffness.

By the fifth day, her overall condition had worsened. She responded incoherently to questions, and her emotional expression was dull. Nevertheless, the occurrence of hallucinations and disordered behaviors had markedly diminished. She engaged in sporadic soliloquies. Due to the substantial risk of EPS, it was deemed essential to start antipsychotic medication at an exceedingly low dose (quetiapine 6.25 mg twice daily). Two days later, she was partially responsive to questions but unable to sustain a conversation. Disorganized speech and behaviors had ceased, and there was no agitation or aggression. No side effects related to the medication were observed. Given the perceived benefit of treatment, the quetiapine dose was increased to 25 mg/day, divided into two doses.

Approximately 11 days after admission, the patient was thoroughly evaluated by multiple departments. CSF and blood cultures, as well as the viral meningitis panel, tests for thyroid autoantibodies, paraneoplastic indicators, limbic encephalitis panel, and viral markers, all yielded negative results. Additionally, other rheumatological factors, including anti-nuclear antibodies, anti-double-stranded DNA antibodies, extractable nuclear antigen profile, complement 3, and complement 4, were also found to be within normal limits. In addition, the patient's amino acid plasma panel and hereditary metabolic screening were also normal. Her symptoms entirely disappeared, and she regained her previous level of functioning.

In this case, comprehensive investigations and evaluations excluded infection, electrolyte imbalance, substance intoxication, excessive or prolonged use of any medication, or any other organic pathology as potential causes. Apart from changes in the patient's condition in response to antipsychotic treatment, no fluctuations in consciousness were observed, and orientation remained intact throughout this process. These findings ruled out delirium and led to the conclusion that the patient's presentation was most consistent with rapid-onset psychosis induced by metoclopramide.

The patient was discharged the following day. During a follow-up visit one week after discharge, the patient's associations were organized and goal-directed, and her affect was appropriate. She could attend school but appeared tired and sluggish during the day. This sedation was considered a side effect of quetiapine, and due to the complete resolution of symptoms, the dose was reduced to 12.5 mg/day. At her one-month follow-up, with no active symptoms or complaints, quetiapine medication was terminated.

The family provided a written consent form for this publication.

Discussion

This case study delves into the rare and remarkable occurrence of rapid-onset psychosis induced by metoclopramide, representing the first reported case of its kind in the pediatric population. The convergence of several factors appears to have facilitated the manifestation of such an atypical condition, particularly in a child. Firstly, the presence of mild intellectual disability and ADHD may have significantly contributed to the patient's susceptibility to this adverse reaction since individuals with neurodevelopmental disorders are at an increased risk of developing other mental health conditions, including psychosis.[18,19] This heightened vulnerability is partly due to the complex interplay between their underlying genetic, neurobiological abnormalities, and environmental stressors.[20,21] Furthermore, these individuals often require pharmacological interventions that can exacerbate or precipitate neuropsychiatric symptoms. Studies have shown that adverse drug reactions are more common in this population, necessitating careful management and monitoring of their pharmacotherapy regimens to mitigate potential risks.[22,23] Additionally, it is worth noting that the two other cases who developed psychosis after the administration of metoclopramide were of elderly age and had already suffered from a cerebrovascular event around 5-10 years earlier.[11] Therefore, each of these cases appear to exhibit some kind of neurological vulnerability, albeit in a distinct manner.

Another potential reason might be the use of a higher-than-usual dosage of metoclopramide. In our case, administering 20 mg of metoclopramide at short intervals on the same day might have increased dopamine receptor blockage, which in turn could have triggered dopamine supersensitivity. Certain factors, including the rapidity of drug withdrawal and the higher dosages, may contribute to the severity of hypersensitivity psychosis.[17,24] Although dopamine supersensitivity is typically observed following prolonged use of antipsychotic medication, literature reports indicate that it can also occur in individuals without a prior history of psychosis, particularly after discontinuation of short half-life drugs like metoclopramide.[17]

The final trigger for the patient's psychosis was probably the concomitant use of methylphenidate. Psychostimulants have been associated with psychotic symptoms in children with ADHD.[25,26] In light of the considerations explained above, their concurrent use with other medications like metoclopramide can further elevate this risk. Despite their divergent pharmacological mechanisms, stimulants and antipsychotics are frequently co-prescribed in clinical practice.[27] Nonetheless, the literature highlights that prolonged administration, abrupt cessation, or even acute exposure to dopamine-enhancing agents may precipitate complex drug interactions and adverse effects.[16,27]

To conclude, this case underscores the potential for serious neuropsychiatric reactions in children, particularly those with underlying neurodevelopmental disorders and concurrent psychostimulant use. It emphasizes the necessity for meticulous pharmacological management in such vulnerable populations.

Ethical approval

A written consent form was obtained from the family for this publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: GIE; data collection: GIE. analysis and interpretation of results: GIE, GSD, ANCK; draft manuscript preparation: GIE. 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

Primary breast Burkitt lymphoma with lactic acidosis in a child: a case report

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ABSTRACT

Background. Primary breast lymphoma is extremely rare and constitutes approximately 1% of all non-Hodgkin's lymphomas (NHL). Only 1-5% of them are Burkitt type. We present a case of childhood primary breast Burkitt lymphoma (BL).

Case presentation. A 16-year-old female patient was referred to our hospital for bilateral breast swelling and respiratory distress. She had lactic acidosis. Despite aggressive dialysis support, lactic acid levels started to decrease only after the combination chemotherapy treatment was started and returned to normal. Histopathological examination of the biopsy was consistent with the diagnosis of BL. The case was classified as stage 4 disease. EICNHL Mature B NHL protocol, Group C3 chemotherapy was given and a very good partial response was achieved. However, the patient died due to fungal septicemia.

Conclusion. Type B lactic acidosis in aggressive malignancies indicates a poor prognosis. In such cases, as in our case, lactic acidosis improves only with appropriate and sufficient chemotherapy, and its improvement is an important indicator that the case is responsive to treatment.

Key words: breast Burkitt lymphoma, lactic acidosis, childhood.

Lymphoma constitutes 17.6% of all childhood cancers in Türkiye.[1] Approximately 20%–40% of patients with non-Hodgkin lymphoma (NHL) arise from primary extranodal sites. The most common site of primary extranodal disease is the gastrointestinal tract followed by skin, testis, bone, and central nervous system (CNS); rarely, the kidney, prostate, bladder, ovary, orbit, heart, breast, salivary glands, thyroid, and adrenal glands may be involved.[2,3]

Primary breast lymphoma (PBL) arises from breast lymphoid tissue. It is an extremely rare disease, which constitutes 2% of extranodal NHLs and approximately 1% of all NHL.[4–6] Diffuse large B cell lymphoma is the most common histopathological subtype of PBL. Only 1-5% of them are Burkitt type.[4,5,7,8]

In this context, herein we present a case of childhood primary breast Burkitt lymphoma (PB-BL).

Case presentation

A 16-year-old female patient was referred to our hospital due to bilateral breast swelling and respiratory distress. It was learned that she had been admitted to a regional hospital with the complaint of swelling in her right breast 40 days

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before. In physical examination, there had been no pathological finding except for swelling, induration and tenderness in the breast. The diagnosis of mastitis had been made and antibiotic therapy started. Since there was no improvement of the symptoms with antibiotics, she underwent to the surgery in that hospital with the diagnosis of a breast abscess, debridement of the tissues thought to be infected, and a tru-cut biopsy was performed. Later on she was referred to our hospital. It was seen that both breasts, especially right one, turned into giant purplish-blue masses with diffuse nodularity. There was a tissue defect and seropurulent discharge on the biopsy area in the right lower lateral quadrant of the right breast. There was lymphadenopathy of 3×2 cm in the right axillary region. In the laboratory examination, hemoglobin was low at 9.5 g/dL, platelet count was $689x10^3$ /mm^3, white blood cells $8.3x10^3$ /mm^3, lactate dehydrogenase (LDH) 3505 U/L, renal and liver function tests were in normal limits. Her blood pH was 7.25, bicarbonate 14.6 mmol/L, lactate 22 mmol/L, anion gap was 20.8. In thorax computed tomography, the size of both breasts was increased and there were diffuse infiltrative soft tissue lesions which was more obvious in the right breast (Fig 1a). Breast ultrasonography demonstrated hypoechoic ill-defined breast masses with soft tissue edema (Fig 1b). Pathological lymphadenopathies along the bilateral parasternal intermammarian chain on the anterior chest wall and in the right axillary region were observed. Abdominal computed tomography revealed prominent diffuse thickening of small intestinal wall and colonic segments (Fig 2), intraabdominal free fluid, and a soft tissue lesion in the left pelvic lesion, consistent with peritoneal thickening.

The patient underwent hemodialysis after sodium bicarbonate deficit treatment for the lactic acidosis. Despite intermittent hemodialysis lactic acidosis persisted and continuous veno-venous hemodiafiltration was started. Daily two sessions of 4 hours intermittent hemodialysis, and 16 hours continuous veno-venous hemodiafiltration was continued for 3 days. Wide spectrum antibiotics were started, since purulent discharge from biopsy site was observed. Despite aggressive dialysis support, lactic acid levels started to decrease only after the cancer specific treatment was started and returned to normal on the third day. Histopathological examination of the biopsy was consistent with the diagnosis of BL. Bone marrow biopsy was normal. Cytological examination of cerebrospinal fluid showed central nervous system (CNS) involvement. The case was classified as stage 4 disease and EICNHL Mature B NHL protocol, Group C3 chemotherapy was started. At the end of the third course very good partial response was achieved. Radiological response evaluation

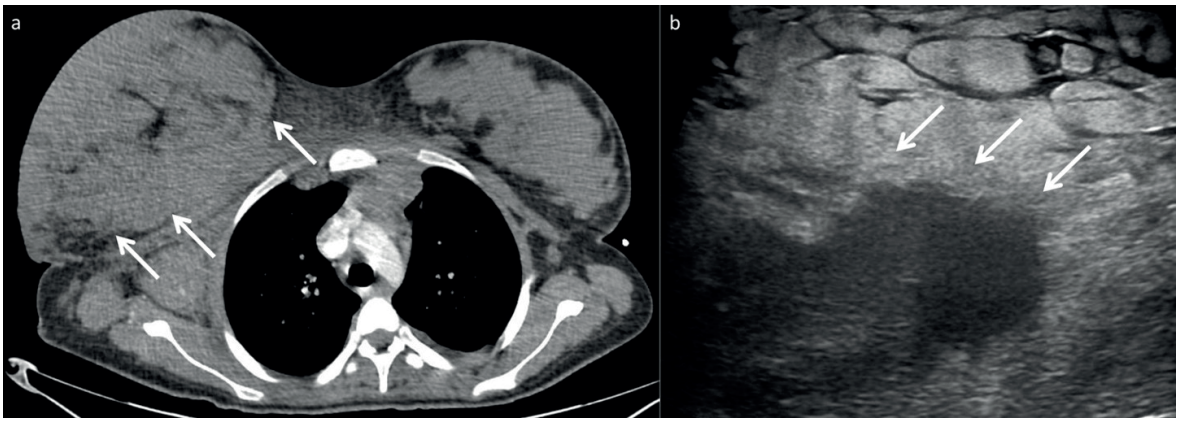

Fig. 1a-b. A 16-year-old girl with Burkitt lymphoma. **(a)** Axial contrast enhanced chest computerized tomography shows large heterogeneous breast mass (arrows). **(b)** Breast ultrasonography demonstrates hypoechoic ill-defined breast mass (arrows) with soft tissue edema.

