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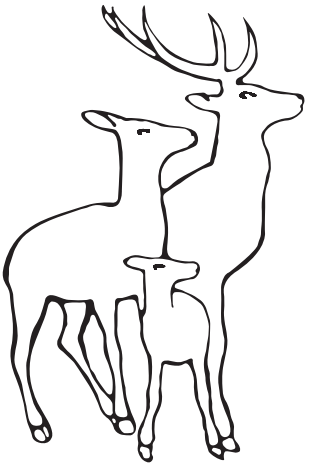

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Original Article

Surface triggering receptor expressed on myeloid cells-1 (sTREM1) in critically ill children: a prospective observational controlled study

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ABSTRACT

Background. In children admitted to the pediatric intensive care unit (PICU), early detection of risk factors and alarming indicators improves the prognosis and may even save lives. Several prognostic markers and scores have been studied in children who are seriously ill. Recently, surface triggering receptor expressed on myeloid cells-1 (sTREM1) has been studied in many infectious and non-infectious settings; however, there is little information on critically ill children. Our aim is to evaluate the sTREM1 level in critically ill children and assess its prognostic role.

Method. A prospective observational study was conducted in a tertiary care hospital. 70 critically ill children and 50 healthy controls were enrolled in the study. Demographic, clinical, and laboratory data were obtained. sTREM1 level was assessed on admission to the PICU. Patients with conditions affecting immunity were excluded. The primary outcome was to assess the level of sTREM1 in both patients and controls. Secondary outcomes were mortality, morbidities as sepsis, need for mechanical ventilation, and PICU stay.

Results. The level of sTREM1 was significantly higher in patients than in controls (850 pg/mL, interquartile range [IQR] 510.0- 1375.0 vs. 67.5 pg/mL, IQR 40.0- 85.0; p<0.001). sTREM1 level was significantly higher in non-survivors (p <0.001), patients with sepsis (p = 0.0028), and in patients who were mechanically ventilated (p <0.001). sTREM1 level had a significant positive correlation with the duration of PICU stay (r=0.624, p <0.001), and the duration of mechanical ventilation (r=0.527, p <0.001). On ROC curve analysis, sTREM1 was the most significant diagnostic marker compared to lactate and procalcitonin, at a cutoff value of 680 pg/mL, with a sensitivity of 93.8% and a specificity of 61.1% with an area under the curve of 0.862.

Conclusion. In critically ill children, sTREM1 has prognostic and diagnostic values. There were associations between sTREM1 and the severity of the disease. To validate our results, subgroup analysis and multicenter trials are necessary.

Key words: sTREM1, critically ill children, sepsis, markers, triggering receptors.

Critically ill children need special care and monitoring, and are usually cared for in the pediatric intensive care unit (PICU). PICU populations are usually heterogeneous, as there will be several medical and surgical disorders, each differing in their hemodynamic, inflammatory state, and presence or absence of infections.[1] Early identification of risk factors and alarming signs in the PICU population helps to save their lives and improve the prognosis.

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Also, risk stratification of the patients helps to intervene early and allocate the appropriate level of care for each patient according to their condition, thus improving the patient outcome and saving resources, especially in resource-limited countries.[1-3]

Numerous prognostic indicators and scores have been examined in critically ill children. Mortality scores have been the most reliable of these indicators, namely Pediatric RISk of Mortality (PRISM) and Paediatric Index of Mortality-2 (PIM2) scores.[2,3] The search for prognostic markers has gained popularity in pediatric research.[1,4] Procalcitonin (PCT) and C-reactive protein (CRP), two frequently used diagnostic markers, are less sensitive and specific. As a result, research is being conducted on novel markers of acute inflammation, among them are surface triggering receptor expressed on myeloid cells-1 (sTREM1) and interleukin-6 (IL-6).[4]

Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily member that is a cell surface protein. The human TREM1 is a type of DAP12-related receptor that is a 30-kDa glycoprotein located on the 6p21 chromosome.[5-7] Mature monocytes, neutrophils, and macrophages all express it. As a pro-inflammatory marker, TREM1 increases inflammation upon exposure to extracellular bacterial and fungal pathogens.[8] TREM1 has been investigated as a marker for pneumonia, neonatal sepsis, neonatal ventilator-associated pneumonia (VAP)[9], pediatric multisystem inflammatory syndrome (MIS-C)[10], pediatric sepsis, and septic shock[11], and in oncology patients with febrile neutropenia.[12] A multitude of research has found that it has a prognostic value in these populations. Additionally, sTREM1 has been investigated in non-infectious situations recently, with varying degrees of efficacy and limited data on pediatric patients. However, sTREM1 has not been investigated in many other critical illnesses in children. Hence, the aim of this study is to assess the role of sTREM1 as a prognostic marker in critically ill children.

Materials and Methods

This was a prospective observational study that was carried out on 70 critically ill children who were admitted to the PICU of Menoufia University Hospital in the period from May 2022 to August 2023 (Fig. 1). Additionally, 50 healthy age- and sex-matched children were enrolled as a control group. A written informed consent was obtained from the parents of the participant children after explaining the aim and procedures of the study. The study was approved by the Ethics Committee of Menoufia University – Faculty of Medicine (IRB 5-20222ped27) Ethics Committee.

All critically ill children admitted to the PICU with infectious and non-infectious conditions, aged 1 month to 18 years were included in this study. Patients with congenital and acquired immunodeficiency, malignancy, acute and chronic liver diseases, and chronic

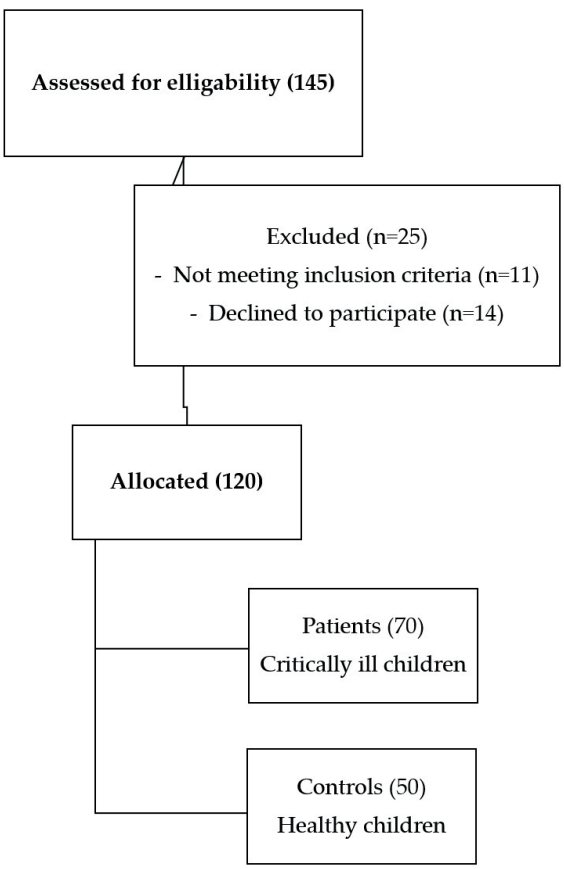

Fig. 1. Flow chart of the included subjects.

inflammatory diseases, and those receiving immunosuppressive medications were excluded.

Detailed history, physical and clinical examination for all participants were recorded. Oxygen saturation (SpO_2), severity of the underlying medical condition, PRISM score, diagnosis category according to primary diagnosis and comorbidities were assessed.

The primary outcome was the level of sTREM1 in patients and controls. Secondary outcomes were the occurrence of mortality, sepsis, need for mechanical ventilation, and length of PICU stay.

Laboratory investigations such as complete blood count, kidney function tests (serum urea and creatinine), liver function tests, serum electrolytes, arterial blood gas, serum lactate, CRP, PCT and sTREM1 levels were conducted.

Serum samples were collected for sTREM1 measurement for all patients on admission to the PICU. For serum separation, two milliliters of blood were drawn from a peripheral vein and placed in special clot activator tubes. The blood was then aliquoted and frozen at -80°C right away for additional analysis. The Human TREM-1 (DuoSet ELISA kit, R and D Systems, Minneapolis, MN, USA) was used to quantify sTREM1 by the manufacturer's instructions. The findings were expressed as pg/mL.

Statistical Package for the Social Sciences (SPSS) version 23 (Armonk, NY: IBM Corp.) was used on a personal computer to gather, tabulate, and statistically analyze the data. There were two sections of statistics, descriptive statistics (the presentation of quantitative data as median and range when data were non-normally distributed) and analytical statistics (chi-square test and Student t test). Mann-Whitney test is a nonparametric test (U) used when data are non-normally distributed. The ROC (receiver operating characteristic) curves were utilized for comparing the diagnostic role of different markers. Logistic regression test was performed for prediction of mortality by sTREM1 and other variables. The P value of < 0.05 was considered a significant level.

Results

The patient group consisted of 70 critically ill children, and the control group consisted of 50 healthy children. The main demographic, clinical, and laboratory characteristics are demonstrated (Table I).

The sTREM1 level was significantly higher in the patient group compared to controls (Table I). sTREM1 level was associated with different outcomes; it was significantly higher in patients who were ventilated than those non-ventilated, and in those who died than those who survived. Also, it was significantly higher in patients who developed sepsis than in those that did not (Table II).

There was a statistically significant positive correlation between sTREM1 with PICU stay ($r=0.624$, $p=0.001$) and with duration of mechanical ventilation ($r^s=0.527$, $p<0.0001$).

On comparing surviving and non-surviving patients regarding different parameters and outcomes, we noticed a significant difference in PRISM score, sTREM1 level, lactate, PCT, CRP, serum transaminases, and need for mechanical ventilation (Table III).

On ROC curve analysis, the area under the curve for sTREM1, PCT, and lactate were 0.862, 0.793, and 0.810, respectively. Additionally, sTREM1 had a higher area under the curve compared to PCT and lactate levels, and it was the most significant diagnostic tool marker in critically ill children at a cutoff value of 680.0 pg/mL, with a sensitivity of 93.8%, and a specificity of 61.1% with an area under the curve of 0.862 (Table IV, Fig. 2).

Binary logistic regression analysis for the parameters affecting survival and mortality rate showed that PRISM score, CRP, lactate, PCT, and sTREM1 were negatively associated with survival (Table V).

Table I. Demographic, clinical, and laboratory data of the patients and controls.

Variable	Patients (N = 70)	Controls (N = 50)	P- value
Age (months)an (IQR)	13 (2.0- 192.0)	18 (4.0- 48.0)	0.270
Male sex	38 (54.2%)	19 (38%)	0.17
Weight (kg)	9 (3.0- 53.0)	11.3 (6.0- 17.0)	0.078
Height (cm)	72.5 (49.0- 162.0)	84 (63.0- 105.0)	0.023*
BMI (kg/m^2)	5.9 (2.7-16.8)	6.9 (4.7- 14.5)	0.115
Primary diagnosis:			
Respiratory disease	32 (45.71%)	NA	NA
Cardiac disease	18 (25.71%)		
Neurologic	11 (15.71%)		
Others	9 (12.86%)		
Hemoglobin (g/dL)	10.2 (2.80- 15.90)	11.9 (10.5- 12.5)	<0.001*
Platelet count (x10^3/µL)	322 (16.0- 797.0)	345 (228.0- 453.0)	0.745
White blood cells (x10^3/µL)	12.8 (4.90-41.00)	9.9 (8.4-12.5)	<0.001*
Urea (mg/dL)	35 (11.0-120.0)	24 (16.0-32.0)	<0.001*
Creatinine (mg/dL)	0.7 (0.20-5.70)	0.5 (0.2-0.7)	0.118
SGOT (U/L)	45 (10.0-454.0)	26 (16.0-65.0)	0.003*
SGPT (U/L)	32 (10.0-539.0)	18 (14.0-25.0)	<0.001*
Na (mEq/L)	139 (126.0-156.0)	139 (136.0-145.0)	0.853
K (mEq/L)	4.2 (1.90-7.20)	4.3 (3.4-4.8)	0.981
Ionized Ca (mmol/L)	0.9 (0.70-9.40)	1.1 (0.90-1.20)	0.005*
sTREM1 (pg/mL)	850 (510.0- 1375.0)	67.5 (40.0- 85.0)	<0.001*
Mortality	16 (22.9%)	NA	NA
Need for MV	29 (41.4%)	NA	NA
Sepsis	25 (35.7%)	NA	NA

Numerical data presented as median (interquartile range), categorical data as n (%). BMI: Body mass index, MV: Mechanical ventilation, NA: Not applicable, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, sTREM1: Surface triggering receptor expressed on myeloid cells-1, *: p<0.05.

Table II. Association between sTREM1 levels and different outcomes.

	sTREM1	P value
Survivor	760 (510-1300)	<0.001*
Non-survivor	1130 (660-1375)	
Sepsis	950 (625-1375)	0.0028*
No sepsis	800 (510-1375)	
Mechanical ventilation	1010 (510- 1375)	< 0.001*
No mechanical ventilation	740 (550- 1125)	

Mann-Whitney U test. sTREM1 levels presented as median (interquartile range) in pg/mL. PICU: Pediatric Intensive Care Unit, sTREM1: Surface triggering receptor expressed on myeloid cells-1, *: Significant.

Table III. Comparison between survived and non-survived children regarding demographic, clinical, and laboratory parameters.

	Survived (n=54) Median (IQR)	Non- survived (n=16) Median (IQR)	P value
Age (months)	12 (2-192)	13.5 (2-160)	0.900
Weight (kg)	8.8 (3-53)	9.5 (3.5-39)	0.905
Height (cm)	72.5 (49-162)	73 (53-152)	0.801
BMI (kg/m^2)	5.9 (2.7-16.8)	6.3 (3.1-12.8)	0.978
Male sex	27 (50.00%)	11 (68.75%)	0.186
PRISM score	3 (0-8)	7 (3-9)	<0.001*
Procalcitonin (ng/mL)	0.7 (0.2-4.5)	1.5 (0.5-18.9)	<0.001*
Lactate (mmol/L)	2.3 (0.5-4.0)	3.5 (1.30-3.80)	<0.001*
sTREM1 (pg/mL)	790 (510-1300)	1130 (660-1375)	<0.001*
Mechanical ventilation	13 (24.07%)	16 (100.00%)	<0.001*
Hemoglobin (g/dL)	10.2 (6.5-15.9)	10.2 (2.8-12.1)	0.675
Platelet count (x10^3/μL)	313.5 (16.0-797.0)	367.0 (45.0-672.0)	0.454
White blood cells (x10^3/ μL)	12.6 (5.5-30.1)	13.0 (4.9-41.0)	0.944
CRP (mg/dL)	24.0 (0.0-158.0)	42.5 (12.0-180.0)	0.296
Urea (mg/dL)	35.0 (11.0-120.0)	35.0 (15.0-69.0)	0.752
Creatinine (mg/dL)	0.6 (0.2-5.7)	0.7 (0.2-2.1)	0.927
SGOT (U/L)	40.0 (10.0-352.0)	72.0 (27.0-454.0)	0.005*
SGPT (U/L)	25.0 (10.0-539.0)	45.5 (18.0-357.0)	0.007*
Na (mEq/L)	139.0 (126.0-156.0)	140.0 (126.0-150.0)	0.580
K (mEq/L)	4.3 (2.2-7.2)	4.0 (1.9-5.2)	0.125
Ca (mmol/L)	0.9 (0.7-9.4)	1.1 (0.7-4.2)	0.566

Numerical data presented as median (interquartile range) and analyzed with Mann-Whitney U test, categorical data presented as n (%) and analyzed with chi-square test. BMI: Body mass index, CRP: C-reactive protein, MV: Mechanical ventilation, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, TREM: Triggering receptor expressed on myeloid cells, *: Significant.

Table IV. ROC curve to detect sTREM1, procalcitonin and lactate levels as a marker in critically ill children.

Variable(s)	AUC	Std Error	P value	Sensitivity	Specificity	Cutoff value	95% CI of AUC	
							Lower	Upper
sTREM1 (pg/mL)	0.862	0.051	<0.001	93.8%	61.1%	680.0	0.76	0.96
Procalcitonin (ng/mL)	0.793	0.057	<0.001	68.80	35.69	1.25	0.682	0.905
Lactate (mmol/L)	0.810	0.059	<0.001	80.13	40.70	14.50	0.713	0.930

AUC: Area under the ROC Curve, CI: Confidence interval, sTREM1: Surface triggering receptor expressed on myeloid cells-1.

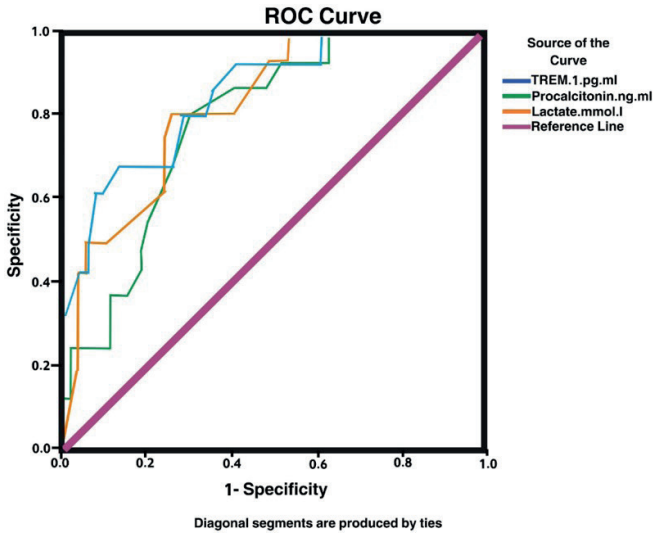

Fig. 2. ROC curve to detect sTREM1, procalcitonin and lactate levels as markers in critically ill children. sTREM1 had a higher AUC and it was the most significant diagnostic tool marker in critically ill children at a cutoff value of 680.0, with a sensitivity of 93.8%, and a specificity of 61.1% with an AUC of 0.862, as compared to procalcitonin and lactate levels. AUC: area under the curve, ROC: receiver operating characteristic, sTREM1: surface triggering receptor expressed on myeloid cells-1.

Table V. Binary logistic regression analysis for the for the parameters affecting survivor and mortality rate.

Variables	B	S.E.	Wald	Sig.	Exp(B)	95% C.I. for EXP(B)	
						Lower	Upper
Age (months)	-0.005	0.007	0.573	0.449	0.995	0.981	1.008
Weight (kg)	-0.024	0.030	0.636	0.425	0.976	0.919	1.036
Height (cm)	-0.013	0.012	1.152	0.283	0.987	0.964	1.011
BMI (kg/m^2)	-0.074	0.098	0.576	0.448	0.928	0.766	1.125
PRISM score	0.720	0.171	17.779	0.0001	2.053	1.470	2.869
Hemoglobin (g/dL)	-0.273	0.145	3.547	0.060	0.761	0.573	1.011
Platelet count (x10^3/µL)	0.002	0.002	0.754	0.385	1.002	0.998	1.005
White blood cells (x10^3/µL)	0.038	0.039	0.950	0.330	1.039	0.962	1.123
CRP (mg/dL)	0.014	0.007	4.063	0.044	1.014	1.000	1.027
Urea (mg/dL)	-0.002	0.013	0.033	0.855	0.998	0.972	1.024
Creatinine (mg/dL)	-0.149	0.372	0.160	0.689	0.861	0.415	1.787
SGOT (U/L)	0.008	0.004	3.299	0.069	1.008	0.999	1.017
SGPT (U/L)	0.001	0.004	0.082	0.774	1.001	0.993	1.010
Na (mEq/L)	-0.061	0.058	1.089	0.297	0.941	0.840	1.055
K (mEq/L)	-0.606	0.407	2.220	0.136	0.545	0.246	1.211
Ca (mmol/L)	-0.004	0.330	0.000	0.990	0.996	0.522	1.900
Procalcitonin (ng/mL)	-0.970	0.323	9.000	0.003	2.637	1.400	4.969
Lactate (mmol/L)	-0.403	0.054	3.613	0.050	1.109	0.997	1.233
sTREM1 (pg/mL)	-0.005	0.002	6.868	0.009	1.005	1.001	1.009

BMI: Body mass index, CI: Confidence interval, CRP: C-reactive protein, PRISM: Pediatric Risk of Mortality Score, SGOT: serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, sTREM1: Surface triggering receptor expressed on myeloid cells-1, *significant.

Discussion

Much research on critically ill children has focused on identifying predictors of mortality and other important outcomes. The quick identification of such people emphasizes how crucial it is to make correct and speedy identification upon admission to the PICU so that proper therapies can be started. This offers the potential to lead to better outcomes.[1-4]

In this study, we investigated the role of sTREM1 in critically ill children. This marker has been investigated in many infectious and inflammatory conditions in children and adults. However, the data on critically ill children is sparse. We found that the sTREM1 level was significantly higher in patients than in controls, and it was also significantly higher in non-survivors than in survivors, which emphasizes its prognostic role. This could be explained by conditions with severe inflammation express much proinflammatory mediators and metalloproteinases that cleaves the soluble form sTREM1 from the membrane anchored TREM1. This sTREM1 can amplify the innate immune response. Neutrophil degranulation, cell survival, and strong production of tumor necrosis factor (TNF)-α, IL-6, IL-1β, IL-8, monocyte chemoattractant protein-1 (MCP-1), IL-12p40, and granulocyte monocyte colony stimulating factor (GM-CSF) by monocytes/macrophages and dendritic cells are all outcomes of activation of the TREM-1 pathways. These processes increase inflammation during infection by various pathogens, including influenza, dengue, and hepatitis C, as well as in chronic inflammatory diseases.

Similar results were illustrated in a study by Şen et al.[8], who showed higher sTREM1 levels in patients with sepsis and septic shock, and this also had a prognostic role with regards to mortality and other outcomes. They explained their findings by the predominant pro-inflammatory mediators in patients with more severe forms of sepsis and septic shock, highlighting the prognostic value of sTREM1 in the early detection of patients requiring special care.

Additionally, Yang et al.[9] demonstrated that sTREM has diagnostic and prognostic roles in children with VAP. Conversely, sTREM1 was also investigated in children with febrile neutropenia; however, other markers, such as IL-8, were found to be superior in predicting the prognosis.[9] Gonçalves et al.[10] investigated the role of sTREM1 in children with MIS-C, and found that it has value as an early screening tool in children with MIS-C.

In relation to different outcomes, the current study investigated the level of sTREM1 in patients who developed sepsis and those who did not. We found that patients with sepsis had significantly higher sTREM1 levels. This indicates that sTREM1 is not only a marker of inflammation but also a marker of infection. This may be explained by the fact that sTREM1 levels are higher in sepsis due to the host organism's cytokine storm, which promotes inflammation that worsens with the severity of sepsis.[10]

Similar results in pediatric sepsis research were found by Leligdowicz et al.[13], who showed that sTREM1 can be used as a rapid triage test in febrile African children at risk of sepsis. Şen et al.[8] investigated the role of sTREM1 and IL-8 in differentiating sepsis, severe sepsis, and septic shock in children, and found that patients with septic shock had higher sTREM1 levels than those with severe sepsis. However, in sepsis, there was no correlation between sTREM1 levels and death, and they recommended that when determining the prognosis of child sepsis, sTREM1 readings should be applied with caution. Conversely, Smok et al.[4] found that PCT and IL-6 had a greater role in systemic inflammatory response syndrome, sepsis detection and prognosis, and further studies are required regarding the role of sTREM1.

Adult studies on patients with COVID-19 have investigated the role of sTREM1 as a prognostic marker and have stated that it is possible to

measure sTREM1 in bodily fluids. Severe cases of inflammatory illnesses generally lead to a rise in this biomarker, which is considered an inflammatory and predictive measure of sepsis. Severe and critical types of COVID-19 are the primary causes of the rise in sTREM1 plasma levels, indicating that this molecule may be a useful predictor of a poor prognosis.[10]

In relation to mechanical ventilation, the current study demonstrated a higher sTREM1 level in patients who were ventilated than those who did not require mechanical ventilation, which indicates that sTREM1 could be used as a marker of poor outcomes. There was a significant positive correlation between sTREM1 and the duration of mechanical ventilation. Similar to our results, much research found that sTREM1 was higher in patients who were ventilated, especially those who had VAP, both in children and neonates.[9]

In relation to PICU length of stay, there was a significant positive correlation between sTREM1 level and duration of PICU stay. This may be related to many factors; those children had more severe disease, more need for mechanical ventilation, and developed sepsis. These results may be utilized to predict critically ill children's prognosis in advance and to guide early intervention to stop their rapid deterioration.

In relation to other biomarkers of infection and inflammation, several markers of inflammation and infection have been used in PICU to predict the severity and prognosis of critically ill children, of these are CRP, albumin, lactate, and PCT besides clinical scores as PRISM. Elevated blood lactate or impaired lactate clearance have been considered prognostic markers in different PICU patients including shock, sepsis, inflammatory conditions, clinically suspected sepsis.[1,14-16] and postoperative cardiac patients with congenital heart disease.[17] CRP has been used as a marker correlating with infection and inflammation. PCT was presented as a novel and innovative indicator of infection. Bacterial endotoxins and exotoxins, together with inflammatory cytokines, are known to induce PCT.[12] Assicot et al.[18] have reported a high serum PCT in patients with sepsis and bacterial infection.

In this study, ROC curve analysis showed that sTREM1 was the most significant diagnostic tool marker in critically ill children compared with lactate and PCT, at a cutoff value of 680 pg/mL, with a sensitivity of 93.8% and a specificity of 61.1% with an area under the curve (AUC) of 0.862, as compared to PCT and lactate levels. However, binary logistic regression analysis indicates that PRISM score, PCT, and lactate in comparison with sTREM1 are statistically significant predictors of the outcome of critically ill children, where every one-unit change in sTREM1 level will increase mortality by 1.005 while the change in PRISM score, PCT, lactate, and CRP are more influential in influencing the outcome in comparison with sTREM1 (odds ratios 2.053, 2.617, 1.109 and 1.014, respectively).

Similar to our study, much research has compared different markers in many conditions in the PICU. Arslan et al.[1] have reported that a lactate cut-off value of 5.55 mmol/L resulted in an AUC of 0.79 in a study performed on 1109 critically ill children.[15] Another study conducted on patients with septic shock showed that lactate levels higher than 4 mmol/L have a good prognostic role in mortality.[16]

Conversely, Miedema et al.[12] investigated the role of multiple markers in diagnosing severe bacterial infection in oncology children with febrile neutropenia, and found that sTREM1 and other markers such as CRP and PCT are less useful markers for early detection compared to IL-8.

Interestingly, combining biomarkers has shown to have a greater value in diagnosis and determining the prognosis of critically ill children; Sdougka et al.[19] mentioned that studies conducted on patients with VAP have shown significant discriminative value of the combined biomarkers in differentiating infectious and non-infectious causes.[20-22]

Additionally, measuring a panel of biomarkers at different times in addition to clinical variables and scores has a better predictive power in those populations. Abdelaziz et al.[17] have shown that combining markers as lactate and central venous oxygen saturation ($ScvO_2$) have a greater prognostic role in children after surgery for congenital heart disease.

We compared survivors with non-survivors in terms of mortality and discovered that, along with other parameters including CRP, PCT, serum glutamate pyruvate transaminase, and PRISM score, non-survivors had considerably greater sTREM1. In order to investigate the factors influencing mortality, we also performed a logistic regression analysis. The results showed that PRISM score, CRP, lactate, PCT, and sTREM1 were all adversely correlated with survival.

Based on the results of our work and previous research, sTREM1 may be considered an accurate triage tool because it is triggered by cytokines and inflammatory mediators and its level increases as the severity of the primary sickness increases. As so, it could facilitate early decision-making and intervention.

Strong points in this study are the precise inclusion criteria, comprehensive data collecting on clinical outcomes and standard-of-care testing. It is remarkable that these biomarkers can predict a patient's likelihood of survival when they have hyperinflammatory conditions like sepsis. The prompt identification of these patients highlights how important it is to accurately and promptly identify them upon PICU admission in order to initiate the appropriate medication. There is a chance that this will produce better results.

The study also has several limitations: Subgroup analysis is required for better interpretation. The study's single-center design limits the findings' applicability to other populations. A multicenter study design is recommended for better validation of the results. Additionally, sTREM1 is better measured serially than in a single measurement; this might correlate better with the disease progression.

Conclusion

sTREM1 has a diagnostic and prognostic value in critically ill children. Compared to other markers of infection and inflammation, sTREM1 has shown comparable correlation with disease severity. Multicenter studies are required to validate our results with subgroup analysis.

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Ethical approval

The study received approval from the Ethical Committee of Faculty of Medicine – Menoufia University (date: 01-05-2022, IRB number: 5-2022ped27).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AAA, ER; Data collection, AAA, ER, SHAE, TAO. Analysis and interpretation of results: ER, AAA, TAO. Draft manuscript preparation: AAA, SHAE, TAO, ER. 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

Evaluation of clinical findings in cases of child sexual abuse

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ABSTRACT

Background. Child sexual abuse (CSA) is a severe problem with physical, emotional, social, moral, cultural, and legal dimensions, which is estimated to be quite common worldwide.

Methods. In this retrospective study including 262 CSA cases admitted to our outpatient clinic between 2013 and 2022, we examine the characteristics of the victim and the abuse and examination findings in cases of CSA.

Results. In our study, most of the victims were females over the age of 12, and most of the suspects were known to the child. Approximately half of the incidents (50.1%) occurred in a home. These data are essential in determining the risk factors of CSA cases and in terms of early detection and timely prevention. Vaginal penetration (40.8%) and anal penetration (32.4%) are frequently present in the history of CSA victims. A history of vaginal penetration was associated with acute and healed laceration findings in the hymen ($p<0.05$). Similarly, a history of anal penetration was associated with decreased sphincter tone, ecchymosis and abrasion in the anal mucosa, reflex anal dilatation, and acute anal fissure findings ($p<0.05$). As the time between the abuse and the examination increased, examination findings decreased in the anogenital region, which can heal quite quickly and without leaving a trace.

Conclusions. Preventing CSA is possible by analyzing risk factors well and reaching at-risk cases as early as possible. Acute and non-acute hymeneal lacerations that may be associated with vaginal penetration, anal fissure and other traumatic findings that may be associated with anal penetration should be carefully reviewed. However, it should be kept in mind that anogenital injuries with rapid regeneration ability may heal without leaving any symptoms and abuse may occur without leaving any symptoms.

Key words: child sexual abuse, anogenital examination.

Child sexual abuse (CSA) is a worldwide severe problem with physical, emotional, social, moral, cultural, and legal dimensions.[1,2] The prevalence of CSA varies according to the characteristics of the population and the definition used. Since it is widespread that many victims of sexual abuse never report the abuse, it is not possible to obtain precise statistical data on the prevalence of CSA.[3,4]

Determining the risk factors of child sexual abuse and taking measures in this direction is very important in terms of preventing the recurrence of the abuses that have occurred and preventing the abuses that are likely to occur. In a meta-analysis conducted by Assink et al.[5] on risk factors for CSA victimization, it was reported that the child being of the female gender, the child and family members being

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victims of forensic cases (sexual assault or other), parental problems such as mental health problems and intimate partner violence, low parental competence, poor quality parent-child relationship, non-nuclear family structure, family problems, and child problems were closely related to CSA.

The findings to be detected in the examination of CSA cases, which require experience and meticulousness from the social and pediatric-gynecological history of the child to anogenital examination, play an essential role in revealing sexual abuse. These findings include concrete medical findings such as ecchymosis, abrasions, acute lacerations, healed (non-acute) lacerations (transection) and decreased anal sphincter tone.[6-9] Adams et al.[10-12] published a series of articles that serve as a guideline for evaluating anogenital findings, which was last updated by Kellogg et al.[13] in 2023. Distinguishing these traumatic findings from normal anatomy and anatomical variations and evaluating them as concrete findings of sexual abuse is of utmost importance for the protection of the victim from possible repeated sexual abuse and more severe physical and psychological consequences and a fair trial process. Factors that may increase the incidence of concrete findings of sexual abuse, such as the time between sexual contact and examination, type of sexual contact (with or without penetration), pain, and bleeding symptoms, have been frequently researched in the literature. In addition, it should be kept in mind that sexual abuse may occur without leaving any traumatic findings. Since it is known that the perianal region heals rapidly without leaving any findings, the anogenital examination may not reveal any findings to support sexual abuse, especially in cases where time has elapsed.[14-16]

In our study, we analyzed the socio-demographic characteristics of the cases that were admitted to our outpatient clinic and the characteristics of sexual abuse. We compared and discussed the medical history and examination findings of the children with the literature data. One of the aims of our study is to prevent CSA by contributing to the early detection of children at risk of CSA. In addition, there are some sexual abuse examination findings in the literature that do not have an expert consensus. This study, in which we present the history and examination findings of CSA, will contribute to the literature in developing these controversial findings. An important hypothesis of our study is to emphasize how useful examinations performed on time and by well-equipped physicians in accordance with the literature information are in revealing children who are victims of sexual abuse.

Materials and Methods

Our study was initiated with the approval of Pamukkale University Non-Interventional Clinical Research Ethics Committee dated 31.05.2023 and numbered E-60116787-020-374178.

CSA suspected cases are referred to our outpatient clinic for CSA examination with an official document by the prosecutor's office or the criminal courts. CSA examinations are performed in cases referred to the outpatient clinic of Pamukkale University Faculty of Medicine, Department of Forensic Medicine after obtaining the consent of the children and the written informed consents of the parents or children's guardians. All archive records in our archive between 2013 and 2022 were retrospectively reviewed. By analyzing the archive files, 262 cases were referred for CSA examination, and the number of cases examined and reported was determined from a total of 1991 cases under the age of 18. Age, gender, degree of closeness of the suspect (intra-familial; father, sibling, husband, relative, extra-familial; boyfriend, engaged, neighbor, friend, stranger), location of the incident, repeated sexual abuse, duration of application to forensic units (0-5 days, 6-10 days, 11-30 days, 1-3 months, 4-12 months and >1 years), type of sexual abuse (including oral, anal and vaginal penetration, not including penetration) and acute (including bleeding, edema or inflammation) or non-

acute (healed trauma findings) anogenital examination findings were categorized. Sample size was calculated using G*Power 3.1.9.7. Using a large effect size (w = 0.50), alpha of 0.05, 95% power and a 5% margin of error, a sample of 119 participants was calculated.

Statistical evaluations were performed using the SPSS (Statistical Package for the Social Sciences) version 21.0 package program. Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as number and percentage. Shapiro Wilk test was used to examine the suitability of the data for normal distribution. Mann Whitney U test was used for the data for which parametric test assumptions were not met. The chi-square test was used for categorical variables. The significance value was accepted as $p<0.05$ at a 95% confidence interval in the analyses.

Results

Of the 262 CSA cases included in our study, 23.7% (n=62) were male and 76.3% (n=200) were female. The mean age of males was 11.68 years (SD=3.458), and the mean age of females was 13.73 years (SD=3.273). A significant difference was found between the ages of male and female cases (U=3735.50, $p<0.001$). When the cases were categorized as 0-2 years, 3-6 years, 7-11 years, and 12-17 years according to their ages, it was seen that the 12-17 age group, which constituted the majority of the cases (77.5%), was the most frequently admitted.

When the cases were analyzed in terms of recurrent sexual abuse and the degree of closeness of the suspects recorded in the history, it was found that 43.1% (n=113) of 262 sexual abuse cases had a history of recurrent sexual abuse. It was observed that 81.8% (n=9) of 11 cases who were alleged to have been sexually abused by their fathers had a history of recurrent sexual abuse. It was found that 83.3% (n=10) of 12 cases who were alleged to have been sexually abused by a stranger had a history of non-recurrent sexual abuse, and these findings were statistically significant ($p<0.05$, Table I).

When the presentation times of the cases were analysed, it was observed that 49.2% (n=129) of the cases were presented more than 1 month later (Fig. 1).

Table I. Distribution of recurrent and non-recurrent sexual abuse cases according to the degree of closeness of the suspects.

Suspect	Non-recurrent, n (%)	Recurrent n (%)	No information, n (%)	Total
Person known by the victim*	54 (48.6)	51 (45.9)	6 (5.4)	111
Intimate partner **	29 (55.8)	22 (42.3)	1 (1.9)	52
Officially married husband	-	1 (100)	-	1
Traditionally married husband	-	2 (100)	-	2
Father	-	9 (81.8)***	2 (18.2)	11
Sibling	6 (40)	8 (53.3)	1 (6.7)	15
Relative	13 (50)	13 (50)	-	26
Stranger	10 (83.3)***	2 (16.7)	0	12
Stepfather	1 (16.7)	5 (83.3)	0	6
No knowledge	6 (19.4)	3 (9.7)	22 (71)	31
Total	117 (44.7)	113 (43.1)	32 (12.2)	262

In some cases, there is more than one suspect. Row percentages are given.
*neighbor, friend etc.
** boyfriend, engaged
***$p<0.05$

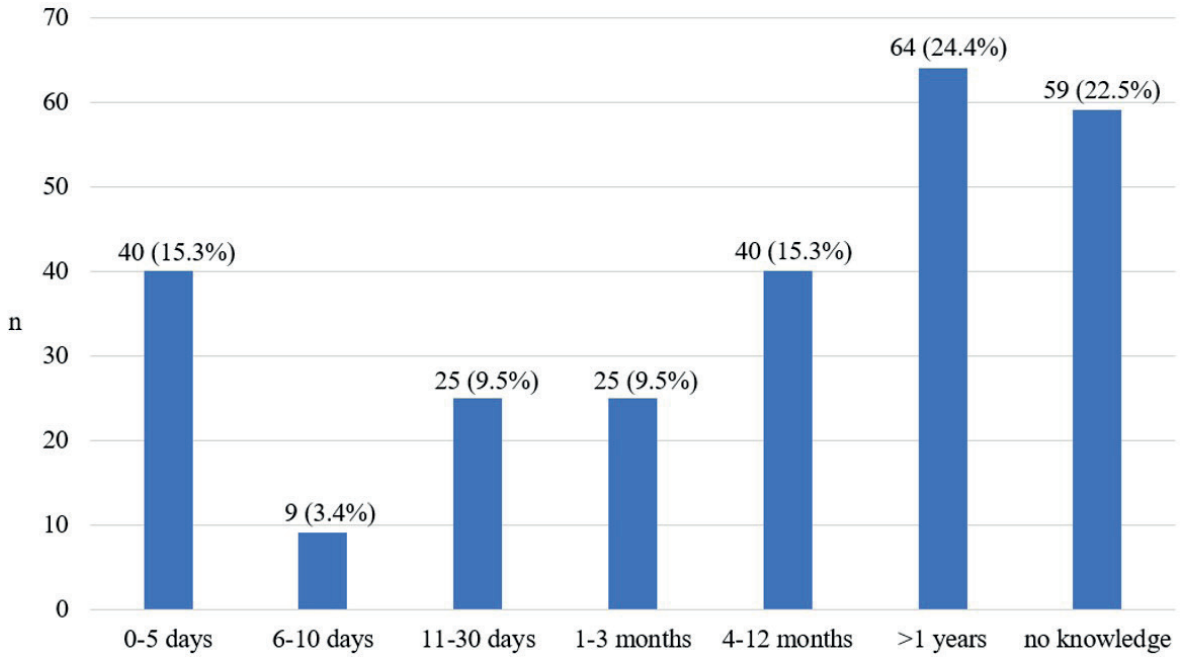

Fig. 1. Distribution of cases based on time of presentation after the sexual abuse.

When the relationship between intra-familial (father, sibling, husband, relative) and extra-familial (boyfriend, engaged, neighbor, friend, stranger) sexual abuse suspects and the duration of presentation of the cases was examined, it was found that 55.7% (n=34) of the intra-familial sexual abuse cases presented to health institutions for sexual abuse examination more than one year later and it was statistically significant ($p<0.05$, Table I).

In the histories obtained from the sexual abuse cases, it was observed that 20.2% (n=56) of the incidents occurred in the suspect's home, 12.6% (n=35) in the homes of friends or acquaintances, 9.7% (n=27) in the joint home of the victim and the suspect, and 7.6% (n=21) in the victim's home.

The type of alleged abuse was categorized into four groups vaginal penetration, anal penetration, oral penetration, and non-penetrative sexual abuse (touching, showing genital organs, watching videos, rubbing). It was found that 40.8% (n=107) of the cases had vaginal penetration and 32.4% (n=85) had anal penetration (Table II).

Vaginal examination findings

In our study, 195 cases who underwent vaginal examination were identified (Table III). When the cases were evaluated according to the presence or absence of vaginal penetration in the history, 59.04% (n=62) of 105 cases with a history of vaginal penetration had lacerations in the hymens (52 complete and incomplete healed lacerations, 10 acute lacerations). In 97% (n=65) of 67 patients without a history of vaginal penetration, there was no sign of injury to the

Table II. Types of alleged abuse.

	n (%)
Vaginal penetration*	107 (40.8)
Anal penetration*	85 (32.4)
Oral penetration	9 (3.4)
Non-penetrative sexual abuse **	56 (21.4)
No knowledge	38 (14.5)

Percentages are given according to the number of cases. In some cases, there is more than one type of sexual abuse allegation.
* One case with a history of anal penetration and two cases with a history of vaginal penetration did not accept the examination.
** Touching, showing genitals, showing videos, rubbing

hymen (Table III). 67.9% (n=36) of 53 cases with non-acute lacerations extending to the base of the hymen were below 3-9 o'clock, 15% (n=8) were at 3-9 o'clock, and 16.9% (n=9) were above 3-9 o'clock. All of the incomplete lacerations were below 3-9 o'clock.

Anal examination findings

It was observed that 75.8% (n=179) of 236 patients who underwent anal examination did not have anal sexual abuse findings (Table IV). When anal examination was analyzed according to the presence or absence of anal penetration history, it was found that 54.8% (n=46) of 84 cases with anal penetration history did not have any findings that could be related to sexual abuse (Table IV). The most common anal finding was 'decreased sphincter tone' seen in 10.6% (n=25) of the cases, followed by 'ecchymosis/abrasion of anal mucosa' seen in 7.2% (n=17) of the cases. While 57.1% (n=8) of 14 patients who had a history of anal penetration and underwent anogenital examination within the first 5 days, 42.3% (n=30) of 71 patients who presented more than 5 days later had findings.

Discussion

The prevention of CSA cases depends on the identification of risk factors and accurate diagnosis of CSA on examination. In this direction, we discussed the sociodemographic characteristics and anogenital examination findings of CSA cases in comparison with the findings in the literature.

It is reported that CSA is significantly more common in women and that women in childhood are 2 to 3 times more at risk of sexual abuse than men. In addition, in a WHO meta-analysis including 65 studies from 22 countries, the prevalence of CSA was reported to be 19.7% in females and 7.9% in males.[17] In our study, 76.3% of the victims of sexual abuse were

Table III. Relationship between vaginal penetration history and examination findings.