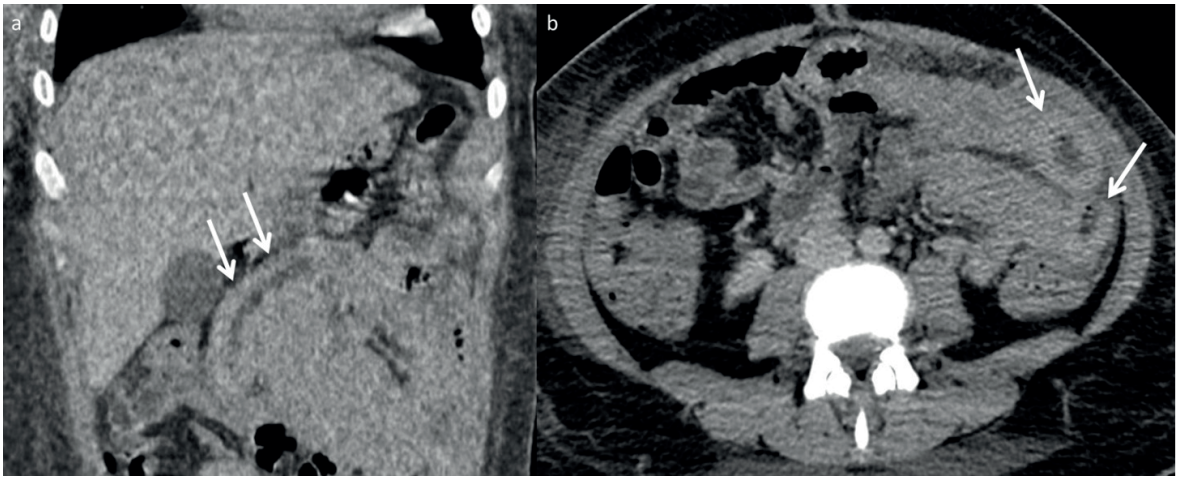

Fig. 2. A 16-year-old girl with Burkitt lymphoma. Coronal reformatted **(a)** and axial **(b)** contrast enhanced abdomen computerized tomography shows diffuse bowel wall thickening (arrows).

showed regression in the entire affected area except for heterogeneous nodularity in the right breast, which was thought to be due to infection rather than tumoral residue. She was diagnosed with neutropenic sepsis at the end of this course. Mold fungus belonging to *Aspergillus spp.* and *Mucoralis spp.* and *Stenotrophomonas maltophilia* were isolated from the pus culture obtained from the biopsy site, where the breast infection had drained through the skin. Amphotericin and voriconazole were started. Wide surgical debridement was planned. However, pulmonary and femoral venous thromboembolism developed during this period. Anticoagulant treatment was started. Respiratory distress and circulatory failure developed, and the patient died from septic shock three months after the diagnosis. Consent was obtained from the patient and the parents for the publication of this case report.

Discussion

Breast cancer arising from epithelial cell components is the most common cancer in women. Lymphoma rarely involves breast tissue either as a part of disseminated disease or as primary location.[4]

Primary breast lymphoma accounts for only 0.04% of breast neoplasms. It mostly affects adult women between fourth and sixth decade.[9] Its onset in childhood is exceptionally rare. Restivo et al.[10] reviewed 11 cases reported in the literature. These were mostly adolescent females with a median age of 14.5 years. There was only one boy. There was a slight right breast predominance and only one bilateral involvement.

Over 90% of reported adult PBL cases are of B cell origin. More than 50% were diagnosed as diffuse large B cell lymphomas, while Burkitt lymphomas were the less common B cell variants. However, anaplastic large cell lymphomas and mature B-cell lymphomas were reported with equal frequency in children.[10]

Burkitt lymphoma in adults is a highly uncommon subtype of PBL. PB-BL primarily affects young women in their reproductive years. It has been observed that PB-BL often presents in pregnancy or post-partum during lactation, which has led to the suggestion that hormonal changes may play a role in its development.[4] Very few cases of PB-BL have been reported in childhood. The most common presentation of PB-BL is a diffuse and rapid bilateral enlargement of the breasts along with associated symptoms such as pain, redness, itching, fever, and axillary lymphadenopathy.[4]

A high incidence of CNS involvement was reported in patients with breast lymphomas.[11]

CNS involvement occurs in 6 percent of pediatric NHL with rates ranging from 8.8% in BL to < 3% in diffuse large B cell lymphoma[12], while this ratio reached 50% in PBL.[11]

Most common presentation of BL is rapidly growing tumor masses and frequent spontaneous tumor lysis. Tumor lysis syndrome, a condition resulting from the massive lysis of tumor cells and the subsequent release of large quantities of potassium, phosphate, and uric acid into the systemic circulation, is a potential oncologic emergency. The deposition of uric acid or calcium phosphate crystals in the renal tubules can lead to acute renal failure.[13] When our patient was admitted to our hospital, serum uric acid, potassium, and phosphate levels were normal, and serum LDH level was 3505 U/L. There was no sign of tumor lysis despite the heavy tumor burden. However, she had severe lactic acidosis. Renal involvement, which is a risk factor for the development and severity of tumor lysis syndrome and common in Burkitt lymphoma, was not present in our case.

Type A lactic acidosis is a serious complication occurring in critically ill patients and is associated with the underlying shock state resulting from tissue hypoperfusion and dysoxia. If lactic acidosis occurs in a person with normal hemodynamic parameters and adequate tissue oxygenation, this condition is called type B lactic acidosis. Type B lactic acidosis is a rare complication in cancer patients. Malignant cells increase glycolysis and lactate production to allow for uncontrolled proliferation, also called the Warburg effect. Lactogenic cancers exhibit increased aerobic glycolysis and lactate production in parallel with the aggressiveness of the cancer.[14] The occurrence of type B lactic acidosis in cancer cases is considered an indicator of a poor prognosis. If the serum LDH level is 2-3 times higher than the standard value, it suggests a poor prognosis.[15] In our case, the serum LDH level was 3505 U/L at the time of admission to our hospital. When appropriate chemotherapy is started, acidosis is corrected.[16] In fact, it is the only treatment method that leads to sustained remission. Lactic acidosis was reported to resolve within 15 h to 3 days after starting chemotherapy. In cases whose tumors are unresponsive to chemotherapy, lactic acidosis will not improve.[16,17] The resolution of lactic acidosis with chemotherapy could be considered a herald of the onset of remission. In the case of lactic acidosis, besides reducing the production of lactic acid, it is important to remove lactic acid extracorporeally in order to keep the patient alive during this period and to prevent irreversible damage to the organs due to acidosis. Although hemodialysis is the best method for this purpose, continuous veno-venous hemodiafiltration may be used in the case of persistent lactic acidosis despite hemodialysis or to prevent recurrence after hemodialysis. In our patient, both dialysis modes were successfully used in combination until the third day when chemotherapy started to take effect.

Surgical interventions should be avoided in these cases because the recovery process after surgery may delay the initiation of systemic therapy, which is needed urgently. Burkitt's lymphoma responds very well to combination chemotherapy. In children and adolescents, risk-adapted treatment approaches are required. These are high-dose intensive chemotherapy protocols that penetrate the CNS. In high-risk patients rituximab improves outcome and should be given to all patients.[13] Although our patient has all kinds of negative risk factors for the outcome; with early initiation of treatment and selected effective chemotherapy, she was in remission rapidly. Unfortunately, she died due to secondary fungal infections and sepsis.

In conclusion, primary childhood breast Burkitt lymphoma (BL) is a very rare disease, frequently associated with tumor lysis syndrome and rarely with type B lactic acidosis. Its prognosis is poor and early initiation of appropriate combination chemotherapy is crucial for the cure of both lactic acidosis and lymphoma.

Ethical approval

Informed consent was obtained from the parents of the child.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EM, NK; data collection: EM; analysis and interpretation of results: EM, NK, TK, HNO, DO, SK; draft manuscript preparation: EM, NK, TK. 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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Case Report

Griscelli syndrome: Erdheim-Chester disease-like local presentation progressing to accelerated phase

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ABSTRACT

Background. Griscelli syndrome (GS) is a rare genetic disorder characterized by oculocutaneous albinism and variable immune dysfunction. Among three distinct types of GS, occurring due to different genetic mutations; GS type 1 presents with neurological manifestations, hemophagocytic lymphohistiocytosis (HLH) generally develops in GS type 2, and GS type 3 primarily exhibits oculocutaneous albinism. HLH, a life-threatening condition with excessive immune activation, may occur secondary to various triggers, including infections, and develop in different tissues, as well as in the testis, similar to Erdheim-Chester disease.

Case. After referral at 19 days of age with restlessness, left testicular swelling, and erythema, an infant was diagnosed with bilateral hydrocele with left testicular torsion by testicular ultrasound, leading to a left orchiectomy. Pathology showed testicle and spermatic cord hemorrhagic necrosis. A week later, the infant presented with right testicular swelling and hepatosplenomegaly. He had silvery gray hair. We administered broad-spectrum antibiotics for increased acute phase reactants. Viral panels, including cytomegalovirus and Epstein-Barr virus, were negative. Laboratory findings indicated cholestasis and disseminated intravascular coagulation. Bone marrow aspiration revealed hemophagocytosis and increased histiocytes. Microscopic hair examination supported the diagnosis of GS. Sanger sequencing revealed the homozygous mutation c.217T>G (p.W73G) in *RAB27A*.

Conclusion. Prompt diagnosis and treatment of HLH are crucial to prevent progression to multi-organ failure and death. This case highlights the diverse tissue involvement and diagnostic challenges in Griscelli syndrome type 2.

Key words: griscelli syndrome, testicular torsion, newborn, *RAB27A*, hemophagocytic lymphohistiocytosis, local HLH.

Griscelli syndrome (GS) is a rare genetic syndrome associated with oculocutaneous albinism.[1,2] The cause of this syndrome was understood with the discovery of mutations of myosin Va, Rab27a, and melanophilin in the years 1997, 2000, and 2002, respectively.[1-3] The clinical phenotype in GS type 1 (OMIM #214450) includes primarily developmental neurological problems. In GS type 2 (OMIM #607624), hemophagocytic lymphohistiocytosis (HLH), cutaneous hypopigmentation, immunodeficiency, and neurological

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involvement due to HLH are primary manifestations. In GS type 3 (OMIM #609227), oculocutaneous albinism is the only symptom.[3]

HLH mainly presents with persistent fever, hepatosplenomegaly, lymphadenopathy, and hemophagocytosis. It can be seen secondary to infections like Epstein-Barr virus (EBV) or cytomegalovirus (CMV), in immunodeficiency syndromes like GS type 2 or Chediak–Higashi syndrome, in lymphoma, autoimmune diseases, and in perforin (*PFR1*), Munc 13-4 (*UNC13D*), syntaxin 11 (*STX11*), syntaxin binding protein 2 or Munc 18-2 (*STXBP2*) deficiencies. For HLH diagnosis, intermittent renewal of criteria occurs due to diagnostic developments.[4] If not treated, uncontrolled inflammation progresses into multiple organ failure and death.[3]

In this report, we present a GS type 2 case who presented in the early neonatal period with testicular torsion and HLH.

Case Presentation

We present an infant who was referred to our clinic on the 19th day of his life with restlessness, swelling, and erythema of the left testicle. He was born as the first child of consanguineous parents. Testicular ultrasound showed left testicular torsion with bilateral hydrocele, and the patient underwent left orchiectomy (pathology presented in Fig. 1A and 1B). We could not perform magnetic resonance imaging or computerized tomography because anesthesia would be required in the newborn period. The pathological specimen showed hemorrhagic necrosis of the testicle and spermatic cord. A week after, during admission for right testicular swelling, clinicians noted silvery gray hair, fever, cytopenia, and hepatosplenomegaly on physical examination (Fig. 1C, Table I). We started broad-spectrum antibiotics (vancomycin, meropenem, and amikacin). Viral panels, including CMV and EBV PCR, were within normal limits. Gamma glutamyl transferase (GGT) and bilirubin levels (direct and total) were slightly high, which may have indicated cholestasis due to drugs or hepatic HLH involvement. High international normalized ratio (INR) and D-dimer levels were compatible with disseminated intravascular coagulation, and vitamin K was administered. Bone marrow aspiration showed hemophagocytosis and increased histiocytes.

Hair microscopy of the patient showed large pigment clusters compatible with GS (Fig. 1D). There was no intracranial involvement, and cerebrospinal fluid parameters were within normal range. We confirmed the GS type 2 accelerated phase diagnosis by Sanger sequencing, which revealed the homozygous mutation c.217T>G (p.W73G) in exon 3 of *RAB27A*. This mutation in *RAB27A* was reported before as a cause of GS.[5]

After the HLH-2004 protocol, the patient underwent an allogeneic hematopoietic stem cell transplantation (HSCT) from his HLA-identical father. Myeloablative regimen was given to the patient. Eight days after HSCT, he developed CMV-related pneumonia and heart failure. With fluconazole, voriconazole and foscarnet treatment, the clinical course regressed. Eighteen months after HSCT, the chimerism was 95% (Table I).

During follow-up, steroids were tapered at the 4th month of HSCT. His evaluation showed hypothyroidism with increased thyroid stimulating hormone. When he was 15 months old, on physical examination, macrocephaly and mild developmental delay (by Denver-II Developmental Screening Test) were detected. Cranial magnetic resonance imaging (MRI) showed increased T2-weighted signal intensity in centrum semiovale and bilateral periventricular regions. The audiological test was bilaterally symmetric, and he achieved his developmental steps late. Now, he attends primary school, and his development is appropriate for his age. He is euthyroid with thyroid hormone replacement.

The family gave written consent for this publication.

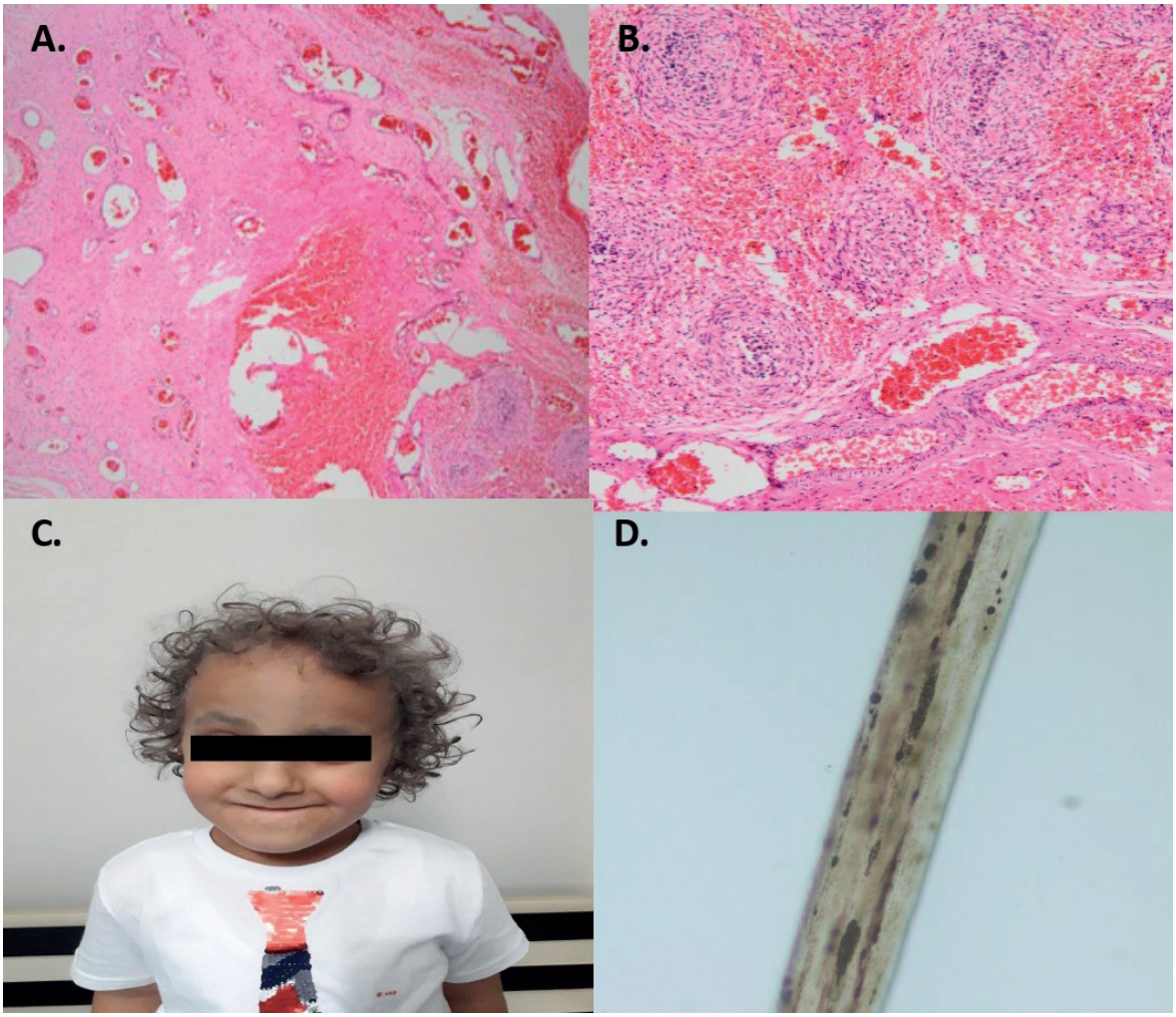

Fig. 1. A and **B)** Light microscopic findings show diffuse hemorrhagic necrosis in left orchiectomy material (Hematoxylin & eosin staining, x10 and x20 magnification, respectively) **C)** Child with obvious grey hair and brows, picture taken by his parents when he was 6-years-old **D)** Microscopic examination of patient's hair, x20 magnification, was compatible with Griscelli syndrome

Discussion

We presented a newborn diagnosed after orchiectomy and a previously reported gene defect for GS type 2.[6]

This case was interesting from two points: He is one of the youngest GS patients whom developed HLH in the literature, and presented with probable testicular involvement not reported before in the medical literature. One study reported testicular involvement in HLH but lacked patient characteristics.[7]

The HLH-2004 protocol (steroids, cyclosporine a, intravenous immunoglobulin, etoposide) and antimicrobial prophylaxis were used to treat GS type 2 as valid treatment at the time of patient presentation. The scheme should be applied until remission occurs, and HSCT should be performed as curative therapy.[3,4] In the current case, after the patient was diagnosed with GS type 2 and HLH by diagnostic criteria, he was treated first for HLH and then with HSCT as curative treatment. We did not find any central nervous system (CNS) involvement. When a

Table I. Laboratory parameters of the patient.