		History of vaginal penetration			Total
		Yes	No	No information	
Hymen with no signs of injury	n	43	65*	21	129
	Row%	33.3	50.4	16.3	
	Column%	41	97.0	91.3	66.2
Acute laceration	n	10*	-	-	10
	Row%	100.0	-	-	
	Column%	9.5	-	-	5.1
Non-acute (healed) complete laceration	n	50*	1	2	53
	Row%	94.3	1.9	3.8	
	Column%	47.6	1.5	8.7	27.2
Non-acute (healed incomplete laceration	n	2	-	-	2
	Row%	100.0	-	-	
	Column%	1.9	-	-	1
Ecchymosis, abrasion	n	3	2	-	5
	Row%	60.0	40.0	-	
	Column%	2.9	3.0	-	2.6
Total	n	105	67	23	195
	%	53.8	34.4	11.8	100.0

Some cases have more than one finding.
*p<0.05

Table IV. The relationship between anal penetration history and examination findings.

		History of anal penetration		No knowledge	Total
		Yes	No		
Decreased sphincter tone	n	20*	4	1	25
	Row%	80.0	16.0	4.0	
	Column%	23.8	3.4	2.9	10.6
Ecchymosis, abrasion of anal mucosa	n	12*	3	2	17
	Row%	70.6	17.6	11.8	
	Column%	14.3	2.5	5.9	7.2
Healed anal fissure	n	9	5	1	15
	Row%	60.0	33.3	6.7	
	Column%	10.7	4.2	2.9	6.4
Reflex anal dilatation	n	12*	3	-	15
	Row%	80.0	20.0	-	
	Column%	14.3	2.5	-	6.4
Acute anal fissure	n	8*	2	-	10
	Row%	80.0	20.0	-	
	Column%	9.5	1.7	-	4.2
Anal dilatation	n	2	-	1	3
	Row%	66.7	-	33.3	
	Column%	2.4	-	2.9	1.3
Erasure of pili	n	3	-	-	3
	Row%	100.0	-	-	
	Column%	3.6	-	-	1.3
No anal findings	n	46	103*	30	179
	Row%	25.7	57.5	16.8	
	Column%	54.8	87.3	88.2	75.8
Total	n	84	118	34	236
	%	35.6	50.0	14.4	100

Some cases have more than one finding.
*p<0.05

female, and this result is consistent with the literature.

It is estimated that the majority of children exposed to sexual abuse were between 12 and 17 years old at the time of the first abuse and that 32.8-63.8% of CSA victims were between 12-17 years of age at the time of the abuse.[18-20] In our study, the most common age range of the cases who presented for sexual abuse examination was 12-17 years (77.5%). Studies show that sexual abuse is more common in children over the age of 12.[19,20]

Most CSA cases are performed by a male who knows the child. In various studies, the rate of CSA performed by a person who knows the children was found to be between 78% and 96%.[21-24] In the present study, 95.5% of the victims stated that they were sexually abused by a person they knew.

Intra-familial abuse is notable in terms of its onset at a younger age, its continuity, and the delay and difficulties in reporting the abuse by the child.[25] In the present study, when the mean ages of intra-familial and extra-familial CSA

cases (12.59 ± 3.5 and 13.77 ± 2.9, respectively) were compared, it was found that intra-familial CSA victims were younger (U=4021.5, p<0.017) and 57.6% of intra-familial cases were reported more than one year after the abuse. In comparison, this rate was 16.9% in extra-familial cases (p<0.05). Magalhães et al.[25] in their study comparing intra-familial and extra-familial CSA cases, reported that intra-familial CSA cases were more likely to be perpetrated at a younger age, usually at home, by abusers with more predominant emotional violence and with a history of sexual offenses; in addition, there was more time between the date of the last abuse and the forensic examination and physical findings were less likely to be detected.

There is a widespread consensus in the literature that children who are sexually abused delay disclosure significantly or do not disclose at all until adulthood, whereas children who are sexually abused intra-familial delay disclosure for a longer period.[4,26-28] In the two surveys conducted, 47-59.5% of those who stated that they had been subjected to sexual abuse disclosed the abuse for the first time during the survey.[29,30] In our study, in the majority of the cases (24.4%), it was observed that more than one year had elapsed between the application to forensic medicine and the onset of sexual abuse. Studies show that a significant number of CSA do not disclose their exposure until adulthood, and even a significant number of adults never disclose the abuse.[4] Educating children who have been victims of sexual abuse and making them feel that they have a safe and supportive environment behind them will facilitate the disclosure of these cases and prevent their recurrence. In addition, late disclosure of sexual abuse may mean the disappearance of concrete findings that can be detected during examination in the anogenital area, which is known to heal quickly and without leaving scars.[7,8,31]

Recurrent CSAs are associated with comorbid illnesses, acute psychotic problems, suicide attempts, and low school achievement in children.[2] In addition, a child who is a victim of sexual abuse is more likely to be sexually abused again.[2] Studies report that 35.2-47.9% of sexual abuse victims are exposed to sexual abuse again.[2,32] In addition, no relationship was found between recurrent sexual abuse and gender.[32] In our study, similar to the literature, the rate of patients who stated that they had been subjected to recurrent sexual abuse was 43.1%, and it was not found to be related to gender (p=0.86). In addition, recurrent sexual abuse was found to be significantly higher in cases of intra-familial CSA. These findings support the idea that children who are subjected to intra-familial sexual abuse hesitate to disclose the abuses and experience recurrent victimization due to reasons such as fear for their safety, shame, self-blame, and loyalty to the perpetrator.[33] These findings makes it imperative for the authorities to take social and legal measures to prevent this abuse from recurring.

In cases of CSA, examination findings are not obtained sufficiently and the fact that the abuse involves penetration does not mean that examination findings will be detected. The reason for this is considered to be delays in disclosing the abuse and the disappearance of physical injuries caused by abuse over time due to the rapid and almost complete regeneration ability of the anogenital region.[6,9,14,34] In a study by Kellogg et al.[13] in which CSA findings were evaluated, it was reported that examination findings were found in 14.2-85% of the studies in which acute cases were evaluated. In contrast, the rate of detection of findings was less than 12% in studies in which non-acute cases were evaluated. It has been reported that healing of the majority of the hymen tissue is possible except for deep lacerations, and genital trauma findings are found more rarely in non-acute cases.[34,35] Anderst et al.[36] found diagnostic examination findings in only 12.6% of the cases in a study consisting almost entirely of cases with a history of non-acute penetration. Gallion et al.[35] did not find genital trauma in the examination of 79% of the penetration histories reported by children. In our study, no traumatic findings were found in the genital examination

of 41% of the cases with a history of vaginal penetration and the majority of these cases (72.1%; 31 of 43 cases) presented more than 5 days later.

In our study, it was found that all of the cases with acute laceration of the hymen had a history of vaginal penetration, while 94.3% of the cases with healed laceration had a history of penetration, and it was statistically significant ($p<0.05$). In addition, there was no sign of injury to the hymen in 94% of the cases without a history of penetration ($p<0.05$). Similar to our study, Gallion et al.[35] reported that 91% of the cases with findings on examination had a history of vaginal penetration, and the most common finding of sexual abuse was healed laceration (32% n=32) in the hymen. According to the literature, the location of non-acute lacerations in the hymen is essential in evaluating the findings.Non-acute laceration extending to the hymen base at the 3 and 9 o'clock positions, which was evaluated among the non-consensus findings in the 2023 update by Kellogg et al.[13], was found in 8 cases in our study, and a history of vaginal penetration was obtained in 87.5%. The finding of non-acute laceration below the 3 and 9 o'clock position, which was evaluated among trauma-related findings, was found in 36 cases, and 94.4% (n=34) had a history of vaginal penetration. All 9 cases with non-acute lacerations above the 3 and 9 o'clock positions had a history of vaginal penetration. According to Kellogg et al.[13] in the reports submitted to the forensic authorities in 9 cases in which non-acute laceration was found at 3-9 o'clock, which was considered as a variant of normal, it was reported that this finding could not be accepted as a finding of penetration alone because of the absence of acute examination findings and the abuse should be clarified by forensic investigation.

In studies conducted in the pediatric age group in which anogenital examination findings were compared in groups with and without anal penetration history, Myhre et al.[37] found total dilatation in 12.1%, anal fissure in 10.7%, anal laceration in 4.6% of children with a history of highly suspected anal penetration and these findings were found to be associated with anal penetration ($p=0.000$). It has been reported that only external dilatation has a weaker association with soiling in the perianal region ($p<0.05$).[37] In the study of Hobbs et al.[38] reflex anal dilatation, anal tonus weakening, redness/erythema, perianal venous congestion, and anal fissure were found to be associated with anal penetration ($p<0.001$). However, in this study, the rate of those who were examined within the first 7 days after sexual abuse was higher (31.5%) compared to previous studies, and therefore, erythema finding was found to be more common compared to the literature, and venous congestion was also reported to be associated with anal penetration contrary to the literature. However, Kellogg et al.[13] in the 2023 update of CSA findings, erythema and venous congestion were evaluated in the 'Category B' group (findings that may have more than one cause and are usually caused by medical conditions other than trauma or sexual contact). Another controversial finding, 'complete anal dilatation,' was evaluated in 'category D' (findings that were associated with a history of sexual abuse in some studies, but there was no consensus among experts about the degree of importance). It is reported that this finding may vary depending on the examination position.[37] In our study, anal fissure, reflex anal dilatation, ecchymosis/abrasion in the anal area, decreased sphincter tone and flattening of the anal pleats were found to be associated with anal penetration, similar to the literature (Table IV).[13,37,38]

The most striking finding in our study was that 54.8% of the children with a history of anal penetration had no findings on anogenital examination. It is known that anal penetration may occur without causing any injury, or the injury may heal without leaving any scar.[14-16] Hobbs et al.[38] reported that 26% of children with a history of anal penetration had no findings on anal examination. Adams et al.[39] did not find any findings in 56% of pediatric cases with a history of anal penetration. In addition, the

detection of physical findings of sexual abuse becomes more complex as time passes since the incident. Smith et al.[15] and Adams et al.[39] reported that more physical findings were found in anogenital examinations performed in the first 72 hours. Studies also indicated more physical findings in examinations performed in the first 7 days.[8,38] In our study, in cases with a history of anal penetration, 57.1% (n=8 of 14) of anal examinations performed within the first 5 days and 44.4% (n=24 of 54) of examinations performed after 5 days were positive.

According to the results obtained in our study, it was observed that CSA cases occurred mainly against a child of the female gender and between the ages of 12-17, by a stranger, in a closed place, most frequently in a house. Intra-familial sexual abuse cases, which start at a young age and are reported late or not reported at all, are in a particular position in the detection and prevention of CSA cases. In our study, findings of acute and healed laceration were found to be associated with the history of vaginal penetration, and findings of decreased sphincter tone, ecchymosis, abrasion, reflex anal dilatation, and acute anal fissure were found to be associated with the history of anal penetration. However, late reporting of CSA stands out as the most crucial factor covering the concrete findings of sexual abuse. It is imperative that efforts be made to facilitate the earlier disclosure of victims of CSA in this regard. Among the cases reported later than 5 days, 37.8% of the cases with a history of vaginal penetration and 53.7% of the cases with a history of anal penetration had no examination findings. It should be kept in mind that sexual abuse may occur without leaving any traumatic findings and the anogenital region may heal rapidly without leaving any findings. Preventing CSA will be possible by analyzing the risk factors and reaching the cases at risk as early as possible. However, the victims need to present in the acute period to prevent the loss of anogenital examination findings. Healthcare workers who encounter a case of CSA should notify the competent authorities as soon as possible and refer for an anogenital examination in order to prevent the disappearance of the findings.

Limitations

Although our study covers a large number of cases, the fact that the data were collected retrospectively from existing medical records can be considered a limitation. This methodology is subject to typical limitations associated with recorded patient information, including a high rate of unreported data and possible errors. Today, there is still a need to update CSA findings and, more structured multidisciplinary prospective studies focusing also on parental education, socioeconomic status, alcohol and drug addiction of victims and, psychiatric outcomes with a higher evidence level in larger populations in different regions of our country are needed.

Ethical approval

The study was approved by Pamukkale University Ethics Committee (date: 31.05.2023, number: E-60116787-020-374178).

Author contribution

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

Eligibility of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulator therapies: cohort of cystic fibrosis registry of Türkiye

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ABSTRACT

Background. Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) variants are essential for determining eligibility for CFTR modulator drugs (CFTRms). In contrast to Europe and the USA, the treatment eligibility profile of cystic fibrosis (CF) patients in Türkiye is not known. In this study we aimed to determine the eligibility of CF patients in Türkiye for the CFTRms.

Methods. The Cystic Fibrosis Registry of Türkiye (CFrT) data was used to determine the age of patients in the year 2021 and the genetic variants they were carrying. Age- and *CFTR*-variant appropriate modulator therapies were determined using the Vertex® algorithm.

Results. Among a total of 1930 registered patients, *CTFR* gene analysis was performed on a total of 1841 (95.4%) patients. Mutations were detected in one allele in 10.7% (198 patients), and in both alleles in 79% (1455 patients) of patients. A total of 855 patients (51.7% for whom at least 1 mutation was detected) were eligible for the drugs. The most appropriate drug among genotyped patients was found to be elexacaftor/tezacaftor/ivacaftor for 486 patients (26.4%), followed by ivacaftor for 327 patients (17.7%) and lumacaftor/ivacaftor for 42 patients (2%).

Conclusions. Only half of patients registered in CFrT were eligible for CFTRms, which is a significant difference from the CFTR variant profile seen in USA and Europe. However, access to treatment is hampered for some patients whose genes are not analysed. Further studies in CF populations, where rare mutations are relatively more common, will contribute to the field of CFTR modulator treatments for such rare mutations.

Key words: cystic fibrosis, cystic fibrosis transmembrane conductance regulator, CFrT registry, modulator, treatment.

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulator drugs (CFTRms) are genome-specific drugs that target the malfunctioning or defective protein produced by the *CFTR* gene.[1] Although cystic fibrosis (CF) affects all races, the distribution of *CFTR* variants varies by race and geographic region.[2,3] The F508del variant accounts for the majority genetic profile in the United States of America (USA) and European CF registries, and can on its own, sufficiently cover 90% and 85% of the CF population eligible for CFTRms, respectively.

Türkiye is a country located in southeastern Europe and western Asia. The peculiarity of this region is that it is home to different ethnic origins. Thus, its location causes a wide spectrum of CFTR variant diversity.[4-7] Previous studies have shown that the most common CFTR variant, F508del, covers less than 30% of the Turkish CF population.[4-7]

Previous treatments for CF disease had only targeted symptoms, but CFTRms, which target the underlying problem, are now successfully used. With the introduction of these therapies, improvements in the health status of the CF cohort have been seen.[8] However, CFTRm therapies are only effective in people with specific CFTR variants. There are four CFTRm drugs approved, which are ivacaftor (IVA), lumacaftor/ivacaftor (LUM/IVA), tezacaftor/ivacaftor (TEZ/IVA), and elexacaftor/tezacaftor/ivacaftor (ETI).[9]

Issues in accessing CFTRms in many countries including Türkiye, currently sets the main agenda of the CF population. In the USA and many countries in Europe, CFTRms are reimbursed, but Türkiye does not cover the costs of these treatments and their cost is far beyond what patients can individually afford. Some eligible patients have access to therapy through lawsuits, which involves a challenging process. The lack of knowledge of *CFTR* variants of the patients with CF is another important issue in the era of CFTRms, and limited access to genetic analysis hinders the knowledge of CFTRm eligibility. Profiling the CF population and their genetic characteristics can enable us to overcome the difficulties encountered in the era of CFTRms.

The purpose of this study was to identify the eligibility of CF patients for CFTRms registered in the Cystic Fibrosis Registry of Türkiye (CFrT). Also, suggestions on how to handle the problems are discussed.

Material and Methods

Study design and population

Cystic Fibrosis Registry of Turkiye (CFrT) is a web based patient registry which was established in 2007 by the Turkish Pediatric Respiratory Diseases and Cystic Fibrosis Society.[10] Data of patients who fulfill the diagnostic criteria of CF are included by each center in a software program that is specifically developed for the CFrT. This registry consists of 25 demographic and 79 annually recorded data for each patient which includes genotyping, sweat test, nutrition, lung function, microbiology, treatments and complications.[11]

In this descriptive study, the data from patients with CF registered in the CFrT in the year 2021 were used. The ages and *CFTR* variants of the patients from 34 CF centers throughout the country were evaluated.

The decision of eligibility of *CFTR* variants for four modulator treatments (ETI, TEZ/IVA, LUM/IVA, IVA) was determined by using the 'Vertextreatment finder' (Finder) on the Vertex® website.[12] The total number and percentages of 'drugs offered by the Finder' was determined. According to 'Finder' more than one drug might be offered to a patient. If more than one drug was recommended, the 'most appropriate drug' (MAD) was determined. This was defined as 'the drug that could be primarily preferred' depending on the literature and Institute for Clinical and Economy Review (ICER) evidence ratings on clinical effectiveness analysis, as follows:

A. For patients who were eligible for both TEZ/IVA and ETI, ETI was determined as MAD because of the clinical superiority of ETI treatment upon TEZ/IVA.[13,14]

B. For patients who were eligible for both LUM/IVA and ETI, MAD was determined according to each drug's recommended starting age.

1. For patients aged between 1-6 years, LUM/IVA was determined as MAD.
2. For patients aged 6 years and above, ETI was determined as MAD, assuming the clinical effectiveness of ETI treatment for patients aged 6 years and above was superior to LUM/IVA.[12]

These assumptions regarding the clinical superiority and effectiveness of the drugs were made based on the literature and ICER evidence ratings.[14] The use of ETI was approved for expanded use from the age of 12 years to age 6 years in 2021, and the use of LUM/IVA was approved for expanded use from the age of 2 years to age 1 year in 2022.[9] We performed the evaluations according to the above regulations. The outcomes are presented as numbers and percentages for patients and eligible drugs.

The study was conducted in accordance with the ethical standards of the institutional research committee (Hacettepe University Ethics Board, date: 12 April 2007, reference number: HEK 07/16-2, Date: 5 June, 2018, reference number: GO 18/473-31) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all patients or their parents/legal guardians.

Statistical analysis

Outcomes were the numbers and percentages of cases, the names of *CFTR* variants and the drugs for which the patients were eligible. Continous variables (age) were presented as mean ± standard deviation for normally distributed variables. Numbers and percentages (*CFTR* variants and CFTRms) were reported for categorical variables. Data were analysed using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic features

There were a total of 1948 patients registered in the CFrT in 2021, 1930 of whom were alive. Among the surviving patients, 1629 (84.4%) were children and adolescents, and 301 (15.6%) were adults. A total of 1841 (95.4%) surviving patients were found to have undergone *CFTR* genetic analysis. Of the surviving patients, 986 (53.5%) were male and 855 (46.4%) were female, with a mean age of 10.66±9.19 years. The number of patients who had not undergone genetic tests was 89 (4.6% of surviving patients).

Genetics

Among the 1841 patients who had been genotyped, two variants were identified in 1455 (79%) patients, only one variant was detected in 198 (10.7%) patients, and no variants were detected in 188 (10.2%) patients (Fig. 1).

A total of 3,108 variants were observed in 1,653 surviving and genotyped patients, with 339 different variants. The most common variant was F508del in 692 alleles (22.2% of all alleles), followed by N1303K in 146 (4.7% of all) alleles and 1677delTA in 127 (4.1% of all) alleles.

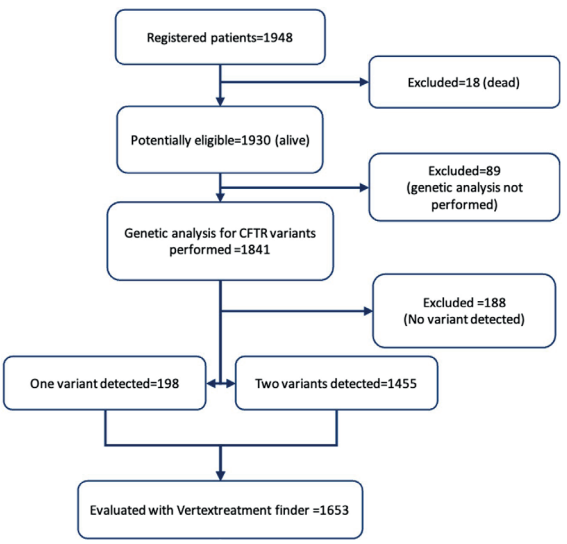

Fig. 1. The flow diagram of the study participants.

Among the 1841 surviving patients who had been genotyped, 483 patients (25%) had the F508del variant on at least one allele. Two hundred patients were homozygous, and 283 patients were heterozygous for the F508del variant (comprising 10.8% and 15.3% of genotyped patients, respectively).

The most common 15 identified variants accounted for 58% of all alleles. Twenty different variants with a frequency of over 1% were identified. These 20 variants accounted for 63.7% of all alleles. The distribution of the most common 15 variants is given in Fig. 2.

Eligibility of the patients with CF for CFTRms

A total of 855 patients (51.7% of the patients for whom at least one allele was known) were eligible for any modulator drug, which constituted 44.3% of the 1930 total surviving patients, 46.4% of 1841 genotyped patients, and 51.7% of 1653 patients with at least one identified variant. The most common recommended drug was ETI (26.4% and 29.4%), followed by IVA (17.7% and 19.8%) and LUM/IVA (2.3% and 2.5%) among the genotyped patients and those with at least one identified variant, respectively. TEZ/IVA treatment was not the drug of choice for any patients (Table I).

Among patients who had at least one identified allele, 43.7% of pediatric patients and 61.0% of adult patients were found to be eligible for a modulator drug. ETI was the most commonly recommended drug in both age groups.

'Finder' recommended a total of 1686 drugs to 855 patients. Among all offered drugs, 633 (37.5%) were ETI, 482 (28.6%) were TEZ/IVA, 375 (22.2%) were IVA, and 196 (11.6%) were LUM/IVA (Table II).

It is noteworthy that TEZ/IVA treatment was offered for 482 patients by 'Finder' for eligible mutations, but was not identified as MAD for the patients (Table I and Table II).

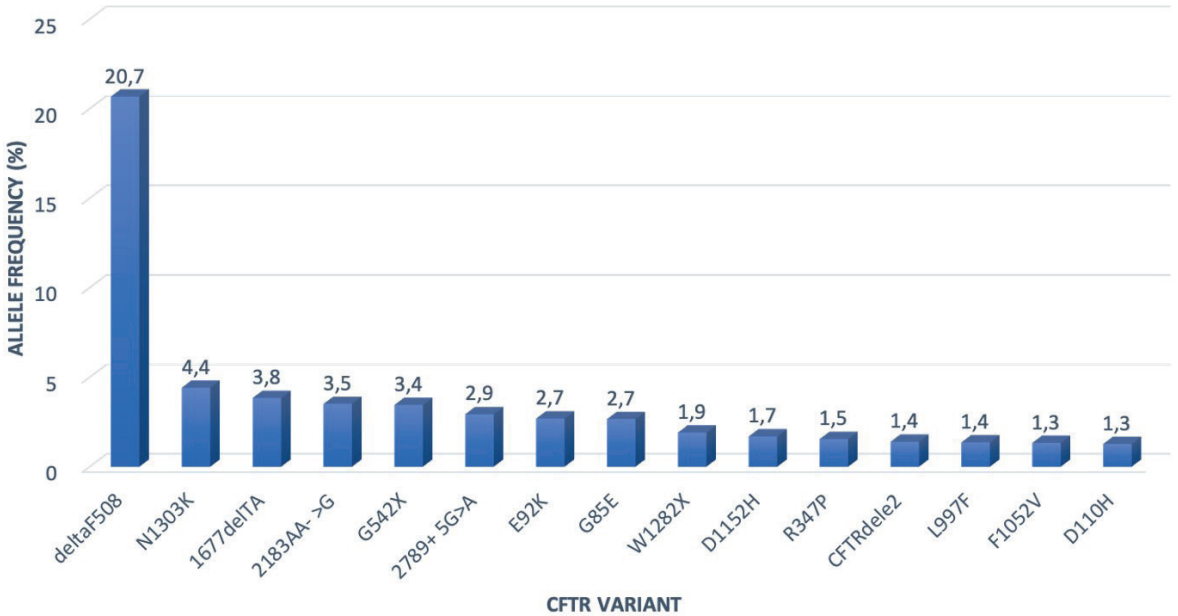

Fig. 2. The most common 15 variants in gene analysis among surviving patients in the CFrT.

Table I. The most appropriate drugs (MAD) for eligible patients by age

Modulator drugs	Age (years)	Number of eligible patients	Percentage among patients with at least one identified variant (N=1653 patients) (%)	Percentage among genotyped patients (N=1841 patients) (%)	Percentage among surviving patients (N=1930 patients) (%)
ETI	<18	369	29.4	26.4	25.1
	≥18	117			
TEZ/IVA	All	0	0	0	0
IVA	<18	267	19.8	17.7	16.9
	≥18	60			
LUM/IVA	<18	42	2.5	2.3	2.17
	≥18	0			
Total	<18	678	51.7	46.4	44.3
	≥18	177			

ETI: elexacaftor/tezacaftor/ivacaftor, IVA: ivacaftor, LUM/IVA: lumacaftor/ivacaftor, TEZ/IVA: tezacaftor/ivacaftor

Table II. The drugs offered by 'Finder'

Modulator drugs	Number of patients offered for drug by 'Finder' (n) (percentage among all recommended drugs) (%)
ETI	633 (37.5)
TEZ/IVA	482 (28.6)
IVA	375 (22.2)
LUM/IVA	196 (11.6)
Total	1686

ETI: elexacaftor/tezacaftor/ivacaftor, IVA: ivacaftor, LUM/IVA: lumacaftor/ivacaftor, TEZ/IVA: tezacaftor/ivacaftor

Discussion

This study demonstrated that almost half of the registered patients were eligible for a CFTRms. The most common variant 'F508del' constituted 22.2% of all identified variants. ETI treatment was found to be the most appropriate drug common in 26.4% of genotyped patients. Genetic analysis of the *CFTR* gene was not performed in 4.6% of surviving patients.

There are some barriers regarding access to CFTRms in Türkiye, like in many other countries. The first step for revealing the obstacles that hinder knowledge of eligibility to CFTRms should be to determine the patient profile of the country. The data from the CFrT, which represents over 60% of the CF population in Türkiye, serves as valuable data for the purpose of drawing realistic conclusions regarding the eligibility status for the CFTRms in our country. Evaluation of the results showed many differences between European, American, and Turkish populations. Here, we would like to identify the genetic characteristics of our patients and highlight the issues that need to be solved.

The first difference between CF patients in Europe, the USA, and Türkiye that we established, was the prevalence of the variant F508del. The last annual reports of Cystic Fibrosis Foundation (CFF) and European Cystic Fibrosis Society (ECFS) patient registries showed that 85.5% and 80.3% of their genotyped patients had the F508del variant, respectively.[8,15] In a 2018 study that evaluated the eligibility of patients in the CFrT from Türkiye, F508del was found in 34.6% of patients who were genotyped.[16] In our study, the most common variant, F508del, accounted for 26.2% of surviving patients. Accordingly, it can be said that while a few *CFTR* variants are sufficient to provide eligibility for CFTRms in USA and Europe, the current qualifying *CFTR* variants for CFTRms only allow for less than 50% of our CF population to be eligible for such therapies.

Secondly, even though verification of *CFTR* variants is mandatory to identify a patient's eligibility for CFTR modulators, *CFTR* variants of approximately 15% of our surviving patients were missing in our cohort. This group consisted of patients who did not undergo genetic testing (4.8%) and those whose mutation could not be detected (10.2%) even though genetic testing was performed. Both 2021 annual reports of the ECFS and CFF showed that 99.4% (50,849 and 48,814 patients) of the registered patients were genotyped.[8,9] According to the ECFS 2021 report, Türkiye was one of the countries ranked at the top with unknown mutations.[15] Nevertheless, the number of patients who were genotyped increased from 87.4% in 2017 to 95.4% in 2021 in patients in the CFrT.[10,16] Despite the improvements in genotyping efforts over the years, we are still behind compared with Europe and USA. The success of genetic tests is important to find patients who qualify for CFTRms. No *CFTR* variant was identified in 25% and 19% of patients according to the 2017 and 2018 annual reports of the CFrT, respectively.[10,16] In 2021, we still failed to detect mutations in 10% of cases.[17] Since genetic analysis methods employed in the CF patients were not collected in the CFrT, it was not possible to draw any conclusions regarding analysis methods used. We speculate that the use of small *CFTR* panels may be the cause of these unknown mutations. Dayangaç-Erden et al.[7] showed that the variant detection rate increased from 49.2% with DNA strip analysis to 76.7% with DNA sequence analysis, and *CFTR* panel use might not be sufficient to achieve the expected success in identifying the *CFTR* variants in our population. Expansion of DNA sequencing analysis, and multiplex ligation-dependent probe amplification (MLPA) throughout the country may help attain the goal of detection of *CFTR* variants. Also, efforts should be increased to ensure that genetic studies can be performed for all patients to identify eligibility for these treatments.

One of the reasons for the relatively low prevalence of drug eligibility in our population is the genetic diversity of the country. According to the worldwide analysis of *CFTR* variants, unlike the homogeneity of *CFTR* variants in central, northern, western, and northeastern European countries, it has been shown that Spain, Greece, Bulgaria, and Türkiye, which are defined as 'gateway' countries across Asia and Europe, show a widespread heterogeneous *CFTR* variant spectrum. Accordingly, whereas 10.2 variants per country constitute 78.9% of all variants in European countries, it has been reported that 25 variants constitute 84% of all variants in 'gateway' countries. A comparison of 'gateway' countries and others showed a statistically significant difference ($p < 0.001$) in the extent of variants.[18] Latter studies also support these results.[4,6] Herein, we identified 339 different *CFTR* variants in surviving patients, and the most common 15 variants accounting for 63.7% of all the alleles. The conclusion here would be that high genetic diversity reduces the eligibility prevalence for CFTRms. Additionally, high genetic diversity may be indicative of a higher prevalence of rare *CFTR* variants that are not amenable to CFTRms. The effect of these drugs on many known mutations has not been studied yet. As highlighted by Fajac and Sermet, there may be *CFTR* variants that are not amenable to but may respond to CFTRms. Ways of assessing the effect of these drugs in people who carry these relatively few variants should be explored. In vitro studies showing its efficacy were sufficient for the United States Food and Drug Administration (FDA) to expand the extent of the mutations for IVA treatment in 2017. Similarly, it is important to find ways to evaluate drugs for this small population.[19] When we looked at the number of eligible patients in the present study, 44.3% of surviving patients were eligible for any type of CFTRm treatment and ETI was the most commonly qualified drug (25% of genotyped patients). Recent data from CFF and ECFS registries showed that 90% and more than 80% of patients with CF were eligible for ETI treatment, respectively.[8,9] Given the genetic profile of Türkiye, expanding the genetic coverage of drugs will enable more of our patients to benefit from these drugs.

Another issue that we would like to highlight is the challenges faced in accessing therapies in Türkiye. The main problems that impede access to the therapies are their cost and the reimbursement policies of the Social Security Institution (SSI) in Türkiye. The annual list prices of CFTRms are between $270,000 and $310,000.[14] Considering that patients with CF must use these drug lifelong, it is not possible to afford them individually unless they are reimbursed by health insurance systems. Some of our patients can access treatment through lawsuits which is a very long, exhaustive and expensive process.

We agree with the idea put forward by Guo et al.[20] who emphasized that efforts to access these treatments should be through global practices rather than individual efforts, and the prices should be reduced. Also, health systems should cover the costs, similarly to methods used in treatments such as HIV and tuberculosis.[20]

In addition, there are other mutations, which are not rare, in which the CFTR protein is not produced in more than ten percent. CFTRms are not effective for these mutations, which are nonsense mutations, frame-shift mutations, large deletions, insertions, and splice-site mutations. Preclinical studies are ongoing for these mutations. However, some issues need to be addressed immediately in the application to daily clinical practices.[19]

Our study has some limitations. First, this is a retrospective study, in which the data of the CFrT belonged only to the year 2021, thus the results do not show the current status of our population, but can only be a reflection of the present. The other limitation is that the type of the genetic testing methods of the CF centers was unknown. Because we did not know genetic analysis methods used, we could not evaluate the exact genetic status of all patients. Additionally, these results cannot be generalized to the whole country or worldwide. The power of our study

is that CFrT data encompasses 60% of the CF population in our country, with data collected from a diverse range of geographical regions. This constitutes a significant proportion of the Turkish CF population. This study can lead each country to take action to determine the status of their patients.

Conclusions

The *CFTR* variant profile of Türkiye is very different from USA and most European countries. Approximately half of the patient population registered in CFrT was eligible for CFTRms. Nevertheless, the inability to perform *CFTR* gene analysis on some patients, even in small numbers, represents a barrier to their access to treatment. A determination of the prevalence of variants and genetic testing in the CF patient community may provide insight into barriers to drug access. Further studies on this subject in populations where rare mutations are relatively more common will contribute to the field of knowledge regarding CFTR modulator treatments for these rare mutations.

Ethical approval

This research was reviewed and approved by the institutional research committee (Hacettepe University Ethics Board, date: 12 April 2007, reference number: HEK 07/16-2, Date: June 5th, 2018, reference number: GO 18/473-31). Informed consent was obtained from all patients or their parents/legal guardians.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MAE, DD; data collection: HNB, VŞ, AAK, HÇ, GD, AİY, GÜ, MS, DUA, EA, ÖK, MMÖ, OK, MK, AEB, EPÇ, YC, AÖ, KH, SU, SEP, MH, HY, GÖ, PK, MK, ZGGA, GÇ, DC, SD, GKÖ, AS, ET, BÖ, MH, ED, HO, ŞB, HY, HSŞ, ADD, EÇ, TŞE, NE, SP, UÖ, DD; analysis and interpretation of results: MAE, DD draft manuscript preparation: MAE, DD. 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

Matrix metalloproteinase-7 and matrix metalloproteinase-9 expression is upregulated in congenital lung malformations

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ABSTRACT

Background. Congenital lung malformations (CLMs) refer to structural abnormalities of the lungs that occur during fetal development. Matrix metalloproteinases (MMPs) constitute a group of zinc-dependent enzymes, with certain members of this family playing pivotal roles in the remodeling of the lungs both prenatally and postnatally. This study aimed to explore expression levels of MMP-2, MMP-7, and MMP-9 in CLMs which are recognized as pivotal contributors to their clinical pathology.

Methods. A total of 41 patients between the ages of 0-17 years that had undergone lung surgery for CLMs between March 2007- July 2023 were analyzed. The demographic features, clinical and pathological findings were recorded. The expression levels of MMP-2, MMP-7 and MMP-9 in patients' tissues were examined by reverse transcription polymerase chain reaction and compared in CLMs and adjacent normal lung tissues.

Results. Among patients with CLMs, 12 patients had congenital pulmonary airway malformations (CPAM, one patient had bilateral lesions), 18 patients had bronchopulmonary sequestration (BPS), 7 patients had congenital lobar overinflation (CLO), and 4 patients had bronchogenic cyst (BC). The higher expression of MMP-7 and MMP-9 in all CLM tissues compared to normal tissue was observed. But, there was a trend in MMP-2 expression in CPAM tissues and MMP-2 showed high expression in the BPS, CLO and BC groups, which was not statistically significant. Upon collective analysis of all groups, it was observed that mRNA expressions of MMP-7 and MMP-9 exhibited greater upregulation in CPAM and BC in comparison to BPS and CLO.

Conclusions. Our findings indicate a specific involvement of MMP-7 and MMP-9 in the pathogenesis of CLMs, particularly in CPAM and BC. To the best of our knowledge, this research represents the initial demonstration of MMP expression in CLMs.

Key words: bronchogenic cyst, congenital lung malformations, congenital pulmonary airway malformations, Matrix metalloproteinase-7 (MMP-7), Matrix metalloproteinase-9 (MMP-9).

Congenital lung malformations (CLMs) have an estimated prevalence of 3.5 per 10.000 births.[1] However, their apparent incidence is increasing with recent data suggesting that CLMs may be identified prenatally in approximately 1 in 2.400 live births.[2] Congenital lung lesions encompass a wide-ranging group of disorders, the most common of which include congenital pulmonary airway malformation (CPAM, previously known as congenital cystic adenomatoid malformation), bronchopulmonary sequestration (BPS),

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congenital lobar overinflation (CLO; previously known as congenital lobar emphysema), and bronchogenic cyst (BC).

The pathogenesis of congenital lung lesions is not yet fully known. CLMs are understood as resulting from a focal development malformation and present as cystic lesions.[3] The hypothesis proposed by Langston suggests that CLMs can be attributed to variations in airway obstruction in utero and that the level, timing, and completeness of the obstruction produce different patterns of malformation.[4,5] Recent studies have shown that bronchial atresia/obstruction is a possible hidden pathology underlying many congenital lung lesions, leading to cystic maldevelopment.[6-8] This focal airway obstruction leads to focal hypoxia, inflammation, and tissue damage. Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent enzymes and several members of the matrix metalloproteinase family are critical in lung remodeling before and after birth.[9] Overexpression of MMPs is shown in inflammatory conditions.[10] Therefore, we hypothesize that overexpression of MMPs in CLMs indicates that CLM may result from tissue repair and remodeling induced by inflammation related to intrauterine airway obstruction. To our knowledge, this is the first study to focus on this subject.

The aim of this study was to investigate the expression of MMP-2, MMP-7 and MMP-9 which have been marked as being critical for the clinical pathology of lung diseases.

Materials and Methods

Clinical characteristics of the patients and study design

Medical records and tissue specimens of 55 patients that had undergone lung surgery for CLMs at the Department of Pediatric Surgery of Bursa Uludag University Hospital between March 2007- July 2023 were retrospectively analyzed. The demographic features, prenatal diagnosis history, clinical and pathological findings were recorded. Cases were considered to be symptomatic if they had acute respiratory distress, pneumothorax, recurrent pneumonia, cough, and inability to feed. Asymptomatic cases were those who did not exhibit these symptoms. In our clinic, we perform surgery in symptomatic cases, even during the neonatal period. In asymptomatic cases, we perform elective surgery after the postnatal 3rd month. We prefer segmentectomy or lobectomy techniques by thoracotomy or thoracoscopy according the computed tomography images.

Fourteen patients were excluded from the study due to lack of data and signs of infection or presence of inflammatory cells in the tissue specimens. A total of 41 patients between the ages of 0-17 years were included in the study. Institutional ethics approval was provided for the study.

RNA isolation and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Formalin-fixed paraffin-embedded (FFPE) cores containing congenital lung malformations were marked and total RNA was isolated from each. Total RNA was isolated from CLM tissue and normal lung tissues using the RNeasy FFPE Kit (Qiagen, USA) as per the manufacturer's protocol. The RNA quality (A_{260}/A_{280} ratios) and quantity (ng/µl) were assessed spectrophotometrically (Beckman Coulter), and samples were stored at -80°C. For cDNA synthesis, 300 ng of RNA was used with the High Capacity cDNA Reverse Transcription Kit protocol (Applied Biosystems), and the resulting cDNA samples were stored at -20°C. Gene expression analyses for MMP-2, MMP-7, and MMP-9 were conducted using the ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems). Each 20-µl reaction contained cDNA, TaqMan™ Gene Expression Master Mix, and TaqMan™ Gene Expression Assays, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as the endogenous control. The amplification process included a melting curve analysis to ensure the specificity of the

reactions, and cycle threshold (*Ct*) values were recorded. Gene expression differences were quantified using the Comparative Cycle Threshold method. According to this method, gene expression levels were calculated using the Δ*Ct* (Delta *Ct*) method, where the *Ct* value of the target gene was normalized to that of the reference gene. The relative gene expression differences between groups were determined using the ΔΔ*Ct* (Delta Delta *Ct*) method and expressed as fold change using the formula $2^{-\Delta\Delta Ct}$.

Statistical analysis

All statistical analyses were performed using SPSS statistical software for Windows, version 28.0 (SPSS, Chicago, IL, USA). Two-tailed Student's *t*-test was used to compare the MMP-2, MMP-7, and MMP-9 levels between CLMs and adjacent normal lung tissues. All data were presented as the mean ± standard deviation, where $p<0.05$ was considered statistically significant.

Results

A total 41 patients with CLMs were included in the study. There were 13 lesions of CPAM (one patient had bilateral lung lesions), 18 lesions of BPS, 7 lesions of CLO, and 4 lesions of BC. Table I describes patient demographic characteristics and clinical features, including the age at the time of operation and operative techniques used. All patients underwent

Table I. Demographic characteristics, clinical features and operative techniques (N:41).

Patients (n)	CPAM (n:12)	BPS (n:18)	CLO (n:7)	BC (n:4)
Prenatal diagnosis	8	15	3	0
Age at operation time Median (Min-Max)	240 days (5-2190)	135 days (4-1095)	4.5 months (1-12)	24 months (1-204)
Gender (Female/Male)	1/1	1/2	2/5	1/1
Symptomatic (n)	5	5	5	3
Asymptomatic (n)	7	13	2	1
Operative technique-1				
Thoracotomy (n)	12	15	7	4
Thoracoscopy (n)	0	3	0	0
Operative technique-2				
Segmentectomy (n)	9*	4**	0	0**
Lobectomy (n)	4*	5**	7	0**
Histopathological type (n)	Stocker's classification*** Type 0: 0 Type 1: 7 Type 2: 5 Type 3: 0 Type 4: 0	Hybrid type****: 3	-	-

* In one patient with bilateral CPAM, right lobectomy and left segmentectomy were performed at different times. The same patient is shown separately as lobectomy and segmentectomy in the table.
**Only lesion excision: 9 for BPS, 4 for BC. The patients with BPS had 8 of extralobar and 10 of intralobar type.
***Classification for CPAM is Stocker's classification. 1 patient with bilateral CPAM is shown separately as type 1 and type 2 in the table, due to having different types. Pathological typing was not performed in 1 patient.
**** Hybrid type refers to the lung lesion comprising both BPS and CPAM type 2.
BC: Bronchogenic cyst, BPS: Bronchopulmonary sequestration, CLO: Congenital lobar overinflation, CPAM: Congenital pulmonary airway malformation.

thoracotomy except 3 patients. Three patients with BPS underwent thoracoscopic surgery. The comparison of MMP mRNA levels across age groups in CPAM, BPS, CLO and BC was currently statistically nonsignificant due to low number of patients. A total of 18 patients had symptoms in all groups. No statistical significance was determined between MMPs and symptoms (p>0.05).

The expression profiles of MMP-2, MMP-7, and MMP-9 in the lesion compared with adjacent normal tissues by comparative *Ct* method ($2^{-\Delta\Delta Ct}$) are shown in Table II.

MMP-7 and MMP-9 mRNA expressions were significantly higher in CPAM tissues as compared to that of adjoining normal lung tissues (MMP-7; $2^{-\Delta\Delta CT}$: 4.103 ± 0.319, p<0.0001, MMP-9; $2^{-\Delta\Delta CT}$: 3.187 ± 0.641, p<0.001). There was a trend in MMP-2 mRNA expression in CPAM tissues compared to normal tissue (MMP-2; 2-ΔΔCT: 0.673 ± 0.164, p=0.06).