Laboratory parameters	On admission	18 months after HSCT
Hemoglobin (g/dL)	6.2 (9.5-16)	13.9 (11-13.9)
WBC (x10^6/L)	4,400 (6,000-18,000)	9,700 (5,500-12,000)
ALC (x10^6/L)	2,800 (900-8,800)	6,000 (5,000-6,000)
ANC (x10^6/L)	600 (1,200-7,500)	2700 (1,500-6,300)
AEC (x10^6/L)	100 (100-1,700)	300 (100-750)
Platelets (x10^9/L)	21 (60-600)	296 (225-470)
Erythrocyte sedimentation rate (mm/h)	2	-
C-reactive protein (mg/dL)	9.87 (0-0.5)	-
Ferritin (mg/dL)	14.4 (20-336)	44.4 (20-336)
Fibrinogen (mg/dL)	253 (180-350)	-
Triglyceride (mg/dL)	88 (<150)	43 (<150)
IgA (mg/dL)	72.9 (11-14.01)	-
IgG (mg/dL)	626 (603-1466)	-
IgM (mg/dL)	74.8 (22-87)	-
CD3 (% - /mm^3)	81 (53-84)	54 (53-75)
	2,270 (2,500-5,500)	3,250 (2,100-6,200)
CD4 (% - /mm^3)	46 (35-64)	28 (32-51)
	1,288 (1,600-4,000)	1,700 (1,300-3,400)
CD8 (% - /mm^3)	41 (12-28)	29 (14-30)
	1,150 (560-1,700)	1,750 (620-2,000)
CD16/56 (% - /mm^3)	9 (4-18)	24 (3-15)
	250 (170-1,100)	1,450 (180-920)
CD19 (% - /mm^3)	7 (6-32)	12 (16-35)
	200 (300-2,000)	700 (720-2,600)

Values in parenthesis for hemoglobin, WBC, ALC, ANC, AEC, AMC and platelet count indicate 5th and 95th percentiles in first column and 25th and 90th percentiles for second column, respectively. Values in parenthesis for immunoglobulin values indicate 15th and 85th percentiles, respectively. Values in parenthesis for lymphocyte subsets indicate 10th and 90th percentiles, respectively. AEC, absolute eosinophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; CD, cluster of differentiation; HSCT, hematopoietic stem cell transplantation; Ig, immunoglobulin; WBC, white blood count.

patient with GS presents with a neurological symptom, CNS involvement in HLH should be excluded.

After HSCT, hypothyroidism was observed, which was related to long-term corticosteroid treatment. MRI showed increased T2-weighted signal intensity in centrum semiovale and bilateral periventricular regions. It may be related to possible CNS involvement of HLH, which was in the beginning phase and had not progressed but was cured during the HLH follow-up after HLH 2004 therapy was started.[8]

Testicular torsion may develop in the early newborn period. Intravaginal testicular torsion usually occurs in adolescents, and the extravaginal testicular tortion generally occurs in prenatal and neonatal periods. Although the most commonly detected etiology of intravaginal torsion is the bell clapper deformity, extravaginal torsion's etiology is still unknown.[9,10] Our patient's specimen was necrotic, and we could not determine the torsion type, which might be an extravaginal torsion according to the medical literature.[9]

We thought that the testicular torsion may be the result of testicular involvement due to HLH. However, we could not confirm this as we could not show hemophagocytosis in the testis

biopsy, as it was necrotic. Hence, testicular torsion in our patient may have been due to the involvement of the testis in the subclinical phase of HLH.

Testicular involvement in this patient is a novel finding. There may be a link between testicular torsion and histiocytic infiltration. Confirmation to this from the medical literature may be the Erdheim-Chester disease, which is characterized with local histiocytic infiltration of the testis and is one of the causes of testicular torsion.[11] Thyroid, CNS, and testicular involvement did not occur at the same time. Central nervous system involvement in HLH can be subtle, as seen in this patient. In a patient with partial albinism, cranial imaging should be conducted before HSCT with a conditioning regimen since intracranial HLH involvement may be the case.

Thyroid function tests were not performed before HSCT and we do not know whether the hypothyroidism that developed after transplantation was autoimmune. The thyroid replacement therapy needed in the course of the disease may be due to thyroid involvement, which may be directly due to the infectious agent triggering the HLH or secondary to the HLH thyroid involvement. We suggest that the local tissue involvement in this disease is step by step and testicular involvement may be the first sign of gradual HLH development as seen in the present case.

Cytopenia and hepatosplenomegaly are common features of HLH in patients with partial albinism. However, various tissues may be affected locally during the progressive course of HLH. Therefore, if a patient with partial albinism presents with atypical local involvement, histiocytosis and HLH may be on the top of the differential diagnosis list.

Ethical approval

Informed consent was obtained from the family for publication of this case report.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: DC, FİT; data collection: TS, GS, AKT; analysis and interpretation of results: HA, HTA; draft manuscript preparation: TA, GS, AKT, DC. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

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Case Report

Anti-SRP myositis: a diagnostic and therapeutic challenge

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ABSTRACT

Background. Anti-signal recognition protein (anti-SRP) myopathy is a rare idiopathic inflammatory myopathy in children. Herein, a 3-year-old patient with severe anti-SRP myopathy showing a rapidly progressive disease course is presented in order to increase the awareness of pediatricians about idiopathic inflammatory myopathies.

Case Presentation. A previously healthy 3-year-old girl presented with progressive symmetrical proximal muscle weakness that caused difficulty in climbing stairs for two months prior to evaluation, and a marked elevation of the serum creatine kinase levels. A skeletal muscle biopsy revealed necrotic and regenerating processes, with mild inflammatory changes. Myositis-specific and associated autoantibodies tested by the immunoblot method were positive for anti-SRP. Pulse corticosteroid, intravenous immunoglobulin, and methotrexate were administered. However, muscle weakness progressed, respiratory distress and dysphagia developed. Rituximab was initiated. While on rituximab treatment, she was able to walk independently and muscle enzymes were within normal range at the 15th month of diagnosis.

Conclusion. Early diagnosis of patients with anti-SRP myositis is important to control inflammation and prevent disease progression and complications. To our knowledge, our patient is the youngest case reported in the literature and was successfully treated with rituximab added to conventional therapy.

Key words: anti-signal recognition protein myopathy, anti-SRP, rituximab, inflammatory myopathy, children.

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of disorders characterized by inflammatory changes in skeletal muscle with muscle weakness more prominent in proximal muscles. Juvenile dermatomyositis (JDM) is the most common subtype of IIM and is relatively easy to diagnose due to specific cutaneous signs. There are other subtypes of IIM, such as immune-mediated necrotizing myopathies (IMNM) and overlap myositis, which are rarer, have a worse prognosis, and are challenging to diagnose.[1]

Immune-mediated necrotizing myopathy is a rare and severe subtype of IIM in children. Anti-signal recognition protein (SRP) and 3-hydroxy-3-methylglutaryl-Co A reductase (HMCGR) antibodies are associated with IMNM.[2] Anti-SRP is a myositis-specific antibody (MSA) which has been well described in adults but rarely in children.[3] It is usually associated with a severe or fulminant course and poor response to therapy.[4] Pediatric patients have a similar clinical spectrum as adults, but only a few cases have been reported.[5-8]

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Herein, a 3-year-old patient with severe anti-signal recognition protein (SRP) myopathy showing a rapidly progressive disease course is presented in order to increase awareness of pediatricians about IIMs other than JDM and to draw attention to the differential diagnosis.

Case Presentation

A previously healthy 3-year-old girl presented with frequent falls, muscle weakness, and difficulty in climbing stairs for two months prior to evaluation. There was neither parental consanguinity nor a family history of muscle disease. Neurological examination revealed nasal speech and proximal muscle weakness which was more prominent in the lower extremities. Motor power measured by the Medical Research Council's (MRC) classification of bilateral proximal and distal upper extremities was 4/5 and 5/5, respectively. The proximal and distal muscle strength of her lower extremities were 3/5 and 4/5, respectively. She had hypoactive deep tendon reflexes and waddling gait pattern. There was no muscle wasting, muscle hypertrophy, or pseudohypertrophy. Our physical examination revealed no skin rash, joint swelling, or other systemic features such as respiratory distress or dysphagia.

Serum inflammatory markers and complete blood count were normal. Muscle enzymes were elevated (creatine kinase, CK: 13552 U/L, lactate dehydrogenase: 3149 U/L, aspartate aminotransferase: 427 U/L), as well as cardiac markers (troponin I: 1064 ng/L and CK-MB: 300 μg/L). Thyroid and parathyroid hormone levels were within the normal range. Metabolic screening (such as organic acids and amino acids) was normal. Ultrasonography of the neck and abdomen and thoracic tomography showed no pathologic findings. Bone marrow aspiration was normal. Electroneuromyography demonstrated an active myopathic process more prominent in the proximal muscles with intermittent spontaneous denervation. The electrocardiogram and cardiac ultrasound were normal. Muscle biopsy from the vastus lateralis revealed widespread variation in fiber size, rounding of fibers, necrosis and regeneration of muscle fibers. There were prominent foci of endomysial and perivascular inflammatory infiltrates, accompanied by endomysial and perimysial fibrosis (Fig. 1). HLA-ABC antigens were not expressed in the sarcolemma or sarcoplasm and most fibers were positive for neonatal myosin. Inflammatory cells were mainly T lymphocytes and macrophages, with rare B lymphocytes. MSA testing by immunoblot method was positive for anti-SRP and other autoantibodies were negative.

A three-day pulse intravenous (IV) methylprednisolone (30 mg/kg/day), followed by oral prednisolone (2 mg/kg/day twice daily) was initiated. Intravenous immunoglobulin (IVIG; 2 g/kg/month) and methotrexate (15 mg/m^2/week) were added. Although CK levels decreased to 1880 U/L in the 2nd week of the treatment, muscle weakness progressed, as she became non-ambulatory, unable to lift her thighs and get up from a supine position. Respiratory distress and dysphagia developed. Rituximab (375 mg/m^2 four times weekly) was initiated. In the first month of treatment, she received the second course of a 3-day IV pulse methylprednisolone. Methotrexate was switched to mycophenolate mofetil (600 mg/m^2/day) because of elevated transaminases. After 2 months of hospitalization, she was discharged with oral prednisolone (1 mg/kg/day), IVIG (2 gr/kg/month), and mycophenolate mofetil. She showed a clinically significant improvement and achieved regain of ambulation in the 5th month of treatment. A second dose of rituximab was given 6 months after the first dose. Oral steroid therapy was tapered. IVIG was discontinued 11 months after the onset of the disease.

At the fifteenth month of diagnosis, she was able to walk independently and climb stairs with mild limitation in jumping and running activities alone. On neurological examination, she had mild proximal muscle weakness in the lower extremities (MRC 4+/5) with hypoactive

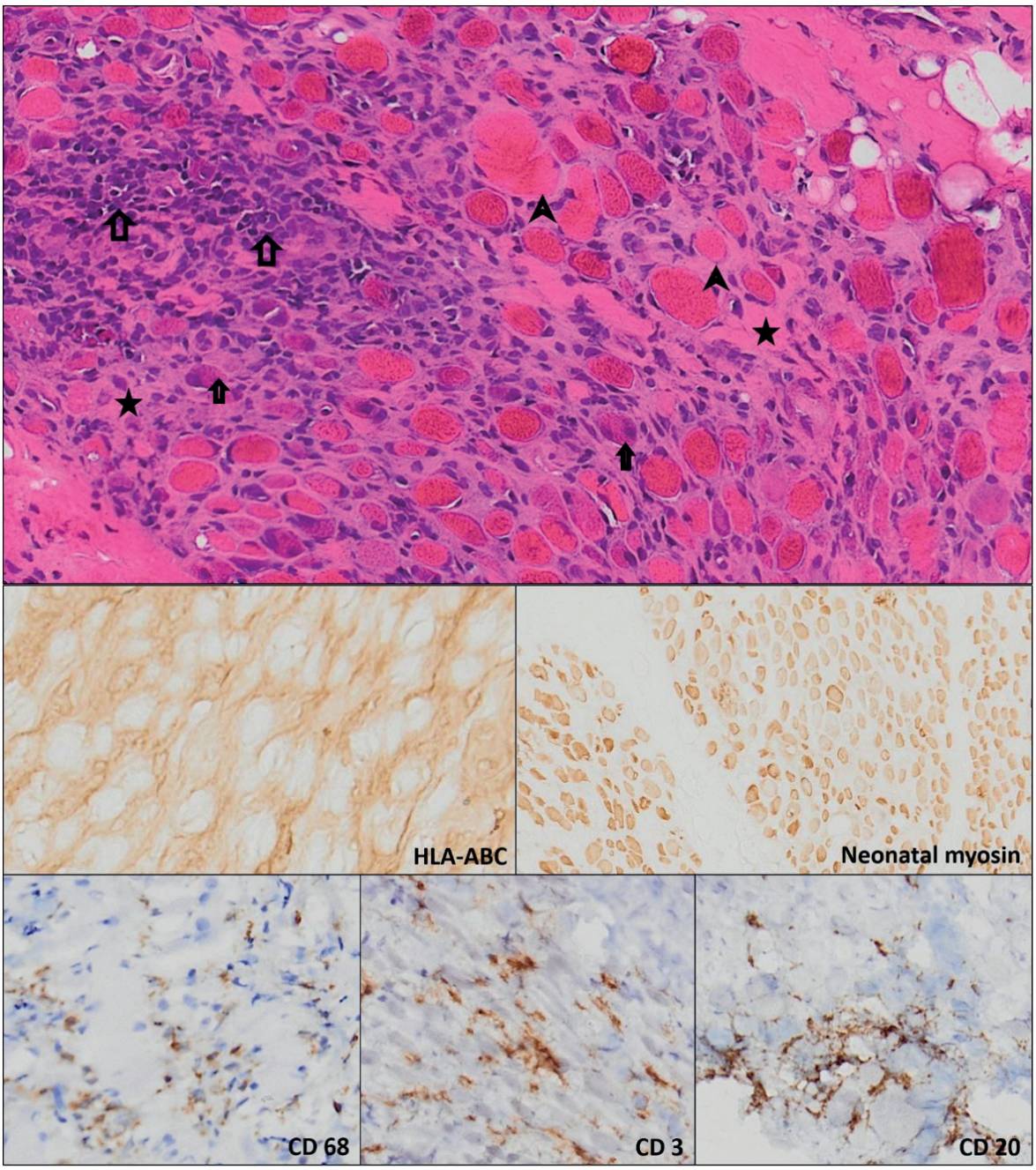

Fig. 1. Top: Muscle biopsy showing many necrotic (arrow head) and regenerating (solid arrow) fibers, prominent inflammatory infiltration (arrow), and severe endomysial and perimysial fibrosis (star), H&E stain. **Middle row:** HLA-ABC is not expressed. Neonatal myosin is expressed in majority of fibers. **Bottom row:** CD 68 and CD 3 positive mononuclear cells are more widespread, while CD 20 positive cells are fewer, in small foci.

patellar deep tendon reflexes. Her muscle enzymes were in the normal range. Asummary of treatment is shown in Fig. 2.

An informed consent was received from the patient's family for the publication of this report.

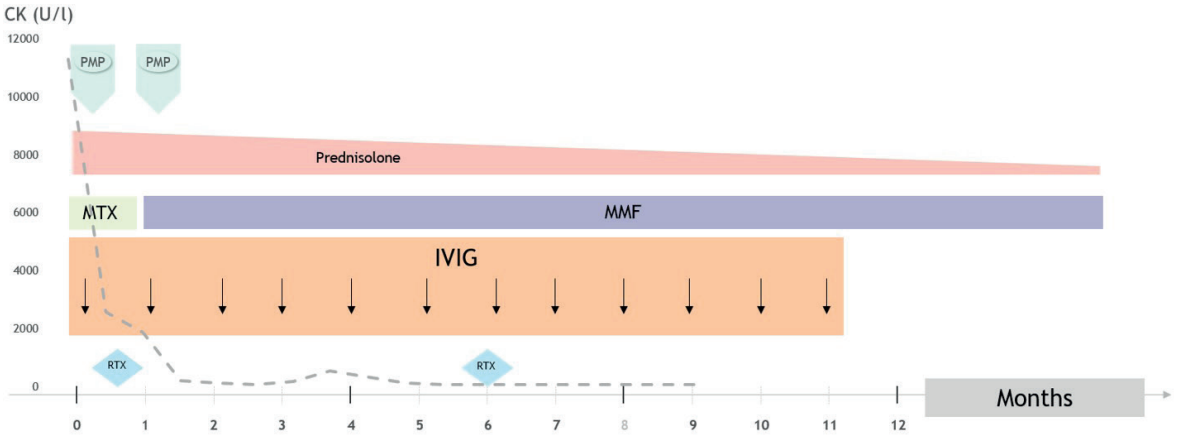

Fig. 2. Summary of treatment. Dashed lines show serum CK levels.

CK, creatine kinase; IVIG, Intravenous immunoglobulin; MMF, Mycophenolate mofetil; MTX, Methotrexate; PMP, Pulse methylprednisolone; RTX, Rituximab.

Search strategy

We screened PubMed/MEDLINE by using the following keywords: "juvenile idiopathic inflammatory myopathy", "pediatric", "anti-signal recognition protein", and "rituximab" and searched the literature from database inceptions to December 31, 2023. The search was limited to English articles. Case reports, original research articles, editorials, and review articles about IIM were analyzed. Two authors independently screened full texts of all relevant articles (Fig. 3). The following parameters were assessed within the included studies: gender, age at diagnosis, clinical and laboratory findings, treatment, and outcome.

Discussion

Immune-mediated necrotizing myopathy is a rare and severe subtype of IIM in children. IMNM has 3 subtypes: anti-SRP myositis, anti- 3-hydroxy-3-methylglutaryl-coenzyme A reductase myositis, and seronegative myositis. Although all subtypes of IMNM are associated with muscle weakness, anti-SRP myositis is associated with more severe muscle weakness and atrophy.[1]

The diagnosis of IIM is based on a combination of medical history, serum muscle enzyme levels, muscle biopsy findings and at times auto-antibodies.[1,9] The broad differential diagnosis of IIM causes diagnostic challenges. The differential diagnosis of muscle weakness includes infectious myopathies, muscular dystrophies, metabolic myopathies, endocrinopathies (such as hypo-hyperthyroidism, hypo-hyperparathyroidism), drug-induced myopathies, and malignancy, in addition to inflammatory myopathies.[9] Metabolic and endocrine tests were normal in this case. There was no drug exposure and no evidence of malignancy. Infective myositis is an acute, self-limiting condition seen in children with a recent history of viral infection; it was most commonly reported in those with influenza, although other viral infections can also cause myositis.[10] Our patient had progressive muscle weakness and no history of previous infections. Muscular dystrophies (MD) are a group of inherited degenerative muscle diseases characterized by progressive muscle weakness and dystrophic findings on muscle biopsy. Muscle weakness can occur at various stages of the clinical course. Congenital MD is characterized by severe and progressive muscle weakness from the neonatal

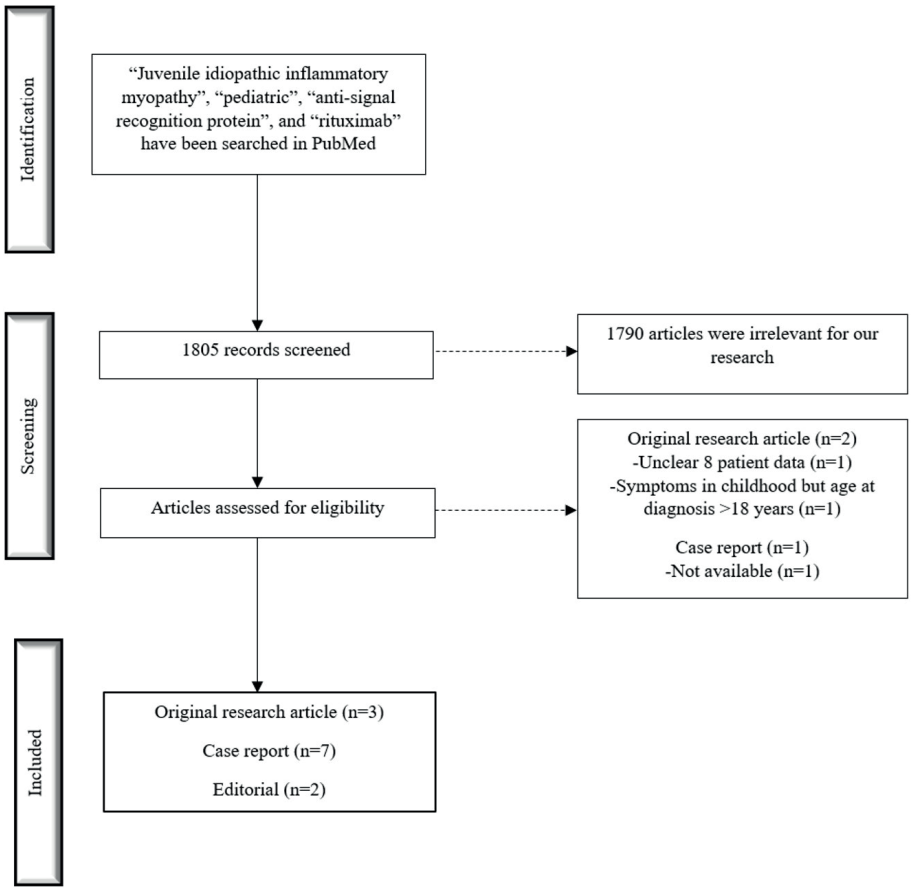

Fig. 3. Search strategy for literature review.

period, whereas in the most common form, Duchenne MD, muscular dystrophy remains relatively stable until approximately 7 years of age.[11] MSAs are powerful diagnostic tools in the slowly progressive form of IMNM that can mimic muscular dystrophy. Suzuki et al.[12] reported that the diagnosis of anti-SRP myositis was confirmed by RNA immunoprecipitation in a patient with childhood-onset myopathy in whom the differential diagnosis of IIM or muscular dystrophy could not be made for 21 years, despite repeated muscle biopsies. It was also shown that none of the 105 patients with various MDs (e.g., Duchenne MD, Becker MD, limb-girdle MD, facioscapulohumeral MD, or Fukuyama-type congenital MD) had anti-SRP antibodies detected in serum samples.[12] In this case, anti-SRP antibody was positive and there were no histopathological findings of muscular dystrophy. Therefore, no further investigation was performed for the diagnosis of MD.