BC tissues also had significantly higher expression of MMP-7 and MMP-9 mRNA compared to the normal lung tissues (MMP-7; $2^{-\Delta\Delta CT}$: 1.993 ± 0.149, p<0.05, MMP-9; $2^{-\Delta\Delta CT}$: 3.373 ± 0.153, p<0.001). Although MMP-2 showed high expression in the BC group, it was not statistically significant (MMP-*2*; $2^{-\Delta\Delta CT}$: 0.567 ± 0.0605, p=0.10).

MMP-2, MMP-7 and MMP-9 expressions were higher in tissues of patients diagnosed with BPS and CLO compared to the normal lung, but this difference was not statistically significant.

When all groups were evaluated together, MMP-7 and MMP-9 mRNA expressions showed higher increases in CPAM and BC compared to BPS and CLO (Fig. 1 and Fig. 2).

Discussion

MMPs are a family of zinc-dependent endopeptidases. They are involved in a number of normal and pathological processes. Degradation of the extracellular matrix was regarded the primary function for MMPs, but several other functions have also been associated with MMPs, including signaling for cell growth, inflammation and angiogenesis.[11] MMP-2 and MMP-14 are constitutively expressed throughout lung development, and their expression diminishes once lung development is completed. These may become upregulated again in response to disease states later in life. Other MMPs (MMP-12, -3, -9, -7, and -21) are not expressed early in lung development, but their expression is induced in response to injury or environmental causes.[9] MMP-2 and MMP-9 are particularly important in the pathogenesis of inflammatory, infectious and neoplastic diseases in many organs including the lung.[10,12] MMP-7 is another subgroup of MMPs, maintaining innate immunity in lungs and is overexpressed in malignant transformations.[13] Here, we investigated the expression levels of MMP-2, MMP-7, and MMP-9 in healthy lungs and CLMs lesion.

The majority of MMPs are not expressed in normal healthy tissues, but are expressed

Table II. The expression profiles of MMP-2, MMP-7, and MMP-9 in the lesion compared with adjacent normal tissues by comparative Ct method ($2^{-\Delta\Delta Ct}$).

	$2^{-\Delta\Delta Ct}$ values		
	MMP-2	MMP-7	MMP-9
CPAM	0.673	4.103	3.187
CLO	0.745	1.145	1.456
BPS	0.678	1.245	1.678
BC	0.567	1.993	3.373

BC: Bronchogenic cyst, BPS: Bronchopulmonary sequestration, CLO: Congenital lobar overinflation, CPAM: Congenital pulmonary airway malformation, MMP: Matrix metalloproteinase.

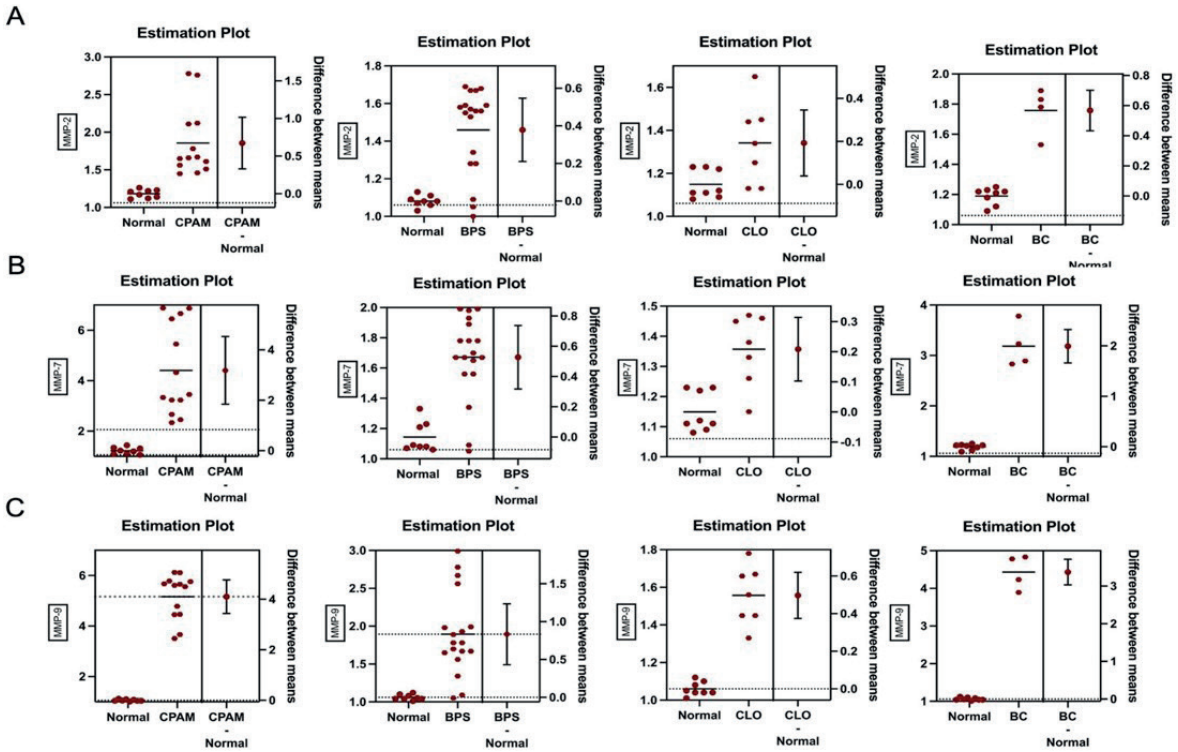

Fig. 1. The expression profiles of MMP-2 (A), MMP-7 (B) and MMP-9 (C) in congenital lung malformations compared to normal lung tissues.

BC: Bronchogenic cyst, BPS: Bronchopulmonary sequestration, CLO: Congenital lobar overinflation, CPAM: Congenital pulmonary airway malformation, MMP: Matrix metalloproteinase.

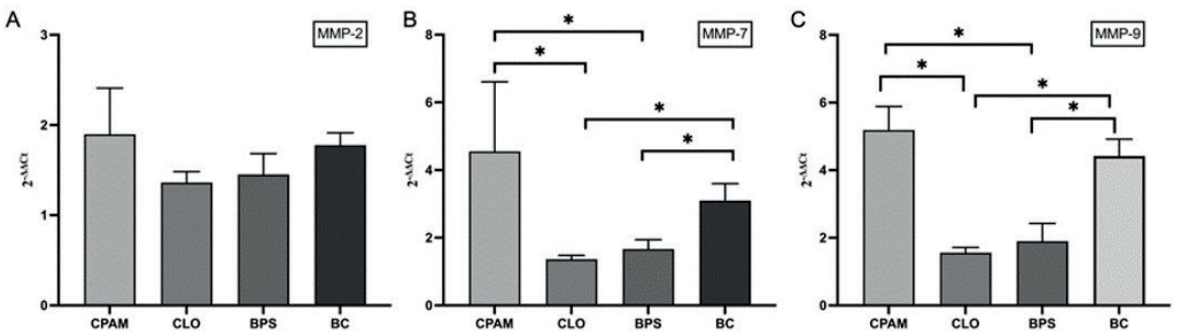

Fig. 2. The expression profiles of A) MMP-2, B) MMP-7 and C) MMP-9 in different congenital lung malformations.

*p value <0.05

BC: Bronchogenic cyst, BPS: Bronchopulmonary sequestration, CLO: Congenital lobar overinflation, CPAM: Congenital pulmonary airway malformation, MMP: Matrix metalloproteinase.

in diseased tissues that are inflamed or undergoing repair and remodeling.[14] Pelizzo et al.[15] reported a 79% incidence of pulmonary inflammation in patients with CPAM, who underwent surgery during early postnatal period and within 3 months of life, and added that early signs of inflammation can be present even in asymptomatic infants. They questioned whether this inflammatory process begins before birth. They suggested that the presence of inflammation without signs of infections suggests a form of inflammatory reaction induced by the malformation itself. In fact, in our study, MMPs which are known to increase

during inflammation were overexpressed in CPAMs and other types of CLMs, although we excluded CLM samples with any sign of infection or presence of inflammatory cells in tissue samples. Furthermore, in our study, no statistically significant relationship was found between MMPs and symptoms ($p>0.05$). The reasons that initiate this inflammatory process are still unclear. Focal airway obstruction may initiate the inflammatory process.

CLMs are a heterogeneous group but hybrid lesion or occasional coexistence of these cystic lesions suggest that there may be a single pathologic mechanism for their development.[16] Level, completeness, and timing of the in-utero airway obstruction determine the different patterns of lung malformation.[4,16] The recent studies have shown that bronchial atresia/obstruction is associated with all types of developmental lung malformations.[6-8] Tang et al.[17] reported a positive correlation between the levels of MMP-9 and the length of foreign body retention. They suggested that mechanical obstruction of foreign bodies leads to the enhanced generation of MMP-9 and also eventual airway remodeling. The overexpression of MMPs leads to matrix breakdown, tissue destruction, and cystic lesions.[18] We found that MMP-7 and MMP-9 mRNA expressions showed higher increases in CPAM, BC, BPS and CLO, which indicated inappropriate remodeling in the lung tissue. The underlying reason is unclear, but a focal obstructive process of the tracheobronchial tree is proposed to explain the pathophysiology.[4]

The management of a newborn with a symptomatic lesion is surgery, but management of an asymptomatic one can be controversial. The main argument in favor of routine resection during infancy is the long term risk of infection or malignancy.[5] All histological types of CLMs may be associated with malignant lung lesions.[19,20] Little is known about underlying pathophysiology of CLMs and the processes that may promote their malignant transformation.[1] In addition, elevated expression levels of MMPs are associated with several cancers. MMP-2, MMP-7 and MMP-9 have essential roles in tumor angiogenesis and in a broader perspective for tumor development. MMPs have been investigated in lung cancer for prognostic factor or potential therapeutic targets.[12,21] In our study, a higher expression of MMP-7 and MMP-9 was found in the all CLM groups but there was a trend in MMP-2 mRNA expression in CPAM tissues compared to normal tissue. MMP-2 showed high expression in the BPS, CLO and BC groups, but was not statistically significant. In their comprehensive review on the association between CLMs and lung tumors in children and adults, Casagrande et al.[20] found that the CLM that was most often associated with lung tumor was CPAM, followed by BC. They also mentioned that malignant malformation of CLMs might be secondary to prolonged inflammatory episodes and metaplastic changes as chronic inflammatory diseases have been recognized to stimulate neuroendocrine cell proliferation. In our study, when all groups were evaluated together, MMP-7 and MMP-9 mRNA expressions showed higher increases in CPAM and BC compared to BPS and CLO. While our findings suggest that an association can be established between congenital lung lesion, inflammation and tumor development, more evidence is needed to explain this association.

Our study highlights the significant overexpression of MMP-7 and MMP-9 in congenital lung malformations, particularly in CPAM and BC, compared to BPS and CLO, suggesting a potential link between inflammation, extracellular matrix remodeling and tumorigenesis. The elevated levels of these MMPs underscore their role in the inappropriate remodeling of lung tissue and raise concerns regarding their contribution to the malignant transformation of CLMs. Although MMP-2 expression showed an upward trend in CPAM, BPS, CLO, and BC, it was not statistically significant.

The results reinforce the hypothesis that inflammation, possibly triggered by focal airway obstruction or intrinsic malformation, could be a driving factor in the pathophysiology of CLMs.

This aligns with prior research suggesting that prolonged inflammatory episodes and matrix breakdown may facilitate tumorigenesis.

Considering the elevated expression of MMP-7 and MMP-9 in CLMs and their established role in tumor development and angiogenesis, our findings provide further support for the potential utility of MMPs as biomarkers or therapeutic targets in lung disease. However, more extensive studies are needed to establish the precise mechanisms linking CLMs, inflammation, and tumor development, as well as to clarify the role of MMP-2 in this process.

Ethical approval

The study was approved by the Bursa Uludağ University Ethics Committee Institutional ethical committee approval was provided for the study (Date: 06.03.2024 Approval no: 2024-3/2).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AP, SAA, BT; data collection: AP, SAA, ME, ÇT, HÖN; analysis and interpretation of results: AP, SAA, BT, ANG; draft manuscript preparation: AP, BT, ANG. 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

DNA damage in children with β-thalassemia minor: genotoxicity assessment by comet assay

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ABSTRACT

Background. In transfusion-dependent forms of β-thalassemia, chronic anemia and iron overload lead to the development of oxidative stress-related DNA damage. In β-thalassemia minor (β-Tm), oxidative stress resulting from an unbalanced globin chain ratio has been documented, even in the absence of anemia and its complications. However, the status of oxidative stress-related DNA damage has not yet been elucidated. The aim of this study was to assess DNA damage in β-Tm in a pediatric population.

Material and Methods. We compared 142 children with β-Tm to 113 healthy controls, including siblings of the β-Tm individuals. The comet assay was used to assess DNA damage in peripheral blood lymphocytes. Additionally, oxidative stress markers and biochemical parameters were measured.

Results. No significant differences were observed between the β-Tm group and controls in terms of demographics, biochemical parameters, or baseline oxidative stress levels ($p>0.05$). In the comet assay, there was no difference in tail intensity (TI) between subjects and controls, nor between siblings with and without β-Tm ($p=0.551$ and $p=0.655$, respectively). However, when the β-Tm group was divided by age, a gradual increase in DNA damage, as measured by TI, was observed. This increase was more pronounced in the β-Tm group compared to controls.

Conclusion. We observed no significant differences in DNA damage between β-Tm individuals and controls. However, TI increased at a faster rate with age in carriers compared to non-carriers, suggesting that environmental factors might exert a more pronounced influence on the genetic integrity of individuals with a β-Tm background. Although β-Tm itself does not seem to pose a substantial genotoxic risk in childhood, our findings underscore the importance of further research into the interplay between β-Tm and other risk factors throughout life. We advocate for long-term monitoring of β-Tm children to assess the health and potential genetic consequences.

Key words: DNA damage, oxidative stress, comet assay, children, β-thalassemia minor.

Thalassemias, resulting from inherited defects in the production of hemoglobin, are among the most common genetic disorders worldwide.[1-4] They are characterized by absent or reduced synthesis of one or more globin chains. Degradation of unpaired chains in erythrocytes causes premature cell death. Thalassemias are designated depending on the affected globin chain. In β-thalassemia, β-globin chain production is impaired due to mutations in one or both of the β-globin genes, each located on a haploid chromosome. Clinically, β-thalassemia may occur in major (homozygous), intermediate (usually compound heterozygous), and minor

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(heterozygous) genetic forms. In β-thalassemia major (TM), the severe form of the disease, both alleles are affected and life-threatening anemia develops. If untreated, anemia leads to tissue hypoxia and resultant redox imbalance.[5,6] TM patients require lifetime blood transfusions, which lead to iron overload. This pathological condition plays a crucial role in the generation of reactive oxygen species (ROS) that are capable of mediating the cellular macromolecules including deoxyribonucleic acid (DNA). Patients suffer from cumulative oxidative damage produced by iron and hypoxia.[5,6] Research on sickle cell disease (SCD) and iron deficiency anemia (IDA) indicates elevated oxidative stress and DNA damage. SCD, which is another hemoglobinopathy, similar to thalassemia, is linked to persistent inflammation and oxidative stress, resulting in increased DNA damage.[7,8] Deficiency of iron, like its overload, although exhibiting less oxidative stress compared to thalassemia or SCD, may still induce genomic instability.[9,10] Similar complications, to a lesser degree, develop in β-thalassemia intermedia. Thalassemia minor (Tm) is the less-severe form of the disorder and is usually expressed as only mild anemia with no clinical disability.[11]The main goal in the detection of Tm in individuals is the opportunity for genetic counseling. However, the potential clinical significance of Tm in some health fields, including DNA damage, has been described.[12-18] There is extensive knowledge about DNA damage in patients with β-thalassemia; however, DNA damage in heterozygotes remains unclear. β-thalassemia is common and ascertaining DNA damage status in β-Tm individuals, the most frequent form, is important worldwide. Determination of potential DNA damage during childhood, known as a critical step in the initiation of cancer, is necessary to enable the regulation of living conditions for those individuals.

DNA damage refers to alterations in DNA structure that may lead to loss of genome integrity.[19,20] Genotoxicity has been shown to be associated with radiation, cigarette smoking and environmental tobacco smoke (ETS) exposure, viral infections, vitamin deficiencies, and some genetic disorders (e.g., ataxia telangiectasia, Bloom's syndrome).[21-23] Oxidation of bases is one of the mechanisms of DNA damage.[19,20] ROS generated by external agents or normal metabolic/cellular processes, as observed in thalassemia, have a genotoxic effect via oxidation reaction leading to DNA strand breaks.[24-27] Among genotoxicity assays, the comet assay is widely used for the detection of DNA damage in several clinical conditions, including childhood diseases.[28-31] Comet assay can directly assess the damage at a single cell level.

The aim of this study was to assess, for the first time, DNA damage by comet assay in a pediatric population with β-Tm using several biochemical parameters including oxidative status tests.

Materials and Methods

Subjects

The present study, assessed DNA damage in β-Tm. 142 children (73 males, 69 females; age: 6.0±3.6 years (mean±SD), range: 1-17 years) with β-Tm from the Pediatric Hematology Department of Gazi University Medical Faculty were recruited between July 1, 2014 and December 31, 2015. β-Tm was diagnosed based on red cell indices on complete blood count (CBC) and hemoglobin (Hb)A2 level in hemoglobin electrophoresis; microcytosis (mean corpuscular volume [MCV]<80 fL), hypochromia (mean corpuscular hemoglobin [MCH]<25 pg) and erythrocytosis (red blood cells [RBC]>5x10^6/L) on CBC, with normal body iron status, and simultaneously increased HbA2 level (≥3.5%) were consistent with the diagnosis. In some individuals with similar results but normal HbA2 levels, the diagnosis was achieved by parental study and molecular analysis of the β-globin gene. As controls, 113 children (50 males, 63 females; age: 8.2±3.4 years, range: 1-17 years) were enrolled. A segment of the control

group was formed from healthy siblings of the β-Tm subjects (n=34, 30% of the controls). For those subjects with no healthy sibling, age- and gender-matched healthy children (n=79, 70% of the controls) were enrolled as their controls. Children with infections, a chronic or genetic disorder, taking any medications, or with a history of blood transfusion for any reason were not included. This study was approved by the ethics committee of Gazi University Faculty of Medicine (No: 162, Date: 24.03.2014) and supported by Gazi University Faculty of Medicine Research Fund (DA, 01/2014-19). The parents of the children were informed about the study and all gave informed consent prior to their children's involvement. Detailed questionnaire forms were completed by the parents, and included age, height, weight, education, cigarette smoking and ETS exposure, recent diagnostic X-ray examination (yes/no; within 3 months prior to the sampling), use of vitamins (e.g., multivitamin, folate, vitamins B and C), viral infections (e.g., varicella, mumps) over the previous 2 years, recent vaccination in the previous year, and physical activity of the children.

Biological sampling

Biological sampling for both the patient and control groups was completed simultaneously between July 2014 and December 2015. Blood samples were collected by two certified phlebotomists by venipuncture to use in the analysis of hematological parameters, biochemical parameters, oxidative status tests, and DNA damage endpoint. In all subjects, hematological parameters of CBC, body iron status (iron, iron binding capacity, and ferritin levels), and hemoglobin electrophoresis as routine thalassemia diagnostic tests and biochemical parameters of serum vitamin B12, folate and C-reactive protein (CRP) were studied. Automated blood counts were performed using a Coulter (LH 780 Hematology Analyzer by Beckman Coulter), and all samples were analyzed on the Bio-Rad Variant TM II high performance liquid chromatography (HPLC) system. Remaining biochemical parameters were analyzed using a hormone analyzer and chemiluminescent immunoassay (Immulite 1000 apparatus [Siemens Health Diagnostics, U.S.]). For analysis of total oxidative status (TOS) and total antioxidant capacity (TAC), as oxidative status parameters, serum was separated by centrifugation for 10 min at 4500 rpm and stored at -80°C until analysis. The levels of TOS and TAC were determined using a commercially available kit (Rel Assay Diagnostics, Gaziantep, Turkey). The results were expressed as mmol Trolox Eqv./L and H_2O_2 Eqv./L, respectively. The levels of vitamins C and E, as antioxidative capacity tests, were measured using commercially available kits (Human Vitamin C, VC ELISA Kit [Cat no: CSB-E08090h] and Human Vitamin E,VE ELISA Kit [Cat no: CSB-E07893h], respectively). The results were expressed as ng/mL and mmol/mL, respectively. Blood samples in heparin-containing tubes were delivered to the laboratory within the same day for genotoxicity testing by comet assay.

Comet assay

The alkaline comet assay was performed on the day of sampling according to Singh et al. with slight modifications.[32] Lymphocytes from 2 mL heparinized whole blood were isolated by density gradient centrifugation using Biocoll (AG Biochrom, Berlin, Germany). Lymphocytes were suspended in 100 μL of 0.65% low melting-point agarose (LMA) at 37°C, and 200 μL of mixture was layered onto a microscope slide precoated with 0.65% high melting–point agarose (HMA). The slide was immediately covered with a coverslip, and the agarose was allowed to solidify for 5 min on ice. Two gels were prepared for each sample. After removal of coverslips, the slides were immersed in light-protected cold lysing solution (89% lysing buffer [2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris-HCl; pH 10], 1% Triton X-100, 10% dimethylsulfoxide) overnight at 4°C for lysis. Slides were pretreated for 20 min in freshly prepared electrophoresis buffer (0.3 M NaOH,

1 mM Na_2EDTA: pH 13) for unwinding, then exposed to 25 V and 300 mA for 20 min at 4°C (Thermo EC250-90). The slides were neutralized three times for 5 min in the neutralizing buffer (0.4 M Tris-HCl: pH 7.5). The gels were then stained with 50 μL ethidium bromide (20 μg/mL), and 50 cells per slide were scored by use of the Comet Assay III image-analysis system (Perceptive Instruments, UK) with a fluorescence microscope (Zeiss Axioscope, Germany). Tail intensity (TI) (percent DNA in the tail) was chosen as the measure of DNA damage.

All parameters were compared between the patient and control groups. The patient group was further divided into age subgroups (<5 years [n=68, 48%], 5-10 years [n=60, 42%], >10 years [n=14, 10%]), and TI values were compared within these subgroups. A similar analysis was conducted for the control group.

Statistical analysis

The Statistical Package for the Social Sciences version 22.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used in the analysis of the data. Receiver operating characteristic (ROC) curve analysis was used for determining the optimal cut-off value of age for discrimination between the β-Tm and control groups. Independent samples t-test was used to compare two independent groups (β-Tm and controls) for normally distributed variables, and Mann–Whitney U test was used for comparison of non-normally distributed variables. Pearson chi-square test was used to compare the two groups (β-Tm and controls) for differences in categorical variables. Arithmetic mean, standard deviation, median, and minimum-maximum values were given as descriptive statistics for quantitative data. Qualitative data were summarized using frequency and percentages. A p value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Some general characteristics of the study population are shown in Table I. The groups were similar according to gender, body mass index (BMI), ETS exposure, viral infections over the previous 2 years, the frequency of use of vitamins, vaccination in the year prior, and X-ray exposure in 3 months prior ($p>0.05$ for all, Table I). Although the age range was similar, the mean age in the control group was found to be higher than that in the β-Tm group (8.2±3.4 versus 6.0±3.6 years, respectively) ($p<0.05$, Table I). The β-Tm group was further divided into 2 age groups according to ROC curve (≤7 years and >7 years) to determine any potential confounding effect of age on the baseline parameters. However, comparison of the age groups showed similar results, excluding an effect of age on these parameters.

Hematological and biochemical parameters of the groups are shown in Table II. Red cell indices of Hb, MCV, MCH, red cell distribution width (RDW), RBC and HbA2 level were significantly different between healthy controls and β-Tm children ($p<0.05$ for all, Table II). With regard to biochemical parameters including serum iron, transferrin saturation, serum ferritin, vitamin B12, folate, and CRP, β-Tm children and controls were similar ($p>0.05$ for all, Table II). According to oxidative status tests of TOS, TAC, and serum levels of vitamins C and E, the β-Tm group and control group were similar ($p>0.05$ for all, Table II).

In the comet assay, TI showed no statistically significant difference between the β-Tm (n=142) and control groups (n=113) (median [min-max], 6.5 [2.6-31.8] versus 6.7 [1.7-40.9], respectively) ($p=0.551$, Fig. 1A). Similarly, TI was not significantly different between the

Table I. General characteristics of the patient and control groups.

	β-thalassemia minor	Control	*P* value
Age (mean±SD)	6.0±3.6	8.2±3.4	<0.05
Gender			
Male, n (%)	73.0 (51.4)	50.0 (44.2)	>0.05
Female, n (%)	69.0 (48.6)	63.0 (55.8)	>0.05
BMI (mean±SD)	17.1±3.3	17.2±3.2	>0.05
ETS exposure, n (%)	25.0 (17.6)	24.0 (21.2)	>0.05
Viral infection, n (%)	7.0 (5.0)	6.0 (5.3)	>0.05
Use of vitamin, n (%)	8.0 (5.7)	3.0 (2.7)	>0.05
Vaccination, n (%)	55.0 (39.0)	26.0 (23.0)	>0.05
X-ray exposure, n (%)	29.0 (20.4)	24.0 (21.2)	>0.05

BMI: body mass index, ETS: enviromental tobacco smoke, SD: standard deviation. For comparisons, Mann Whitney U test, Pearson chi square and Fisher's excact tests were used. Differences were accepted as statistically significant at a *p* value of <0.05.

Table II. Descriptive statistics of hematological, biochemical, and oxidative status parameters in the patient and control groups.

	β-thalassemia minor		Control		P value
	Median	(min-max)	Median	(min-max)	
Hb (g/dL)	11.6	(8.1-14.7)	13.0	(10.2-17.0)	<0.001
MCV (fL)	65.0	(53.4-79.8)	83.0	(57.9-89.7)	<0.001
RBC (x10^6/L)	5.5	(4.4-7.1)	4.7	(4.0-6.2)	<0.001
RDW (%)	15.8	(12.2-30.9)	13.2	(11.8-16.4)	<0.001
MCH (pg)	20.8	(16.2-27.2)	28.0	(18.4-30.1)	<0.001
Hb A2 (%)	3.5	(2.1-8.7)	3.0	(2.0-6.0)	<0.001
Ferritin (ng/mL)	33.4	(7.8-223.7)	34.5	(10.4-149.3)	>0.05
Transferrin saturation (%)	26.3	(3.4-104.2)	26.8	(6.8-106.1)	>0.05
C-reactive protein (mg/L)	1.9	(0.3-12.7)	2.0	(1.0-16.6)	>0.05
Vitamin B_{12} (pg/mL)	396.0	(117.3-1208.0)	364.5	(166.2-1240.0)	>0.05
Folate (ng/mL)	11.2	(4.9-80.0)	11.2	(4.9-48.7)	>0.05
TAC (mmol/L)	2.2	(0.2-4.0)	2.2	(0.2-4.2)	>0.05
TOS (μmol/L)	6.4	(1.3-51.1)	6.6	(0.3-67.0)	>0.05
Vitamin C (ng/mL)	63.2	(6.4-313.9)	69.4	(9.7-312.1)	>0.05
Vitamin E (nmol/ml)	30.9	(10.3-130.3)	37.7	(10.4-128.9)	>0.05
	Aritmetic Mean	Std. deviation	Aritmetic Mean	Std. deviation	
Iron (μg/dL)	77.4	30.6	85.2	32.5	>0.05

Hb: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, RBC: red blood cell, RDW: red celrl distribution width, TAC: total antioxidant capacity, TOS: total oxidative status. Independent samples t-test was used to compare two independent groups that are β-thalassemia minor and control for serum iron, and Mann–Whitney U test was used for comparison for other variables.

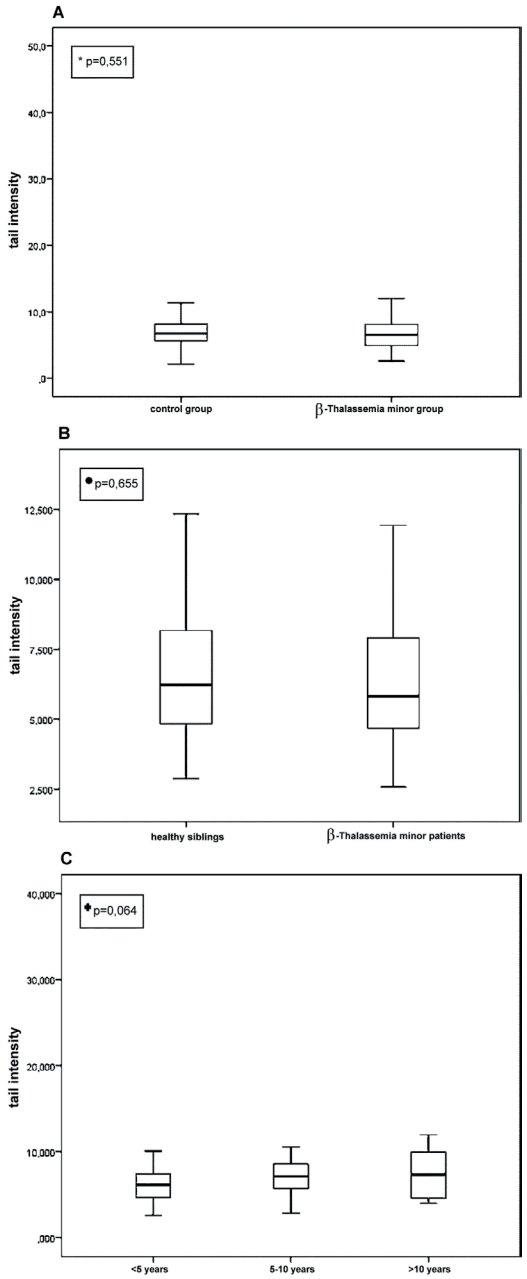

Fig. 1. Box plots showing the DNA damage measured by comet assay in lymphocytes. **A)** The results of beta thalassemia minor children and of controls. **B)** The results of beta thalassemia minor children and of their healthy siblings. **C)** The results of age subtypes of beta thalassemia minor group.

* β-Thalassemia minor group (n=142) vs control group (n=113).

• β-Thalassemia minor patients (n=34) vs their healthy siblings (n=34).

+Among age groups of β-thalassemia minor children (<5 years [n=68, 48%), 5-10 years [n=60, 42%], >10 years [n=14, 10%]).

β-Tm children (n=34) and their healthy siblings (n=34) (median [min-max]; 5.8 [2.6-11.3] versus 6.2 [2.9-12.4], respectively) (p=0.655, Fig. 1B). After further dividing the β-Tm group into three subgroups (<5 years [n=68, 48%], 5-10 years [n=60, 42%], >10 years [n=14, 10%]), TI was not significantly different among age subgroups, but a gradual increase by age was observed (median [min-max]; 6.1 [2.6-12.0], 7.1 [2.8-31.8], and 7.3 [4.0-11.9], respectively) (p=0.064 for all) (Fig. 1C). A similar increase was observed when the control group was divided into age subgroups, and this increase was also not statistically significant (median [min-max]; 6.2 [1.7-9.0], 6.3 [2.8-40.9], and 6.9 [3.1-12.3], respectively) (p >0.05 for all). The increased rate of TI in β-Tm group was higher than that in the control group.

Hb levels did not exhibit a significant correlation with TI or oxidative status parameters (TOS, TAC, vitamins C and E) (p>0.05 for all).

Discussion

Hemoglobinopathies, including thalassemia and SCD, are genetic disorders affecting hemoglobin structure or synthesis. In severe forms, such as transfusion-dependent thalassemia, chronic hypoxia due to anemia and iron overload from transfusions contribute to oxidative stress and subsequent DNA damage. This cumulative oxidative stress is a key mechanism underlying genotoxicity in these conditions.[5-7,10,33-39] Although β-Tm is the silent form of the disorder, β-Tm individuals have also been shown to be at a risk of oxidative stress generated from unpaired globin chains.[40-43] In addition, lower antioxidative capacity has been shown in heterozygotes.[44,45] Though the oxidative stress-related DNA status in heterozygotes is as yet unknown. By investigating DNA damage in children with β-Tm, the present study might help enlighten this. To our knowldge, to date only two genotoxicity studies have examined DNA damage status in β-Tm. In both studies, adult subjects were assessed, the sample size was small, and none or only a few biochemical

parameters were studied. In the study by Al-Sweedan et al., for example, only 18 individuals were assessed. No parameter in the study by Krishnaja et al. and only a single parameter in the study by Al-Sweedan et al. (8-hydroxy-2′-deoxyguanosine [8-OHdG] level in urine, as oxidative stress parameter) was studied.[12,17] Our study was carried out in a large population of children enabling appropriate statistical analysis using comet assay, and was strengthened by the use of several biochemical parameters. In addition, unlike in the previous two studies, our control group included healthy siblings of the subjects. Comet assay is widely used for genotoxicity assessment in various disorders and particularly in transfusion-dependent thalassemia.[5,6,28,32-37] However, to date, it has not been used for genotoxicity assessment in Tm individuals. Considering these factors together, our study design is unique, with no comparison in the literature.

In this study, children with similar demographic features, socioeconomic conditions and habits were enrolled in the β-Tm and control groups. Although the mean age was found to be higher in the control group, further evaluation after controlling for the effect of age on other variables revealed no additional influennce on the results. It can, therefore, be said that the only distinctive feature between the patient and control groups was the presence of β-Tm. As a natural consequence, erythrocyte indices and HbA2 levels were significantly different between the groups, which also served the purpose of distinguishing the heterozygotes and healthy children. In children with β-Tm, a disorder characterized with hypochromic microcytic anemia, red cell indices of Hb, MCV and MCH were lower, and RBC and HbA2 levels, as expected, were higher.

Iron, folate and vitamin B12 have a povital role in maintaining healthy cell division and DNA synthesis. Deficiencies of these micronutrients inhibit purine and thymidylate synthesis.[46,47] Impaired DNA synthesis can cause genomic instability, some of which may give rise to cancer. Results of studies investigating the levels of serum iron, folate, and vitamin B12 in heterozygotes have been inconsistent.[44,48-51] Despite their certain effect on DNA synthesis and uncertain levels in heterozygotes, these micronutrients were not measured in the two previous studies investigating DNA damage in β-Tm. In our study, the levels of folate and vitamin B12 were assessed and found to be within normal limits. In all studies investigating DNA damage in patients with transfusion-dependent thalassemia, iron was measured because of its genotoxic effect when accumulated.[5,6] Our study population, however, consisted of subjects with transfusion-free Tm. We measured serum iron as part of the diagnostic tests and because its deficiency might affect DNA synthesis. Our results with regard to all parameters of body iron status were similar with those of the controls and were within normal limits. The results for CRP, a marker for inflammation and infection commonly used in genotoxicity studies, were within normal limits and similar to those of the controls.[52,53]

Oxidative stress plays a pathological role in the development of various diseases, including cancer. Systemic oxidative stress results from an imbalance between oxidant derivatives production and antioxidants defenses. In β-Tm, there is an unbalanced globin chain synthesis, which results in excess α-globin chains in erythrocytes. This has been shown to generate ROS, which put the patients at a high risk of oxidative stress.[40-42,44,45] In addition, it has been shown that heterozygotes have increased non-heme iron content of erythrocytes.[54] Non-heme iron can inhibit the action of various cytoplasmic enzymes, thereby altering cellular homeostasis. Thus, the cell experiences oxidative stress and possibly significant protein degradation and lipid peroxidation in the cell membrane.[55] Increased oxidative stress in heterozygotes may hypothetically be exacerbated by a decreased level of natural antioxidants, which was previously documented.[37] Antioxidant defense can be evaluated by measurement of either individual antioxidant levels in cell and serum or TAC. In our study, oxidative stress

was double-checked by testing both oxidants (measured as TOS) and antioxidants defenses (by measurement of the individual antioxidant levels of vitamins C and E in serum and also the TAC), and the results were found to be similar in heterozygotes and healthy controls. Those measurements were not taken in the two previous studies. In the study by Al-Sweedan et al., the observation of increased DNA damage was speculated to be related with the lower serum levels of these vitamins in heterozygotes; however, vitamin levels in their study were estimated based on literature knowledge and not measured directly.[17] In our study, serum levels of vitamins C and E were measured and were not found to be significantly different from those in controls, probably due to our selection of the subjects and controls from the same environment and/or family (Table II).

Genotoxicity refers to all types of alterations in DNA sequence, and genotoxic events have been known to be a critical step in the initiation of cancer.[21,25,27] The assessment of cancer risk using genotoxicity assays is of utmost importance. Patients with TM and SCD have an increased risk of cancer compared to the general population. While the underlying mechanisms of cancer development in these patients are not fully understood and are likely multifactorial, disease-related DNA damage is a highly probable contributing factor.[56-60] Among the various methods employed in the estimation of DNA damage, the comet assay used in our study is proven to be a relatively simple, effective and versatile tool, and is a validated biomarker assay.[28,31,32] With regard to DNA damage, we found no significant difference between the β-Tm and control groups (Fig. 1A). Further, there was no significant difference in DNA damage between the β-Tm children and their healthy siblings (Fig. 1B). However, after further dividing the β-Tm group into three age subgroups, TI was found to gradually increase with age. The most striking finding was that the control group had a higher mean age compared to the β-Tm group, and healthy siblings had a higher mean age than β-Tm children. Moreover, the TI value increased with advancing age in all age subgroups of the control group. These findings suggest that age has an impact on the TI value. Interestingly, the age-related increase in TI value was more rapid in β-Tm children compared to the control group. Although carrier children initially had numerically lower TI values than controls, they increased more rapidly within the same age range, even surpassing control TI values. These results indicate that while age has an increasing effect on the TI value, environmental factors may exert a more pronounced influence in the context of β-Tm. This supports the notion that β-Tm children should be monitored at appropriate intervals throughout adulthood and protected against certain risks.

In the two previous studies, micronuclei frequency in lymphocytes and chromosomal aberrations, sister chromatid exchanges and 8-OHdG assays were used to investigate DNA damage in adult heterozygous subjects.[12,17] Thus, we cannot compare our study with these except for the fact that subjects were β-Tm individuals. In both studies, increased DNA damage was found in heterozygotes compared to healthy controls. The most likely explanation is age. Although there were several disadvantages regarding their study design, particularly their small population size, it may be speculated that the age factor and its related conditions (e.g., cigarette smoking, alcohol consumption, and environmental and occupational factors) might have contributed to the potential genotoxicity in adult subjects with Tm and augmented it to a detectable level. In accordance, the division of our β-Tm group into three groups according to age revealed numerical differences in DNA damage among the subgroups, suggesting that reassessment beyond the pediatric age range is necessary. A more severe effect of some mutations might also be responsible for the different results.[61]

The micronucleus (MN) assay in a thesis study investigating DNA damage in children with β-Tm found similar levels of genotoxicity,

cytotoxicity, and oxidative stress parameters compared to controls. However, β-Tm children had a higher frequency of MN values above the cut-off level (39.2% vs. 21.6% in controls, $p<0.05$).[62] This result obtained with a method that shows DNA damage at the cytome level, as in the comet assay, suggests a potential effect of β-Tm on DNA damage and emphasizes the need for monitoring carrier children.

Our study benefits from a substantial sample size and the evaluation of multiple factors linked to DNA damage. Additionally, we included a significant number of unaffected siblings in the control group. However, we were unable to measure intracellular vitamin levels and did not follow up on TI parameters in adulthood, which are our limitations.

In conclusion, our study, the first to our knowledge to investigate DNA damage in a large pediatric population with β-Tm using the comet assay, found no significant differences in DNA damage levels between β-Tm patients and healthy controls, nor between siblings with and without β-Tm. However, TI, a marker of DNA damage, increased more rapidly with age in β-Tm patients compared to healthy controls. These findings suggest that regular monitoring of DNA damage in β-Tm patients throughout childhood and adulthood, along with examining the relationship between TI and age-related risk factors, could provide valuable insights. Future studies employing diverse genotoxicity testing methods may help to further clarify the status of DNA damage in β-thalassemia.

Ethical approval

This study was approved by the Ethics Board of Gazi University Faculty of Medicine (No: 162, Date: 24.03.2014).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: DA; data collection: DM; analysis and interpretation of results: DM, EE, TG, GÇ, DA; draft manuscript preparation: DM, GÇ, DA. 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

Inflammatory myofibroblastic tumors in children: clinical characteristics and treatment outcomes with a focus on targeted therapies

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ABSTRACT

Background. Inflammatory myofibroblastic tumors (IMTs) are rare neoplasms in children. Traditionally, surgical resection has been the primary treatment modality with limited efficacy reported for conventional chemotherapy and radiation therapy. Recently, targeted therapies have emerged as potential options for selected cases. This study aimed to evaluate the demographic, clinical, laboratory, and radiological characteristics, as well as treatment outcomes, in children diagnosed with IMTs.

Methods. This study involved a retrospective review of medical records for eight children diagnosed with IMTs between 1990 and 2022. We collected demographic, clinical, laboratory, and radiological data, as well as treatment outcomes. Data on tumor characteristics, surgical procedures, and chemotherapy or targeted therapy treatments were extracted.

Results. The mean age at diagnosis was 9 years. None presented with metastatic disease at the time of diagnosis. Anaplastic lymphoma kinase (ALK) positivity was identified in tumor tissue from five patients. Among the six patients who underwent surgical resection, three achieved negative surgical margins. Of the three patients with positive surgical margins, one underwent re-resection, local and metastatic recurrences were noted in another, and one was started on crizotinib. A patient with an inoperable tumor at diagnosis was initiated on crizotinib and achieved complete remission. Ceritinib was administered to a patient with YWHAE-ROS fusion, resulting in more than 90% reduction in tumor volume. The median follow-up time was 67.5 months. The five-year overall survival and event-free survival rates for the cohort were 85.7% and 72.9%, respectively.

Conclusions. While surgical resection remains the cornerstone of treatment for IMTs, favorable outcomes can be achieved with chemotherapy and targeted therapies in selected cases. Increasing the utilization of targeted therapies may be beneficial, particularly through molecular studies aimed at minimizing the side effects associated with conventional chemotherapy.

Key words: inflammatory myofibroblastic tumor, ALK inhibitor, crizotinib, ceritinib, childhood.

Inflammatory myofibroblastic tumors (IMTs) are rare tumors that typically occur in soft tissues, particularly in children and young adults, although they can arise at any age. They were previously known as inflammatory pseudotumors and are characterized by a proliferation of mesenchymal spindle cells along with a prominent inflammatory cell component. IMTs may lead to various clinical courses due to tumor size, localization and capacity

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to invade neighboring tissues. Epithelioid inflammatory myofibroblastic sarcoma (EIMS), which is a variant of IMT, is characterized by the proliferation of epithelioid and spindle-shaped myofibroblasts within a background of inflammatory cells. While IMTs are generally benign or low-grade tumors, EIMS presents with more aggressive features and a higher potential for recurrence and metastasis.[1,2-4]

Historically, surgical resection has been the primary treatment of IMTs, with little documented efficacy of traditional chemotherapy or radiation therapy.[1-3,5]

Comprehensive genomic analyses have identified various gene rearrangements, most notably involving the anaplastic lymphoma kinase *(ALK)* gene, which occurs in over 40% of IMT cases.[6,7] Different patterns of ALK staining can be observed in both IMTs and EIMS, but the RANBP2-ALK fusion is specific to EIMS. Cases that do not exhibit ALK fusion are considerably less common and may involve translocations associated with ROS1, PDGFRB, NTRK3, RET, and IGF1R.[8]

In recent years, targeted therapies have been utilized for inoperable cases with identifiable targetable fusions. Therefore, the identification of specific molecular markers not only helps in diagnosing IMTs and differentiating them from other tumors with similar histological features but also is crucial for yielding new therapeutic targets and further refining existing treatment strategies. In this study, we share the treatment strategies applied to our patients diagnosed with IMT.