Cardiac symptoms such as chest pain, palpitations, and congestive heart failure are observed in some patients with anti-SRP myositis.[1] Although our patient had no cardiac symptoms or cardiac ultrasound or ECG findings, her cardiac enzymes were quite high, which was accepted as subclinical cardiac involvement. Dysphagia occurred in the 2nd week of follow-up with our patient's, and she had difficulty swallowing solid foods in the swallowing test. A recent study reported dysphagia in 30–69% of anti-SRP myositis cases.[13] The frequency of dysphagia is significantly more common than in other types of myositis. Therefore, dysphagia should

be investigated in IMNM cases to avoid life-threatening respiratory complications.

High-dose corticosteroids, IVIG, and methotrexate are the first-line treatments for patients with juvenile IIM. Similar to adults, cyclosporine, azathioprine, mycophenolate mofetil, and cyclophosphamide can also be used to treat refractory disease in children.[9] It has been shown that pediatric patients without clinical improvement with IVIG, methotrexate, infliximab, or cyclophosphamide have been successfully treated with rituximab.[3] A recent review reported that rituximab is the best alternative biologic agent when combinations with conventional drugs are inadequate in IIM.[14]

In our literature search, we reviewed 31 pediatric cases of anti-SRP myositis reported to date. The majority of the patients were girls (80.7%). The age range at diagnosis was 4-16 years (minimum-maximum). Only 4 patients had nonspecific skin manifestations. Cardiac and pulmonary evaluation results were obtained in 28 and 27 patients, respectively. 7 (25%) patients had abnormal electrocardiograms or echocardiograms and 18 (66.7%) patients had abnormal pulmonary function tests. In addition, three patients had thoracic computed tomography findings compatible with interstitial lung disease. Dysphagia was described in 13 patients. All patients received IVIG or at least one cytotoxic drug in addition to corticosteroid treatment. Despite intensive treatment, 14 patients used wheelchairs. Clinical, laboratory, treatment, and outcome data of anti-SRP myositis patients are summarized in Table I.

Early diagnosis of patients with anti-SRP myositis is important to control inflammation and prevent disease progression and complications. To our knowledge, our patient is the youngest case reported in the literature and was successfully treated with rituximab added to conventional therapy.

Ethical approval

Informed consent was obtained from the parents of the child.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: NŞ, DA, EÇ; data collection: MCP; analysis and interpretation of results: MCP, BT, BÇA; draft manuscript preparation: MCP, EÇ. 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.

Table I. Clinical characteristics of pediatric anti-SRP myositis patients.

Reference	Number of patients	Gender	Age at disease onset	CK levels (U/L)	Cutaneous involvement	Cardiac involvement	Pulmoner involvement	Dysphagia	Treatment	Clinical course
Binns et al.[3]	3	F	14 years	23111	-	-	Abnormal PFTs	+	CS, MTX, IVIG, CYC, RTX	She was at school full-time and able to re-join her physical education and dance classes.
		F	13 years	25937	Periorbital swelling, RP	-	-	-		She had returned to school.
		F	11 years	19808	-	-	Abnormal PFTs, Ground glass opacification on thorax CT	-		NA
Luca et al.[5]	1	F	12 years	8826	RP	-	Abnormal PFTs	+		She was able to participate in dance classes.
Suzuki et al.[6]	2	F	10 years	2467						Wheelchair use
		F	6 years	4629	NA	NA	NA	NA		Not able to get up from a supine position
Rouster-Stevens et al.[7]	3	F	16 years	22155	RP	Abnormal ECHO	Abnormal PFTs, Honeycombing at both bases on thorax CT	-		Wheelchair use
		F	14 years	22857	-	-	Abnormal PFTs, Linear opacities at the bases on thorax CT	-		Wheelchair use
		F	11 years	33000	RP	Abnormal ECHO	Not tested	+		She was unable to run well, but otherwise had normal function.
Kawabata et al.[8]	1	F	15 years	20375	-	-	-	-		She recovered sufficiently to resume normal daily activities.
Kishi et al.[15]	8	5F; 3M	14.9 (10.7-16) years*	NA	RP (50%)	Abnormal ECG or ECHO (50%)	Abnormal PFTs (75%)	50%	NA	Wheelchair use (75%), Devices for mobility (62.5%)

*,Median (IQR).

AZA, Azathioprine; CK, Creatine kinase; CS, Corticosteroid; CYC, Cyclophosphamide; CT, Computed tomography; DLCO, Diffusing capacity of lungs for carbon monoxide; ECG, electrocardiogram; ECHO, Echocardiogram; F, Female; Hq, Hydroxychloroquine; IFX, Infliximab; IVIG, Intravenous immunoglobulin; LFM, Leflunomide; M, Male; MMF, Mycophenolate mofetil; MTX, Methotrexate; NA, Not available; PE, Plasma exchange; PFT, Pulmonary function test; RP, Raynaud's phenomena; RTX, Rituximab; TAC, Tacrolimus.

Table I. Continued.

Reference	Number of patients	Gender	Age at disease onset	CK levels (U/L)	Cutaneous involvement	Cardiac involvement	Pulmoner involvement	Dysphagia	Treatment	Clinical course
Della Marina et al.[16]	1	F	8 years	10710	-	-	Abnormal PFTs	+	CS, MTX, IVIG, RTX	She was able to stand up from the sitting position unsupported, but had to hold onto a railing when climbing stairs.
Momomura et al.[17]	1	F	15 years	20375	-	NA	NA	+	CS, AZA, CYC, PE	She was able to jog.
Zhao et al.[18]	3	F	4 years	4020				-	CS, IVIG	She was able to get up from the floor without assistance.
		F	11 years	4660	NA	-	-	-		She was able to walk without assistance
		F	12 years	13265				+		
Kobayashi et al.[19]	1	M	8 years	5896	-	-	-	-	CS, MTX, IVIG, TAC	He had no weakness.
Rider et al.[20]	1	F	10 years	8316	Erythema of cheeks	-	Abnormal PFTs	NA	CS, MTX, IVIG	NA
Rider et al.[21]	6	4F; 2M	15.1 (12.1-16.2) years*	18544 (9111-22857)*	Edema (16.7%) Cuticular overgrowth (16.7%) RP (50%)	Abnormal ECG or ECHO (50%)	Abnormal PFTs (83.3%)	50%	NA	Wheelchair use (83.3%)
Present case	1	F	3 years	13552	-	-	-	+	CS, IVIG, MTX, MMF, RTX	She was able to walk independently and climb stairs with mild limitation in jumping and running activities

*,Median (IQR).
AZA, Azathioprine; CK, Creatine kinase; CS, Corticosteroid; CYC, Cyclophosphamide; CT, Computed tomography; DLCO, Diffusing capacity of lungs for carbon monoxide; ECG, electrocardiogram; ECHO, Echocardiogram; F, Female; Hq, Hydroxychloroquine; IFX, Infliximab; IVIG, Intravenous immunoglobulin; LFM, Leflunomide; M, Male; MMF, Mycophenolate mofetil; MTX, Methotrexate; NA, Not available; PE, Plasma exchange; PFT, Pulmonary function test; RP, Raynaud's phenomena; RTX, Rituximab; TAC, Tacrolimus.

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Case Report

Polyarteritis nodosa with life-threatening intracranial aneurysms in a child, and treatment with infliximab

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ABSTRACT

Background. Polyarteritis nodosa (PAN) is a rare and serious form of systemic necrotizing vasculitis that predominantly affects medium and small-sized arteries, with central nervous system involvement being particularly uncommon. Treatment strategies are tailored according to the extent and severity of the disease. While conventional therapy includes glucocorticoids and conventional disease-modifying-rheumatic drugs (cDMARDs), biologic agents may be critical for severe and refractory cases.

Case. We report a case of systemic PAN in a 7-year-old girl with no prior medical history, who presented with fever, abdominal pain, and altered mental status. Initial investigations with cranial MRI and echocardiography suggested encephalitis and myocarditis, respectively. Positive SARS-CoV-2 antibodies in both cerebrospinal fluid and serum oriented the diagnosis towards multisystem inflammatory syndrome in children. Despite intensive conventional therapies with glucocorticoids, cDMARDs, and intravenous immunoglobulins, the patient's condition deteriorated. Elevated von Willebrand factor levels, hypertension, and proteinuria emerged, along with stable intracranial hemorrhage and abdominal organ infarctions on imaging, leading to the diagnosis of PAN. Cyclophosphamide was added to the treatment regimen. Three cranial aneurysms were identified on selective conventional cranial angiography. Following angiography, severe intraparenchymal bleeding was detected, leading to emergency cranial surgery. Unresponsiveness to conventional therapeutics led to treatment escalation with a tumor necrosis factor inhibitor, infliximab, resulting in clinical stabilization and allowing for successful endovascular coil embolization.

Conclusion. This case highlights the importance of considering a tumor necrosis factor inhibitor, infliximab, in severe PAN with involvement of intracranial aneurysm.

Key words: polyarteritis nodosa, PAN, infliximab, intracranial aneurysm, COVID-19.