Materials and Methods

Medical records of children with IMT diagnosed and treated between 1990-2022 at the pediatric oncology clinic of a referral hospital were retrospectively reviewed. Demographic, clinical and radiological characteristics, treatment and outcome of the patients were evaluated. Follow-up time was recorded as the period from diagnosis to February 2023 or until the last visit. All patients were diagnosed histopathologically. In recent years, ALK has been investigated by immunocytochemistry (IHC) and ALK and other fusions were investigated by flourescence *in situ* hybridization (FISH) and real time polymerase chain reaction (RT-PCR).[9]

Non-mutilating surgical complete resection was performed whenever possible. In unresectable cases or in cases where surgery would lead to unacceptable morbidity, diagnosis was established by a tru-cut biopsy and neoadjuvant chemotherapy was initiated. In cases with incomplete resection, chemotherapy was used postoperatively. Chemotherapy consisted of adriamycin and ifosfamide.

The patients were evaluated by physical examination, complete blood count and biochemical tests before and during treatment and as necessary. None of the patients received radiation therapy.

This study was reviewed and approved by the Institutional Ethics Committee of İstanbul University, Oncology Institute (2023/1627454).

Statistical analyses

Statistics were calculated using IBM SPSS® 26 (Armonk, New York, U.S.). Kaplan-Meier method was used for survival analysis. Overall survival was calculated from the date of diagnosis to the date of last information on follow-up or death. Event-free survival was calculated from the date of diagnosis to the date of the first event, such as progression, relapse, or death from any cause.

Results

Patient characteristics are summarized in Table I. There were eight patients (5 male, 3 female), with a mean age at diagnosis of 9 years (range: 8 months to 17 years). Six patients had IMT and two had EIMS. None of the patients presented with metastatic disease at the time of diagnosis.

Three of the six patients (including one with EIMS) who underwent surgical resection alone

Table I. Patients' characteristics

Age/ Sex	Histopathology	Localization	ALK status	Metastasis at diagnosis	Treatment	Surgical margin	Recurrence / Progression	Further treatment	Latest status	Follow-up period (months)	Event-free survival (months)
12 y/M	IMT	Intestine	Positive	No	Resection	Negative	No		NED	163	163
10 y/M	IMT	Retroperitoneum	Positive	No	Resection	Positive	Local and metastatic (lung, omentum, liver, bone)	4 courses with VAC, and 12 courses with ICE after progression with VAC	EX	14	-
8 y/M	EMS	Back	Unknown	No	Resection	First positive. Negative after re-resection	No		NED	124	124
7 y/M	IMT	Retroperitoneum	Positive	No	Crizotinib after resection for 12 mo.	Positive	No		NED	99	98
12 y/F	IMT	Right lower extremity	Positive	No	Crizotinib for 7 mo.	Only biopsy	Local (2 mo after stopping crizotinib)	Crizotinib	NED	23	4
7 y/M	IMT	Liver	Unknown	No	Resection	Negative	No		NED	174	174
8 mo/F	EMS	Intestine	Positive	No	Resection	Negative	No		NED	36	35
17 y/F*	IMT	Right lower extremity	Negative*	No	Resection	Negative	Local (At 16 mo)	One course with IA and ceritinib after toxicity with chemotherapy	AWD	9	4

*The patient with a YWHAE-ROS fusion.
ALK: anaplastic lymphoma kinase AWD: alive with disease, EMS: Epithelioid inflammatory myofibroblastic sarcoma, F: female, IA: ifosfamide, adriamycine, ICE: ifosfamide, carboplatinum, etoposide, IMT: Inflammatory myofibroblastic tumor, M: male, NED: no evidence of disease, VAC: vincristine, adriamycine, cyclophosphamide.

achieved negative surgical margins and are currently under follow-up with no evidence of disease (NED). Among the other two patients who underwent surgical resection alone, both had positive surgical margins. One of these patients underwent re-resection for EIMS and is currently under follow-up without any events. A patient who underwent surgical resection at diagnosis and had positive surgical margins received crizotinib treatment for one year and is currently under follow-up in complete remission. Crizotinib treatment was initiated for a patient diagnosed with an inoperable tumor. After seven months of treatment, the patient achieved complete remission as confirmed by magnetic resonance imaging (MRI) (Fig. 1). Following evaluation by the multidisciplinary tumor board, it was decided to discontinue the treatment. However, local recurrence was detected on MRI two months after the cessation of crizotinib (Fig. 2). Although the patient reported no complaints and exhibited no significant findings on physical examination, crizotinib was restarted due to the infiltrative nature of the tumor and the potential morbidities associated with surgical intervention. Two months after resuming crizotinib, MRI showed a complete response (Fig. 3). The patient has been continuing treatment for four months.

Another patient diagnosed with ALK-negative IMT, who had undergone surgical resection with negative margins, was subsequently treated with conventional chemotherapy consisting of ifosfamide and doxorubicin following disease recurrence. The patient presented with symptoms including cachexia, fever, and hypercalcemia after the recurrence of the disease. The etiological factors for the fever and hypercalcemia were investigated, and it was ultimately determined that these symptoms might be related to malignancy. Despite treatment with intravenous fluids and furosemide, the hypercalcemia remained refractory. After the first cycle of chemotherapy, the patient developed febrile neutropenia, which progressed to life-threatening acute respiratory distress syndrome (ARDS), necessitating 10 days of intensive care and the hypercalcemia was refractory to chemotherapy. Molecular analysis of the tumor tissue via next-generation sequencing (NGS) revealed the presence of a YWHAE-ROS fusion. Consequently, the patient was initiated on ceritinib treatment, which has demonstrated comparable activity to crizotinib against ROS1. As a result, treatment with ceritinib was initiated, which has shown comparable efficacy to crizotinib against ROS1. Within the first week of treatment, hypercalcemia improved, and the patient's appetite significantly increased by the second week. Remarkably, the previously bedridden patient was able to mobilize. A follow-up MRI conducted three months post-initiation of treatment demonstrated a reduction of over 90% in tumor size. The patient has continued on ceritinib for six months. The patient has been using ceritinib for 6 months.

The median follow-up time for the entire cohort was 67.5 months (range: 9–174 months). The five-year overall survival and event-free survival rates for the group were 85.7% and 72.9%, respectively.

Discussion

Inflammatory myofibroblastic tumors, first described by Brunn et al. in 1939, are rare neoplasms that primarily occur in the lungs, abdomen, and orbit.[10,11] They most commonly present within the first two decades of life, although they can arise at any age and can vary widely in size and location.[1-4] Histologically benign, IMTs may exhibit locally aggressive behavior and, in rare cases, can metastasize.[3,5]

The primary treatment for IMTs has historically been complete surgical resection, although this can often be challenging and associated with significant morbidity. Incomplete resection has been linked to a high recurrence rate. Other treatment options, such as nonsteroidal anti-inflammatory drugs (NSAIDs), high-dose corticosteroids, chemotherapy, and radiotherapy, may carry serious side effects and their efficacy remains unclear.[2,3]

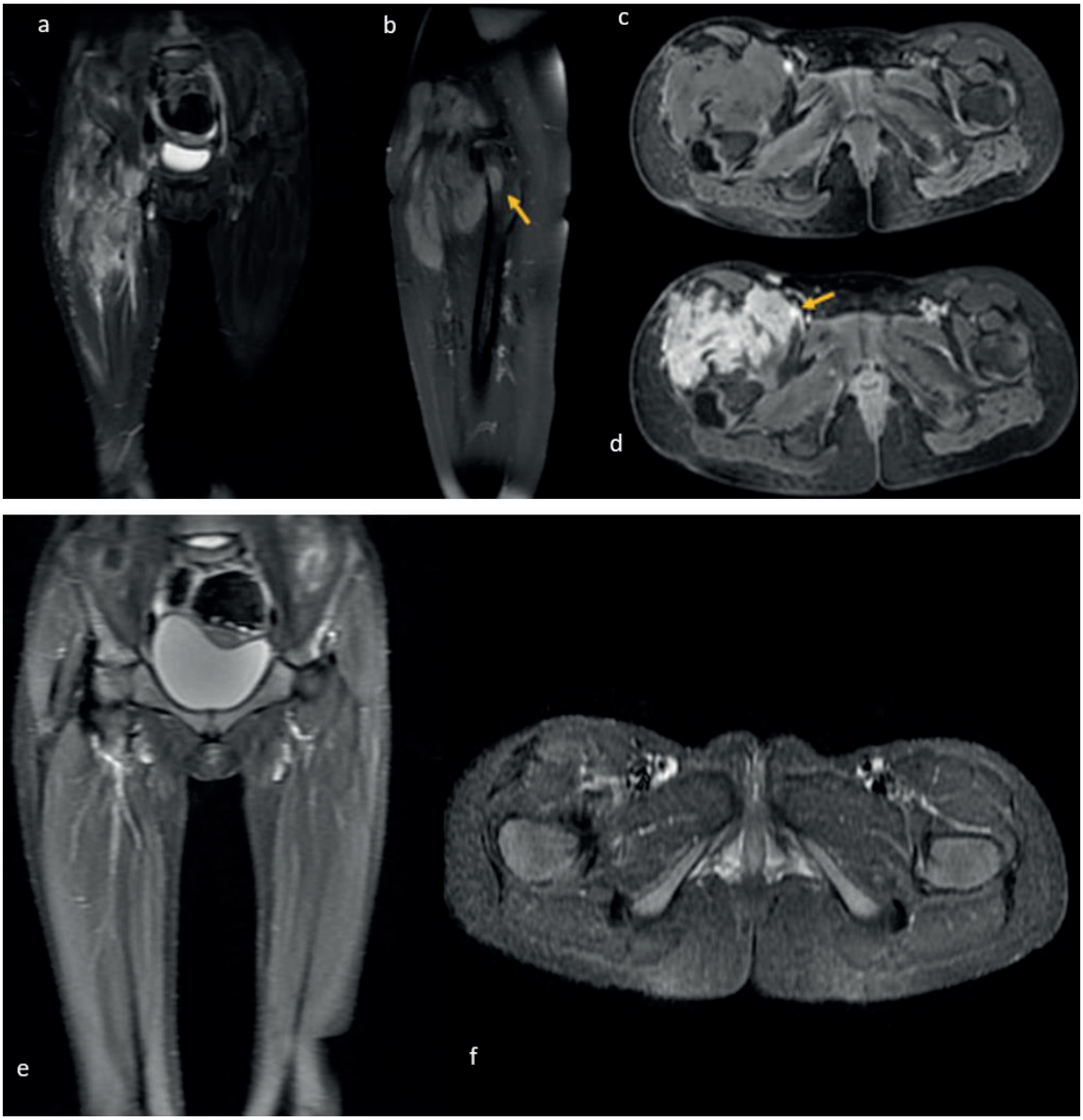

Fig. 1. MRI findings before treatment and complete regression of mass after treatment. **a)** Coronal T2 weighted image showed large lobulated soft tissue mass infiltrating the muscles in the anterior part of right lower extremity, extending into the groin. **b)** Sagittal T1-weighted image with fat suppression after intravenous administration of gadolinium showed homogenous enhancement of the mass and bone involvement (arrow). Contrast TSE image demonstrated a hypointense intermuscular mass. **c,d)** Axial fat supressed T1 weighted images before (c) and after (d) intravenous administration of gadolinium showed homogenous enhancement of the mass surrounding the femoral neurovascular bundle. **e)** Coronal and **f)** axial T1-weighted image with fat suppression after intravenous administration of gadolinium showed complete regression of mass after treatment.

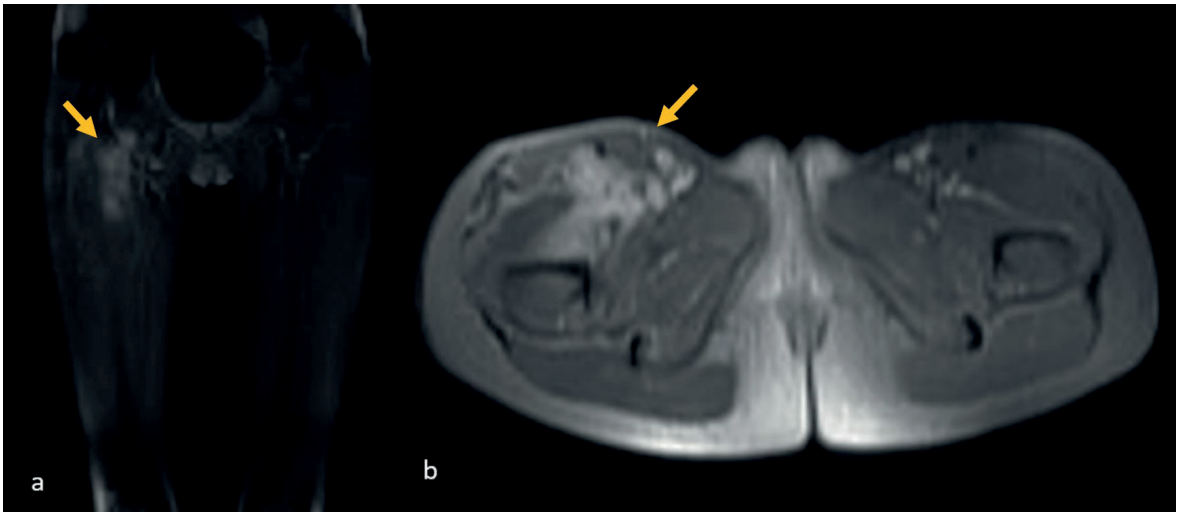

Fig. 2. MRI images at local recurrence. **a)** Coronal and **b)** axial T1-weighted image with fat suppression after intravenous administration of gadolinium showed homogenous enhancement of the mass (arrows) without bone involvement infiltrating the muscle plane in the proximal right lower extremity.

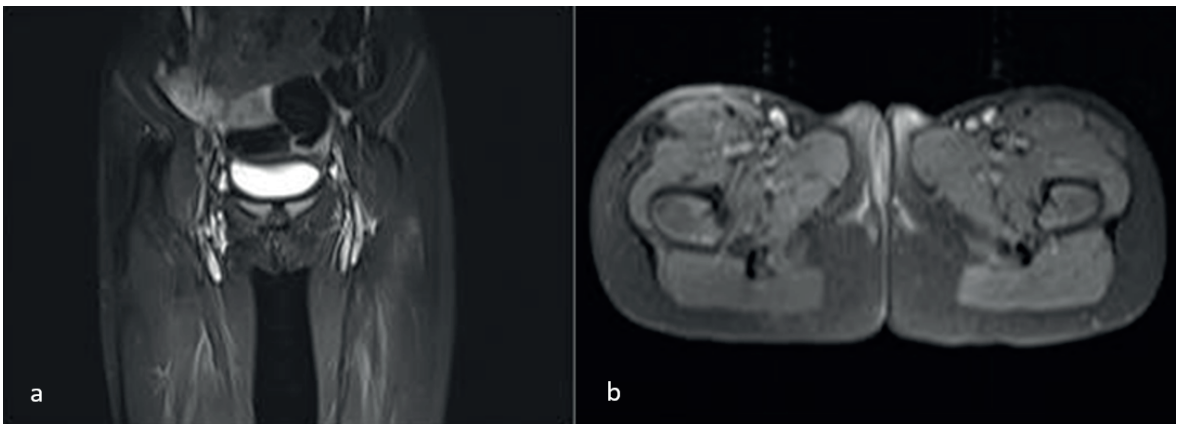

Fig. 3. Complete regression of the mass two months after re-treatment. **a)** Coronal and **b)** axial T1-weighted image with fat suppression after intravenous administration of gadolinium showed complete regression of the mass two months after re-treatment.

Approximately half of the observed IMTs involve a clonal translocation that activates the ALK receptor tyrosine kinase gene located at chromosome band 2p23, in conjunction with various partner genes. The frequency of ALK rearrangements is notably high among pediatric and young adult patients with IMT.[6,12]

In cases of IMTs that are negative for ALK fusion genes, other fusion genes such as c-ros oncogene 1 (ROS1), neurotrophin tropomyosin receptor kinase (NTRK), platelet-derived growth factor receptor (PDGFR), and RET have been identified.[9] Additionally, certain partner genes associated with ALK or other fusion genes may correlate with clinical features. For example, EIMS is a subtype of IMT that exhibits aggressive behavior and is often associated with specific fusion genes like RANBP2-ALK and RRBP1-ALK. While numerous potential fusion genes have been identified in IMT, conducting gene panel testing at the initial stages can be cost-prohibitive. If the IHC method can help narrow down the candidates for fusion genes, it could be advantageous in reducing both the cost and time required for diagnosis.[13-16]

Following the study by Mosse et al.[17] indicating the effectiveness and safety of crizotinib in pediatric patients with ALK-positive tumors, we began to consider its use in patients with inoperable tumors where ALK status was known at the time of diagnosis, as well as in cases with positive surgical margins. In one of the patients in this study, complete remission was achieved after seven months of crizotinib used as neoadjuvant therapy, leading to the discontinuation of treatment. However, a local recurrence was noted two months later, and a complete response was achieved two months after resuming crizotinib.

According to the existing literature, responses to crizotinib can vary among tumors, and there is no definitive guidance on the optimal duration of treatment for patients who respond positively.[18-27] Tumor regression is often observed early in the treatment course.[4,18] In a study involving eight cases of ALK-positive IMTs, crizotinib was discontinued in five patients after a median treatment duration of one year (range: 0.2 to 3.0 years). These patients were subsequently followed for a median of 1.7 years (range: 0.3 to 3.7 years), during which four achieved complete remission (CR) and one had stable disease (SD). This suggests that treatment may be safely discontinued without a rapid recurrence, in contrast to observations in anaplastic large cell lymphoma.[17,28]

Disease progression or recurrence has also been reported both during and after the cessation of crizotinib treatment. Cases of progression or recurrence occurring during or after crizotinib treatment are summarized in Supplementary Table 1.[25-34]

Following the previous reports by Lovly et al.[12] and Comandini et al.[35], here we represent the third case in the literature carrying YWHAE1-ROS1 fusion detected by NGS. Ceritinib, a selective oral tyrosine kinase inhibitor (TKI) of ALK, operates similarly to crizotinib but lacks MET-inhibiting capabilities and received FDA approval for treating ALK-positive non-small cell lung cancer (NSCLC) resistant to crizotinib. In enzymatic assays, ceritinib demonstrated 20 times greater potency against ALK compared to crizotinib and exhibited comparable efficacy against ROS. Additionally, ceritinib exhibited comparable efficacy to crizotinib against ROS1. In an open-label multicenter phase II study, ceritinib demonstrated robust clinical activity in NSCLC patients with ROS1 rearrangement, achieving a 62% objective response rate. The median progression-free survival was 9.3 months for all patients and 19.3 months for those who were crizotinib-naïve.[36-38] Ceritinib has also exhibited clinical effectiveness in patients with IMT. Li et al.[39] reported the first instance of using ceritinib to treat a ROS1-rearranged IMT, resulting in a partial response. By opting for ceritinib in our case, we were able to avoid the severe side effects associated with chemotherapy while achieving a high success rate in a remarkably short period.

This study has a few limitations. The small sample size (eight patients) limits generalizability, and the retrospective design introduces potential biases in treatment protocols and follow-up care. Not all patients underwent comprehensive molecular profiling, which could have missed other therapeutic targets. Additionally, the lack of long-term data on the side effects of targeted therapies like crizotinib and ceritinib is a significant gap. Lastly, a direct comparison between traditional chemotherapy and targeted therapies was not included.

Future studies should aim to include larger, multi-center cohorts to validate these findings. Expanding molecular profiling using next-generation sequencing could uncover additional therapeutic targets. Long-term studies are needed to assess the durability and safety of targeted therapies, and research should focus on identifying optimal treatment durations. Prospective trials comparing targeted therapies with conventional chemotherapy regimens would provide valuable insights into the best treatment approaches for IMTs.

In conclusion, the identification of molecular alterations in rare malignancies, such as IMTs, is essential for guiding personalized treatment strategies with targeted therapies. Tailoring treatment based on specific molecular profiles allows for the use of TKIs, which have demonstrated significant efficacy while often reducing the adverse effects associated with conventional chemotherapy. This personalized approach not only enhances treatment outcomes but also improves the overall quality of life for patients. By prioritizing a comprehensive diagnostic workup and focusing on individualized treatment plans, we can ensure that each patient receives the most appropriate and effective care, ultimately advancing the management of rare oncological conditions.

The authors declare that there is no conflict of interest to disclose.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5463

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Ethical approval

This study was approved by İstanbul University, Oncology Institute Ethics Committee (13.02.2023-1627454). Informed consent was obtained from the parents.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: RK; data collection: ÜMY, BB, BZ; analysis and interpretation of results: ÜMY; draft manuscript preparation: ÜMY. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Treatment of systemic juvenile idiopathic arthritis: conventional treatment versus biologics

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ABSTRACT

Objective. We aimed to identify and compare systemic juvenile idiopathic arthritis (sJIA) patients receiving treatment with either glucocorticoids and/or conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) or biologic drugs.

Methods. This was a retrospective cross-sectional study. sJIA patients (n=138) were categorized into two groups: Group A (n=51) consisted of individuals who received only glucocorticoids and/or csDMARDs, while Group B (n=87) included those who received at least one biologic drug.

Results. Group B patients exhibited a higher prevalence of macrophage activation syndrome (MAS) (p=0.001) at presentation. C-reactive protein (CRP) levels and systemic Juvenile Arthritis Disease Activity Scores (sJADAS) at diagnosis were significantly higher in Group B (p<0.001). A higher proportion of Group B were able to discontinue glucocorticoid treatment in a shorter timeframe (p<0.001), and a higher number of patients in this group successfully discontinued glucocorticoids within the first year (p<0.001). Presentation with MAS (odds ratio [OR] 3.419, 95% confidence interval [CI] 1.194-9.792; p=0.022), polycyclic disease course (OR 4.351, 95% CI 1.329-14.240; p=0.015), CRP levels >13.6 mg/dL (OR 2.838, 95% CI 1.182-6.815; p=0.020) and sJADAS >24.1 (OR 4.490, 95% CI 1.725-11.684; p=0.002) at diagnosis were independent predictors of biologic requirement in treatment.

Conclusion. Patients with a history of MAS, polycyclic disease course, elevated CRP, and high sJADAS at diagnosis may require biologic drugs in the treatment. This observation could help clinicians tailor treatment according to the individual needs of sJIA patients.

Key words: biologic drugs, disease-modifying antirheumatic drugs, glucocorticoids, systemic juvenile idiopathic arthritis.

Systemic juvenile idiopathic arthritis (sJIA) is a subtype of JIA characterized by arthritis as well as systemic symptoms that can affect various organs and systems.[1] The sJIA treatment aims at reducing inflammation, relieving symptoms, and preventing complications. sJIA treatment may include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), and in some cases, biologic drugs.[2,3]

In the past, high-dose glucocorticoids were the first choice for the treatment of sJIA to suppress the cytokine storm.[4,5] Recently, treatment strategies such as targeted therapy and early aggressive use of biologics have been available for these patients and have improved outcomes

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for patients with sJIA. The American College of Rheumatology (ACR) recommends csDMARDs or biologics following systemic glucocorticoids in patients with glucocorticoid resistance or severe systemic and joint findings.[6] The choice between conventional therapy (glucocorticoids ± csDMARD) and biologic therapies depends on the severity of the disease and the response to treatment.[7] In many cases, a step-up approach is employed.[8]

In this study, we aimed to compare patients using biologic drugs with patients on glucocorticoids ± csDMARD among patients with SJIA and identify associated factors for the treatment with biologic drugs.

Materials and Methods

This is a retrospective cross-sectional study. It was approved by the ethics committee of Hacettepe University (date: 15.06.2021, number: 2021-12). Informed consent was acquired from both parents and patients before they participated in the study. The study adhered to the ethical principles laid out in the 1964 Declaration of Helsinki and its subsequent revisions.

Patients

All patients with sJIA from May 2011 to June 2023 were included in the study. The patients before the biologic treatment era were excluded from the study. All participants fulfilled the ILAR classification criteria for sJIA.[9] Demographics, clinical and laboratory features, disease courses, treatments, and outcomes of all patients were evaluated. Additionally, the systemic Juvenile Arthritis Disease Activity Score (sJADAS) was calculated at diagnosis and last visit.[10] Disease courses were defined as monocyclic (only one-time flare lasting up to 24 months), polycyclic (with multiple flares separated by inactive periods), or persistent (marked by unceasing inflammation and progressive arthritis, often affecting multiple joints).[11] For the clinically inactive disease, the following ACR definition was used: no active arthritis, a physician's global assessment of disease activity score of 0, normal levels of erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP), the absence of sJIA features (fever, rash, serositis, splenomegaly, or generalized lymphadenopathy), the absence of uveitis, and duration of morning stiffness lasting less than 15 minutes.[12] Remission was evaluated as on-drug and off-drug, and considered to be a clinically inactive disease for at least six months.

All patients were initially administered high doses of pulse intravenous glucocorticoids (10-30 mg/kg/day) for three days, and treatment was continued with oral glucocorticoids at 1-2 mg/kg/day in the follow-up. Methotrexate (MTX, 15-20 mg/m^2/week subcutaneously) or cyclosporine-A (Cyc-A, 3-5 mg/kg/day orally) was frequently used as csDMARDs. In cases of severe disease, incomplete response, or relapse, biologic drugs were added (Fig. 1). The patients were divided into two groups: those who received glucocorticoids and/or csDMARDs alone (Group A) and those who received at least one biologic drug (Group B). Differences between these two groups were analyzed. Factors determining the requirement for the biologic treatment were identified.

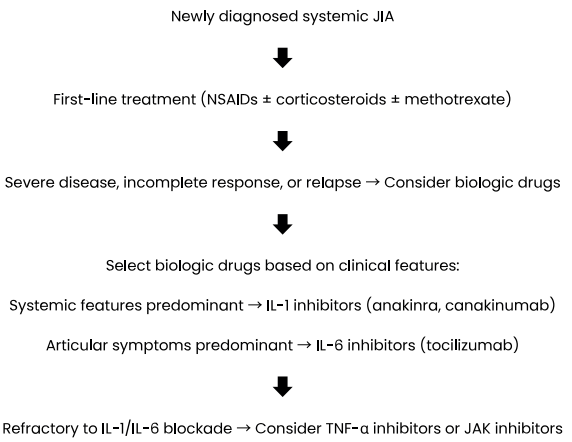

Fig. 1. Indication and selection protocol for biologic agents in the treatment of systemic juvenile idiopathic arthritis.

IL, interleukin; JAK, Janus kinase; JIA, juvenile idiopathic arthritis; NSAIDs, nonsteroidal anti-inflammatory drugs; TNF-α, tumor necrosis factor-alpha.

Statistical analysis

To assess variable distribution, both visual methods (histograms and probability graphs) and analytical tests (Shapiro-Wilks) were utilized. The descriptive statistics were presented as frequency (n) and percentage (%) for categorical variables and as median (25^{th} percentile [Q1] and 75^{th} percentile [Q3]) for continuous variables. Categorical variables were compared using the chi-square test or Fisher's exact test, while non-normally distributed continuous variables were compared using the Mann-Whitney U-test. Univariate analysis was employed to identify predictors of the requirement for biologic therapy. Continuous variables were dichotomized through ROC analysis, and variables with an unadjusted p-value below 0.05 in the logistic regression analysis were identified as potential predictive markers and included in the full model. The model was subsequently refined through multivariate logistic regression analyses during retrospective elimination. Significance was established at a p-value below 0.05, with a 95% confidence interval (CI) applied.

Results

A total of 138 patients with sJIA were included in the study (F/M=0.8). The median age of the patients at diagnosis was 5.5 (1.9-11.6) years. In the initial treatment, after glucocorticoids, 65 patients (47.1%) were administered csDMARDs and 45 patients (32.6%) received biologic drugs (Fig. 2). In the median third month of the csDMARD therapy, a biologic agent was added to the treatment, if the disease was not clinically inactive. Therefore, 42 of 65 patients (64.6%) receiving csDMARDs were switched to biologic drugs. Twenty-eight patients (20.3%) received only glucocorticoids, while 23 patients (16.7%) received only csDMARDs (Fig. 2).

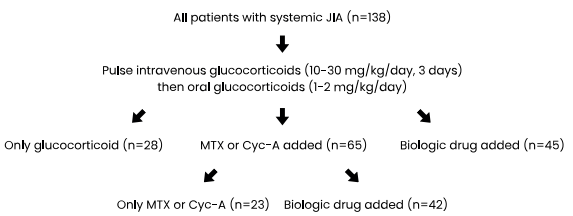

Fig. 2. Treatment chart in systemic juvenile idiopathic arthritis patients.

Cyc-A, cyclosporine-A; JIA, juvenile idiopathic arthritis; MTX, methotrexate.

CsDMARDs were initiated for various reasons such as prominent articular symptoms (n=22), macrophage activation syndrome (MAS, n=12), and systemic symptoms that could not be controlled with glucocorticoids (n=11) at disease onset. Interleukin (IL)-1 inhibitors were used in 45 patients who had severe systemic symptoms and/or presented with MAS at disease onset. Anakinra was initiated in most of them, then it was switched to canakinumab. In 29 patients using csDMARDs, biologics were introduced due to a persistent polyarticular disease course. On the other hand, for 13 patients, the reason for initiating biologics was the resistant/recurrent systemic inflammation. Notably, all patients with persistent polyarticular course were treated with tocilizumab.

As a result, 51 patients (36.9%) were treated with only glucocorticoids and/or csDMARDs (Group A), while eighty-seven patients (63.1%) were treated with biologic drugs (Group B) (Table I). Of note, there were no patients who were treated with only NSAIDs.

Group B patients more frequently had MAS at disease presentation than Group A patients (p=0.001). In addition, acute phase reactant (CRP and ESR) levels and sJADAS at diagnosis in Group B were higher than in Group A (p<0.001 for both). While monocyclic disease course was frequently observed in patients of Group A (p<0.001), polycyclic disease course was more common in patients of Group B (p=0.027). Most of the patients in Group B were able to discontinue glucocorticoids in a shorter period (p<0.001), and the number of patients who discontinued glucocorticoids in the first year was higher (p<0.001).

In the univariate and multivariate logistic regression analyses, history of MAS (OR 3.419,

Table I. Characteristics of systemic juvenile idiopathic arthritis patients treated with glucocorticoids ± conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) (Group A) vs. those treated with biologics (Group B).

	All patients (n=138)	Group A (n=51)	Group B (n=87)	P value
Age at diagnosis, year, median (Q1-Q3)	5.5 (1.9-11.6)	5.7 (2.1-11.8)	5.2 (1.8-11.4)	0.114
Sex, female, n (%)	63 (45.7)	25 (49.1)	38 (43.7)	0.543
Clinical findings at diagnosis, n (%)				
Fever	138 (100)	51 (100)	87 (100)	-
Rash	82 (59.4)	30 (58.8)	52 (59.8)	0.913
Arthritis	78 (56.5)	29 (56.9)	58 (66.7)	0.249
Lymphadenopathy	71 (51.4)	26 (50.9)	45 (51.7)	0.933
Hepatomegaly/splenomegaly	6 (33.3)	18 (35.3)	28 (32.2)	0.708
Serositis	15 (10.9)	5 (9.8)	10 (11.5)	0.758
MAS	53 (38.4)	12 (23.5)	45 (51.7)	0.001
Laboratory findings at diagnosis, median (Q1-Q3)				
Hemoglobin, gr/dL	10 (8.9-12.7)	10.5 (9.2-13)	9.9 (8.7-12.5)	0.116
Leukocyte count, $x10^3/mm^3$	14.9 (4.3-24.5)	15 (4.9-23.9)	14.6 (3.9-24.8)	0.208
Platelet count, $x10^3/mm^3$	411 (132-585)	417 (144-523)	405 (124-598)	0.341
CRP, mg/dL (<0.5)	13.1 (1.5-26.8)	10.7 (1.1-23.1)	15.3 (2.1-28.3)	<0.001
ESR, mm/hour (0-20)	56.7 (33-97)	55.4 (31-94)	58.5 (37-101)	0.056
sJADAS at diagnosis, median (Q1-Q3)	24.8 (15.1-31.2)	19.5 (14.3-19.5)	28.7 (17.9-34.8)	<0.001
Treatment, ever, n (%)				
NSAIDs	64 (46.4)	25 (49.1)	39 (44.8)	0.634
Glucocorticoid	138 (100)	51 (100)	87 (100)	-
Methotrexate	48 (34.8)	17 (33.3)	31 (35.2)	0.784
Cyclosporin-A	17 (12.3)	6 (11.8)	11 (12.6)	0.879
IVIG	29 (21.1)	10 (19.6)	19 (21.8)	0.756
Biologic drugs	87 (63.1)	0	87 (100)	-
Anakinra	70 (50.7)	0	70 (80.4)	-
Canakinumab	45 (32.6)	0	45 (51.7)	-
Tocilizumab	29 (21.1)	0	29 (33.3)	-
Anti-TNF-α agents	7 (5.1)	0	7 (8.1)	-
Disease course, n (%)				
Monocyclic	76 (32.8)	32 (62.7)	11 (11.5)	<0.001
Polycyclic	33 (18.1)	5 (9.8)	22 (25.3)	0.027
Persistent	74 (40.4)	14 (27.5)	38 (43.7)	0.058
Glucocorticoid withdrawal time, months, median (Q1-Q3)	5 (1.5-40)	7 (2-48)	3 (1-30)	<0.001
Number of patients who discontinued glucocorticoid in the first year, n (%)	98 (71.1)	26 (50.9)	72 (82.8)	<0.001

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; IVIG, intravenous immunoglobulin; MAS, macrophage activation syndrome; NSAIDs, nonsteroidal anti-inflammatory drugs; sJADAS, systemic Juvenile Arthritis Disease Activity Score; TNF-α, tumor necrosis factor-alpha

Table I. Continued.

	All patients (n=138)	Group A (n=51)	Group B (n=87)	P value
Transition time to biologic drug, months, median (Q1-Q3)	3 (3-12)	-	3 (3-12)	-
sJADAS at last visit, median (Q1-Q3)	0.4 (0-4.6)	0.4 (0-4.2)	0.5 (0-4.9)	0.283
Duration of follow-up, years, median (Q1-Q3)	6.4 (1.5-8.8)	6.1 (1.7-8.5)	6.7 (1.3-9.1)	0.472
Outcome, n (%)				
Remission on-drug	44 (31.9)	13 (25.5)	31 (39.1)	0.217
Remission off-drug	92 (66.7)	37 (72.5)	55 (63.2)	0.262
Exitus	2 (1.4)	1 (1.9)	1 (1.1)	1.000

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; IVIG, intravenous immunoglobulin; MAS, macrophage activation syndrome; NSAIDs, nonsteroidal anti-inflammatory drugs; sJADAS, systemic Juvenile Arthritis Disease Activity Score; TNF-α, tumor necrosis factor-alpha

Table II. Univariate and multivariate regression analysis for predictive factors associated with the requirement of biologic drugs in the treatment of patients with systemic juvenile idiopathic arthritis.

Variables	OR (%95 CI)	P value
Univariate analyses		
MAS at diagnosis	3.482 (1.610-7.533)	0.002
Polycyclic disease course	3.114 (1.099-8.826)	0.033
CRP at diagnosis >13.6 mg/dL	2.092 (1.036-4.226)	0.040
sJADAS at diagnosis >24.1	3.760 (1.803-7.838)	<0.001
Multivariate analyses		
MAS at diagnosis	3.419 (1.194-9.792)	0.022
Polycyclic disease course	4.351 (1.329-14.240)	0.015
CRP at diagnosis >13.6 mg/dL	2.838 (1.182-6.815)	0.020
sJADAS at diagnosis >24.1	4.490 (1.725-11.684)	0.002

CI, confidence interval; CRP, C-reactive protein; MAS, macrophage activation syndrome; sJADAS, systemic Juvenile Arthritis Disease Activity Score

95% CI 1.194-9.792; p=0.022) and polycyclic disease course (OR 4.351, 95% CI 1.329-14.240; p=0.015), CRP levels of >13.6 mg/dL (OR 2.838, 95% CI 1.182-6.815; p=0.020), and sJADAS levels of >24.1 (OR 4.490, 95% CI 1.725-11.684; p=0.002) at diagnosis were associated with the requirement of biologic drugs in the treatment (Table II).

Discussion

In our study, prominent articular symptoms, MAS, and severe/resistant systemic symptoms were major indications for using csDMARDs or biologics in SJIA treatment. History of MAS and polycyclic disease course, CRP >13.6 mg/dL, and sJADAS >24.1 at diagnosis were independent predictors of the requirement for biologic drugs in treatment. Also, in most patients receiving biologic treatment, glucocorticoids were discontinued in a shorter period, and a higher percentage of patients discontinued glucocorticoids within the first year.

In the initial treatment of sJIA, glucocorticoids serve as the first step.[13] However, there is currently no consensus regarding the appropriate dosage and duration of steroid therapy. Before biologic drugs were available, sJIA patients were mainly treated with glucocorticoids and csDMARDs.[5,6]

IL-1 inhibitors (anakinra, canakinumab) and IL-6 inhibitors (tocilizumab) are significantly effective in patients with sJIA.[14,15] In the most recent ACR guideline, it is recommended to give anti-IL-1 to patients with predominant systemic findings, and tocilizumab to patients with severe arthritis.[6] Even in the biologic treatment era, some sJIA patients are still treated with glucocorticoids alone or in combination with csDMARDs. Glucocorticoids alone are generally preferred in patients with monophasic disease, and the treatment can be discontinued in 3-6 months.[7,16] In patients with severe arthritis, MTX is usually added to glucocorticoids, and in those presenting with MAS, Cyc-A is often added.[17,18] If there is no response to these treatments during follow-up, it is recommended to switch to biologic treatments.[6] In our study, all patients with sJIA were initially given glucocorticoids. Of patients, 20.3% achieved remission with glucocorticoid therapy alone. csDMARDs were started after glucocorticoids in 47.1% of the patients, and biologic drugs were started in 32.6%. While csDMARDs were mostly started for reasons such as significant joint symptoms, MAS at diagnosis, and systemic symptoms that could not be controlled with glucocorticoids, IL-1 inhibitors were also given to patients with severe systemic symptoms and those presenting with MAS. During follow-up, some of the patients (64.6%) using csDMARDs were switched to biologic treatment due to a persistent polyarticular unresponsive course to csDMARDs or resistant/recurrent systemic inflammation.

Adiguzel Dundar et al.[7] observed 58 disease episodes in 50 sJIA patients. Forty-one (70.6%) of these episodes were controlled with MTX, following the discontinuation of glucocorticoids. However, a biologic drug was needed in the remaining 17 (29.4%) episodes. Patients receiving MTX were stratified into two groups: Group I (n=36) comprising patients treated with MTX alone, and Group II (n=14) consisting of patients treated with MTX in combination with a biologic agent; Group I patients had mainly a monocyclic disease course (56.1%), while Group II exhibited a higher prevalence of a persistent course (70.6%). Notably, the initial ESR and the neutrophil/lymphocyte ratio (NLR) were found to be significantly elevated in Group II than in Group I (p=0.003 and p=0.007, respectively).[7] Similar to this study, there was a significant elevation in acute phase reactants at the time of diagnosis in our patients treated with biologic drugs, and a monocyclic disease course was frequently detected in patients treated with glucocorticoids ± csDMARDs, in our study. However, arthritis and MAS were more common at diagnosis and there were high sJADAS values in the patients treated with glucocorticoids ± csDMARDs. In addition, polycyclic course was more common in our patients in this group.

Biologic agents have demonstrated remarkable efficacy in the treatment of sJIA, effectively reducing the need for glucocorticoids and their associated adverse events.[19] Consistent with these, the patients treated with biologics discontinued glucocorticoids in a shorter period in our study. Aydın et al.[20] evaluated the treatments and outcomes of 36 sJIA patients in 2020. All patients had received glucocorticoids. Twenty-six (72.2%) were treated with biologics. They reported that the duration of glucocorticoid exposure was significantly reduced after the use of biologic agents (p=0.001).

Finally, in our study, we revealed the necessity of using biologic drugs in initial or follow-up treatments in patients with a history of MAS and polycyclic disease course, CRP >13.6 mg/dL, or sJADAS >24.1 at diagnosis. As far as we know, there has been no study before on this subject in the literature. It was not surprising that these patients had a history of MAS at diagnosis and polycyclic disease course. However, we recommend closer observation and follow-up, especially in patients with significant CRP elevations at diagnosis and high sJADAS scores, because in sJIA, as in many diseases, early treatment is very important to prevent serious morbidity and mortality. It would be beneficial

in sJIA patients with these characteristics not to wait too long before initiating biologic treatment.

The primary limitation of this study was its retrospective nature, which makes it vulnerable to potential inaccuracies and erroneous assumptions due to incomplete or incorrect medical records. Another important limitation is the lack of randomization in this study. Future randomized controlled trials will make the results clearer. In addition, the study was a single-center study, which may limit the generalizability of its findings. Finally, we revealed our clinical experiences in our study, but it should not be forgotten that the treatment approach is heterogeneous and individual.

Conclusion

In conclusion, sJIA patients with a history of MAS, polycyclic disease course, significantly high CRP levels and sJADAS values at the time of diagnosis are probably more likely to require biologic drugs in the treatment. In addition, biologic drugs protect patients from long-term glucocorticoid exposure. However, it is also important to point out that sJIA is a complex and variable disease, and the treatment approach needs to be adjusted over time based on the patient's response and the course of the disease.

Ethical approval

The study was approved by Hacettepe University Ethics Committee (date: 15.06.2021, number: 2021-12). Informed consent was obtained from all parents/patients before inclusion in the study.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: Seher Şener, Ezgi Deniz Batu, Özge Başaran, Yelda Bilginer, Seza Özen; data collection: Seher Şener, Zeynep Balık, Emil Aliyev, Yağmur Bayındır, Veysel Çam; analysis and interpretation of results: Seher Şener; draft manuscript preparation: Seher Şener, Ezgi Deniz Batu, Özge Başaran, Yelda Bilginer, Seza Özen. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

Ezgi Deniz Batu received payment for speakers' bureaus from Novartis. Seza Özen received consultancy fees and payment for speakers bureaus from Novartis and Sobi. Other authors did not declare any conflicts of interest.

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Original Article

Value of ultrasound in predicting the outcome of conservative treatment of testicular appendage torsion in children

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ABSTRACT

Background: The treatment modalities for testicular appendage torsion in children are classified as conservative or surgical, and the choice is controversial.

Objectives: This study aimed to identify ultrasound-based indicators influencing the outcome of conservative treatment in children with testicular appendage torsion to divide the boundaries between conservative and surgical management.

Methods: A retrospective analysis was conducted on testicular appendage torsion data of children from November 2022 and November 2023 in the Children's Hospital of Zhejiang University School of Medicine to compare the conservatively successful and conservatively unsuccessful groups' clinical and ultrasound characteristics. Furthermore, we constructed a logistic regression model and evaluated its predictive ability.

Results: We observed 405 (88.62%) and 52 (11.38%) cases of conservative success and failure, respectively. Univariate analysis indicated significant differences between these groups in testicular appendage torsion nodule size, terminology for hydrocele, scrotal wall edema, and increased epididymal blood flow ($p<0.05$). Additionally, binary logistic regression analysis indicated that the testicular appendage torsion nodule size was an independent risk factor influencing conservative treatment outcomes. There was a 12.3% rise in the incidence ratio of conservative failure (Odds ratio [OR] 1.123, 95% confidence interval [CI] 1.055-1.195, $p<0.05$) for every 1 mm^2 increase in nodule size. The receiver operating characteristic (ROC) curve revealed an optimal critical value of 23.56 mm^2, corresponding to an area under the curve (AUC) of 0.810, a sensitivity of 0.692, and a specificity of 0.798 (95% CI 0.752-0.867, $p<0.05$).

Conclusions: When the size of the testicular appendage torsion node measured by ultrasound exceeds 23.56 mm^2, conservative treatment is predicted to fail, and surgical treatment may be considered. This result has certain clinical application potential and can be used as one of the important indicators to assist decision-making.

Key words: child, scrotum, testicular appendage torsion, ultrasonography, surgery.

Testicular appendages are the remnants of the mesonephric or paramedian ducts, typically ovoid and pedunculated, commonly located at the upper pole of the testis or adjacent to the epididymis. Testicular appendage torsion is a common cause of scrotal emergencies in children.[1] Children and adolescents tend to present with sudden scrotal pain, while infants present with crying and refusal to be touched on one side of the scrotum. Physical examination

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may reveal tenderness and a palpable nodule above the testicle.[2] Since it lacks specific clinical manifestations, ultrasonography is the preferred diagnostic tool, offering non-invasive, rapid and accurate results.[3-5] Typical ultrasound findings include a torsion nodule and absent blood flow on color Doppler ultrasound.[6] The treatment modalities for testicular appendage torsion are classified as conservative or surgical.[7] Historically, conservative treatment was favored, as the condition was considered self-limiting. However, recent studies have demonstrated the advantages of surgical treatment, particularly in cases with severe inflammatory manifestations, intractable pain, or recurrent pain after remission, and that surgical treatment can ensure that the testicular appendage torsion on that side will not recur and shorten the time for pain relief and disappearance of edema.[7] The study of Shi et al.[8] made a small scrotal incision for testicular appendage resection after precise positioning of testicular appendage torsion, which shortened the operation time and reduced intraoperative bleeding and postoperative pain compared with traditional surgery. Although surgery is a good solution to the problem, there are currently no objective screening indicators to use as an indication for surgery, which may lead to questions about the existence of over-surgery. Furthermore, a report by Horiike et al.[9] suggested that if the testicular appendage torsion is more than 10 mm in diameter, it may wrap around the testis or epididymis, resulting in necrosis due to obstruction of blood flow to the testis. Although the importance of ultrasound in the diagnosis of testicular appendage torsion is widely recognized, there is a relative lack of studies on the relationship between ultrasound characteristics and treatment decisions, which makes it difficult for specialists to derive more clues from the ultrasound report when determining treatment options. In practice, ultrasound can clearly reveal not only the testicular appendage nodules but also the morphology and blood flow in the testis and its surrounding structures. Therefore, based on the above studies, we hypothesized that the existence of certain ultrasound indices, such as size and blood flow, can be used as a basis for surgery.

In this study, we aimed to identify ultrasound-based indicators influencing the outcome of conservative treatment in children with testicular appendage torsion to divide the boundaries between conservative and surgical management.

Materials and Methods

Patients

Subjects: The study was approved by the Ethics Committee of the Affiliated Children's Hospital of Zhejiang University School of Medicine, and the requirement for informed consent was waived (Ethics No. 2024-IRB-0167-P-01). We retrospectively analyzed 457 patients clinically diagnosed with testicular appendage torsion from November 2022 to November 2023 at the Affiliated Children's Hospital of Zhejiang University School of Medicine.

The inclusion criteria were: 1. Patients with the clinical diagnosis of testicular appendage torsion; 2. Those with complete medical records, and all patients received conservative treatment after diagnosis, including oral or intravenous antibiotics; 3. Patients with clear ultrasound images. The exclusion criteria were: 1. Patients with congenital genitourinary anomalies; 2. Those with other grave systemic diseases; 3. Patients with scrotal disease requiring urgent surgery such as suspected or combined testicular torsion.

The patient's age, height, weight, time of examination (time from the initial onset of symptoms to the time of ultrasound examination), and treatment modality were collected from the electronic case system. Treatment modalities were categorized as conservative success or conservative failure, with the latter defined as the need for conversion to surgery (hereafter referred to as conservative failure). Patients were assessed by specialists

on the first day and every two days thereafter. The criteria for determining the failure of conservative treatment include persistent or worsening pain following conservative management, necessitating surgical intervention for resolution. Conversely, pain alleviation after conservative treatment was considered the criterion for the success of conservative management. The pathological diagnosis of the operated patients was confirmed.

Equipment

We used a Logiq E9 ultrasound machine (GE, USA), and the line array probe's frequency was 10 MHz.[4] The ultrasound parameters were set to small organ mode, and the grey scale image scanning depth, gain, and focus point were adjusted until the scrotal structures were distinct and uniformly indicated. While the mechanical and thermal indexes were <0.4, the color Doppler ultrasound flow velocity was 3-6 cm/s.

Imaging analysis

Patients with clear ultrasound images: two-dimensional including testicular appendage torsion nodules, bilateral testes, epididymis, spermatic cord, scrotal wall, and inguinal area, as well as color Doppler ultrasound images of testicular appendage torsion nodules and epididymal blood flow. Two senior ultrasonographers who worked in the hospital for >10 years analyzed the patients. In the event of a disagreement in their conclusions, a third experienced sonographer was invited to participate in the evaluation.

Our observation indexes were: 1. The testicular appendage torsion nodule's location, echo, size, and blood flow. Since the testicular appendage tended to be ovoid, the oval area was used as a proxy for size. The left and right diameters were measured along with the upper and lower diameters in the section with the largest area of the torsion nodule. Size = π × left and right diameter/2 × upper and lower diameter/2 mm^2. 2. Other signs of inflammation, including the presence or absence of enlargement of the affected epididymis compared with the healthy side, enlargement of the testis and spermatic cord, presence or absence of the terminology for hydrocele, edema of the scrotal wall, and increased blood flow to the epididymis.

Statistical analysis

We used SPSS 26.0 software for data analysis. Count and metric data were expressed as rate (n, %) and $\bar{\chi} \pm s$, respectively. An independent sample t-test was used to compare metric data between the two groups. At the same time, the $\chi 2$ test was utilized for count data. All values of $p<0.05$ were considered statistically significant. After constructing the binary logistic regression model, the Wald $\chi 2$ test was used to estimate the regression parameters, whereas the likelihood ratio test evaluated the model's fit. The receiver operating characteristic (ROC) curve was employed to evaluate the logistic regression model's forecasting ability.

Results

Comparison of general data

We included 457 cases, of which 425 (93.00%) had no obvious causative factors, and 32 (7.00%) were associated with sports, trauma, or surgical injury. Age at onset was 0.5-17.0 years (8.99±2.51 years), height and weight were 102.0-164.0 cm (139.32±15.22 cm) and 8.0-95.5 kg (35.10±12.77 kg), respectively. The examination time was 0-14 days (2.60±2.49 days), and the left-sided onset was seen in 229 (50.11%) cases. Moreover, right-sided lesions were found in 228 (49.89%) cases. There were 405 (88.62%) and 52 (11.38%) cases of conservative success and failure, respectively.

In pediatric testicular appendage torsion patients, ultrasound images revealed a testicular appendage torsion nodule in the affected scrotum, frequently accompanied by enlarged epididymis on the affected side, terminology for hydrocele, and edema of the affected or

bilateral scrotal walls; the echogenicity, size, and position of the testes and spermatic cords were not visibly altered. The color Doppler ultrasound revealed absent blood flow in the torsion nodules and increased blood flow in some epididymis, respectively (Fig. 1 and Fig. 2).

Comparison of general information and ultrasound image characteristics in conservative Success and Failure Groups

The patients' general information in the two conservative success and conservative failure groups included age, height, weight, and examination time, without any significant differences between the groups (p>0.05, Table I).

The ultrasound image characteristics showed the torsion nodule's location, echo, and epididymal enlargement, without any significant differences between the groups (p>0.05, Table II). However, the differences in the four ultrasound features, i.e., testicular appendage torsion nodule's size, terminology for hydrocele, scrotal wall edema, and increased blood flow in the epididymis, respectively, were statistically significant between the groups (p<0.05, Table II).

Logistic regression analysis of factors influencing the failure of conservative testicular appendage torsion treatment

With the failure of conservative treatment as the dependent variable and statistically

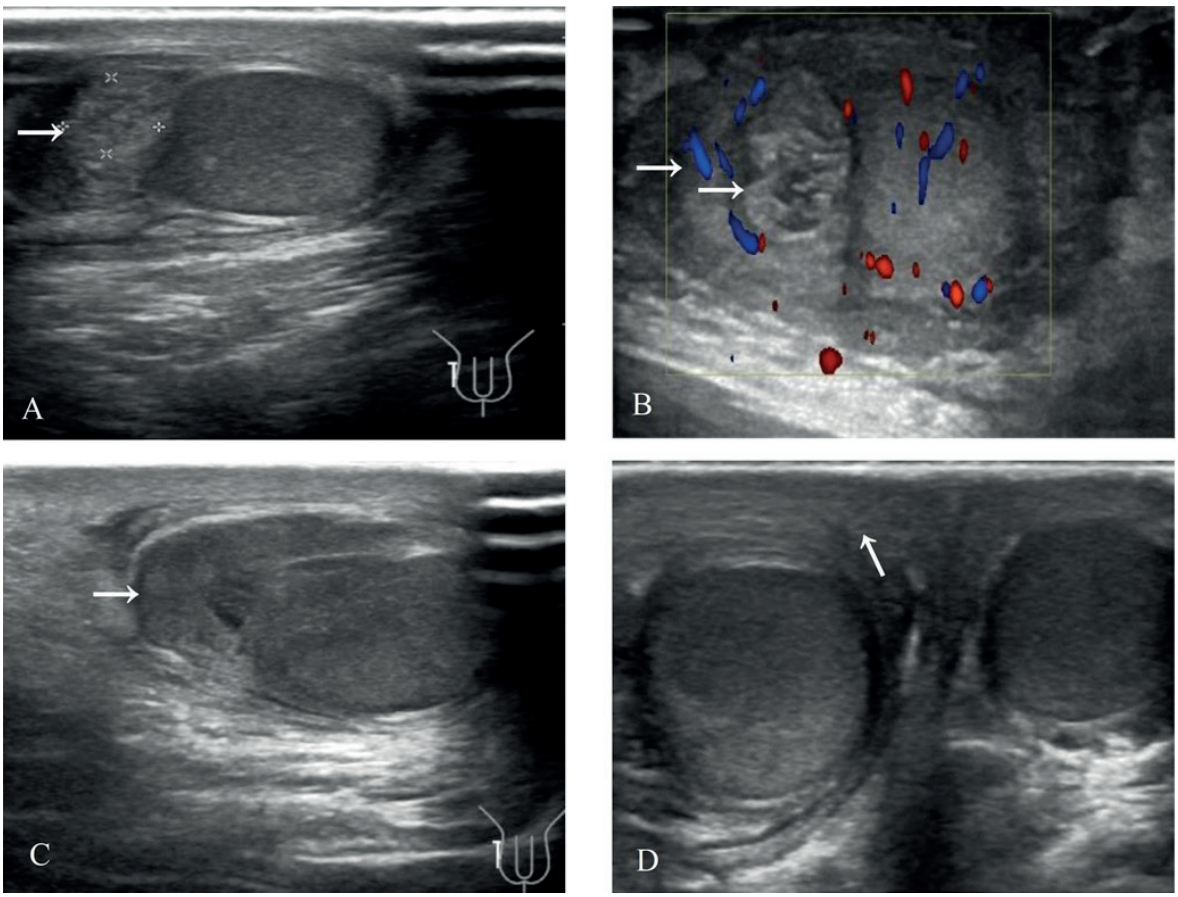

Fig. 1. A 10-year-old boy with right scrotal pain for 5 days. **A:** The testicular appendage torsion nodule between the right testis and epididymis was an ovoid heterogeneous structure with well-defined borders and heterogeneous internal echogenicity; **B:** No blood flow was seen within the torsional node; however, there was increased blood flow to the epididymis on that side; **C:** The affected epididymis was enlarged; **D:** Edema of the scrotal wall on the affected side.

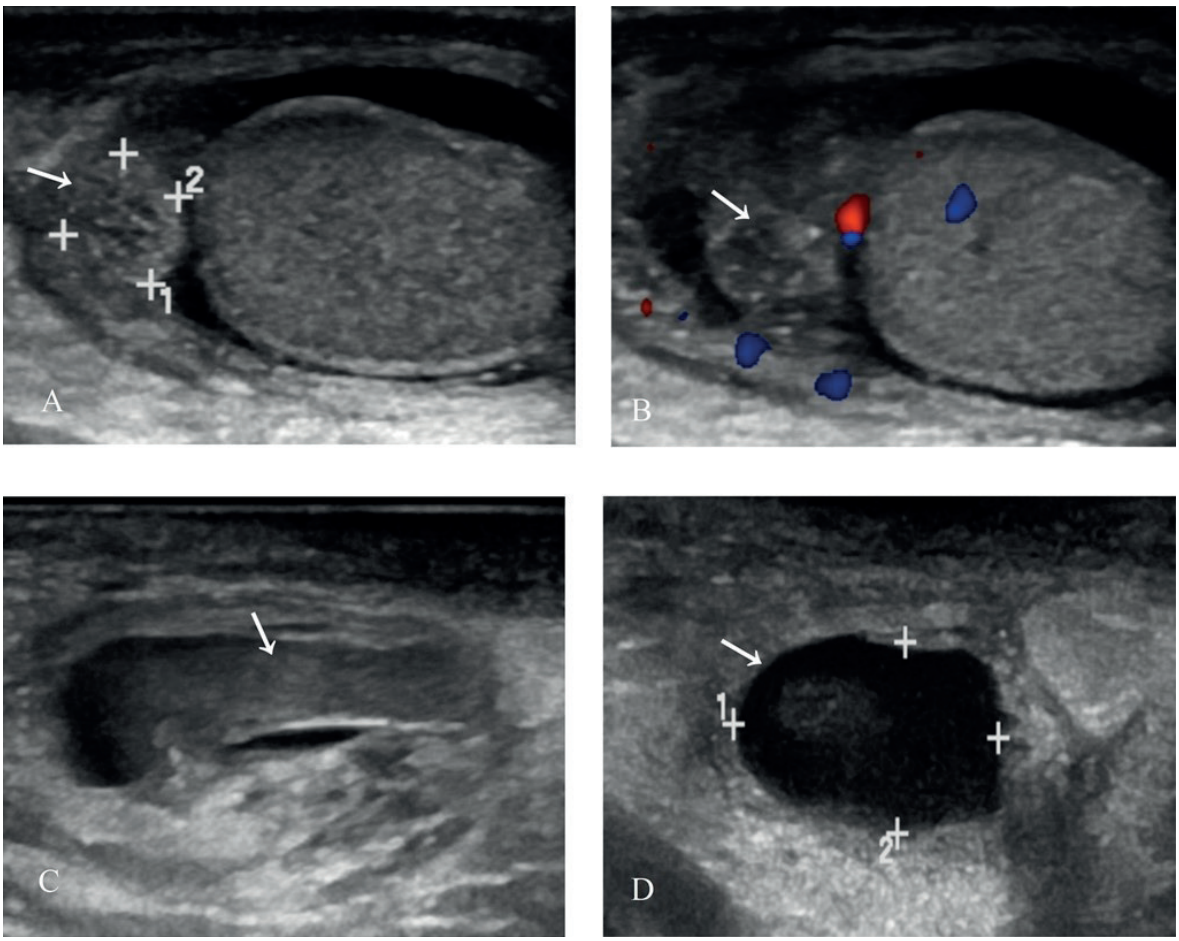

Fig. 2. An 8-year-old boy with left-sided scrotal pain for 2 days. **A:** The testicular appendage torsion nodule at the upper pole of the left testis; **B:** No blood flow within the twisted nodule; **C:** The affected epididymis was enlarged; **D:** Hydrocele of the affected testis.

Table I. Comparison of general information between conservative success and failure groups in testicular appendage torsion.

Group	Conservative success (n=405)	Conservative failure (n=52)	T value	P value
Age (years)	8.97±2.53	9.15±2.29	1.505	0.221
Height (cm)	138.94±14.64	139.52±15.66	0.217	0.643
Weight (kg)	34.83±12.91	37.21±11.60	0.686	0.408
Inspection time (days)	2.60±2.44	2.63±2.23	1.220	0.270
Torsion of nodule size (mm^2)	17.21±11.52	33.83±19.37	22.438	<0.001

Data presented as mean ± standard deviation. All values of $p<0.05$ were considered statistically significant.

significant variables in the univariate analysis as independent variables, including testicular appendage torsion nodule size (mm^2), terminology for hydrocele, scrotal wall edema, and increased epididymal blood flow, the stepwise regression was used to incorporate all four of these indicators, and the results revealed that testicular appendage torsion nodule size had an independent predictive value for conservative failure ($\chi 2=24.436$, $p<0.05$, Table

Table II. Comparison of ultrasound characteristics between conservative success and failure groups in testicular appendage torsion.

Group		Conservative success (n=405)		Conservative failure (n=52)		χ2 value	P value
		n	%	n	%		
Position of torsion of nodule	Left	201	49.6	28	53.8	0.328	0.567
	Right	204	50.4	24	46.2		
Torsion nodule echo	High	231	57.0	37	71.2	3.787	0.052
	Low	174	43.0	15	28.8		
Epididymal swelling	Yes	334	82.5	48	92.3	3.252	0.075
	No	71	17.5	4	7.7		
Terminology for hydrocele	Yes	166	41.0	34	65.4	11.145	0.001
	No	239	59.0	18	34.6		
Edema of the scrotal wall	Yes	182	44.9	38	73.1	14.615	<0.001
	No	223	55.1	14	26.9		
Increased blood flow in epididymis	Yes	234	57.8	46	88.5	18.284	<0.001
	No	171	42.2	6	11.5		

χ^2, chi-square. Data presented as counts and percentages. All values of $p<0.05$ were considered statistically significant.

Table III. Multifactorial stepwise regression analysis of ultrasound-related indicators with regards to conservative failure of testicular appendage torsion

Variant	B value	SE	P value	OR (95% CI)
Torsion of nodule size (mm^2)	0.116	0.032	<0.001	1.123 (1.055-1.195)
Constant	-1.843	0.662	0.005	-

B value, Beta value; SE, standard error; OR, odds ratio; CI, confidence interval.

III). The incidence ratio of conservative failure increased by 12.3 percent ((e0.116-1)*100%) for each 1 mm^2 increase in the size of the testicular appendage torsion nodule.

ROC analysis of testicular appendage torsion nodule size as a diagnostic indicator

Fig. 3 reveals that the ROC curve was plotted using the testicular appendage torsion nodule size as the diagnostic outcome value and the conservative outcome as the 'gold standard'. The Jordon's index (J = Sensitivity + Specificity - 1) was calculated to be 0.49, which corresponded to an optimal threshold for the size of testicular appendage torsion nodules of 23.56 mm^2, with an area under the curve (AUC) of 0.810, a sensitivity of 0.692, and a specificity of 0.798, with a 95% confidence interval of 0.752-0.867.

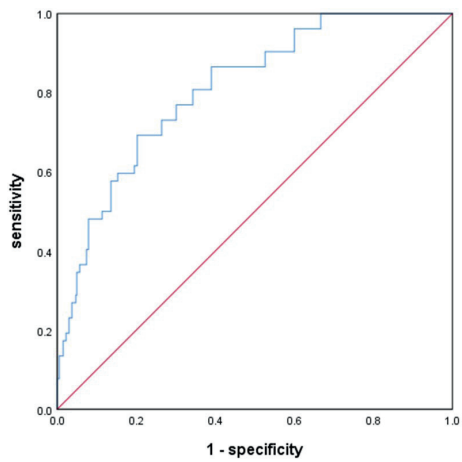

Fig. 3. Receiver operating characteristic (ROC) curve for predicting conservative outcomes for testicular appendage torsion nodule size. The best cut-off value for testicular appendage torsion nodule size was 23.56 mm^2 (area under the curve = 0.810), with a sensitivity of 0.692, specificity of 0.798, and a 95% confidence interval of 0.752-0.867.

Discussion

Testicular appendage torsion is a common cause of scrotal emergencies in children, with a peak incidence between the ages of 6-12 years old, with androgen and estrogen receptors in the testicular adnexal structures, and the occurrence of torsion may be related to increased stimulation by sex hormones before puberty.[10] Most cases have no obvious trigger; a few may be associated with exercise, trauma, or surgery. It can develop on both the left and right sides without obvious favoritism. Its clinical presentation is sudden scrotal pain, but in infants, unexplained crying and refusal to be touched in the scrotum should also rule out testicular appendage torsion. Diagnosis can be aided by identifying a small nodule over the testicle and increased pain on palpation. Although conservative treatment is often effective, failure may cause aseptic inflammation of the epididymis, potentially causing infertility due to vas deferens obstruction.[11,12] However, no studies have indicated how to assess the risk of conservative failure of testicular appendage torsion. Gopal et al.[13] believe that the full use of clinical and ultrasound data should be made to limit emergency scrotal exploration to children at higher risk. Ultrasound is of great interest as the examination of choice for this disease, from which more useful information can be obtained to screen patients at high risk of conservative failure to assist in clinical assessment.[14]

Our study retrospectively analyzed 457 patients with testicular appendage torsion, of whom 52 (11.38%) experienced conservative treatment failure. Difficulty in relieving pain may be related to the extent of testicular appendage torsion, and ultrasound can reveal the torsion of the testicular appendage itself and associated inflammatory changes, such as hydrocele, edema of the scrotal wall, and increased blood flow to the epididymis. The study identified the nodule size as an independent predictive value for conservative failure, with the incidence ratio of conservative failure increasing by 12.3% for each 1 mm^2 increase in nodule size. The risk of conservative failure was high when nodule size exceeded 23.56 mm^2 (AUC = 0.810). Compared to the case report of Horiike et al.[9], who used the diameter of the testicular appendage torsion nodule, our study selected the area of the largest section of the torsional nodule, which is perhaps more accurate. Hydrocele, scrotal wall edema, and increased blood flow to the epididymis are signs of inflammation, which aligns with Tanaka et al.[7], who recommended surgical treatment for severe inflammatory symptoms. In addition, some scholars have recommended surgical treatment for patients with recurrent pain after improvement with conservative treatment.[15]

Differentiating testicular appendage torsion from testicular torsion is critical, and the key distinctions lie primarily in the age of presentation and symptom severity. Testicular torsion is more common in adolescents and typically prompts immediate evaluation due to its severity. In contrast, testicular appendage torsion often presents with milder symptoms, leading to potential delays in assessment and a higher likelihood of localized inflammatory signs.

This study's strength lies in its substantial number of clinical cases and the development of a logistic regression model to quantitatively evaluate the ultrasound characteristics, providing a scientific basis for clinical decision-making. Upcoming studies can explore more factors that might influence treatment choices to improve the prediction model's accuracy and efficiency. Since this was a single-center study, our model's applicability in different healthcare institutions requires further validation. Future studies should explore additional predictive factors, and conduct multi-center validation to improve the model's accuracy and generalizability.

Conclusion

This study has identified ultrasound indicators that significantly influence the outcomes of conservative treatment in children with testicular adnexal torsion. Notably, the size of

the testicular adnexal torsion nodule emerged as an independent predictor of the failure of conservative management. This pivotal finding would offer a quantitative foundation for distinguishing between cases suitable for conservative versus surgical interventions, thereby addressing the prevailing gap in objective ultrasound criteria guiding surgical indications.

Ethical approval

The study was approved by the Ethics Committee of the Affiliated Children's Hospital of Zhejiang University School of Medicine, and informed consent was waived (date: January 24th, 2024, number: 2024-IRB-0167-P-01).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: DW, XY; data collection: DW, CW; analysis and interpretation of results: DW, XY; draft manuscript preparation: DW; Review conception and design: JY, JM; 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 relationship between microRNA-155-5p and postoperative inflammatory markers in children with acute suppurative appendicitis and its role in predicting postoperative complications

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ABSTRACT

Background. The prevalence of acute suppurative appendicitis (ASA) is the highest among pediatric cases of acute abdominal conditions. This research examined the stress response linked to surgical techniques and identified potential biomarkers that could predict postoperative complications to enhance clinical treatment strategies.

Methods. This study involved a selection of 166 ASA patients who underwent laparoscopic appendectomy (LA), and 150 patients who underwent open appendectomy (OA), based on data collected from 2020 to 2023. Comprehensive documentation of clinical and pathological characteristics, as well as postoperative complications, was conducted following patient enrollment. Quantitative polymerase chain reaction (qPCR), enzyme-linked immunosorbent assay (ELISA), and blood smear techniques were employed to assess the levels of microRNA (miR)-155-5p, C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), superoxide dismutase (SOD), and malondialdehyde (MDA), as well as changes in leukocytes, neutrophils, and lymphocytes at preoperative and postoperative 0 h, and 24 h. A logistic regression model was utilized to identify risk factors associated with the development of complications. Furthermore, receiver operating characteristic (ROC) curve analysis was performed to assess the predictive capacity of miR-155-5p for the occurrence of complications.

Results. The study revealed that the levels of miR-155-5p postoperatively in patients undergoing OA was significantly greater than that observed in patients undergoing LA (P<0.001). The expression levels of miR-155-5p exhibited a significant positive correlation with inflammatory markers (CRP, r=0.546; IL-6, r=0.628; TNF-α, r=0.808; leukocytes, r=0.778; neutrophils, r=0.718; and lymphocytes, r=0.820), indicators of oxidative stress (SOD, r=0.671; and MDA, r=0.489), and visual analog scale (VAS, r=0.671) scores (P<0.001). Furthermore, miR-155-5p might influence the concentrations of inflammatory and oxidative stress markers in the serum of patients both preoperatively and postoperatively (P<0.05). Notably, miR-155-5p was identified as risk factors for the development of postoperative complications (OR=8.331, P=0.008) and had a high predictive value for the occurrence of postoperative complications (area under the curve=0.926, P<0.001).

Conclusion. miR-155-5p might play a role in modulating the body's stress response, subsequently impacting the postoperative complications incidence of ASA patients.

Key words: acute suppurative appendicitis, laparoscopic appendectomy, open appendectomy, miRNA, complication.

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Acute suppurative appendicitis (ASA) in children represents the most prevalent acute abdominal condition among children in China.[1] The disease is characterized by atypical clinical presentations, rapid progression, and severe manifestations.[2] Open appendectomy (OA) remains a conventional treatment approach for ASA in pediatric patients; however, this surgical method is associated with considerable physical trauma, excessive intraoperative bleeding, and prolonged recovery periods.[3] In light of advancements in laparoscopic and minimally invasive surgical techniques, laparoscopic appendectomy (LA) is gaining traction due to its advantages, including smaller incisions, enhanced safety, and expedited postoperative recovery.[4] Nonetheless, postoperative complications following ASA in children are not infrequent.[5] Consequently, the pursuit of specific biomarkers capable of predicting the occurrence of complications is essential for facilitating timely management, thus reducing the risk of postoperative complications.

MicroRNA (miRNA, miR) represents a class of multifunctional non-coding RNA.[6] It is estimated that over 30% of human genes are modulated by miRNAs.[7] Recent investigations have identified miRNA as a pivotal component in the mechanisms of oxidative stress and inflammatory responses across a range of diseases.[8,9] Previous research has demonstrated that miR-155-5p is instrumental in macrophage polarization and plays a critical role in the regulation of inflammatory responses.[10] miR-155-5p exhibits diverse functions and is implicated in numerous biological processes.[11] For instance, in individuals with rheumatoid arthritis, elevated levels of miR-155-5p can trigger the expression of inflammatory mediators, and serve as a crucial predictor of disease onset.[12] Furthermore, increased expression of miR-155-5p in the serum of patients with sepsis and tuberculosis has been identified as a diagnostic, prognostic, and predictive infection occurrence biomarker.[13,14] Given the broad applicability of gene function, it is speculated that miR-155-5p may also contribute to the promotion of pediatric ASA. However, there is a paucity of research regarding miR-155-5p in this disease.

This study focuses on miR-155-5p as the primary subject of investigation to assess its expression variations and its association with clinical pathological characteristics in pediatric patients diagnosed with ASA who are undergoing either OA or LA. The study examines the relationship between the incidence of postoperative complications and various clinical parameters, aiming to identify independent factors that may influence the risk of postoperative infections. Ultimately, the study seeks to elucidate the predictive value of miR-155-5p concerning postoperative complications.

Materials and Methods

Study participants

A total of 316 pediatric patients who were diagnosed with ASA and underwent surgical intervention at Xingtai People's Hospital between 2020 and 2023 were selected for this study. Among these patients, 150 underwent OA, while 166 underwent LA. A comparative analysis was conducted between the two patient groups. The inclusion criteria for this study were as follows: (a) patients aged 14 years or younger; (b) patients diagnosed with ASA and fulfill the criteria for surgical intervention; (c) patients possessing clear consciousness, with consent from both themselves and their guardians to participate in the study, and completion of the informed consent form; and (d) ability to cooperate in completing the visual analog scale (VAS) assessment.

The exclusion criteria included: (a) complicated cases, such as necrosis or perforation; (b) occurrence of concurrent infections in other body regions post-surgery; (c) pre-existing infections prior to surgery or inflammatory disease in the patient's history; (d) patients with congenital conditions such as congenital heart disease or trisomy 21; (e) individuals with blood, immune system, or neurological disorders; and

(f) patients exhibiting severe liver and kidney dysfunction or malignant tumors.

Preoperative clinical baseline characteristics were collected for all patients, encompassing age, gender, and course of the disease. Perioperative data such as duration of surgery, anal exhaust time, and length of stay, as well as status of complications were recorded. The total intraoperative blood loss is measured by subtracting the total weight of gauze from the combined weight of gauze with blood and adding the volume of blood in the suction bottle. A VAS score was used to assess the level of pain in the children preoperatively and postoperatively. The score of which ranged between 0–10, with higher scores indicating more severe pain. The final VAS score was assessed by the patient in conjunction with two medical workers, and the mean value was subsequently calculated to exclude evaluation bias. This study received approval from the Ethics Committee of Xingtai People's Hospital and adhered strictly to the principles outlined in the Helsinki Declaration.

Serum collection

2.5-3.5 mL of peripheral venous blood was obtained from participants at three time points: prior to surgery (following the patient's admission and enrollment in the study), immediately post-surgery, and 24 hours post-surgery. The samples were subjected to centrifugation using a low-temperature ultracentrifuge. Subsequently, the supernatant serum was preserved in a -80 °C freezer for subsequent analysis.

RNA extraction and complementary DNA synthesis

The RNAeasy™ Blood RNA Isolation Kit with Spin Column (Beyotime, China) was employed to extract total RNA. The purity and concentration of the extracted RNA was assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). A total of 1 μg of the extracted RNA was utilized as a template for the synthesis of the first strand of complementary DNA (cDNA) using the BeyRT™ III First Strand cDNA Synthesis Kit (Beyond, China).

Quantitative polymerase chain reaction (qPCR)

The HRbio™ miRNA qPCR gene expression detection kit (HeRui, China) was employed to assess the expression levels of miR-155-5p. The primer sequences (5′-3′) utilized for miR-155-5p were as follows: GCGGCTCCTACTATTAGATTAAC (forward) and CAGTGCAGGGTCCGAGGTAT (reverse). Detection was performed using a LightCycler 480 real-time fluorescence qPCR instrument (Roche Applied Science, Switzerland). The primers were synthesized by Genscript Biotechnology Co., Ltd. in Nanjing, China.

Enzyme-linked immunosorbent assay (ELISA) and blood smear

The human interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) detection kits were from Nanjing Jiancheng Bioengineering Institute, the human C-reactive protein (CRP) detection kit from Wuhan Yunclonal Technology Co., Ltd., and the human malondialdehyde (MDA) and superoxide dismutase (SOD) detection kits from Shanghai Coibo Biotechnology Co., Ltd. Following the cessation of color development, the blank well was calibrated to zero, and the optical density (OD) value was measured using an ELISA reader at a wavelength of 450 nm, with each well being measured in triplicate.

Additionally, leukocyte, neutrophil, and lymphocyte counts were performed using the blood smear method. A drop of blood from the patient was placed on a glass slide, which was then tilted at a 45° angle to ensure an even distribution of the specimen across the slide. After allowing the slide to air dry, Wright's staining was applied. The staining solution was allowed to cover the blood film completely and was left to stand for 5 min. Subsequently, a phosphate buffer solution was added, and the slide was maintained for 10 min. The slide was

then rinsed with sterile double-distilled water, air-dried, and examined under a microscope. Each field of view was counted in triplicate to ensure accuracy.

Statistical analysis

This research employed SPSS 23.0 and GraphPad Prism 9.0 for the statistical analysis. Measurement data were presented as mean ± standard deviation (SD), with independent sample t-tests utilized for between group comparisons. Categorical data were expressed as percentages (%), and chi-square tests were applied for group comparisons. The comparison of VAS scores, serum miR-155-5p expression levels, levels of inflammatory factors, and oxidative stress indices between the laparotomy and laparoscopy groups at specific time points, and comparisons across different time points within the laparotomy and laparoscopy groups were performed using two-way analysis of variance. The correlation between preoperative miR-155-5p expression levels and various indicators measured 24 h post-surgery was assessed using Pearson correlation analysis. Independent sample t-tests and chi-square tests were employed to examine the relationship between it and preoperative clinical and pathological characteristics. Multiple logistic regression analysis was utilized to identify risk factors for postoperative complications, calculating the odds ratio (OR) and corresponding 95% confidence interval (CI) for each factor. The predictive efficacy of miR-155-5p for postoperative complications was evaluated through receiver operating characteristic (ROC) curves, with specificity and sensitivity of the detection indicators calculated.

Results

General characteristics of the study groups

The analysis revealed no statistically significant differences in terms of age distribution, gender ratio, and course of the disease between the two groups ($P>0.05$). However, the laparoscopy group exhibited significantly reduced duration of surgery, intraoperative bleeding, anal exhaust time, and length of stay when compared to the laparotomy group ($P<0.001$, Table I).

Relative expression levels of miR-155-5p and VAS score

The results of the qPCR analysis indicated that there was no statistically significant difference in the expression levels of miR-155-5p in preoperative serum between the laparotomy and laparoscopy groups ($P>0.05$). The postoperative expression levels of miR-155-5p in the serum of the laparoscopy group were found to be significantly lower than those in the laparotomy group ($P<0.001$, Fig. 1A). Notably, the expression levels of miR-155-5p in the serum of patients in both groups exhibited

Table I. Participant characteristics.

Factors	Laparotomy group (n=150)	Laparoscopy group (n=166)	*P* value
Age, years	8.12 ± 3.31	8.18 ± 3.36	0.872
Gender, n (%)			0.404
Male	73 (48.67%)	73 (43.98%)	
Female	77 (51.33%)	93 (56.02%)	
Course of the disease, days	1.20 ± 0.61	1.27 ± 0.69	0.367
Duration of surgery, min	62.36 ± 7.21	48.89 ± 7.17	<0.001
Intraoperative bleeding, mL	27.01 ± 3.51	11.78 ± 3.35	<0.001
Anal exhaust time, hours	3.79 ± 0.66	2.45 ± 0.72	<0.001
Length of stay, days	8.59 ± 2.65	6.91 ± 2.60	<0.001

$P<0.05$ indicates a significant difference. Data are presented as mean ± standard deviation, except for gender.

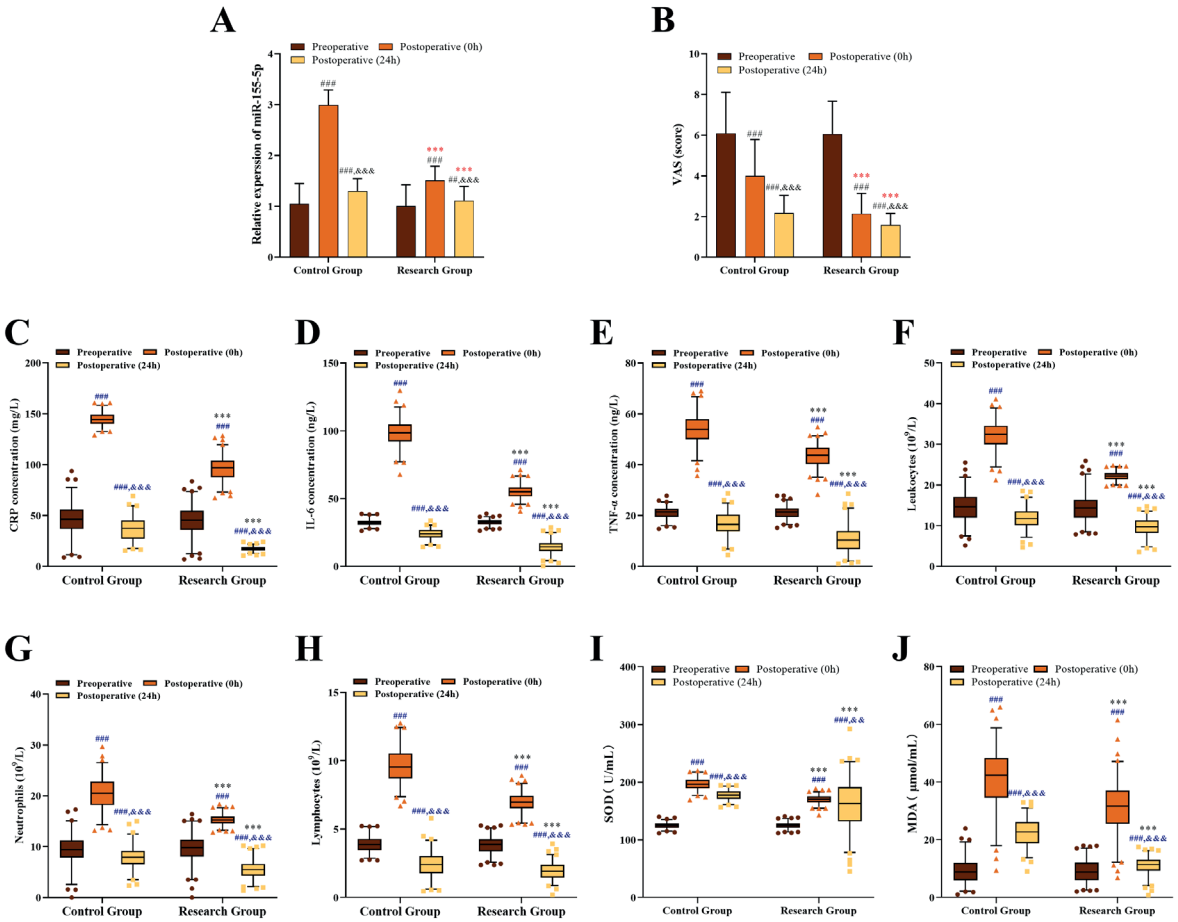

Fig. 1. The comparison of preoperative, postoperative 0 h, and postoperative 24 h miR-155-5p expression levels, VAS score, inflammatory factor level, SOD, and MDA levels in the laparotomy and laparoscopy groups of patients. (A) Comparison of miR-155-5p expression level. (B) Comparison of VAS score. (C) Comparison of CRP. (D) Comparison of IL-6. (E) Comparison of TNF-α. (F) Comparison of leukocytes. (G) Comparison of neutrophils. (H) Comparison of lymphocytes. (I) Comparison of SOD level. (J) Comparison of MDA level. miR-155-5p, microRNA-155-5p.

***, *P*<0.001, vs laparotomy group; ##, *P*<0.01, ###, *P*<0.001, vs preoperatively; &&, *P*<0.01, &&&, *P*<0.001, vs postoperative 0 h.

CRP, C-reactive protein; IL-6, interleukin-6; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; VAS, visual analog scale.

a significant increase immediately following surgery (*P*<0.001), followed by a significant decrease 24 h post-surgery (*P*<0.001), although these levels remained significantly elevated compared to preoperative values (*P*<0.01, Fig. 1A).

Furthermore, there was no significant difference in preoperative VAS scores between the laparotomy and laparoscopy groups (*P*>0.05). The VAS scores for the laparoscopy group were significantly lower than those of the laparotomy group at both 0 and 24 h post- surgery (*P*<0.001, Fig. 1B). In comparison to preoperative levels, VAS scores for patients in both groups demonstrated a significant reduction following surgery (*P*<0.001), with the scores at 24 h post-surgery being significantly lower than those recorded at 0 hours post-surgery (*P*<0.001, Fig. 1B).

Levels of inflammatory factors and oxidative stress indexes

The results of the analysis conducted using ELISA and blood smear techniques indicated comparable alterations in the levels of CRP (Fig. 1C), IL-6 (Fig. 1D), TNF-α (Fig. 1E), as well as the counts of leukocytes (Fig. 1F), neutrophils (Fig. 1G), and lymphocytes (Fig. 1H) in the serum of both patient groups. No statistically significant difference was observed in the concentration of inflammatory markers in the preoperative serum between the laparotomy and laparoscopy groups (P>0.05). However, the levels of inflammatory markers in the serum of the laparoscopy group were significantly lower than those in the laparotomy group at both 0 and 24 h post-surgery (P<0.001). In comparison to preoperative levels, the serum concentrations of inflammatory markers in both the laparotomy and laparoscopy groups exhibited a significant increase immediately following surgery (P<0.001), followed by a significant decrease at 24 h post-surgery (P<0.001), ultimately significantly lower than preoperative levels (P<0.001).

The results of the ELISA analysis indicated that there was no statistically significant difference in the levels of SOD and MDA in the preoperative serum between the laparotomy and laparoscopy group patients (P>0.05). The serum levels of SOD and MDA in the laparoscopy group patients were found to be significantly lower than those in the laparotomy group at both 0 and 24 h post-surgery (P<0.001, Fig. 1I and Fig. 1J). In comparison to preoperative levels, both groups patients exhibited a significant increase in SOD and MDA levels immediately following surgery (P<0.001), followed by a significant decrease at 24 h post-surgery (P<0.01). Nevertheless, the levels at this time point remained significantly elevated compared to preoperative measurements (P<0.001, Fig. 1I and Fig. 1J).