Polyarteritis nodosa (PAN) is a rare and serious systemic necrotizing vasculitis that predominantly affects medium and small-sized arteries.[1-2] Although PAN can manifest at all age groups, its occurrence in children is particularly rare. The incidence of pediatric systemic PAN is estimated to be 0.9 to 1.8 per million.[3] The condition is characterized with a wide range of systemic symptoms that affect multiple organ systems, often resulting in complex complications. Involvement of central nervous system is relatively uncommon, nearly 2-10 % of cases.[4]

Treatment strategies are tailored according to the extent and severity of the disease. For non-severe PAN cases, the standard treatment typically involves glucocorticoids and conventional disease-modifying anti rheumatic drugs (cDMARDs) as the first-line approach.[5] In contrast, for severe and refractory cases, the use

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of biologic agents has shown promising results in the treatment regimen.

Multisystem inflammatory syndrome in children (MIS-C) was observed in patients exposed to SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19). MIS-C is a post-infectious inflammatory syndrome characterized by multiorgan involvement and elevated inflammatory markers. Its presentation might overlap with vasculitis, however, unlike severe vasculitis, the clinical response to the conventional therapy in MIS-C is generally favorable, and the clinical course is mostly mild and short-lasting.[6]

In this report, we present a severe case of PAN following COVID-19 infection, characterized by abdominal organ infarctions and multiple cranial aneurysms that resulted intraparenchymal hemorrhage. Remarkably, the patient's symptoms were alleviated following the initiation of a biologic agent. Our aim is to highlight the complex course and emphasize the potential role of tumor necrosing factor-α (TNF-α) inhibitor, infliximab, in treating severe systemic PAN.

Case Report

A 7-year-old girl, with no prior medical history, was referred to our pediatric emergency department with a six-day history of fever, abdominal pain, and altered mental status. On admission, the patient was pale, disoriented, confused and hypotensive. Her physical examination showed abdominal discomfort, neck stiffness, and nonpalpable purpuric rash on her palms and soles. Initial laboratory investigations revealed leukocytosis with neutrophilic predominance as 29,460 cells/μL, along with elevated acute phase reactants: C-reactive protein 438 mg/L (normal: < 5 mg/L), ferritin 778 ng/mL, procalcitonin 22 ng/mL (normal: <0.5 ng/mL), and fibrinogen 521 mg/dL (normal: 200-400 mg/dL). D-dimer levels exceeded 4400 ng/mL. Echocardiography indicated myocarditis with a reduced ejection fraction and grade 2 mitral valve regurgitation. Cranial MRI revealed widespread scattered millimetric ischemic and hemorrhagic signal changes both in the cerebral and cerebellar hemispheres, consistent with viral encephalitis. A lumbar punction deferred due to the patient's unstable condition and prolonged coagulation parameters. She was admitted to the pediatric intensive care unit with a probable diagnosis of meningoencephalitis and MIS-C. Treatment was initiated, including intravenous immunoglobulin (IVIG) at 2 g/kg, intravenous pulse methylprednisolone at 30 mg/kg/dose, enoxaparin, ceftriaxone, vancomycin, and acyclovir.

Upon achieving stabilization, follow-up cranial MRI and MRI angiography were performed, revealing an increased ischemic area size and a millimetric hemorrhagic focus in the left posterior parietal lobe. Cranial MRI angiography did not reveal any vascular abnormalities. Subsequently, a lumbar punction was performed, showing pleocytosis with lymphocytes (135 cells/mm^3, with 59% of leukocytes), elevated cerebrospinal fluid (CSF) protein levels (0.98 g/L), negative CSF cultures, and a negative meningitis PCR panel. Although the patient and her family had no recent history of COVID-19, and the patient's COVID-19 PCR test result was negative, serological tests revealed a positive SARS-CoV-2 S antibody CSF index of 34, (negative <8, positive >11), and serum levels of 9.19 COI (negative <1.0 COI, positive >1.0 COI).

Serologic tests for antinuclear antibody (ANA), antineutrophilic cytoplasmic antibody (ANCA), and anti-double-stranded DNA (anti-dsDNA) were all negative. Positive results were obtained for anti-cardiolipin IgM (14.57 PL-IgG-U/mL, normal: <12) and anti-beta-2 glycoprotein 1 IgM (44.95 U/mL, normal: <20), while C3 (0.88 g/L, normal: 0.9–1.8) and C4 (0.09 g/L, normal: 0.1–0.4) levels were just below the normal limits, and von Willebrand factor (vWF) antigen levels were elevated at 398% (normal: <160%). The levels of immunoglobulin G, A, and M before IVIG treatment were within normal limits for

the patient's age. Hepatitis B markers, including surface antigen, were negative. The patient had not previously received the COVID-19 vaccine. Serum adenosine deaminase 2 (ADA2) enzyme activity levels were within normal range and no mutations were found in the *ADA2* gene.

The patient became hypertensive, 127/98 mmHg, and developed elevated levels of proteinuria, spot urine protein/creatinine as 0.41 mg/mg, and 24-hour protein levels as 7.8 mg/m^2/hour, with normal serum creatinine, 0.32 mg/dL. Oral cyclosporine, with a dose of 2 mg/kg/day, was added to the treatment regimen to address a probable diagnosis of systemic vasculitis. The presence of elevated vWF, negative ANCA, diastolic hypertension and proteinuria raised a clinical suspicion for PAN. To confirm this diagnosis, abdominal angiography was performed to detect any aneurysmatic alterations, as aneurysms are a hallmark feature of PAN imaging studies. Abdominal computed tomography angiography revealed scattered wedge-shaped non-contrast-enhancing areas in both the kidneys and spleen, and abdominal MRI angiography showed diffusion restrictions in the intestinal walls of the proximal jejunum and ileum. A new parenchymal hemorrhage at the left posterior parietal lobe, measuring 15 x 10 mm, was observed in a follow-up cranial MRI, in addition to the previously noted abnormalities (Fig. 1). A skin biopsy for histopathological evidence was not performed during follow up.

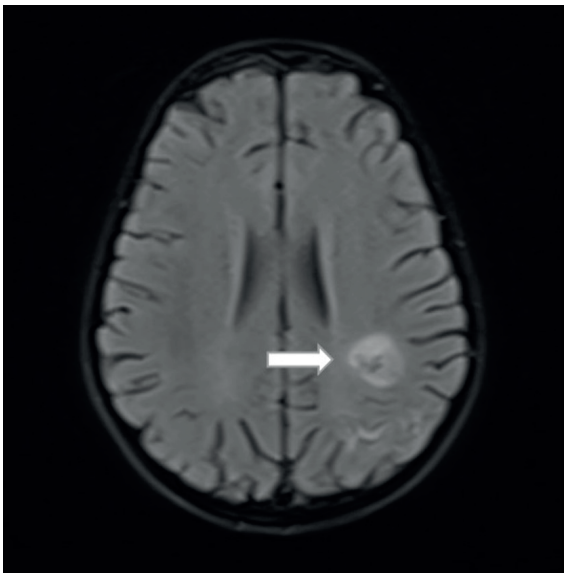

Fig. 1. Cranial magnetic resonance imaging. Axial FLAIR T2-weighted image illustrates newly developed intraparenchymal hemorrhage in the left posterior parietal lobe (arrow).

Given the progressive nature of the vasculitis despite concurrent IVIG administration (1 gr/kg for two days) and six doses of intravenous methylprednisolone (three doses on consecutive days and three doses on every other day), cyclophosphamide at a dose of 500 mg/m^2/dose was initiated, and intravenous prednisolone at 2 mg/kg/day continued. Subsequent selective conventional cranial and abdominal angiography revealed two dissecting aneurysms at the left anterior cerebral artery branches of precentral and precuneal arteries measuring 4 x 3.3 mm and 1.5 x 1 mm consequently and a bilobed dissecting aneurysm at the left medial cerebral artery (measuring 4.5 x 3.6mm, Fig. 2). No other aneurysms were shown in the mesenteric and renal arteries.

Six hours after angiography, the patient developed slurred speech and right hemiparesis. A non-enhanced brain computed tomography confirmed the presence of a large 45 x 30 mm intraparenchymal hematoma in the periventricular white matter at frontoparietal region (Fig. 3). Emergency craniotomy and intracranial hematoma drainage surgery were performed. Postoperatively, the patient was transferred to the pediatric intensive care unit, and prophylactic levetiracetam treatment was initiated. On postoperative day 1, the patient was extubated, and her right hemiplegia showed significant improvement within a week. Subsequent postoperative cranial MRI indicated regression of the intracranial hemorrhage.

Given the presence of two additional intracranial aneurysms in critical locations, a decision was made to initiate biologic therapy. Infliximab, a TNF-α inhibitor, was administered at a dose of 5 mg/kg with biweekly intervals in the first three doses, followed by a subsequent monthly regimen. Following the second infliximab dose,

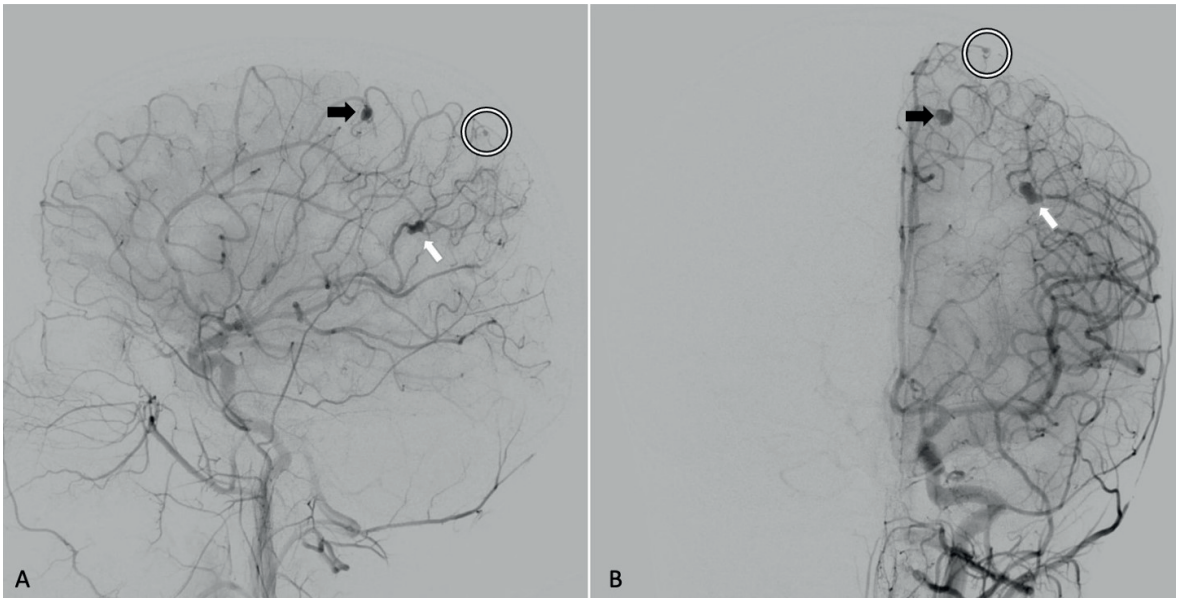

Fig. 2. Selective conventional cranial angiography (A, B). A total of three intracranial aneurysms, which are demonstrated with sagittal **(A)** and coronal **(B)** view images, two at the left anterior cerebral artery within the branches of the precuneal artery (circle), and the precentral artery (black arrow) and one at the branches of left middle cerebral artery (white arrow).