Relationship of miR-155-5p expression level with inflammatory factors, oxidative stress indexes, and VAS score

The results of the Pearson correlation analysis are presented in Table II. The expression levels of miR-155-5p in the preoperative serum of the two patient groups demonstrated significant positive correlations with various inflammatory factors measured 24 h post-surgery, including CRP (r=0.546, P<0.001), IL-6 (r=0.628, P<0.001), TNF-α (r=0.808, P<0.001), leukocytes (r=0.778, P<0.001), neutrophils (r=0.718, P<0.001), and lymphocytes (r=0.820, P<0.001). Additionally, significant positive correlations were also observed with oxidative stress markers, specifically SOD (r=0.671, P<0.001) and MDA

Table II. Correlations between miR-155-5p and various inflammatory markers, oxidative stress indexes, and VAS score.

Factors	Correlation coefficient (r)	*P* value
CRP (mg/L)	0.546	<0.001
IL-6 (ng/L)	0.628	<0.001
TNF-α (ng/L)	0.808	<0.001
Leukocytes (×10^9/L)	0.778	<0.001
Neutrophils (×10^9/L)	0.718	<0.001
Lymphocytes (×10^9/L)	0.820	<0.001
SOD (U/mL)	0.671	<0.001
MDA (μmol/mL)	0.489	<0.001
VAS (score)	0.366	<0.001

P<0.05 indicates a significant difference.
CRP, C-reactive protein; IL, interleukin; MDA, malondialdehyde; SOD, superoxide dismutase; TNF, tumor necrosis factor; VAS, visual analog scale.

(r=0.489, P<0.001), as well as with VAS scores (r=0.671, P<0.001). Therefore, the expression level of miR-155-5p in serum might serve as a crucial predictive parameter for preoperative inflammatory factors, oxidative stress indicators, and VAS scores.

Relationship between miR-155-5p expression level and complication occurrence

A total of 16 patients experienced complications, with 13 from the laparotomy group and 3 from the laparoscopy group (Table III). The occurrence of postoperative complications was higher in patients who underwent OA (P<0.01). The predominant complications identified in the laparotomy group included mesenteric adhesions and bowel obstruction. Further analysis using the chi-square test indicated demographic and clinical characteristics—such as age, gender, course of the disease, intraoperative bleeding, length of stay, and VAS scores between the two groups concerning the incidence of complications were not significantly different (P>0.05). Notably, the duration of surgery, anal exhaust time, levels of inflammatory markers (including CRP, IL-6, TNF-α, leukocytes, neutrophils, and lymphocytes), and indicators of oxidative stress (SOD and MDA) demonstrated a significant correlation with the occurrence of complications (P<0.01, Table IV). Patients who developed complications exhibited prolonged surgery and anal exhaust times, elevated levels of inflammatory cytokines and MDA, and reduced levels of SOD.

Logistic regression analysis revealed that several indicators were significant risk factors for postoperative complications in patients. Specifically, CRP exhibited an OR of 5.043 (95% CI: 1.224-20.772, P=0.025), IL-6 had an OR of 3.922 (95% CI: 1.016-15.145, P=0.047), and TNF-α demonstrated an OR of 4.618 (95% CI: 1.054-20.242, P=0.042). Additionally, leukocytes (OR=4.831, 95% CI: 1.141-20.465, P=0.032), neutrophils (OR=4.927, 95% CI: 1.090-22.265, P=0.038), lymphocytes (OR=4.468, 95% CI: 1.109-18.005, P=0.035), and MDA levels (OR=4.724, 95% CI: 1.026-21.754, P=0.046) were also identified as significant risk factors. Conversely, SOD was found to be a protective factor, with an OR of 0.268 (95% CI: 0.062-1.164, P=0.079). Notably, miR-155-5p emerged as the most influential factor regarding the occurrence of complications (OR=8.331, 95% CI: 1.746-39.747, P=0.008, Fig. 2A). Further analysis using ROC curves indicated that the area under the curve (AUC) for miR-155-5p in predicting complications occurrence was 0.926 (95% CI: 0.868-0.984), demonstrating high sensitivity (90.67%) and specificity (81.25%, Fig. 2B).

Discussion

Due to the incomplete development of the appendix in children, their ability to limit inflammation is poor. In instances where the appendix becomes inflamed and is not addressed promptly, there is a risk of developing significant lymphedema in the lymph nodes, as well as the rapid onset of purulent appendicitis.[15] Open appendectomy, a traditional and well-established procedure, is associated with considerable trauma and physical damage.[16] Conversely, laparoscopic appendectomy, characterized as a minimally invasive technique

Table III. Complications after resection in pediatric patients with acute suppurative appendicitis based on group.

Factors	Total (N=316)	Laparotomy group (n=150)	Laparoscopy group (n=166)	P value
Incision infection, n (%)	5 (1.58%)	3 (2.00%)	2 (1.20%)	0.572
Mesenteric adhesions, n (%)	5 (1.58%)	5 (3.33%)	0 (0.00%)	0.018
Bowel obstruction, n (%)	6 (1.90%)	5 (3.33%)	1 (0.60%)	0.076
All complications, n (%)	16 (5.06%)	13 (8.66%)	3 (1.80%)	0.005

P<0.05 indicates a significant difference.

Table IV. Relationship between complications and clinicopathological variables.

Factors	Complications		*P* value
	Yes (n=16)	No (n=300)	
Age, years	9.06 ± 2.64	8.10 ± 3.36	0.262
Gender, n (%)			0.304
Male	5 (31.25%)	141 (47.00%)	
Female	11 (68.75%)	159 (53.00%)	
Course of the disease, days	1.35 ± 0.45	1.23 ± 0.66	0.443
Duration of surgery, min	62.68 ± 6.68	54.89 ± 9.84	0.002
Intraoperative bleeding, mL	21.88 ± 9.91	18.86 ± 8.25	0.160
Anal exhaust time, h	3.69 ± 0.72	3.05 ± 0.97	0.009
Length of stay, days	8.27 ± 2.10	7.67 ± 2.78	0.395
CRP, mg/L	76.55 ± 6.89	44.04 ± 13.65	<0.001
IL-6, ng/L	37.41 ± 0.94	32.23 ± 2.03	<0.001
TNF-α, ng/L	26.09 ± 0.98	20.90 ± 2.15	<0.001
Leukocytes, $\times 10^9$/L	22.58 ± 1.64	14.10 ± 3.11	<0.001
Neutrophils, $\times 10^9$/L	15.06 ± 1.01	9.26 ± 2.60	<0.001
Lymphocytes, $\times 10^9$/L	5.07 ± 0.13	3.80 ± 0.55	<0.001
SOD, U/mL	114.18 ±1.41	125.47 ± 4.95	<0.001
MDA, μmol/mL	18.22 ± 1.91	8.72 ± 3.81	<0.001
VAS score	6.06 ± 1.95	6.11 ± 2.03	0.237

P<0.05 indicates a significant difference. Data are presented as mean ± standard deviation, except for gender.
CRP, C-reactive protein; IL, interleukin; MDA, malondialdehyde; SOD, superoxide dismutase; TNF, tumor necrosis factor; VAS, visual analog scale.

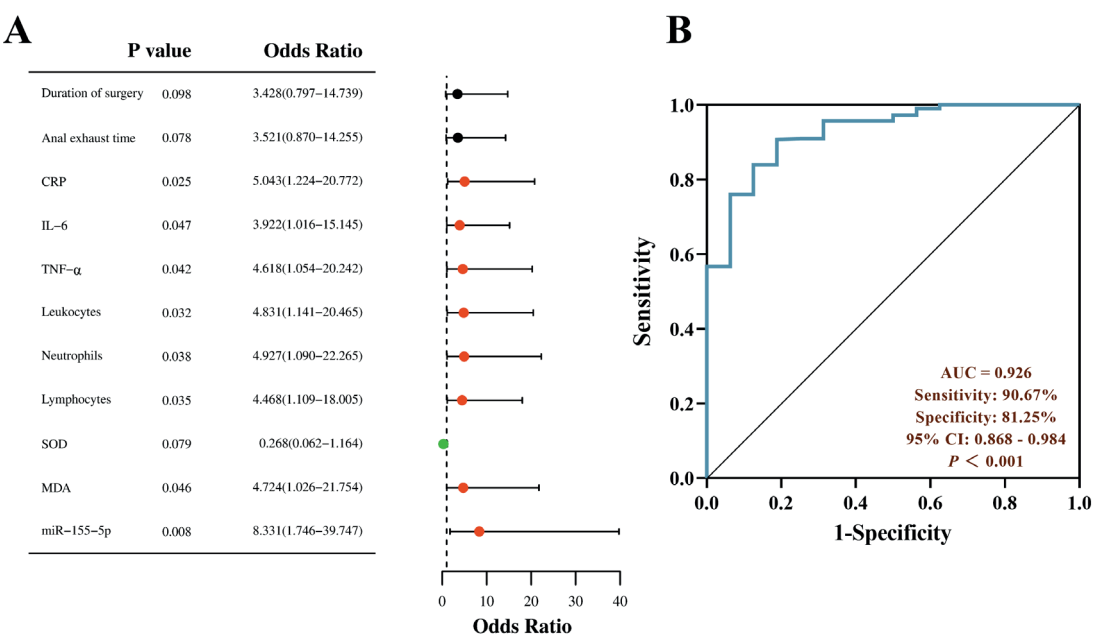

Fig. 2. Prediction of risk factors for postoperative complications occurrence and the predictive capability of miR-155-5p for occurrence of complications. (A) Logistic regression analysis. (B) ROC curve analysis.
AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin-6; MDA, malondialdehyde; miR-155-5p, microRNA-155-5p; ROC, receiver operating characteristic; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

that integrates contemporary surgical and endoscopic methods, offers a broader surgical field of view, as well as less trauma and damage.[17] This study indicated that patients undergoing LA experienced shorter operation times, and reduced intraoperative blood loss than OA, was consistent with previously reported data.[18] The surgical process can lead to significant alterations in gene expression levels post-surgery, which might play a role in the body's inflammatory response and immune regulation. These genes influence the release of inflammatory mediators and immune processes by modulating the activation or inhibition of associated pathways and the expression of target genes.[19] Numerous studies have identified a variety of abnormally expressed miRNAs in organisms or cells undergoing inflammatory responses.[20] As mentioned earlier, miR-155-5p has been implicated in the regulation inflammatory responses in various diseases.[12-14] Furthermore, research utilizing a rat model of inflammation has demonstrated that miR-155-5p functions as a pro-inflammatory factor, inhibiting the nuclear factor kappa-B (NF-Kb) signaling pathway, which in turn exacerbates inflammatory damage.[21] This study found similar results, revealing that the expression of miR-155-5p was pronounced fluctuation observed in patients undergoing OA relative to those undergoing LA. Additionally, inflammatory markers, indicators of oxidative stress, and VAS scores exhibited a significant positive correlation with miR-155-5p. Therefore, we preliminary speculated that the variations in these indicators during the perioperative period in patients with appendicitis might not solely be attributable to the smaller incision associated with LA, but might also be influenced by the activity of miR-155-5p.

Inflammatory indexes such as CRP, TNF-α, and IL-6 are both pro-inflammatory cytokines, and their elevated levels serve as indicators of inflammation within the body.[22] Leukocytes, neutrophils, and lymphocytes play crucial roles in the inflammatory process, exhibiting phagocytic, immune, and tissue-damaging activities.[23] The findings of this study indicated that both patient groups experienced an increase in postoperative inflammatory markers; however, patients who underwent LA exhibited a lower change of these markers compared to those who underwent OA. This suggested that LA might mitigate the inflammatory response elicited by surgical intervention.

Superoxide dismutase, an active compound in biological systems, is secreted in substantial amounts to safeguard cells in response to injury or damage.[24] Malondialdehyde is a widely recognized indicator of stress response. An increase in MDA levels signifies the oxidative stress state of cells.[25] On the first postoperative day, SOD levels in LA patients exhibited significant yet relatively minor fluctuations compared to their immediate postoperative levels, whereas SOD levels in OA patients demonstrated considerable variability. MDA levels in LA patients in postoperative 24 h were significantly lower than immediately after surgery. Although MDA levels in patients undergoing OA also showed a significant decrease, they remained markedly elevated compared to those in LA patients, indicating that OA exerts a more pronounced effect on oxidative stress in pediatric patients, while LA appears to promote stabilization.

Surgical interventions inherently involve a certain level of trauma, which can potentially lead to various complications.[26] The LA mitigates the drawbacks associated with manual exploration and extensive incisions typical of traditional OA.[27] The use of laparoscopy decreases the duration of appendectomy procedures, helps prevent infections.[28] The findings of this study indicate that the incidence of short-term complications among patients undergoing LA was significantly lower compared to those undergoing OA, which is consistent with previous research findings. This study affirmed that LA was superior to OA concerning perioperative and postoperative complications. Furthermore, our findings indicated that inflammatory factors, MDA, and miR-155-5p served as risk factors for the

development of complications, whereas SOD functioned as a protective factor. miR-155-5p possessed significant predictive capability regarding the occurrence of complications, suggesting that preoperative levels of miR-155-5p could aid in determining the appropriate surgical approach for patients. Elevated levels of miR-155-5p could facilitate the secretion of inflammatory mediators, augment the organism's inflammatory and stress responses, and impede the healing processes of tissues and blood vessels in the vicinity of surgical incisions. Consequently, this might elevate the likelihood of bleeding complications in patients. Furthermore, if preoperative levels of miR-155-5p are markedly elevated, these patients may be better suited for LA, as this approach may mitigate substantial fluctuations in miR-155-5p during the perioperative period, thereby reducing inflammation, oxidative stress, and the associated complications that contribute to patient discomfort. This approach offers a more straightforward, scientifically grounded, and cost-effective method for selecting surgical techniques.

This research also has certain limitations, while it encompasses various aspects and indicators, including clinical, preoperative, and postoperative comparisons, it represents merely an initial exploration into the relationship between miR-155-5p and the methods and complications associated with appendectomy. The findings indicated that the expression of miR-155-5p correlated with levels of inflammation and oxidative stress in patients with ASA, as well as the onset of complications. This also suggests that clinical samples could be further expanded to explore the potential molecular mechanisms of miR-155-5p regulation through the integration of in vitro cellular experiments and in vivo animal models.

In conclusion, miR-155-5p played a role in modulating the body's stress response, which in turn influenced the levels of inflammation and oxidative stress markers. The variability in miR-155-5p expression levels of patients undergoing OA was significantly greater than LA. Furthermore, miR-155-5p demonstrated considerable predictive value regarding the likelihood of complications and might serve as a reference indicator for determining appropriate surgical approaches for patients.

Ethical approval

This study received approval from the Ethics Committee of Xingtai People's Hospital and adhered to the principles outlined in the Helsinki Declaration.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: CD, LZ; data collection: CD, WL, JM; analysis and interpretation of results: CD, YS, LZ; draft manuscript preparation: CD, LZ. Author. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Should we give priority to plasma exchange and hyperbaric oxygen treatment before deciding on amputation for severe crush injuries?

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ABSTRACT

Background. The most common medical sequelae after earthquakes are crush injuries and syndromes that require urgent and well-organized care, which further complicates the approach in the face of overstretched resources. The 2023 Kahramanmaraş earthquake, with a magnitude of 7.7, was one of the largest disasters in Türkiye, affecting 11 cities with a population of about 13.5 million people and claiming more than 50,000 deaths. Approximately 4.6 million pediatric patients were affected, with over 500 children undergoing amputations. The overwhelming number of cases rendered further treatment efforts nearly unfeasible.

Cases. Here we present three cases of severe crush injuries in which amputation was initially considered in both primary and our tertiary centers but was prevented by a protocol that included therapeutic plasma exchange (TPE) and intensive hyperbaric oxygen treatments (HBOT). Based on our review of the literature, this appears to be the first case series documenting the use of therapeutic plasma exchange (TPE) in the management of crush injury.

Conclusion. In extremities at risk for amputation, TPE therapy is crucial to preventing disseminated intravascular coagulation, systemic inflammatory response syndrome, and the accompanying multiorgan failure. It has been shown that extremities at risk for amputation due to poor perfusion can be managed confidently during the safe recovery period of daily TPE therapy with frequent HBOT, anticoagulant and vasodilator treatments, frequent wound care to prevent the development of infection, prophylactic antibiotics, vacuum-assisted closure therapy, and debridement when necessary.

Key words: amputation, crush injury, hyperbaric oxygen treatment, therapeutic plasma exchange, pediatric protocol.

More and more medical professionals are being called upon to treat crush injuries and syndromes following human-made or natural disasters.[1] These disasters can devastate numerous lives in an instant, significantly diminishing the affected individuals' will to survive. The Kahramanmaraş earthquake of 2023 has been one of the clearest and most painful examples of this concept of the struggle for life. A magnitude of 7.7 was enough to destroy 11 cities in Türkiye, where approximately 13.5 million people lived.[2]

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Numerous children underwent amputations as the possibility of further treatment was deemed unattainable. In this article, we aim to share the invaluable lessons learned by Turkish healthcare professionals through these challenging experiences, hoping they will serve as guidance. A team of healthcare professionals from our hospital was dispatched to the earthquake's epicenter to provide care for pediatric victims. While they treated numerous patients on-site, three children were subsequently transferred to our hospital after achieving stable hemodynamics and metabolic balance. However, Doppler ultrasound evaluations revealed an absence of blood flow to the injured extremities in these cases. The aim of this article is to provide healthcare professionals with a pediatric protocol that includes therapeutic plasma exchange (TPE) and intensive hyperbaric oxygen treatments (HBOT) on how to manage these patients at high risk of amputation in both primary and tertiary care centers.

Additionally, hyperbaric oxygen therapy (HBOT) is documented in the literature to aid cellular respiration and prevent anaerobic infection in trauma cases, as well as reduce edema through increased extravascular fluid resorption.[3,4] HBOT has also demonstrated effectiveness in severe limb injuries, where it supports wound healing and potentially mitigates the need for amputation in patients with high Mangled Extremity Severity Scores (MESS) (≥7).[5] To our knowledge, this study represents the first cases series to investigate TPE in the context of crush injury.

Case Presentations

We present three cases of severe crush injuries in which amputation was initially considered but ultimately avoided through the implementation of a comprehensive treatment protocol that included TPE and intensive HBOT (Fig. 1).

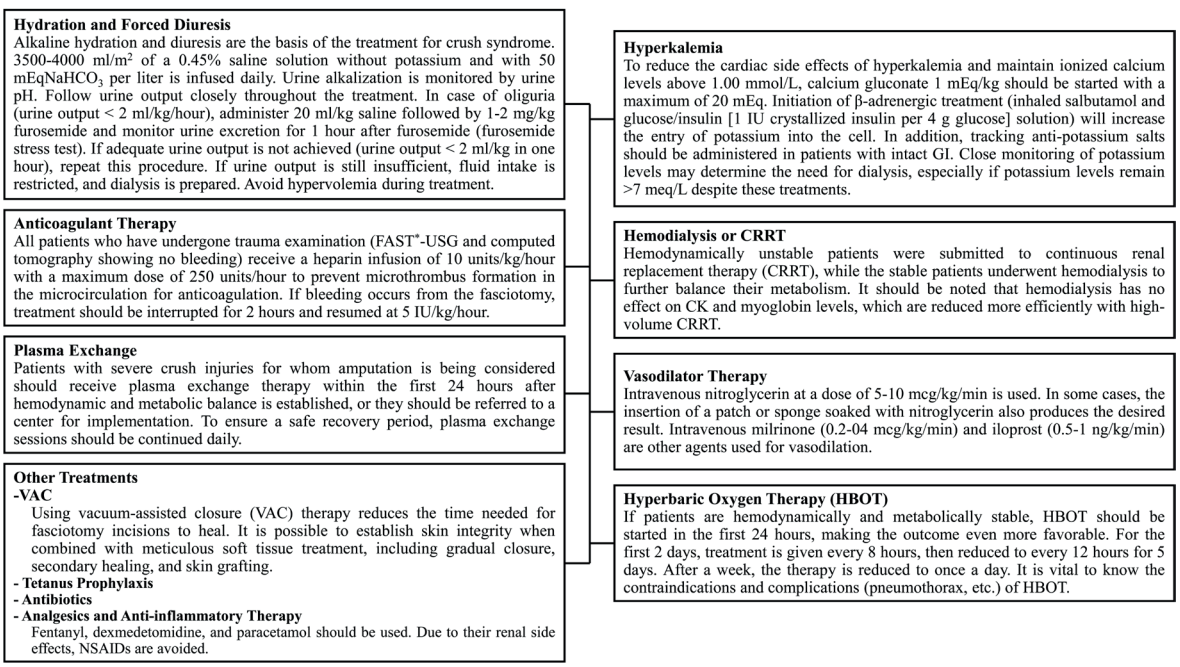

Fig. 1. Pediatric treatment protocol for severe crush injuries.

CK: creatine kinase, CRRT: continuous renal replacement therapy, FAST: focused assessment with sonography, GI: gastronitestinal tract, HBOT: hyperbaric oxygen therapy, NSAID: non-steroid anti-inflammatory drug, USG: ultrasonography, VAC: vacuum-assisted closure.

Patient-1

A 16-year-old girl was rescued 38 hours after the earthquake and presented with acute kidney injury and significant damages in the left lower limb, which was ecchymotic, edematous, and cold. The left leg had been subjected to crushing and compartment syndrome, and there were notable injuries, including an L1 vertebra fracture and a left fibula fracture. Three fasciotomy sites were noted, and Doppler ultrasonography revealed no detectable flow in the dorsal pedal artery. Initial recommendations by the orthopedic and trauma department included below-knee amputation. However, after implementing our treatment protocol on the 3rd day post-earthquake, the limb was salvaged through five debridement procedures, hemodialysis, 22 TPE sessions, and 33 HBOT sessions. The patient was discharged from the pediatric intensive care unit (PICU) after 24 days.

Patient-2

A 10-year-old boy was rescued 24 hours after the earthquake and admitted with an otherwise normal physical examination, except for three prior fasciotomy procedures on his left lateral gastrocnemius and both medial and lateral cruris. His left leg had also been subjected to crushing and compartment syndrome. He exhibited bullous lesions on his left foot, and his left toes were ecchymotic and anesthetic. The treatment protocol was initiated on the 2nd day after the earthquake. A week later, a Doppler ultrasound revealed a patent left dorsal pedal artery with biphasic-triphasic flow on spectral Doppler analysis, indicating significant improvement in vascular status. The patient underwent 3 debridement proceduress, 14 TPE sessions, and 45 HBOT sessions and was discharged after a 33-day PICU stay.

Patient-3

A 6-year-old male, was rescued 42 hours after the earthquake. He had undergone fasciotomies at a local PICU before being admitted to our facility. Upon admission, the left leg exhibited ecchymosis below the knee, with weak pulses in the left foot and an absent dorsal pedal pulse. The left toes showed signs of necrosis. The treatment protocol in our institution started on the 6th day after the earthquake. Doppler ultrasonography at admission indicated reduced peak systolic velocity in the left posterior tibial artery and no flow in the dorsal pedal and digital arteries. Initially, an above-knee amputation was considered. However, after following our treatment protocol, which included 3 debridement proceduress, 27 TPE sessions, and 30 HBOT sessions, the demarcation line previously suggested for below-knee amputation regressed to the dorsum of the foot, demonstrating significant improvement. On days 24 and 25, a Chopart amputation was performed.

Details of the interventions and other patient characteristics are outlined in Table I, Fig. 2 and Fig. 3. Written informed consent was obtained from the parents for this publication.

Discussion

The first treatments given in primary centers to patients rescued from the earthquake zone with severe crush syndrome are intravenous (IV) alkaline hydration, forced diuresis, correction of electrolyte imbalances, assessment of urgent need for dialysis, and ensuring hemodynamic and metabolic stability.[6]

In our protocol, in addition to these treatments, heparin infusion was started (in patients who underwent trauma work-up and were not found to have hemorrhage in US and tomography) in order to prevent microthrombus formation in the extremities with severe crush injuries and decreased or absent blood flow in the Doppler. Treatment-related bleeding or new thrombi were not observed in any patient when the heparin doses specified in the protocol were used. Similarly, vasodilating treatments such as nitroglycerin (IV, topical), milrinone, and iloprost (in patients with decreased or absent

Table I. Demographic and clinical characteristics of the patients.

	Patient 1	Patient 2	Patient 3
Age	16 years 5 months	10 years 4 months	6 years 1 months
Sex	Female	Male	Male
Weight (kg)	59	60	21
BMI (kg/m^2)	20.4	21.3	12.4
Rescue time	38 hours	24 hours	42 hours
The day of the start of treatment protocol	3rd day after the earthquake	2nd day after the earthquake	6th day after the earthquake
Extremities subjected to crushing	Left leg	Left leg	Left leg
Compartment syndrome	+	+	+
Fracture	L1 vertebra/left fibula	-	-
Lung injury	Pneumomediastinum	-	-
Liver injury	-	-	-
AKI	+	-	-
Local infection	-	*Acinetobacter baumannii*	*Stenotrophomonas maltophilia*
Systemic infection	-	-	-
DIC	-	-	-
Echocardiogram	Normal	Normal	Normal
Fasciotomy	+	+	+
Amputation	-	-	The left tarsometatarsal joint.
Number of debridement procedures	5	3	5
Hemodialysis	+	-	-
Hemofiltration	+	-	-
Number of PE	22	14	27
Number of HBOT	33	45	30
VAC	+	+	+
MV support	-	-	-
Tetanus prophylaxis	Fully vaccinated	Vaccine +/Ig G+	Fully vaccinated
Length of stay in ICU	24 days	33 days	31 days
Patient outcome	Inpatient service	Inpatient servise	Inpatient servise
Laboratory test results on admission			
HGB (g/dL)	17.4	9.6	15.6
PLT (10^3/ μL)	384	260	508
WBC (10^3/ μL)	21.29	12,57	23.4
NEU (10^3/ μL)	17.75 (83.4%)	8.60 (68%)	19.2 (82%)
LYM (10^3/μL)	1.46 (6.8%)	2.9 (23%)	2.0 (9%)
BUN (mg/dL)	22	11	27
Urea (mg/dL)	47	24	58
Creatinine (mg/dL)	1	0.4	0.4

AKI: acute kidney injury, ALT: alanine transaminase, AST: aspartate transaminase, BMI: body mass index, BUN: blood urea nitrogen, DIC: disseminated intravascular coagulation, HBOT: hyperbaric oxygen therapy, HGB: hemoglobin, ICU: intensive care unit, LDH: lactate dehydrogenase, LYM: lymphocytes, MV: mechanical ventilation, NEU: neutrophils, PE: plasma exchange, PLT: platelets, VAC: vacuum-assisted closure, WBC: white blood cells.

Table I. Continued.

	Patient 1	Patient 2	Patient 3
Uric acid (mg/dL)	6.8	5.6	9.1
Sodium (mmol/L)	133	132	136
Potassium (mmol/L)	5.4	4.3	5
Calcium (mg/dL)	8.4	1.1	10.2
Phosphorus (mg/dL)	7.2	2.9	5
Albumin (g/dL)	26.8	23.9	4.5
AST (U/L)	1910	565	285
ALT (U/L)	695	264	131
LDH (U/L)	4500	564	>750
Amylase (U/L)	19	N/A	24
Creatine kinase (U/L)	134117	603	>1000
Lipase (U/L)	13	11	6
Total bilirubin (mg/dL)	0.9	0.23	0.6
Direct bil. (mg/dL)	0.2	0.16	0.2
Urine analysis			
pH	6	7	9
Protein (mg/dL)	100	30	0
Hemoglobin	+++	-	-
Color	dark brown	straw color	Straw color
Density	1037	1037	1009
Myoglobin (μg/L)	80500	65	32
Laboratory test results of the last workup			
HGB (g/dL)	10.5	9.7	7.7
PLT (10^3/ μL)	326	440	420
WBC (10^3/ μL)	4.97	7.64	6.72
NEU (10^3/ μL)	2.7 (54%)	3.9 (51%)	3.6 (53%)
LYM (10^3/μL)	1.5 (30%)	3 (39%)	2.1 (31%)
BUN (mg/dL)	7	11	18
Urea (mg/dL)	14	23	39
Creatinine (mg/dL)	0.7	0.4	0.2
Uric acid (mg/dL)	4	4.9	1.2
Sodium (mmol/L)	140	138	140
Potassium (mmol/L)	4	4.3	3.6
Calcium (mg/dL)	9.8	10.3	9.6
Phosphorus (mg/dL)	4.1	5.8	4.5
Albumin (g/dL)	42.4	38.7	37.1
AST (U/L)	35	27	30
ALT (U/L)	31	30	25
LDH (U/L)	209	250	212
Creatine kinase (U/L)	101	154	63

AKI: acute kidney injury, ALT: alanine transaminase, AST: aspartate transaminase, BMI: body mass index, BUN: blood urea nitrogen, DIC: disseminated intravascular coagulation, HBOT: hyperbaric oxygen therapy, HGB: hemoglobin, ICU: intensive care unit, LDH: lactate dehydrogenase, LYM: lymphocytes, MV: mechanical ventilation, NEU: neutrophils, PE: plasma exchange, PLT: platelets, VAC: vacuum-assisted closure, WBC: white blood cells.

Table I. Continued.

	Patient 1	Patient 2	Patient 3
Total bilirubin (mg/dL)	0.33	0.3	0.23
Direct bilirubin (mg/dL)	0.18	0.16	0.13
pH	8	7	8
Protein (mg/dL)	0	0	0
Color	Straw color	Straw color	Straw color
Density	1013	1014	1004
Myoglobin (μg/L)	< 8	< 8	< 8

AKI: acute kidney injury, ALT: alanine transaminase, AST: aspartate transaminase, BMI: body mass index, BUN: blood urea nitrogen, DIC: disseminated intravascular coagulation, HBOT: hyperbaric oxygen therapy, HGB: hemoglobin, ICU: intensive care unit, LDH: lactate dehydrogenase, LYM: lymphocytes, MV: mechanical ventilation, NEU: neutrophils, PE: plasma exchange, PLT: platelets, VAC: vacuum-assisted closure, WBC: white blood cells.

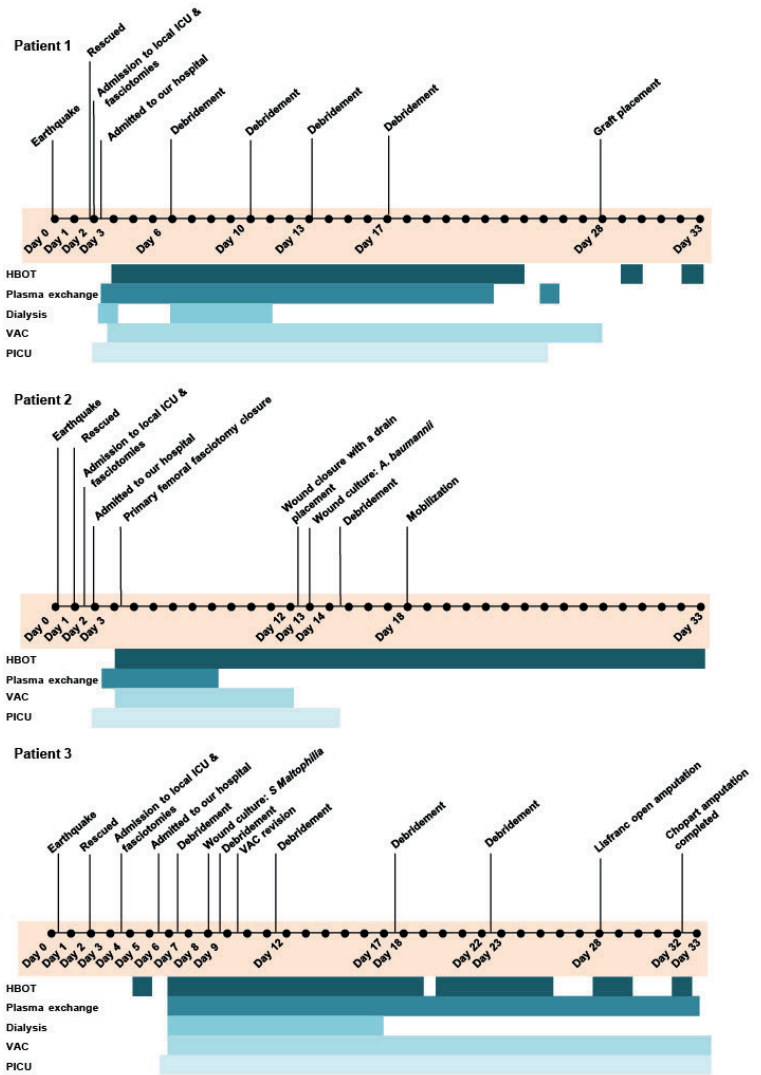

Fig. 2. Clinical timeline.

HBOT: hyperbaric oxygen therapy, ICU: intensive care unit, PICU: pediatric intensive care unit, VAC: vacuum-assisted closure.

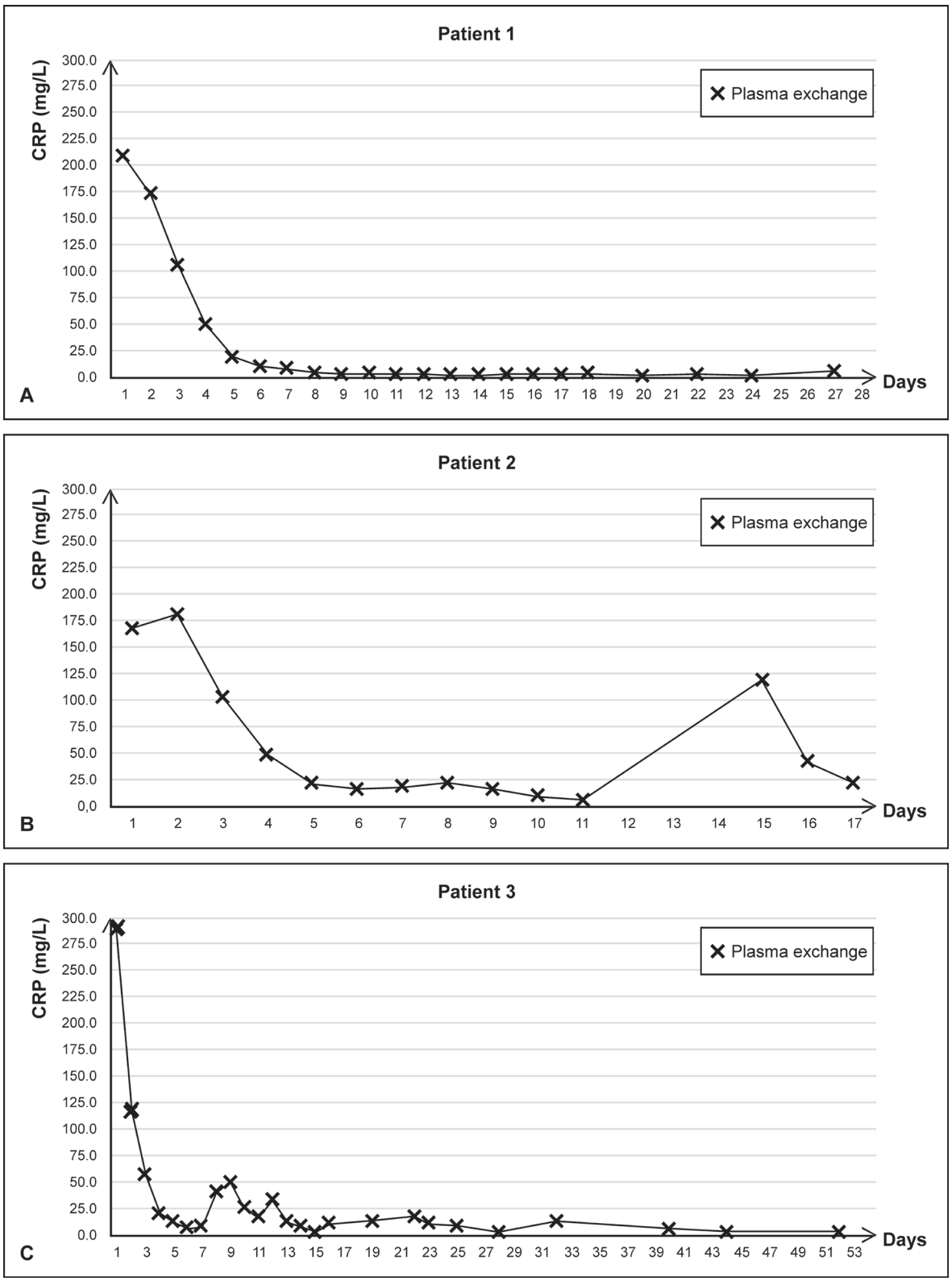

Fig. 3. Daily changes in C-reactive protein (CRP) levels during plasma exchange therapy.

arterial phase currents) were initiated to maintain microcirculation and increase perfusion in the ischemic ends of the extremities. Treatment-related complications and hypotension were not observed in any patients when used at the doses indicated in the protocol.

In the case of a stable patient with crush injuries, where the affected extremities present as ecchymotic, edematous, and ischemic with no detectable pulses or blood flow on Doppler ultrasound, the critical question arises: should amputation be performed promptly in the primary earthquake region, or should the patient be given the opportunity to recover from both the physical and emotional toll, provided their condition remains stable? This article will attempt to find answers to these questions. These extremities that remain ischemic with no blood flow can cause many serious systemic and life-threatening events such as thrombocytopenia associated multi-organ failure (TAMOF), systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation (DIC), sepsis, and gas gangrene.[7] Continuing to treat these patients without amputation in the primary setting is a very daring decision given the high volume of patients, as there is a risk of patient loss due to potential systemic complications. Even transferring the patient in this condition to a tertiary center may pose a great risk in terms of systemic complications that may develop along the way. At this point, TPE therapy provided a very safe recovery period, allowing both safe transfer of the patient to a tertiary center and the possibility of performing limb-preserving surgery with complete recovery or elimination of the demarcation line through more frequent prolonged use of HBOT in tertiary centers.

In recent years, extensive experience has been gained with the utility of TPE therapy in the treatment of sepsis, TAMOF, and DIC.[8,9] The experience with TPE in crush injury has not yet been reported. Considering the pathophysiology of the event, crush injury leads to severe endothelial damage.[7] This endothelial damage may trigger the inflammatory pathway, leading to the formation of a cytokine storm and the development of SIRS.[7] The developing endothelial damage may also trigger the microthrombotic pathway, leading to excessive endothelial exocytosis and consequent release of large amounts of ultralarge von Willebrand factor (uLVWF) into the circulation.[7] The enzyme ADAMTS13 is required to degrade the uVWF protein.[7] If there is not enough ADAMTS13 enzyme to degrade the uLVWF, which enters the bloodstream in large quantities, the uLVWFs attach to the endothelium in the microcirculation, leading to the formation of microthrombi with the accumulation of platelet aggregates on them and compromising organ perfusion.[7] Multiple organ failure occurs when other organs are also affected. The usual treatment is TPE when these two pathways are activated. Plasma exchange ensures both a decline in proinflammatory mediators of the inflammatory pathway and also clear and refreshing the elements of microtrombotic pathway.[9] The timing of initiating treatment is critical to see the beneficial effect of TPE. Patient-1 who spent similar period under the debris and had even more severe crush injuries than Patient-3, did not need amputation due to early initiating TPE and the protocol, whereas partial amputation was needed in Patient-3 (Fig. 2).

The rationale for utilizing TPE in our patient cohort stems from the potential to mitigate the sequelae of severe crush injuries, including SIRS, DIC, and TAMOF. While our patients exhibited clinical stability, some presented with concerning signs indicative of potential extremity compromise, such as coldness, ischemia, and Doppler-confirmed absent blood flow. However, we refrained from immediate amputation, opting instead for comprehensive management in tertiary care facilities equipped to monitor these patients closely.

The implementation of TPE was particularly critical in the high-risk, post-earthquake environment, where the increased patient load posed significant challenges to effective monitoring for systemic complications.

Prophylactic use of TPE aimed to prevent the onset of SIRS, DIC, and TAMOF, allowing time for effective interventions like HBOT, which was also employed early in treatment protocols.

Despite the absence of overt clinical manifestations of SIRS, DIC, or TAMOF in many patients, TPE therapy was maintained for approximately one month. This prolonged intervention was justified by the need to prevent potential complications associated with the underlying pathophysiological processes of crush syndrome, which include significant endothelial injury and inflammatory responses leading to microthrombi formation. The benefits of TPE in clearing inflammatory mediators and pro-thrombotic elements underscore its role in facilitating recovery and possibly enhancing outcomes when combined with other treatments.

Additionally, we observed that the trend of acute phase reactants in these patients could serve as an important indicator of inflammatory status and response to therapy (Fig. 3). Future analyses could provide insights into how different biomarkers correlate with clinical outcomes, thus reinforcing the rationale for the ongoing use of TPE in this patient population.

Furthermore, our experience aligns with literature suggesting the efficacy of TPE in similar scenarios, albeit less explored in crush injuries specifically. For instance, existing studies have illustrated the benefits of TPE in severe burn injuries, where similar pathophysiological (endothelial injury) mechanisms are at play.[10] By addressing the cytokine storm and mitigating microthrombi formation through early intervention, TPE has demonstrated potential to avert drastic outcomes, such as limb amputation, as exemplified by our patients.

HBOT increases not only the dissolved oxygen partial pressure in the blood, but also ensures mitochondrial oxygenation.[11] This allows aerobic respiration to continue in all cells and keeps tissues alive.[11,12] HBOT has an anti-edematous effect by lowering capillary blood pressure and increasing extravascular fluid reabsorption.[12] Since it keeps aerobic metabolism alive, it has an antibacterial effect by preventing the development of anaerobic organisms.[5,12] One of its most important effects is the acceleration of wound healing by stimulating collagen synthesis and fibroblast migration.[5,12] In our protocol, HBOT was administered to patients with extremities initially considered for amputation. The treatment schedule included sessions every 8 hours for the first two days, every 12 hours for the subsequent five days, and then once daily thereafter. Each treatment provided in monoplace hyperbaric chambers a pressure of 2.4 atmospheres absolute (ATA) for 90-120 minutes with compression and decompression periods.

We had one patient who was on dialysis due to acute kidney injury. We think that by choosing the high-volume continuous renal replacement therapy method in addition to TPE therapy in the first two days, it also contributed to cytokine clearance.

Using vacuum-assisted closure (VAC) for fasciotomies was one of the treatment's most crucial components. It has been demonstrated that VAC therapy reduces the time needed for fasciotomy incisions to heal.[13] Meanwhile, it should be remembered that a healed wound is merely the first stage of salvaging. Long-term rehabilitation procedures and other interventions will be required for a functional limb.

Patients at risk of amputation should be referred to tertiary care centers in a coordinated manner following metabolic and hemodynamic stabilization at primary centers, which experience high patient volumes in the aftermath of an earthquake. TPE plays a crucial role in preventing DIC, SIRS, and associated multiorgan failure in extremities at risk of amputation.

Conclusion

It has been shown that extremities at risk for amputation due to poor perfusion can be managed confidently during the safe recovery period of daily TPE therapy with frequent HBOT, anticoagulant and vasodilator treatments, frequent wound care to prevent the development of infection, prophylactic antibiotics, VAC therapy, and debridement when necessary.

In conclusion, our approach underscores the importance of a multifaceted treatment protocol that incorporates TPE, HBOT, and supportive measures to optimize patient outcomes in the face of severe crush injuries. Early intervention and close monitoring in specialized settings are vital to navigating the complexities associated with these critical conditions.

Ethical approval

Written informed consent was obtained from the parents for this publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MT, IB, OO; data collection: KS, AC, LO, AC; analysis and interpretation of results: MT, OO, IE, AC, LO; draft manuscript preparation: MT, IE, KS, AC, IB. 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

Drug-induced enterocolitis syndrome in children: report of two cases and a literature review

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ABSTRACT

Background. Drug-induced enterocolitis syndrome (DIES) is a recently defined clinical entity, first described in 2014. DIES is a hypersensitivity reaction with non-IgE mechanisms involving the gastrointestinal tract, occurring 1 to 4 hours after drug ingestion. Antibiotics are most commonly responsible, particularly amoxicillin or amoxicillin / clavulanic acid (AMX/CL). The main criterion is recurrent, often uncontrollable vomiting occurring 1-4 hours after drug ingestion, without classic IgE-mediated allergic symptoms such as cutaneous or respiratory reactions. To the best of our knowledge, 10 pediatric cases of DIES have been described in the literature.

Case Presentations. A 4-year-old male and a 14-year-old male presented to our pediatric allergy clinic with a suspected hypersensitivity reaction to AMX/CL, and their specific IgE tests for penicillin G and penicillin V were negative. The younger patient was also tested for specific IgE against amoxicillin and ampicillin, which were also negative. Skin prick tests and intradermal test with AMX/CL were negative in both patients, but oral provocation testing with AMX/CL resulted in abdominal pain, vomiting and lethargy, confirming the diagnosis of DIES.

Conclusions. DIES should be considered in patients presenting with vomiting and lethargy following drug ingestion, particularly when IgE-mediated allergies have been ruled out. Early recognition and appropriate management, including drug provocation testing in a controlled setting, are crucial to ensure optimal patient outcomes. By presenting these two rare cases, we aim to raise awareness and deepen the understanding of DIES among healthcare professionals, which could contribute to earlier diagnosis and better patient outcomes.

Key words: amoxicillin/clavulanic acid, children, drug-induced enterocolitis syndrome, rare allergic diseases.

Drug hypersensitivity reactions (DHRs) are increasing in both adult and pediatric populations, especially in recent years. While IgE-mediated mechanisms are the most common cause of DHRs, non-IgE-mediated reactions involving drug-specific T-cell responses can also occur.[1]

Drug-induced enterocolitis syndrome (DIES) is a non-IgE-mediated, drug-specific T-cell-mediated hypersensitivity reaction, primarily affecting the gastrointestinal system. The syndrome typically manifests 1 to 4 hours after drug ingestion and is characterized by symptoms such as nausea, vomiting,

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abdominal pain, diarrhea, pallor, lethargy, and dehydration. Hypovolemic shock has been reported in some cases.[2]

The non-immediate adverse drug reaction known as DIES is not well understood. It is very important that this clinical picture, whose pathogenic mechanism is still unclear, is recognized early and treated correctly by physicians.[2,3] We present two pediatric cases to raise awareness of this rare clinical entity, first described in 2014.

Case Presentations

Patient 1

A 4-year-old boy was admitted to our pediatric allergy outpatient clinic with a suspected hypersensitivity reaction (HSR). At 15 months of age, he developed maculopapular exanthema on the third day of treatment with amoxicillin / clavulanic acid (AMX/CL), prompting discontinuation of the medication. The rash disappeared within 2-3 days. The patient had not used AMX/CL again since the initial reaction.

The patient was evaluated in our pediatric allergy clinic and amoxicillin, ampicillin, penicillin G, and penicillin V specific IgE (sIgE) tests were negative. Skin prick test (SPT) and intradermal test (IDT) with AMX/CL (20 mg/mL) were negative. With his family's informed consent, the patient was set up for an open drug provocation test (DPT). The AMX/CL oral suspension was divided into two doses, each representing a portion between 10-90% of the total therapeutic dose and administered at 30-minute intervals (reaching a total of 40 mg/kg/day).

One and a half hours after the last dose, the patient developed severe abdominal pain, four episodes of intermittent severe vomiting, pallor, and lethargy, without cutaneous, mucosal, respiratory, or systemic symptoms. Vital signs revealed an oxygen saturation of 98%, respiratory rate of 22/min, blood pressure of 80/50 mmHg, and heart rate of 105/min.

Intravenous (IV) 20 mL/kg 0.9% NaCI and ondansetron were administered. Laboratory tests, obtained three hours after the onset of vomiting, showed white blood cells (WBC): $12.98x10^9$/L (normal range 5.5-15.5), neutrophils: $9.15x10^9$/L (normal range 1.5-8.5), platelets (PLT): $401x10^9$ /L (normal range 300±50), C-reactive protein (CRP): 0.3 mg/L (<5 mg/L), blood urea nitrogen (BUN): 30 mg/dL (0-23), creatinine: 0.69 mg/dL (0.3-1.2); and normal serum electrolytes. Acidosis was detected in blood gas analysis; pH: 7.29 (7.35-7.45), HCO_3: 18.6 mmol/L (18-23), PCO_2: 38 mmHg (32-46). The patient was closely monitored, and lethargy improved after 12 hours. After 24 hours of observation, oral intake improved. On the day of the challenge test, there were no confounding variables or infectious contexts. No mast cell degranulation was observed (tryptase levels: 3.78 ug/L [normal: <11.4]) three hours after reaction). The patient was diagnosed with DIES, fulfilling the diagnostic criteria (Table I).[3] Afterwards, the patient was evaluated for beta-lactam cross-reactivity, and he tolerated cefuroxime without any problem.

Patient 2

A 14-year-old male patient was referred to our pediatric allergy outpatient clinic for recurrent vomiting episodes starting one hour after AMX/CL ingestion at 3 and 11 years of age. No other AMX/CL exposures were reported.

Specific IgE tests for penicillin G and V were negative. SPT followed by IDT were performed using AMX/CL (20 mg/mL), and both were negative. The patient was scheduled to undergo an open DPT with informed consent from his family. The AMX/CL tablet was administered in three doses, each representing 10-40-50% of the total therapeutic dose, at 30-minute intervals (reaching a total of 40 mg/kg/day). One hour and 15 minutes after the last dose, the patient developed severe abdominal pain, three episodes of severe vomiting, lethargy,

Table I. Diagnostic Criteria for Patients Presenting with Possible DIES.

Major criterion
1. Vomiting in the 1- to 4-h period after ingestion of the suspected drug and absence of classic IgE-mediated allergic skin or respiratory symptoms
Minor criteria
1. A second episode of repetitive vomiting after ingestion of the same drug
2. Repetitive vomiting episode 1-4 h after ingestion of a different drug
3. Extreme lethargy
4. Marked pallor
5. Need for emergency department visit
6. Need for intravenous fluid support
7. Diarrhea in 24 h (usually 5-10 hours) after ingested drug
8. Hypotension
9. Hypothermia

DIES: Drug-induced enterocolitis syndrome.

and pallor. IV hydration with 0.9% NaCl, methylprednisolone, and ondansetron were administered. There were no cutaneous or respiratory system findings other than vomiting. Upon assessment of vital signs, oxygen saturation was 97%, respiratory rate was 20 breaths per minute, blood pressure was 100/60 mmHg, and heart rate was 95 beats per minute. Laboratory results revealed leukocytosis (WBC: $13.34x10^9$/L [normal range 4.5-13]) and neutrophilia ($12.53x10^9$/L [normal range 1.8-8]), PLT: $314x10^9$/L (normal range 300±50), BUN: 11 mg/dL (normal range 0-23), creatinine: 0.51 mg/dL (normal range 0.3-1.2); and serum electrolytes were within normal physiologic range. Tryptase levels were normal (0.98 μg/L). On the day of the challenge test, there were no signs of infection or any other potential confounding factors. The patient, who met the diagnostic criteria, was diagnosed with DIES (Table I).[2] SPT/IDT and DPT with cefuroxime were planned for alternative beta-lactam antibiotics.

Discussion

Drug-induced enterocolitis syndrome is a drug-induced non-IgE mediated HSR, primarily affecting the gastrointestinal system and typically occurring 1 to 4 h after drug administration. This clinical picture, which can cause severe clinical manifestations such as lethargy, hypotension and hypovolemic shock, is poorly recognized worldwide.[4] Timely diagnosis and appropriate management of DIES are crucial. In this paper, we present two cases of DIES to highlight the need for increased awareness of this condition.

In 2014, an Italian research group was the first to describe a case of a 6-year-old girl who developed vomiting 1-2 hours after AMX ingestion. They coined the term DIES to describe this clinical picture, as its clinical presentation and laboratory findings were reminiscent of food protein-induced enterocolitis syndrome (FPIES). The immunologic mechanisms underlying DIES are still not fully understood. It has been suggested that metabolites formed after the drug passes through hepatic and intestinal processes may cause drug-induced intestinal damage.[2] Reports of DIES cases were published from Spain in 2017,[5] Netherlands in 2019[3], and most recently from Türkiye in 2024.[6] The fact that all of the 10 pediatric cases described were from European countries suggests that awareness of DIES is very low worldwide (Table II).[3-10] The incidence is unknown, but one Italian children's hospital reported a 0.4% prevalence of DIES among pediatric patients referred for suspected DHRs.[8]

Table II. Review of the published cases according to the last search of PubMed in September 2024*.

Age (yr), sex, country	Triggering Drug	History of drug reaction	DPT			Suspicious Drug Laboratory Findings		Ref.
			Latency, hr	Signs/Symptoms	Treatment	Laboratory Findings	SpIgE	SPT and IDT	
6, F, Italy	AMX	1. Vomiting 2. Morbilliform rash (one day later)	2	1. Vomiting 2. Diarrhea 3. Pallor 4. Lethargy 5. Hypotension	1. Saline solution infusion 2. Steroid	1. Leukocytosis 2. Neutrophilia	Negative	Negative	10
3, M, Spain	AMX	1. Urticaria	4	1. Vomiting 2. Diarrhea 3. Abdominal pain	1. Saline solution infusion 2. Steroid 3. Antiemetics	1. Leukocytosis 2. Neutrophilia	Negative	Negative	5
4, M, Netherlands	AMX	1. Erythematous skin reaction (When 2 years old) 2. Vomiting (When 4 years old) 3. Lethargy (When 2 years old)	1.5	1. Vomiting 2. Pale 3. Abdominal pain 4. Lethargy	1. Adrenalin 2. Steroid 3. Ondansetron	1. Leukocytosis 2. Neutrophilia	Negative	Negative (only SPT) IDT was not performed	3
10, F, France	AMX	1. Vomiting 2. Pallor 3. Diarrhea	3	1. Vomiting 2. Pallor 3. Abdominal pain 4. Lethargy 5. Diarrhea	1. Only rehydrated orally	n.s	n.a	n.a	7
6, M, Italy	AMX/CL	1. Angioedema	2.5	1. Vomiting 2. Pale 3. Lethargy	1. Saline solution infusion	1. Leukocytosis 2. Neutrophilia	Negative	Negative	8

* Review of the published pediatric cases according to the last search of PubMed in September 2024 (The case described from Türkiye is not available on PubMed)
AMX: Amoxicillin, AMX/CL: Amoxicillin/clavulanate, DPT: Drug provocation test, SPT: Skin prick tests, IDT: Intradermal tests, sIgE: Serum-specific IgE, n.s: Not specified, n.a: Not applied, Ref: Reference

Table II. Continued.

Age (yr), sex, country	Triggering Drug	History of drug reaction	DPT			Suspicious Drug Laboratory Findings		Ref.
			Latency, hr	Signs/Symptoms	Treatment	Laboratory Findings	SpIgE	SPT and IDT	
14, F, Italy	AMX/CL	1. Vomiting 2. Lethargy	2.5	1. Vomiting 2. Pallor 3. Lethargy 4. Abdominal pain 5. Malaise	1. Saline solution infusion 2. Ondansetron	1. Leukocytosis 2. Neutrophilia	n.a	Only IDT	8
9, F, Switzerland	AMX/CL	1. Maculopapular exanthema	3	1. Vomiting 2. Pallor 3. Lethargy	n.s	n.s	n.a	Only IDT	8
0.8, M, France	Paracetamol	1. Vomiting 2. Asthenia 3. Pallor 4. Tachycardia	4	1. Vomiting 2. Pallor 3. Lethargy	1. Rehydration 2. Corticosteroid	n.s	Negative	Negative	9
4, M, France	AMX	1. Progressively extensive erythematous rash 2. Eyelid edema (On the seventh day of treatment)	2	1. Vomiting 2. Abdominal pain 3. Pallor 4. Tachycardia	1. Adrenalin 2. Saline solution infusion 3. Ondansetron	n.s	n.a	Negative (Only IDT)	4
7, M, Türkiye	AMX/CL	1. Vomiting 2. Lethargy	2.5	1. Abdominal pain 2. Vomiting 4. Lethargy 5. Pallor	1. Saline solution infusion	1. Neutrophilia	n.a	n.a	6

* Review of the published pediatric cases according to the last search of PubMed in September 2024 (The case described from Türkiye is not available on PubMed)
AMX: Amoxicillin, AMX/CL: Amoxicillin/clavulanate, DPT: Drug provocation test, SPT: Skin prick tests, IDT: Intradermal tests, sIgE: Serum-specific IgE, n.s: Not specified, n.a: Not applied, Ref: Reference

The low number of reported cases suggests that DIES may be underdiagnosed, particularly outside Europe.

The primary diagnostic challenge in DIES lies in differentiating it from IgE-mediated hypersensitivity reactions. The absence of skin and respiratory symptoms, along with normal tryptase levels, are the most critical points in differentiating it from an IgE-mediated reaction.[1,2] Upon evaluating the clinical findings following DPT in 10 cases from the literature and 2 cases presented by us, vomiting was observed in all cases (12/12 patients). Pallor (11/12 patients) and lethargy (10/12 patients) were the other most common findings. Vomiting after drug ingestion may develop both with an IgE-mediated mechanism and with a non-IgE-mediated mechanism (as in DIES). The key distinguishing factor is the timing of vomiting onset following drug ingestion in DIES, it begins 1.5 to 2 hours after ingestion, whereas in IgE-mediated reactions, it occurs within the first hour of exposure.[2]

Among the pediatric DIES cases in the literature, five were caused by AMX, 4 by AMX/CL, and 1 by paracetamol. The two cases we presented were AMX/CL-induced, which is consistent with previous reports.[2] Nausea, vomiting, and diarrhea are reported to be common side effects of AMX.[11] For these reasons, in patients who develop nausea, vomiting, and diarrhea after AMX, DPT should be performed under hospital conditions to diagnose DIES.

Diagnostic criteria for DIES were proposed by Van Thuijl et al. (Table I).[3] For the diagnosis, 1 major and 3 minor criteria must be met (in addition, IgE-mediated cutaneous and respiratory symptoms must be absent). As in the 10 cases described in the literature, these diagnostic criteria were met in the two cases we presented.

There are no specific or diagnostic laboratory parameters for DIES. There is no increase in tryptase levels. The most notable laboratory finding is "neutrophilic leukocytosis". The neutrophil peak in DIES is expected approximately 6 hours after drug ingestion due to increased IL-8 and cortisol levels.[2] In the described cases of DIES, there is no information about the time of collection of blood tests. Leukocytosis and neutrophilia were described in 6 cases in the literature, while in other 4 cases, no information about these tests was provided. In the first case we presented, blood tests were taken 3 hours after drug intake, and although leukocytosis was absent, neutrophilia was present. In the second case, blood tests were taken 6 hours after drug ingestion, and marked leukocytosis and neutrophilia were detected. To support the diagnosis, obtaining a complete blood count 6 hours after drug intake may be useful in confirming DIES, as this timing correlates with the characteristic laboratory findings.

In the cases we have presented, type A reactions are the most likely to be confused with DIES. Isolated gastrointestinal symptoms, such as vomiting, abdominal pain, and diarrhea, can occur as side effects after the use of certain medications. These symptoms are typically mild and transient, leading to their interpretation as pharmacological reactions.[11] However, a key distinction between DIES and drug side effects lies in the severity, timing of onset, and the presence of associated systemic symptoms. In DIES, gastrointestinal symptoms are more severe, with vomiting often leading to significant dehydration and accompanied by systemic symptoms such as lethargy. This contrasts with the mild and self-limiting nature of typical drug side effects. Additionally, DIES symptoms usually begin 1 to 4 hours after drug ingestion, which represents a delayed onset compared to the immediate onset typically seen with drug side effects.[2] Severe gastrointestinal distress, along with systemic symptoms like pallor and lethargy, suggests DIES rather than a simple drug side effect. Moreover, once IgE-mediated reactions (e.g., anaphylaxis) have been ruled out, DIES should be considered in the differential diagnosis.

Patch tests are used in the evaluation of delayed-type hypersensitivity reactions; however, their sensitivity is limited in reactions associated with the gastrointestinal system.[12,13] In the majority of DIES cases reported in the literature, patch testing was not performed. Similarly, in our study, the diagnosis was confirmed based on clinical findings and DPT. Patch testing presents practical challenges in pediatric patients and may not be adequate for assessing delayed reactions in the gastrointestinal tract, particularly in systemic conditions like DIES. However, we think that whether patch tests play a role in understanding DIES pathophysiology may be investigated in future studies.

The management of DIES is primarily supportive care. Since the most prominent clinical finding in the acute phase of DIES is vomiting and the resulting fluid loss, treatment is mainly focused on addressing these symptoms.[2] In nearly all cases reported in the literature, IV 0.9% NaCl and IV ondansetron were administered, leading to clinical improvement. Both IV 0.9% NaCl and IV ondansetron were also administered in the cases we presented. While the efficacy of corticosteroids has not been proven, they may be considered in patients with severe symptoms, as cell-mediated inflammation is suspected in FPIES.[2] We administered IV steroids in our second case. Adrenaline is not recommended in the management of DIES, which shares a similar pathophysiology with FPIES.[2] However, intramuscular adrenaline was given in the two cases from the literature because it was difficult to distinguish DIES from anaphylaxis. Despite adrenaline administration, vomiting persisted in both patients and only resolved after ondansetron was given.[3,4]

In conclusion, the identification of all reported cases of DIES in Europe underscores the need for increased global awareness of this condition. DIES should be considered in patients presenting with vomiting and lethargy following drug ingestion, particularly when IgE-mediated allergies have been ruled out. Early recognition and appropriate management, including DPT in a controlled setting, are crucial to ensuring optimal patient outcomes. We hope that by presenting these two rare cases, we contribute to greater awareness and understanding of DIES.

Ethical approval

All the studies were performed by the Declaration of Helsinki and guidelines for good clinical practice. All legal representatives of the patients were informed, and their informed consent was obtained.

Author contribution

The authors confirm their contribution to the paper as follows: Study conception and design: MÖ, EB, BCD, CA, SÖ; data collection: MÖ, EB, BCD, CA, SÖ; analysis and interpretation of results: MÖ, EB, BCD, CA, SÖ; draft manuscript preparation: MÖ, EB, BCD, CA, SÖ. 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

Three locally invasive infantile fibrosarcoma cases treated with larotrectinib

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ABSTRACT

Background. Infantile fibrosarcoma is a rapidly growing soft tissue tumor, often managed by surgical resection, with chemotherapy and radiotherapy as additional options. Due to the high local aggressiveness and surgical morbidity, targeted therapies like larotrectinib can enhance quality of life and preserve organs, particularly in limb-threatening cases. Here, we present three cases where larotrectinib prevented mutilating surgeries.

Cases. The first patient presented antenatally with a 75 x 48 mm oral floor mass, severely narrowing the airway. Surgery was unfeasible due to invasion of vital organs, and complications arose with conventional chemotherapy. Following detection of an ETV6-NTRK3 fusion, larotrectinib was initiated, resulting in complete regression after two years. The second patient had a 42 x 37 mm right-hand tumor confirmed as infantile fibrosarcoma, for which initial treatment suggested amputation. After identifying an ETV6-NTRK3 fusion and failing to respond to chemotherapy, larotrectinib led to significant regression by year two, preserving hand function. The third patient presented with a 56 x 55 mm right foot mass at birth. Chemotherapy proved ineffective, and larotrectinib was initiated due to an ETV6-NTRK fusion signal, ultimately achieving near total regression within one year and avoiding amputation. All three cases demonstrated successful outcomes with targeted therapy.

Conclusions. These cases emphasize the importance of advanced molecular studies, like next-generation sequencing, for childhood tumors and integrating research with clinical trials. tropomyosin receptor kinase inhibitor larotrectinib may offer a safe and effective alternative to chemotherapy for NTRK fusion-positive, metastatic, or unresectable tumors.

Key words: infantile fibrosarcoma, larotrectinib, TRK inhibitors.

Fibrosarcoma, a rare malignant soft-tissue tumor, is classified into infantile (congenital) and adult types. Infantile fibrosarcoma (IFS) typically presents in children under 1 year, most often in the extremities but also in the head, neck, or abdomen.[1] The five-year survival rate for localized IFS is 89%, with an event-free survival rate of 81%.[2] Treatment aims to achieve a cure with minimal long-term side effects.

The primary treatment for IFS is conservative surgical resection. When direct surgery is not feasible, systemic treatments, such as chemotherapy, are used to reduce tumor size and facilitate secondary resection. Standard chemotherapies like vincristine and actinomycin-D are commonly used in Europe; however, they have limitations, highlighting the need for safer alternatives.

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Aggressive therapies, including alkylating agents, anthracyclines, mutilating surgeries, and radiotherapy, are generally avoided due to severe long-term effects and are reserved for relapse or recurrence.[3,4]

The ETS Variant Transcription Factor 6-Neurotrophic Receptor Tyrosine Kinase 3 (ETV6-NTRK3) fusion is a major oncogenic driver in up to 90% of IFS cases.[5] Initially used for diagnostic purposes, it has become a valuable therapeutic target. Selective tropomyosin receptor kinase (TRK) inhibitors are particularly promising for pediatric patients with neurotrophic tyrosine receptor kinase (NTRK) fusion-positive tumors, where chemotherapy and radiotherapy are less ideal. These inhibitors are recommended when tumors are metastatic, resection would result in significant morbidity, or alternative treatments are unsatisfactory or have failed. TRK inhibitors are now increasingly favored as the primary treatment for most IFS patients.[6]

Randomized controlled trials are challenging for rare conditions like IFS, limiting robust evidence. Consequently, recommendations for the TRK inhibitor larotrectinib rely on cumulative case reports.[4,7-12] Even a small case series, such as three patients, can offer meaningful insights and enrich the literature. We report three cases showing that larotrectinib may be a valid therapeutic option for newborn and infant patients with IFS.

Case Reports

Case 1

The first patient was diagnosed antenatally with a suspicious neck mass extending from the tongue root. Fetal magnetic resonance imaging (MRI) suggested a sarcomatous tumor (Fig. 1A), and postnatal tru-cut biopsy confirmed a spindle-cell mesenchymal tumor. An ETV6-NTRK3 fusion was detected in 30% of tumor cells, leading to a diagnosis of IFS.

Primary surgery was not feasible due to vital organ invasion. Vincristine and actinomycin D treatment began immediately, but severe airway narrowing at 18 days required an open tracheostomy. After the third chemotherapy cycle, the regimen was changed to vincristine and cyclophosphamide due to veno-occlusive

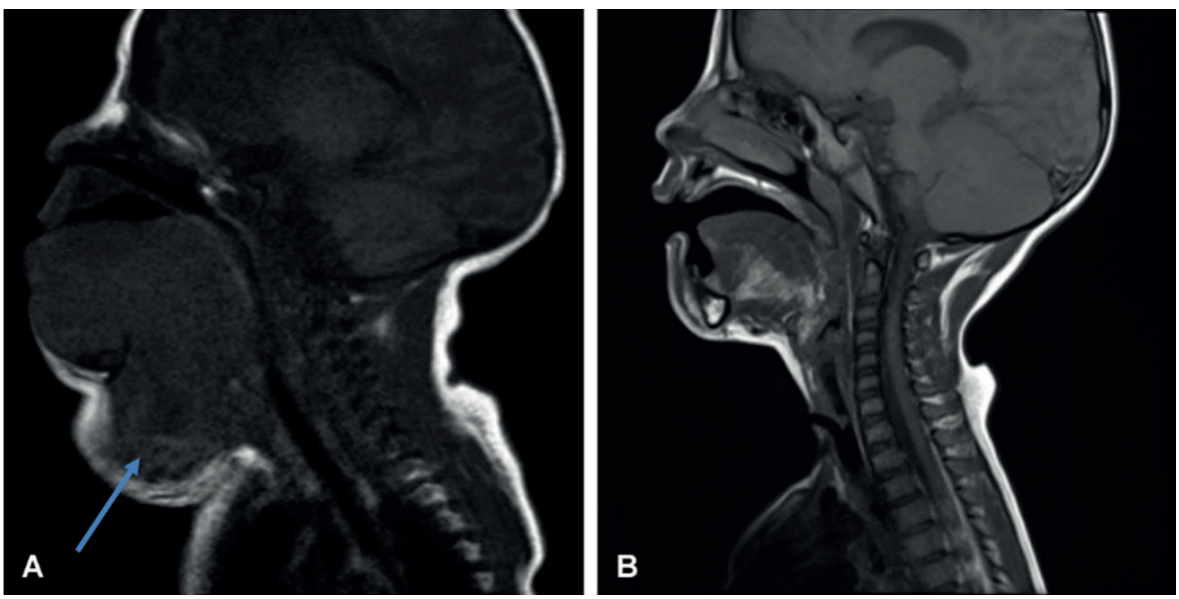

Fig. 1. Infiltration of the anterior commissure and thyroid cartilage extending to the oropharynx, hypopharynx, and parapharyngeal region of the left hypopharynx. (A) Infiltrative mass lesion of 75 × 48 × 42 mm. (B) Nearly complete regression of the tumor and patency of the tracheostomy cannula.

disease (VOD) and slow response, but treatment was discontinued soon because of toxicity.

Larotrectinib was initiated as a single agent three months after diagnosis, following the identification of the ETV6-NTRK3 fusion. The patient's clinical condition significantly improved, with the best response noted by week 8 of larotrectinib.

Larotrectinib treatment was continued for two years. After stopping the treatment, the patient was evaluated for closure of the tracheostomy and could be fed orally. The patient has since been followed up for one year and has shown a complete response (Fig. 1B and Table I).

Case 2

The second patient was a 20-day-old female admitted with a large mass in her right hand. Ultrasound identified a mass with vascular structures, and MRI revealed a 42 x 37 mm lesion surrounding the second metacarpal and infiltrating the adductor pollicis muscle group and distal carpal bones (Fig. 2A).

Diagnosis of IFS was confirmed by biopsy, revealing an ETV6-NTRK3 fusion in 100% of examined cells. The patient began treatment with vincristine and actinomycin D. Follow-up MRI after three chemotherapy cycles showed residual tumor without mass regression.

Table I. Clinical and radiological features of three cases.

Features	Case 1	Case 2	Case 3
Age at diagnosis	2 days old	20 days old	First day of birth
Sex	Male	Female	Female
Tumor localization	Floor of mouth and tongue muscle	Surrounding second metacarpi on the hand	Starting from tarsal bone level in right foot
	Oropharynx	Infiltrating adductor pollisus muscle group, intraosseous muscles	Extending distally, surrounding tarsal and metatarsal bones 360 degrees
	Deep lobe of left parotid gland to hypopharynx	Ending with distal row of carpal bones	
	Narrowing in airway at these levels		
Size	75 x 48 mm	42 x 37 mm	56 x 55 mm
Previous treatment	Vincristine	Vincristine	Vincristine
	Cyclophosphamide	Cyclophosphamide	Cyclophosphamide
	Actinomycin D	Actinomycin D	Actinomycin D
Initiation of larotrectinib	Third month of chemo	After 3 cycles of chemo	After 2 cycles of chemo
Larotrectinib dosage	100 mg/m^2 twice daily	100 mg/m^2 twice daily	100 mg/m^2 twice daily
Chemo complications	VOD	None	None
Reason for chemo discontinuation	VOD and Slow response	Inadequate response	Inadequate response
Larotrectinib related side effects	None	None	None
Outcome	CR	CR	Near CR
Duration of larotrectinib treatment	2 years	2 years	1 year
Follow-up	NED	NED	NED

Chemo: chemotherapy, VOD: veno-occlusive disease, CR: complete remission, NED: no evidence of disease.

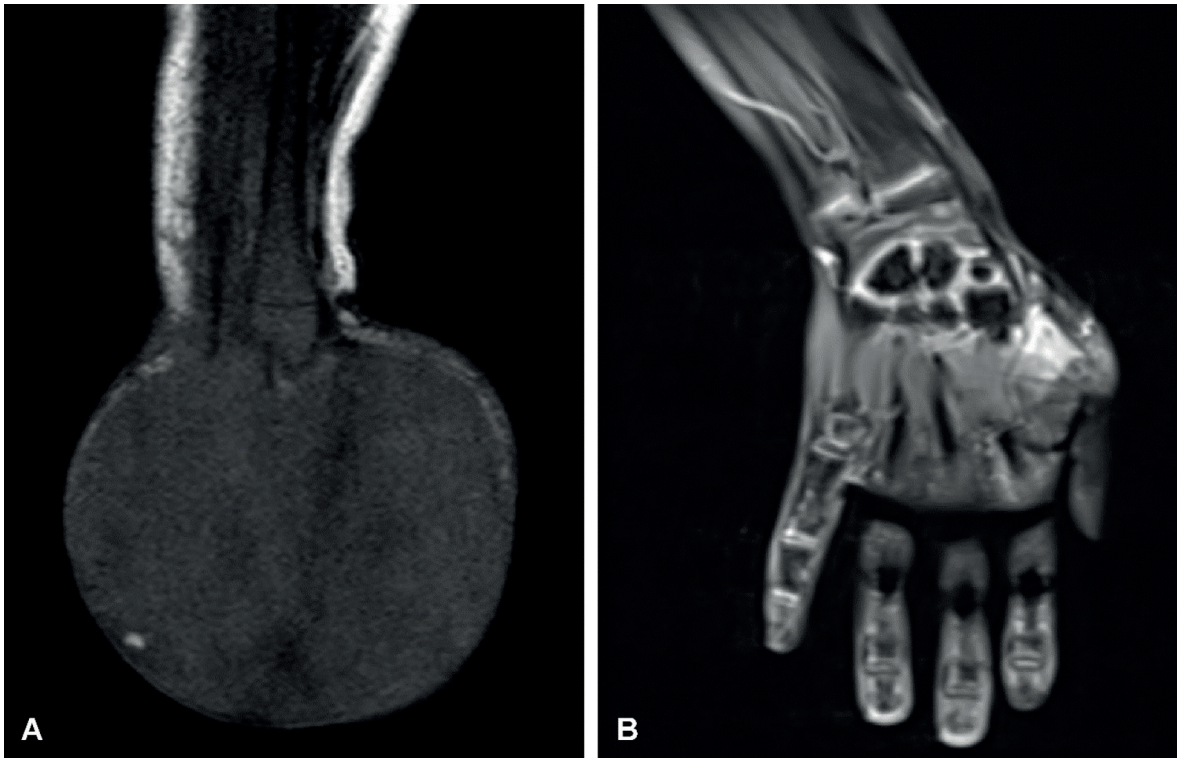

Fig. 2. (A) Giant mass detected surrounding 270 degrees of the metacarpal from the lateral, influencing the adductor pollicis muscle group within the intraosseous muscles in the right hand. (B) Nearly complete regression of the tumor was detected after treatment.

Surgical evaluation suggested potential limb amputation; however, larotrectinib was initiated, successfully preserving the limb (Fig. 2B). One month after the initiation of treatment, a noticeable improvement in response was observed. With 2 years of larotrectinib treatment, the residual lesion completely disappeared. The patient is now 3 years old, and even at 1 year following discontinuation of treatment, she can use her extremity functionally, and there are no signs of the tumor (Table I).

Case 3

On the first postnatal day, the third female patient was treated for a mass with necrotic areas on the dorsum and sole of the right foot. Computed tomography angiography indicated a heterogeneous hypervascular lesion surrounding the tarsal and metatarsal bones, consistent with a malignant mesenchymal tumor.

MRI revealed a 56x55 mm mass originating at the tarsal bone in the right foot, surrounding the tarsal and metatarsal bones by 360 degrees (Fig. 3A). Tru-cut biopsy indicated spindle cell sarcoma, and an ETV6-NTRK fusion confirmed the diagnosis of IFS.

The treatment regimen initially included vincristine, actinomycin D, and cyclophosphamide; however, the tumor did not regress and was deemed unsuitable for surgery. After two cycles, the treatment was switched to larotrectinib. A reduction in macroscopic mass dimensions was noted by the second month, with the best response achieved by month three. Larotrectinib was used for one year and spared the patient from mutilating surgery (Fig. 3B and Table I). The patient no longer required surgical intervention. The larotrectinib was discontinued when nearly total regression was detected. The patient has been followed up for

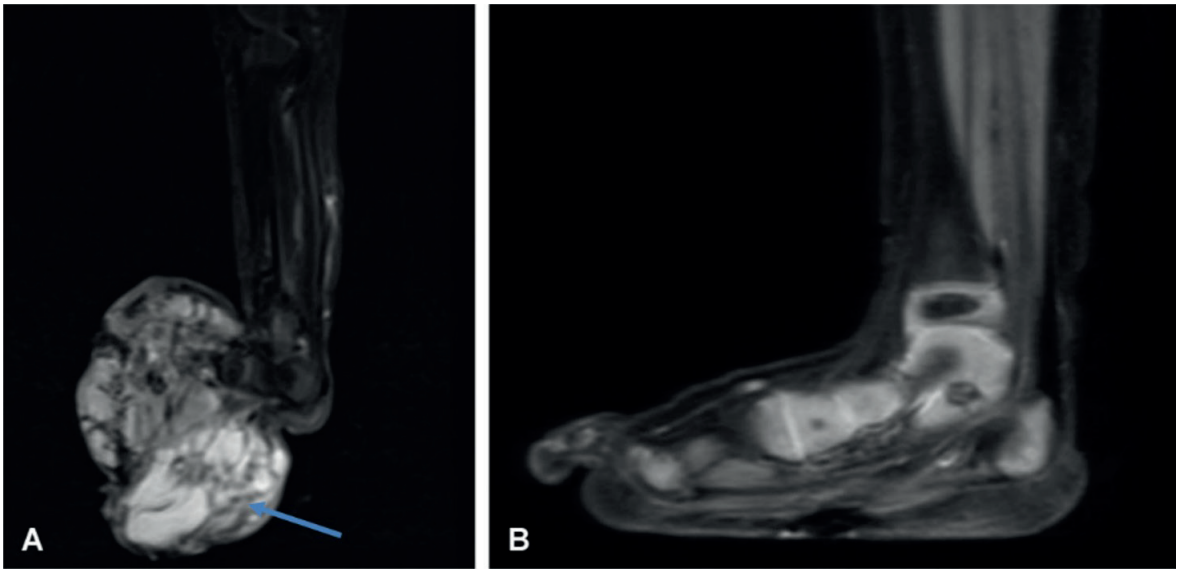

Fig. 3. (A) Heterogenous internal structure hypervascular mass starting from the tarsal bone level in the right foot and extending distally, surrounding the tarsal and metatarsal bones by 360 degrees, and extending to the subcutaneous fatty tissue on the dorsal and plantar faces. (B) Nearly complete regression of the tumor was detected after treatment.

2 years without treatment and can successfully use her extremity.

All families provided written informed consent for the publication.

Discussion

The results presented showed rapid decreases in tumor size during the first 3 months of treatment and the achievement of tumor remission with larotrectinib in three infants with IFS. Surgery was the primary approach in the 1980s for IFS and is still the mainstay of therapy, but it is now treated with a more multidisciplinary approach, and surgery is applied as the final step.[13-15] Chemotherapy can be used as the first step, and curative effects have been observed with vincristine, actinomycin D, cyclophosphamide or ifosfamide, and etoposide.[2]

In 2003, Russell et al.[3] conducted a literature review on IFS and identified 22 cases treated with chemotherapy either preoperatively or as the sole treatment. Among the chemotherapy agents mentioned in the studies, vincristine, cyclophosphamide, actinomycin D, and doxorubicin were the most commonly used. Additionally, six infants were treated with chemotherapy protocols that included ifosfamide and etoposide.[3,16]

Loh et al.[17] also treated patients with similar chemotherapy. The regimens included vincristine, adriamycin, and cyclophosphamide (adria-VAC); vincristine, actinomycin-D, and cyclophosphamide (actino-VAC); and etoposide and ifosfamide. They described 11 infants who were treated for IFS over a 16-year period at the Dana-Farber Cancer Institute and Children's Hospital, Boston. All of these regimens were tolerated well. A recent multi-institutional European study concluded that conservative surgery is the mainstay of treatment, and alkylating agent and anthracycline-free first-line chemotherapy should be used to treat unresectable tumors.[2,18]

Larotrectinib is a selective TRK inhibitor that can be given orally in cases of ETV6-NTRK3 fusion. In our cases, we used larotrectinib due to unresponsiveness or discontinuation of chemotherapy (one of our cases developed VOD), and two of them showed no response to

chemotherapy). All of our cases were positive for ETV6-NTRK3 fusion and showed complete responses to larotrectinib. Additionally, two of our patients were saved from amputation by larotrectinib. In the other case with head and neck localization, airway patency was provided with tracheostomy, and effective results were obtained with larotrectinib treatment without the need for mutilating surgery.

The EPI VITRAKVI study was a retrospective, observational investigation (NCT05236257) that utilized historical data to evaluate the effectiveness of larotrectinib in comparison to chemotherapy-based treatments.[4] Among the patients treated with larotrectinib, 49% experienced a complete response, 41% had a partial response, and 9.8% had stable disease. None of the patients discontinued treatment permanently due to drug-related side effects. Patients with IFS demonstrated significant and durable responses to larotrectinib.

The findings indicated that larotrectinib offers a therapeutic advantage over the conventional chemotherapy treatments for pediatric patients with locally advanced or metastatic IFS.[4] The study revealed that larotrectinib notably extended the time until treatment failure (defined as the need for subsequent systemic therapy, radiotherapy, surgery, or death, whichever occurred first) when compared to external historical controls who were only treated with chemotherapy. The results indicate that treatment with larotrectinib reduced morbidity and the need for aggressive local therapies compared to conventional chemotherapy in children with IFS.[4]

Consequently, this treatment method is particularly promising for cases that are treatment resistant and ineligible for surgery. Clinical trials have already demonstrated the pantumour efficacy of larotrectinib with a favorable safety profile in patients as young as 1 month of age.[19-22]

The drug was administered orally on a continuous 28-day schedule to pediatric patients, primarily at a dose of 100 mg/m² (up to 100 mg) twice daily. Treatment continued until a complete response was achieved. Common adverse events reported in the literature include elevated alanine aminotransferase, anemia, and neutropenia, with no treatment-related deaths noted. We observed no drug-related side effects or compliance issues in our patients. It is necessary to consider that TRKs are involved in neural development, and the long-term neurologic effects of larotrectinib have not been clearly shown.[23]

Locally advanced TRK fusion sarcomas have significant morbidity with surgical resection, and larotrectinib continues to be evaluated as a presurgical therapy for children who are newly diagnosed.[24,25] Larotrectinib treatment should be attempted for patients who are diagnosed with IFS and have ETV6-NTRK3 fusion, especially if the response to chemotherapy is insufficient. Nevertheless, multi-center studies are needed, and the duration of treatment should be decided according to radiological and clinical findings. Although there are case reports of limb salvage for patients with IFS in the literature, there are no robust data on the duration of treatment or the risk of recurrence after discontinuation of larotrectinib.[25]

Orbach et al.[26] developed a consensus strategy with the Children's Oncology Group (COG) after analyzing retrospective data for all reported patients with IFS from the European Paediatric Soft Tissue Sarcoma Study Group and *Cooperative Weichteil Sarkomstudien Gruppe* (CWS). The median duration of conventional chemotherapy in patients with localized disease has been reported to be 4–6 months. For patients who are initially unresectable, the optimal duration of larotrectinib administration has been defined as 4 months to 1 year, and drug discontinuation is recommended if total surgical resection is feasible.[26] It is also recommended that the duration of larotrectinib use be extended for metastatic patients or patients with localized tumors that are still not suitable for resection despite tumor regression.[26] It is not yet known how long larotrectinib therapy

should be continued in cases of complete clinical and radiological response of the tumor without surgery.[26-28]

The COG ADVL1823 trial administered larotrectinib continuously in 28-day cycles until the disease progressed, the tumor became surgically resectable following a minimum of 6 cycles of therapy, or the completion of 12 cycles (for those with complete remission) or 26 cycles (for those with partial remission) of therapy.[20,29] In all three of our cases, a significant response to larotrectinib was observed within the first three months. The treatment duration can vary between clinical trials. The results of attempts to discontinue therapy in these situations are not yet available.

In conclusion, our case series has presented the successful treatment of patients with IFS in different locations using a targeted agent without the need for mutilating surgery. We continued larotrectinib until complete clinical and radiological response of the tumor occurred without surgery. The medication also has a safe side effect profile. Thus, larotrectinib could emerge as an efficacious and safe alternative to chemotherapy for NTRK fusion-positive and metastatic or unresectable tumors.

Ethical approval

Informed consent was obtained from the parents or legal guardians of the patients for the publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: HGT, DD, DT, ZK; data collection: YY, ŞŞ; analysis and interpretation of results: ZK, AÜ; draft manuscript preparation: HGT, SK, DT. 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

Acute promyelocytic leukemia presenting as isolated femoral granulocytic sarcoma

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ABSTRACT

Background. Granulocytic sarcoma (GS), or myeloid sarcoma or chloroma, is a tumoral mass containing myeloblasts and immature granulocytes in an anatomic site other than the bone marrow. GS is very rare in children with acute promyelocytic leukemia (APL). This case report presents a rare case of GS manifesting as a solitary bone mass.

Case. A 15-year-old female presented with left knee pain. Complete blood count and biochemistry were normal. No blasts or early granulocytic elements were observed in the peripheral blood smear. Magnetic resonance imaging (MRI) revealed a 4x4-cm solid lesion extending to the physis line in the distal metaphyseal section of the left femur. A Tru-cut biopsy of the mass confirmed GS with immature promyelocytic cell infiltration containing Auer rods and immature myeloid cells. The t(15;17) mutation was highly positive in the tissue suspension. Bone marrow aspiration performed afterward showed no abnormalities, and acute myeloid leukemia and acute lymphoblastic leukemia mutation panels were negative. The patient was diagnosed as having APL presenting as GS of isolated femoral origin. Treatment with standard-risk chemotherapy, including all-trans retinoic acid (ATRA) according to the BFM 2013 protocol, was initiated. After 2 months, a repeat biopsy showed no pathologic promyelocytic infiltration and a negative t(15;17) mutation. However, the patient died of severe neutropenia, sepsis, and typhoid fever.

Conclusion. This case contributes to the literature as a rare presentation of APL as isolated femoral GS. It is the first reported case of an isolated femoral mass in this context to the best of our knowledge.