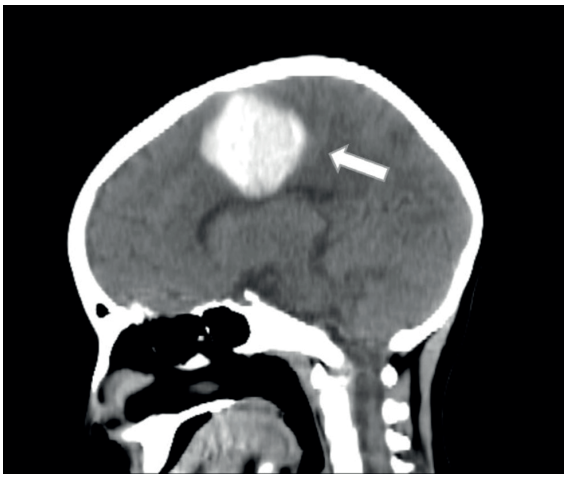

Fig. 3. Non-enhanced brain computed tomography (CT): Sagittal view of brain CT showing a parenchymal hematoma of 45x30 mm opening into the left ventricle in the left centrum semiovale region (arrow).

von Willebrand factor levels notably decreased. A second selective conventional cranial angiography was performed at another facility, and the two remaining cranial aneurysms were successfully coil embolized with no further complications.

The patient's condition remains stable after 7 months of diagnosis, biologic treatment with infliximab continues monthly, while oral prednisolone being tapered down and discontinued within two months. Parental permission for publication was obtained.

Discussion

This case is a rare and life-threatening manifestation of pediatric systemic PAN associated with COVID-19, leading to the development of intracranial aneurysms and subsequent intraparenchymal hemorrhage despite aggressive treatment with high-dose corticosteroids and cyclophosphamide. Infliximab emerged as a critical intervention, leading to clinical improvement where standard treatment failed, suggesting a potential role for biologic agents in managing severe and refractory cases of pediatric PAN cases.[7,8]

The main therapeutic options of PAN

include high-dose corticosteroids and cyclophosphamide, however, their efficacy is less certain in the presence of intracranial aneurysm, due to increased risk of rupture and hemorrhage.[9] Furthermore, significant adverse effects of these medications, such as steroid toxicity and infertility, requires a careful balance between aggressive immunosuppression and the minimalization of treatment related morbidity.[10]

In literature, Ginsberg et al.[7] reported on 26 pediatric and adult PAN patients, among whom 9 were refractory and treated with infliximab following the failure of conventional therapy. Among these 9 patients, only one, a 16-year-old boy, had a cerebral aneurysm. His condition was stabilized with infliximab treatment after four years of follow-up; however, the patient was later found to have an *ADA2* mutation. In that report, it is demonstrated that four months of infliximab is an adequate duration to assess the efficacy of infliximab response.[7] Toyoda et al.[11] highlighted the crucial part of pediatric PAN follow-up with a case of an 8-year-old who, after two years of PAN diagnosis, presented with headache and altered mental status, ultimately experiencing a ruptured superior cerebellar artery, indicating the unpredictable and severe trajectory of PAN. In literature, there are limited number of case reports on refractory systemic pediatric PAN cases with intracranial aneurysms[7,11-14], as demonstrated in Table I.

Table I. Review of pediatric refractory systemic PAN cases with intracranial aneurysm.

Reference, Year	Age / Sex	Clinical Manifestations	Angiography	Treatment	Outcome
Lee[11], 2021	7.7 / M	Fever, myalgia, arthralgia, cutaneous lesions (purpura, skin infarct)	Small microaneurysms in the distal cerebral artery branches	High dose MPS, CYC (6 doses) IFX (5 mg/kg/dose, every 2 weeks)	Remission
Toyoda[12], 2012	8 / F	New onset symptoms: Headache, LOC, right hemiparesis Known systemic PAN at the age of 6: fever, livedo reticularis, subcutaneous nodules, abdominal pain, hypertension	4 mm aneurysm in the left superior cerebellar artery	Emergency craniotomy	Remission
Ginsberg[7], 2019	16 / M	Cutaneous lesions, digital ulcers, seizures	Cerebral aneurysms	CS, CYC, MTX IFX (5 mg/kg/dose, at 0, 2, 6 weeks and every 6 weeks)	Remission
Oran[13], 1999	10 / M	Headache, fever, weakness of both arms, right sided hemiparesis	Two small aneurysms in the right anterior inferior and superior cerebellar arteries Multiple aneurysms in the branches of SMA	CYC	Remission
Sharma[14], 2010	13 / M	Seizure, LOC, facial weakness, fever, weight loss, abdominal pain	Multiple microaneurysms at the distal branches of ICA	CS, CYC	Remission

CS: corticosteroids, CYC: cyclophosphamide, ICA: internal carotid artery, IFX: infliximab, LOC: loss of conciseness, MPS: methylprednisolone, MTX: methotrexate, PAN: polyarteritis nodosa, SMA: superior mesenteric artery.

In the present case, the catastrophic presentation with high fever, altered mental status, multiorgan involvement, with myocarditis, encephalitis and cranial hemorrhage, with unresponsiveness to traditional treatment of pulse MPS and cDMARDs, suggested vasculitis over MIS-C. The elevated SARS-CoV-2 antibody levels in both CSF and serum indicate a recent COVID-19 infection as the cause of the vasculitis. To confirm the diagnosis, a selective angiography was conducted, revealing intracranial aneurysms and fulfilling the mandatory criterion of the Ankara 2008 criteria.[3] Additional criteria met include myalgia, diastolic hypertension, proteinuria and skin involvement in our case. Consequently, this report presents an exceedingly uncommon instance of PAN manifesting in conjunction with COVID-19.

The mechanism of COVID-19-related vasculitis primarily involves the downregulation of angiotensin-converting enzyme 2 by the SARS-CoV-2 virus, which subsequently results in increased levels of angiotensin II. Additionally, the interferon-1 response to the virus decreases levels of nitric oxide.[15] Both of these actions trigger vasoconstriction and endothelial dysfunction. Moreover SARS-CoV-2 triggers an inflammatory response, elevating pro-inflammatory cytokines such as IL-6, TNF-α, and activates thrombotic cascade.[15]

In the largest multicenter cohort study by Batu et al., among 41 patients with COVID-19-related vasculitis, with excluding Kawasaki disease and MIS-C, the most common type of vasculitis observed was immunoglobulin A vasculitis, followed by chilblains, post viral graft vasculitis, ANCA-associated-vasculitis, CNS vasculitis, retinal vasculitis, urticarial vasculitis, cutaneous leukocytoclastic vasculitis and acute hemorrhagic edema of infancy, respectively.[16] There were no cases of COVID-19-related PAN reported in this cohort series. This highlights the diverse nature of vasculitis associated with COVID-19. In literature, there are a limited number of adult cases reports documenting PAN occurring after COVID-19 vaccination.[17]

The differential diagnosis included consideration of the deficiency of adenosine deaminase 2 (DADA2), a rare autosomal recessive genetic disorder presenting with PAN-like vasculopathy. DADA2 leads to an autoinflammatory condition with intermittent fevers, early-onset lacunar strokes, livedoid skin rash, hepatosplenomegaly, and PAN-like vasculopathy with a positive family history.[18] The mutations in *ADA2* gene leads to DADA2 disease and TNF-α inhibitors are the first line therapy.[5,18,19] Although our patient exhibited similar symptoms, the normal ADA2 enzyme levels with absence of *ADA2* gene mutation,DADA2 diagnosis were ruled out.

The mechanism of action of infliximab, which is a monoclonal antibody, is through by blocking both soluble TNF-α and transmembrane TNF-α. Both actions reduce inflammation and modulates the immune responses. In the study by Eleftheriou et al.[20], the evaluation of biologic treatment regimens for primary systemic vasculitis of childhood revealed therapeutic responses with infliximab showed a significant decline in the median daily prednisolone doses and showed reduced vasculitis activity scores. In a narrative review evaluating PAN cases treated with biologic therapy, a total of 30 cases received infliximab.[21] Of these cases, only 3 did not achieve complete remission, and 4 experienced adverse effects, including cerebral abscess, bacterial sepsis, pertussis, and allergic reaction. Despite these side effects, infliximab's efficacy as a therapeutic option in managing resistant cases of systemic PAN patients is promising.

The multidisciplinary management approach was essential for pediatric PAN patients. Individualized step-up treatment regimens and close monitoring are crucial in optimizing for the treatment success of cases. The attempt at endovascular coiling following the stabilization of necrotizing vasculitis with infliximab provided a favorable prognosis and procedural success, as evidenced by the absence of complications post-embolization in our case.

In conclusion, this case demonstrates the complexity of managing severe pediatric PAN with multiple intracranial aneurysms. It supports the consideration of infliximab as an effective alternative option in children with refractory PAN. Additionally, this case also advocates for a reevaluation of the potential etiologies for PAN in the post-pandemic era. Further research is warranted to enhance this rare condition and refine treatment strategies.

Ethical approval

Informed consent was obtained from the legal guardians of the patient for the publication of the case report.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SAU, BS; data collection: SAU; analysis and interpretation of results: SAU, MD, BS; draft manuscript preparation: SAU, BS. 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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