Key words: granulocytic sarcoma, myeloid sarcoma, chloroma, acute promyelocytic leukemia, neutropenia.

Granulocytic sarcoma (GS), also known as myeloid sarcoma or chloroma, is defined as the appearance of a tumoral mass containing myeloblasts and immature granulocytes in an anatomic site other than the bone marrow[1] GS can occur before, concurrently, or after a diagnosis of acute myeloblastic leukemia (AML). It may develop as a relapse in the setting of myeloproliferative diseases such as myelodysplastic syndrome, chronic myeloid leukemia, or even after achieving remission with AML treatment.[1] APL occurs in about 7% of children with AML. APL is a distinct subtype of AML.[2] In children with acute promyelocytic leukemia (APL), GS is rare and typically involves tissues such as skin, gingiva, central nervous system, lung, mediastinal lymph nodes, and testis in relapse cases.[3,4] This case report presents a rare case of GS manifesting as a solitary bone mass.

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Case Presentation

A 15-year-old female patient presented with a 3-week history of left knee pain and swelling. Her medical history revealed no specific signs. She had no fever on admission, and her vital signs were stable. A firm, slightly tender mass approximately 4x5 cm in size, located 5 cm proximal to the left knee joint was observed after physical examination. No redness or temperature increase was observed in the mass. No organomegaly or lymphadenopathy was observed.

A complete blood count revealed the following: white blood cells (WBC), 4800/mm^3; neutrophils, 2800/mm^3; hemoglobin, 14.4 g/dL; and platelets, 303,000/mm^3. A peripheral blood smear showed 60% neutrophils, 30% lymphocytes, and 10% monocytes. Erythrocytes were normochromic and normocytic, platelets were abundant and clustered, and no blasts were observed.

The chest X-ray and abdominal ultrasonography were normal. Direct radiography showed millimetric lytic lesions medial to the distal metaphysis of the left femur with a surrounding lamellar periosteal reaction (Fig. 1). The mass was evaluated to be a primary bone tumor. Magnetic resonance imaging (MRI) of the left knee revealed a 4x4-cm solid lesion extending to the physis-line in the distal metaphyseal section of the left femur with medial cortical destruction. The mass extended into the soft tissue (Fig. 2).

A Tru-cut biopsy of the femoral bone mass was performed under general anesthesia as the first diagnostic step. A pathology examination confirmed GS with immature promyelocytic cell infiltration containing Auer rods and immature myeloid cells (Fig. 3). Pathologic promyelocytes containing Auer rods were observed in the imprint smear prepared from the Tru-cut biopsy material. The t(15;17) translocation mutation was highly positive in the tissue suspension prepared from the biopsy material. Based on these findings, the patient was diagnosed as having APL, presenting as GS of isolated femoral origin.

To eliminate systemic involvement, bone marrow aspiration and biopsy were performed from the pelvic bone marrow. Standard bone marrow smears demonstrated no systemic myeloid disease; repeated biopsies and aspirations from this site revealed a regular cellular pattern without any malignancy. The

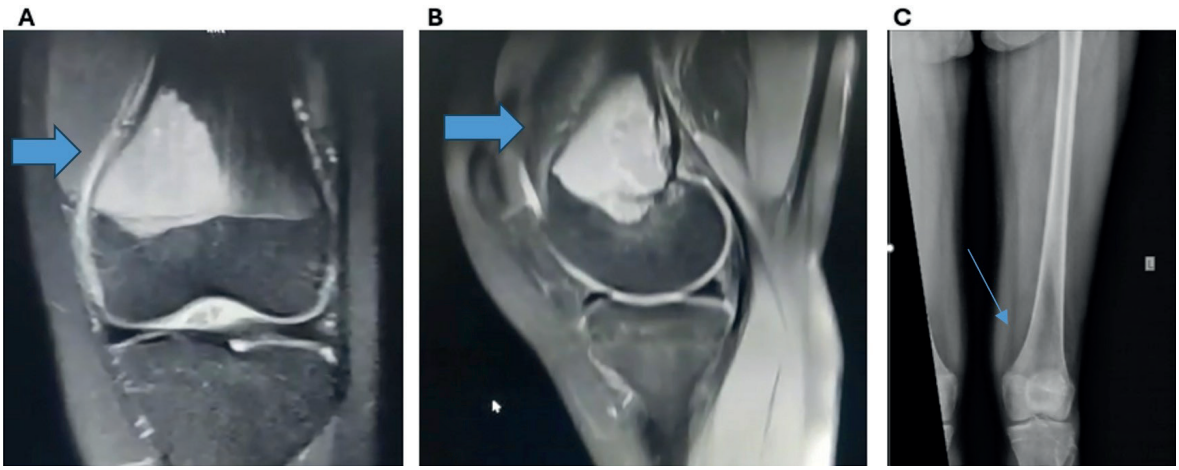

Fig. 1. Radiological appearance of the granulocytic sarcoma lesion. T2-weighted coronal **(A)** and sagittal **(B)** magnetic resonance imaging sequences show a hyperintense lesion (arrows) in the metaphyseal region, partially extending into the epiphysis, accompanied by periosteal reaction and minimal soft tissue components surrounding the lesion. Direct radiograph **(C)** obtained before chemotherapy shows millimetric lytic lesions medial to the distal metaphysis of the left femur with a surrounding lamellar periosteal reaction (arrow).

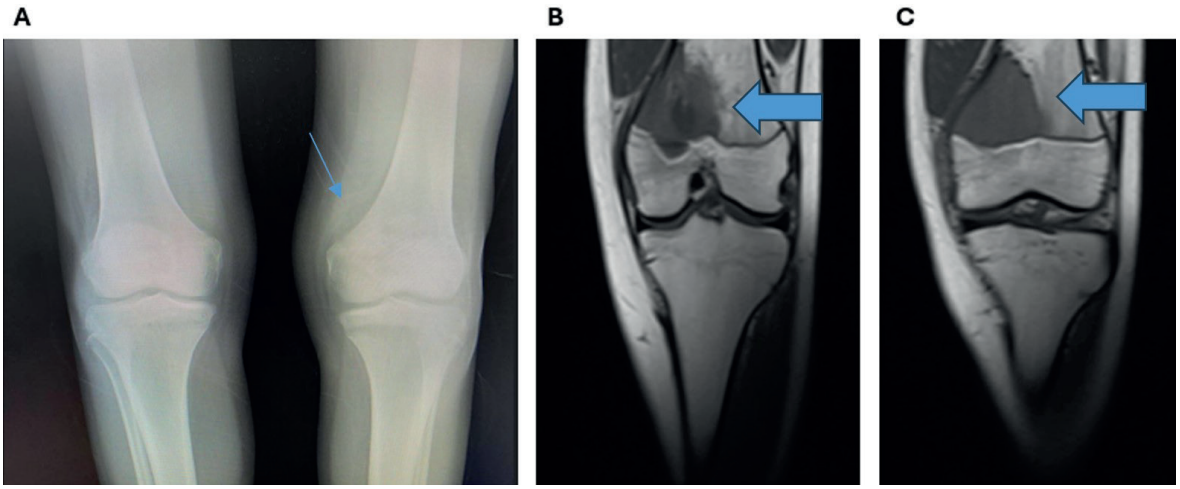

Fig. 2. Images obtained after chemotherapy. **A:** X-ray; **B** and **C:** T1-weighted coronal magnetic resonance images, showing a hypointense appearance within the lesion (arrows), corresponding to the intervention tract.

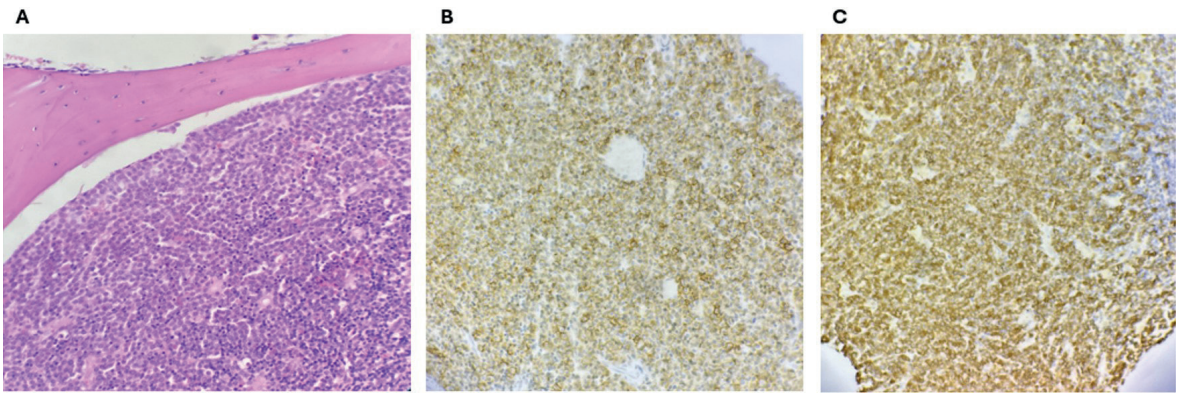

Fig. 3. Histopathological findings of the lesion. **A:** Infiltration of atypical cells located in the paratrabecular area of the bone tissue (hematoxylin eosin, original magnification x200); **B:** Positive immunohistochemical staining with CD43 (original magnification x200); **C:** Positive immunohistochemical staining with myeloperoxidase (MPO) (original magnification x200).

t(15;17) mutation was also negative in the bone marrow aspirate.

A standard chemotherapy protocol, including all-trans-retinoic acid (ATRA) treatments according to the AML Berlin-Frankfurt-Munich (BFM) 2013 guideline, was initiated. An ATRA regimen was administered 3 days prior to intravenous (IV) chemotherapy because the WBC count was lower than 10,000/mm^3. The protocol consisted of single induction therapy with anthracycline (daunorubicin or idarubicin) plus cytarabine, followed by three courses of consolidation therapy. MRI showed no significant change in the mass size 2 months after chemotherapy. Therefore, another Tru-cut biopsy was performed. The pathology examination revealed no pathologic promyelocytic infiltration and a negative t(15;17) mutation. Based on the biopsy results, the treatment was thought to be beneficial and continuation of chemotherapy along with surgical resection of the mass was planned.

The patient received AIE (cytarabine, idarubicin, etoposide) and AI (cytarabine, idarubicin) chemotherapy blocks as per the protocol. Following these blocks, the patient experienced deep and prolonged cytopenias, neutropenic fever, and feeding problems. Unfortunately,

the patient died of severe neutropenia, sepsis, and septic shock after the second block of chemotherapy. Informed consent was obtained from the patient's family for the publication of this case report.

Discussion

The prognosis for pediatric AML has improved, and the long-term survival rate has now approached 70%. Approximately 95% of children with APL achieve a complete response (CR), and the event-free survival (EFS) and overall survival (OS) rates are 80-90% and approximately 90%, respectively. Patients often present with fatal disseminated intravascular coagulation (DIC) in induction chemotherapy. Chloromal involvement is seen in 2-5% of patients with childhood AML, typically in AML M2, M4, and M5 subtypes, regardless of sex.[1,5] Therapy restricted to local procedures, even those that appear to be cured by resection or irradiation, increases the risk of systemic disease. When left untreated, most cases of primary GS progress to overt leukemia.[6]

Worch et al.[4] reported a similar case. Their patient was a 16-year-old boy with lesions on the femur, tibia, and humerus. However, reverse transcription polymerase chain reaction (RT-PCR) demonstrated the presence of t(15;17) PML/RARα fusion mRNA from the pelvis in both peripheral blood and bone marrow. We were unsuccessful in investigating t(15;17) using fluorescence *in situ* hybridization (FISH) only in pelvic bone marrow. Accordingly, we recommend using RT-PCR to investigate t(15;17) in bone marrow. Shimizu et al.[7] retrospectively analyzed 434 consecutive patients with AML. Forty-five (10.4%) patients with GS at diagnosis were younger and were more likely to conform to the French–American–British M4 and M5 classifications than those without GS. The site of GS was bone in four cases (8.9%), and one was APL. Complete remission rates did not differ significantly between the GS and non-GS groups. The GS group had a significantly higher relapse rate than the non-GS group and a significantly lower 5-year disease-free survival (DFS) rate. However, acute promyelocytic leukemia was excluded from the survival analysis. Thirty-nine (15%) patients achieved CR after the second induction therapy. Although Shimizu et al.'s[7] study was conducted on adult patients, it may also provide a guide for child patients because of the large patient sample. Patients did not achieve CR after the first induction therapy but did so after the second, like our patient.

Harrer et al.[8] reported a 67-year-old male patient with APL presenting with a tumor in the right piriform sinus, accompanied by a mini-review of 16 similar cases of extramedullary APL manifestations. Among these, three cases involved bone lesions, specifically located in the vertebrae, sternum, and shoulder. These cases provide additional context for understanding the variability of extramedullary APL presentations. In the patient with a sternal lesion, blood counts were normal, coagulopathy was absent, and bone marrow was not affected. In contrast, the patient with a vertebral lesion exhibited leukopenia, absent coagulopathy, and t(15;17) was identified in the bone marrow. The patient with a shoulder lesion had anemia; although coagulopathy was not mentioned, t(15;17) was also detected in the bone marrow. In contrast to these cases, our patient presented with a femoral mass, normal blood count, no evidence of coagulopathy, and no detectable t(15;17) in the bone marrow. This combination of findings is exceptionally rare and adds to the diversity of extramedullary APL presentations. The absence of coagulopathy and normal hematologic parameters in our patient highlights the diagnostic challenges in identifying APL in such atypical scenarios.[8]

The early use of ATRA can improve prognosis, regardless of whether sarcoma is the first or a recurrent manifestation in APL. A misdiagnosis of lymphoma may occur, and diagnostic distinction can be difficult in the isolated presentation of myeloid sarcoma without any signs of leukemia.[4,9] Tissue examination plays a very important role in the diagnosis because some patients have no bone marrow

involvement at the time of onset. When fresh tissue samples cannot be obtained, cytogenetic abnormalities of fixed and paraffin embedded sections can be detected using FISH.[10] When the PML/RARα fusion gene is found, it is recommended to use ATRA treatment and monitor the condition through peripheral blood and bone marrow.

The optimum therapy is also unclear. Wang et al.[11] published a review of cases, reporting that 20 APL patients with myeloid sarcoma received ATRA with chemotherapy; 16 achieved CR, two achieved partial remission (PR), and the other two died of sepsis and cerebral hemorrhage. In the same review, eight patients were treated with chemotherapy only without ATRA or ATO; two achieved CR, one achieved PR, and the remaining five died. The longest follow-up was 96 months. In this case, the patient was treated with radiotherapy + ATRA + chemotherapy, although radiotherapy or tumor resection together with ATRA and chemotherapy may improve the prognosis of myeloid sarcoma (MS)/APL. MS/APL has diverse clinical manifestations, molecular biology, and cytogenetics, is easily confused with stromal tumor, lymphoma, and carcinoma, and is therefore associated with a high misdiagnosis rate, poor prognosis, and high recurrence rate.[11]

There is limited information about APL with GS in the literature; therefore, our case presentation makes a valuable contribution to existing knowledge. However, given the isolated nature of the bone mass, it was thought that this treatment regimen alone would be insufficient. Therefore, ATRA treatment was combined with standard-risk chemotherapy per the AML BFM 2013 protocol. Evaluating remission solely based on morphologic and cytogenetic features was because the bone marrow was not leukemic. In our case, the MRI performed after AIE and AI blocks showed no significant change in the mass size. Conversely, the biopsy revealed extensive necrosis within the mass and no tumoral tissue. Therefore, relying solely on mass size reduction to assess treatment response in patients with GS, similar to soft tissue sarcomas, may be misleading. The plan was to continue chemotherapy without additional radiotherapy and consider a bone graft if needed. Unfortunately, the patient died of neutropenic sepsis and septic shock. Although similar outcomes are observed with classic APL treatment in patients with GS, OS and DFS are significantly lower in these cases. This suggests the need for alternative treatment strategies specifically tailored for patients with GS.[1,7]

Conclusion

This case report underscores the rare presentation of APL as an isolated femoral GS without classic hematologic abnormalities, coagulopathy, or detectable t(15;17) in the bone marrow. This atypical case adds to the spectrum of APL manifestations in pediatric patients and highlights the diagnostic challenges associated with such presentations. Further research is essential to optimize treatment strategies and improve outcomes for rare extramedullary APL cases.

Ethical approval

Written informed consent was obtained from the patient's family.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ÜÇ, HT, AŞ; analysis and interpretation of results: ÜÇ, HT, AŞ; draft manuscript preparation: HT, AŞ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A case of neonatal gastric teratoma complicated with occult gastrointestinal hemorrhage misdiagnosed as lymphangioma

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ABSTRACT

Background. Gastric teratoma is a rare neoplasm, particularly in neonates, and usually presents as a palpable abdominal mass. However, severe occult gastrointestinal bleeding is uncommon and often misdiagnosed. Imaging studies are crucial for the preoperative diagnosis of neonatal teratoma, but definitive diagnosis relies on pathological examination.

Case presentation. A 28-day-old boy presented with abdominal distension accompanied by vomiting for 2 days without hematemesis or melena. A complete blood count upon admission showed a hemoglobin level of 37 g/L. Ultrasound and computed tomography scans indicated a large cystic, solid mass in the abdominal cavity (approximately 9.8 × 8.8 × 11.2 cm), containing nodules, septa, calcification, and fat, and causing gastrointestinal compression. The mass was misdiagnosed as lymphangioma with hemorrhage before surgery. During surgery, the upper pole of the tumor was found to be fused with the gastric wall of the greater curvature of the fundus of the stomach, with most of the tumor growing exophytically and a small portion growing into the gastric lumen. The tumor, along with part of the gastric wall at the attachment site, was completely removed. Postoperative pathological examination revealed an immature teratoma grade 1. After discharge, the patient's growth and development were normal, with no adverse manifestations.

Conclusions. Neonatal gastric teratoma with severe occult gastrointestinal bleeding is extremely rare and hence must be on the list of differential diagnoses of neonatal abdominal mass when a cystic solid mass is found, especially when accompanied by severe anemia without obvious gastrointestinal bleeding. Attention should be paid to the location of the lesion, which is predominantly in the left upper abdomen and has been significantly pushed and displaced by the gastrointestinal tract, and to the imaging characteristics of teratoma such as fat and calcification, which help to exclude other palpable masses encountered during the neonatal period.

Key words: gastric teratoma, neonatal, immature teratoma, occult gastrointestinal hemorrhage.

Gastric teratomas are extremely rare, accounting for less than 1% of all teratomas. The first case of gastric teratoma was reported by Eustermann and Sentry in 1922. These rare tumors primarily occur in boys younger than 3 months of age.[1] Most gastric teratomas are benign and asymptomatic, often manifesting as an abdominal mass, abdominal distention, vomiting, hematemesis, or melena. Severe occult gastrointestinal bleeding is rare and easily misdiagnosed. Radiological examinations are crucial for the preoperative diagnosis of neonatal teratoma. However, the definitive diagnosis relies on histopathological examination revealing embryonal neuroepithelial tissue accompanying three-germ layer structures. This report details

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a case of neonatal immature gastric teratoma with severe occult gastrointestinal hemorrhage, initially misdiagnosed as a lymphangioma with hemorrhage. The clinical and imaging characteristics are summarized, causes of misdiagnosis are analyzed, and literature is reviewed to enhance the understanding of this condition among clinicians and radiologists and mitigate future misdiagnosis and mismanagement.

Case Presentation

The patient, a 28-day-old male, was admitted to the hospital due to abdominal distension accompanied by vomiting for 2 days. One day before admission he experienced abdominal distension and vomiting without obvious inducement. The vomiting consisted of stomach contents, and his stool was smooth, yellow, and soft, without blood. On physical examination upon admission, he was alert but exhibited poor mental response, pale face, abdominal distention, a palpable mass in the left upper abdomen with an unclear boundary, and high mobility.

The laboratory examination on the day of admission revealed a white blood cell (WBC) count of 21.3 × 10^9/L, hemoglobin (Hb) of 37 g/L, procalcitonin (PCT) of 0.77 ng/mL, and C-reactive protein (CRP) of 89.48 mg/L. Color ultrasound showed a large cystic mixed mass in the abdominal cavity (Fig. 1a), measuring 9.8 × 8.8 × 11.2 cm. Multiple bands with uneven thickness, separated echoes, and dotted blood flow signals could be detected along the compartments, and nodular protrusions could be seen in some compartments, causing compressive displacement of the gastrointestinal tract in the abdominal cavity. A computed tomography (CT) scan revealed a large mixed-dense mass in the abdominal cavity, containing calcification, fat, and some solid components (Fig. 1b). Some branches of the superior mesenteric artery were deformed, and the gastrointestinal tract was displaced. The clinical diagnosis was lymphangioma complicated by hemorrhage.

Due to severe anemia, four routine blood tests were performed to evaluate anemia indicators, and 0.25 units of red blood cells were transfused to correct anemia after each test. The huge abdominal mass showed significant extrusion on the surrounding gastrointestinal tract with no suitable needle biopsy path. Concurrently, the persistence of anemia symptoms signified ongoing bleeding within the patient. Therefore, the patient underwent laparoscopic exploratory surgery on day 14 following admission after anemia correction, anti-infection and other symptomatic treatments.

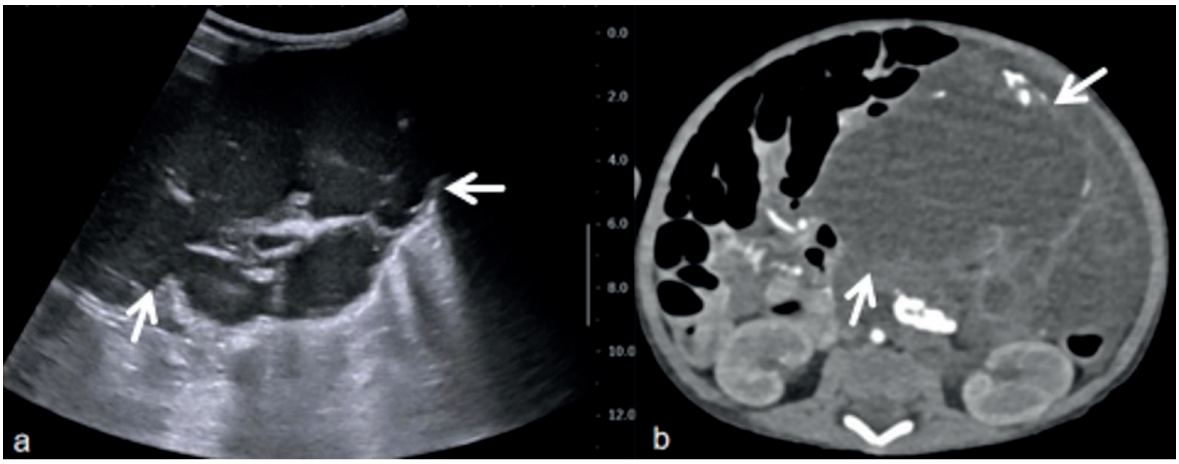

Fig. 1. Ultrasonography **(a)** and computed tomography **(b)** revealed a large cystic, solid mass (white arrows) in the abdominal cavity.

During the operation, the upper pole of the tumor was found to fuse to the gastric wall of the greater curve of the fundus of the stomach (Fig. 2). The gastric wall was cut 1 cm away from the tumor, and most of the tumor was found to be exogenous, with a small part extending into the gastric lumen, measuring approximately 4 × 4 × 3 cm. Dark brown blood clots were observed on the tumor surface and in the gastric lumen. The tumor, along with part of the gastric wall at the attachment site, was completely removed.

Postoperative pathological biopsy revealed predominantly mature tissues, with some areas containing immature mesenchymal components, leading to a diagnosis of grade 1 immature teratoma (Fig. 3). No tumor tissue was observed on the resection margins. Immunohistochemical staining showed tumor cells positive for CD56 (partial +), S-100 (partial +), and negative for OCT4 and SALL4, with a Ki-67 index of 20%. Postoperatively, the patient received treatments, including fasting water, gastrointestinal decompression, nutritional support, and other treatments. He gradually resumed oral water intake, recovered well, and was discharged. Three months later, follow-up indicated normal growth and development with no adverse manifestations.

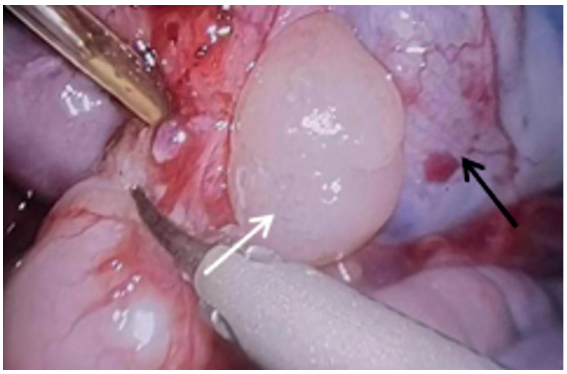

Fig. 2. During surgery, the upper pole of the tumor was found to be fused with the gastric wall of the greater curvature of the fundus of the stomach, with most of the tumor growing exophytically (black arrow) and a small portion growing into the gastric lumen (white arrow).

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

Discussion

Teratoma is a germ cell tumor formed by the abnormal development of at least two tissues in the outer, middle, and inner layers, occurring both in and outside the gonads. In neonates, teratomas are mostly located outside the gonads, with the sacral tail being the most common site.[1] Gastric teratomas are rare in clinical settings, first reported by Eusteman and Sentry in 1922. They mainly occur in boys within 3 months of age and account for <1% of pediatric teratomas.[2,3] Over 90% of gastric teratomas occur in the greater curvature of the stomach, with exophytic growth being more common (approximately 60%)[1,4] compared to endophytic growth (approximately 30%). Combined exophytic and endophytic growth patterns are rare, and such cases are rarely reported. In this case, the neonatal gastric teratoma occurred in the gastric wall of the

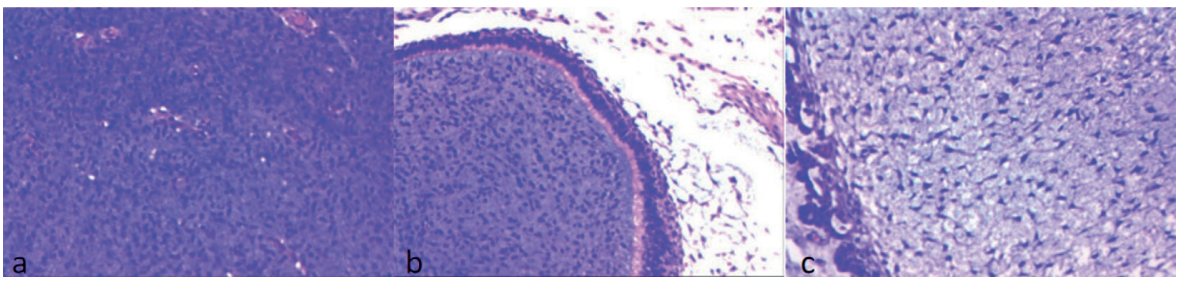

Fig. 3. Postoperative pathological biopsy revealed predominantly mature tissues, including mature respiratory epithelial tissue (b and c), with some areas containing immature mesenchymal components, mainly primitive mesenchymal immature cartilage (a-c, H&E staining).

greater curvature of the fundus of the stomach. Most teratomas are exogenous, and a few are located in the stomach. Furthermore, most pediatric teratomas are benign and mature, but this patient's postoperative pathology revealed a grade 1 immature teratoma, which is relatively rare and malignant. The etiology of this condition involves many factors, and its pathogenesis remains unclear.[5]

The clinical manifestations of gastric teratomas are related to the mass's size, location, ulceration, and bleeding. Most of them are asymptomatic in the early stages, often presenting later as abdominal mass, abdominal distension, vomiting, hematemesis, and melena. In this case, the gastric teratoma primarily manifested as an abdominal distention accompanied by vomiting, with the vomitus consisting of gastric contents without hematemesis or melena. Upon admission, routine blood examination revealed severe anemia (Hb 37 g/L). During surgery, dark brown blood clots were found on the mass surface and in the gastric lumen, indicating severe occult gastrointestinal bleeding. The probable causes of this bleeding include gastric acid erosion leading to vascular rupture within the tumor, rapid tumor growth causing necrosis and hemorrhage due to insufficient blood supply, or tumor invasion into the gastric mucosa resulting in vascular rupture and hemorrhage. Additionally, the high activity of the abdominal tumor, causing repeated displacement and traction of the gastric wall, may have exacerbated the bleeding, leading to severe anemia in the child. Because gastric bleeding in neonates is relatively hidden, early symptoms are not obvious, resulting in severe hemorrhagic anemia. Emergency surgery should be performed as soon as possible to prevent serious complications.

Immature teratomas contain various amounts of fat, hair, skin, brain tissue, bone, cartilage, and other mature tissues, as well as immature neural and embryonic tissues, such as primitive neural tubes, whose complexity determines the diversity of imaging manifestations.[6] Preoperative diagnosis is often based on intratumoral calcification and a mixed cystic-solid mass. Ultrasonography and CT scans can reveal not only heterogeneous masses with varying amounts of cystic and solid components but also fat and calcification, the latter suggesting the diagnosis of teratoma. Differential diagnosis should include other cystic-solid abdominal masses in the child, such as lymphangioma.

The ultrasonographic manifestations of cystic-solid lymphangioma were as follows[7]: (1) the boundary of the lesion was unclear, and most cases lacked a capsule; (2) irregular shape; (3) uneven internal echo, no echo area of different sizes can be seen, surrounding high or strong echo, the focus is tortuous and expanded tubular structure or honeycomb; (4) color Doppler imaging showed punctate blood flow signal within the lesion. CT findings also have certain characteristics, and the diagnosis of lymphangioma is strongly suggested when the following signs appear[8]: (1) The shape of the lesion is irregular or bag-like structure, and there are many compartments in the cyst; (2) The volume is huge, but the occupying effect is not apparent; (3) The lesion exhibited "crawling growth" and presents a plastic change with the surrounding tissues. A "vessel crossing sign" can be seen inside the cyst, Indicating poor blood supply. The "creeping growth" of the lesion and the large size with a slight disproportion of the mass effect are the most valuable for diagnosing the disease. The formation of fat signs in lymphangioma may be caused by the growth of surrounding adipose tissue or the accumulation of specific lipid components in lymphatic fluid.

In this report, both CT and ultrasonography indicated a large cystic, solid intraperitoneal mass with a septum, calcification, and a small amount of fat. However, the tumor source could not be determined due to the large mass and insufficient fat in the neonatal abdomen. The child also had severe anemia without hematemesis or melena, which did not conform

to the typical characteristics of gastrointestinal bleeding in a neonatal teratoma. This led to a preoperative misdiagnosis of lymphangioma with hemorrhage by clinicians.

Upon reviewing the imaging data, our case showed a regular mass with no "creeping growth" and significant gastrointestinal extrusion, inconsistent with a lymphangioma. At the same time, we found that this cystic-solid mass contained fat and calcification, which was rare in lymphangioma but characteristic in teratoma. Additionally, the tumor was mainly located in the left upper abdomen, suggesting a possible gastric wall origin. Surgery and pathology confirmed that the mass was an immature teratoma originating from the gastric wall.

Currently, the primary treatment for gastric teratoma is complete surgical resection as soon as possible, even if histological examination reveals immature or malignant components and metastasis.[9] In this case, the tumor was completely removed via laparoscopy, and the gastric wall was carefully sutured with an enlarged resection margin. Neonatal teratoma generally has a good prognosis, and postoperative transformation is typically not necessary.

However, immature teratomas contain immature components derived from the germ cell layers most commonly neuroectodermal tissue. A few postoperative cases may experience local recurrence, malignant transformation, and even distant metastasis.[10,11] The degree of immaturity is correlated with the ultimate prognosis of children. Grade 1 is immature with <10% microscopic foci containing immature elements, grade 2 is immature with 10%–50% of immature elements, and grade 3 is immature with 50% of immature elements. Grade 1 and 2 teratomas may become malignant (grade 3), and malignant teratomas have the potential to metastasize.[12] Our case belonged to the grade 1 immature teratoma group. Therefore, the long-term postoperative follow-up is crucial.

In most cases of gastric teratoma, the tumor marker alpha-fetoprotein (AFP) is elevated, which can decrease to normal after surgery. Currently, serum AFP is a monitoring indicator for postoperative tumor remnant and recurrence. Elevated serum AFP levels may also be the only alerting signal for the presence of malignant yolk sac components. However, the diagnostic utility of AFP is low in young infants because of the physiologically elevated levels.[12] Therefore, AFP monitoring in this case was not conducted at the first review before surgery and three months after surgery.

In conclusion, neonatal gastric teratoma with severe occult gastrointestinal bleeding is extremely rare and hence, must be on the list of differential diagnoses of neonatal abdominal mass when a cystic solid mass is found, especially when accompanied by severe anemia without obvious gastrointestinal bleeding. Attention should be paid to the location of the lesion, which is predominantly in the left upper abdomen and has been significantly pushed and displaced by the gastrointestinal tract, and to the imaging characteristics of teratoma such as fat and calcification, which help to exclude other palpable masses encountered during the neonatal period.

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Ethical approval

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

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BL, KW; data collection: BL, KW; analysis and interpretation of results: BL, KW; draft manuscript preparation: BL, KW. 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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Adolescent medicine in the first 100 years of the Republic of Türkiye

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Dear Editor,

We read the article titled "Child Health in the First 100 Years of the Republic of Türkiye: A Story of Hope, Labor, and Success" by Seren with great interest.[1] We congratulate the author on their thorough exploration of the advancements in child health during the first century of the Republic. This era has witnessed remarkable progress in healthcare systems, policies, and the overall well-being of children. However, we observed that while the article provides a detailed overview of child health, it does not extensively address the developments in adolescent medicine, a pivotal yet distinct phase of child health. To offer a more holistic perspective, we would like to emphasize the key milestones and achievements in adolescent medicine in Türkiye.

While adolescence is defined within the field of pediatrics, it has long been distinguished from childhood and is defined by the World Health Organization as the period between the ages of 10 and 19.[2] Adolescent medicine focuses on addressing the unique physical, psychological, and social health needs of this developmental stage, including growth and development, mental health, chronic illness management, sexual and reproductive health, and the prevention and treatment of risky behaviors such as substance use and unsafe sexual practices. This field bridges the gap between pediatric and adult medicine, providing holistic and developmentally appropriate care tailored to adolescents. Adolescent medicine specialists aim to promote the optimal health and well-being of adolescents and young adults, provide necessary services during the transition from adolescence to adulthood, and increase public awareness of adolescent medicine.

Establishment of Adolescent Medicine Divisions in Türkiye

The first Division of Adolescent Medicine was established in the United States in 1951 by Dr. J. Roswell Gallagher at Boston Children's Hospital, Harvard University.[3] Inspired by this pioneering initiative, Prof. Dr. İhsan Doğramacı sent Prof. Dr. Mithat Çoruh to train at the same clinic. Upon his return, Dr. Çoruh founded Türkiye's first Division of Adolescent Medicine within the Department of Pediatrics at Hacettepe University Faculty of Medicine in 1963.[4] Subsequently, the second division was founded in 1988 at the Institute of Child Health, İstanbul University, followed by the third in 1996 at İstanbul University Cerrahpaşa Faculty of Medicine.[5]

In 2009, the Council of Higher Education *(Yükseköğretim Kurulu)* initiated efforts to expand adolescent medicine divisions across Türkiye, beginning with major healthcare institutions in Ankara and İstanbul. Since then,

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these divisions have proliferated, establishing a greater presence, and clinics offering adolescent medicine services are now accessible in several regions.

Doctoral Education in Adolescent Medicine

Despite its critical importance, adolescent medicine is not yet recognized by the Ministry of Health as an official subspecialty in Türkiye. Historically, pediatricians interested in adolescent health faced limited access to specialized training. To address this gap and accommodate the unique healthcare needs of adolescents, Hacettepe University's Institute of Child Health launched Türkiye's first doctoral (PhD) program in Adolescent Medicine in 2004. This program has trained professionals who have since established adolescent medicine clinics nationwide. Subsequently, doctoral programs were established at Istanbul University Faculty of Medicine (2014), Ankara University (2020), and Dicle University (2022). Within the scope of this program, 14 pediatricians have graduated from doctoral education at Hacettepe University Faculty of Medicine to date, and the programs continue to advance the field by cultivating expertise and fostering research.

Adolescent Medicine Associations in Türkiye

Türkiye is home to two active adolescent medicine associations: the first Adolescent Health Association (*Adolesan Sağlığı Derneği*-ASD), established in Istanbul in 2004, and subsequently the Adolescent Health Association (*Ergen Sağlığı Derneği*-ESD), established in Ankara in 2007. ESD organized the inaugural National Adolescent Medicine Congress in 2006, which has since been held biennially. ASD began organizing meetings in 2022, further enriching the professional discourse in the field.

Initiatives by the Ministry of Health

Since 2000, the Ministry of Health has launched initiatives to prioritize adolescent health. Forty-one Youth Centers were established within Mother and Child Health and Family Planning (*Ana Çocuk Sağlığı ve Planlaması*-AÇSAP) Centers, with specialized training provided to multidisciplinary teams. These centers remained inactive after being affiliated with Community Health Centers.

Key milestones include the Adolescent Health Development Workshop in 2013 and the integration of adolescent-specific assessments into the Infant, Child, and Adolescent Monitoring Protocols in 2015, which were updated in 2018.[6] In addition, a "Pocket Book for Approaching Adolescent Medicine Problems in Primary Health Care" was disseminated in 2017 to support healthcare providers and is undergoing revision.[7]

The establishment of Child and Adolescent Substance Addiction Research, Treatment, and Education Centers (*Çocuk Ergen Madde Bağımlılığı Araştırma, Tedavi ve Eğitim Merkezi*-ÇEMATEM) further underscores the Ministry's commitment to adolescent health. First launched in 1995 as Volatile Substances Research, Treatment and Education Centers (*Uçucu Maddeler Araştırma, Tedavi ve Eğitim Merkezi*-UMATEM), these centers now provide specialized care for adolescents across Türkiye.

In 2021, Türkiye opened its first smoking cessation clinic exclusively for individuals under 18 years of age at Ankara Bilkent City Hospital. This initiative expanded to include Hacettepe University and Istanbul Training and Research Hospital in 2023, followed by Ankara University, Başkent University, and other centers in 2024.

Adolescent Health Data

Adolescents (ages 10-19) currently constitute 13 million of Türkiye's population, while 6.8 million youth fall within the 19-24 age group. While the proportion of youth in the overall population has remained stable, their absolute numbers have increased.[8] Mortality trends show a slight decrease in youth mortality rates

from the 1980s to 2022, although a significant rise was observed in 2023 (Table I).[9-11] External causes of injuries and poisonings, which are the most common cause of death in the 15-24 age group, increased from 2692 (44%) to 9220 (70.7%) from 2022 to 2023. According to the data, the increase in the number and rate of deaths among 15-24 age group in the last year was stated to be caused by the Kahramanmaraş earthquake in 2023.[11]

According to UNICEF's State of the World's Children 2024, Türkiye's adolescent mortality rate was 3% in 2022. According to the Global Health Estimates for 2019 of the World Health Organization the top mortality cause of adolescents aged 15-19 years is interpersonal

Table I. Youth population and death statistics of Republic of Türkiye[8-10]

Year	Total population	Youth population (15-24 age)	Proportion of youth population in total population (%)	Total death	Youth death (15-24 age)	Proportion of youth deaths in total deaths (%)
1935	16 158 018	2 433 916	15,1			
1940	17 820 950	2 568 914	14,4			
1945	18 790 174	3 461 047	18,4			
1950	20 947 188	4 350 499	20,8			
1955	24 064 763	4 650 353	19,3			
1960	27 754 820	4 607 042	16,6	96 403	2 961	3,1
1965	31 391 421	5 254 191	16,7	95 427	2 878	3,0
1970	35 605 176	6 545 971	18,4	104 556	3 184	3,0
1975	40 347 719	7 796 643	19,3	120 302	3 654	3,0
1980	44 736 957	9 016 986	20,2	130 062	3 860	3,0
1985	50 664 458	10 191 944	20,1	141 324	3 441	2,4
1990	56 473 035	11 311 973	20,0	150 292	3 455	2,3
1995				169 856	4 620	2,7
2000	64 729 501	12 575 362	19,4	174 315	4 072	2,3
2007	70 586 256	12 397 606	17,6	212 731	3 228	1,5
2008	71 517 100	12 441 662	17,4	215 562	3 109	1,4
2009	72 561 312	12 514 737	17,2	369 440	7 052	1,9
2010	73 722 988	12 545 094	17,0	366 187	6 657	1,8
2011	74 724 269	12 542 174	16,8	375 923	6 813	1,8
2012	75 627 384	12 591 641	16,6	376 000	6 608	1,8
2013	76 667 864	12 691 746	16,6	372 094	6 069	1,6
2014	77 695 904	12 782 381	16,5	383 639	5 446	1,4
2015	78 741 053	12 899 667	16,4	397 037	5 076	1,3
2016	79 814 871	12 989 042	16,3	420 189	5 976	1,4
2017	80 810 525	12 983 097	16,1	423 878	5 839	1,4
2018	82 003 882	12 971 396	15,8	426 785	5 905	1,4
2019	83 154 997	12 955 672	15,6	436 624	5 486	1,3
2020	83 614 362	12 893 750	15,4	509 147	5 547	1,1
2021	84 680 273	12 971 289	15,3	566 624	5 877	1,0
2022	85 279 553	12 949 817	15,2	505 269	6 092	1,2
2023	85 372 377	12 872 039	15,1	525 814	13 038	2,5

violence, followed by road injuries and self-harm in both sexes. Additionally, tobacco use among adolescents aged 13-15 years was reported at 17.9%, while alcohol use among adolescents aged 15-19 years was 6% for males and 2% for females in Türkiye. The adolescent birth rate was 1.2% for ages 15-19 years in 2022.[12]

The Future of Adolescent Medicine in Türkiye

While Türkiye has made significant progress in adolescent health, there remains a need for further advancements, particularly in recognizing adolescent medicine as an official subspecialty. This recognition will enable the training of more dedicated physicians and enhance pediatricians' ability to address the specific health concerns of adolescents. The continued efforts of adolescent medicine specialists are essential, as the health and well-being of today's youth lay the foundation for a healthier future society.

We hope this contribution highlights the importance of adolescent medicine in Türkiye's healthcare journey and inspires further discussions in this field.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SA; draft manuscript preparation: EŞK, MPK, SA; supervision: MPK, SA. 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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Corrigendum

Author Correction to: "Disseminated cryptococcosis in a child with liver transplantation: a case report." [Turk J Pediatr 2024; 66: 499-504.]

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Following publication of this article, the editors of the Turkish Journal of Pediatrics have been notified that Gözde Kayalı Akkuş has been mistakenly included as an author instead of Ayça Aydın Uysal.

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The authors apologize for any inconvenience this error may have caused, and state that this does not change the scientific conclusions of the article in any way. The original publication has been corrected.

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CASE REPORTS

LETTER TO THE EDITOR

CORRIGENDUM

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