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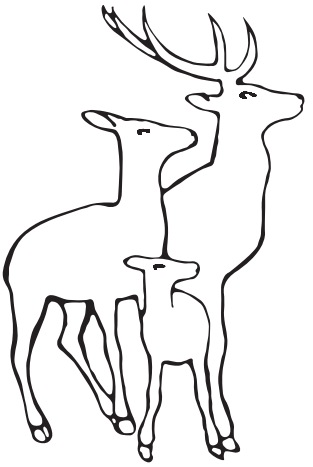

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Review

Management of pediatric hemolytic uremic syndrome

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ABSTRACT

Classical clinical triad of hemolytic uremic syndrome (HUS) is microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury associated with endothelial cell injury. Several situations, including infections, medications, malignancies, and transplantation can trigger endothelial damage. On the HUS spectrum, atypical hemolytic uremic syndrome (aHUS) deserves special attention in pediatric patients, as it can cause end-stage kidney disease and mortality. A dysfunction in the alternative complement pathway, either acquired or genetic, has been shown to be the main underlying cause. In the last decades, breathtaking advances have been made in understanding the pathophysiology of this rare disease, which has led to more efficient treatment. Recent studies have implicated genes in pathways beyond the alternative complement system, such as *DGKE*, *TSEN2*, and *INF2* highlighting the importance of personalized management. Eculizumab has brought about dramatic improvements in the treatment of aHUS. Beyond eculizumab, there are many alternative therapeutics in the pipeline that target the complement system. Because of the rarity of aHUS, data from multiple patient registries are very important. The present report aimed to summarize the most important aspects of diagnosing and treating aHUS based on the Turkish national registry and the literature so as to improve clinical practice.

Key words: hemolytic uremic syndrome, shiga toxin-producing *Escherichia coli*, TRACK syndrome, monoclonal complement C5 antibody.

Hemolytic uremic syndrome (HUS) is an important differential diagnosis in pediatrics as it has a significant potential for morbidity and even mortality. It is clinically characterized by microangiopathic hemolytic anemia, low platelet count and acute kidney injury.[1] Although kidney involvement takes an important place both in diagnosis and prognosis, other vital organs can also be affected.[2]

Thrombotic thrombocytopenic purpura (TTP), a major differential diagnosis among this group of disorders, is characterized by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin motif type 1, member 13) deficiency. ADAMTS13 breaks down the large multimers of von Willebrand Factor (vWF) on endothelial cells. In TTP patients, ADAMTS13 activity is <10%. The incidence of HUS in pediatric thrombotic microangiopathy (TMA) patients is higher than that of TTP.[2]

HUS has attracted significant attention in terms of etiologic diversity, pathogenesis and management in recent years.[3] Since its first description in 1955, our understanding of its pathophysiology has vastly improved. For many years, it was investigated mainly in two categories; "diarrhea-positive" (typical) and "diarrhea-negative" (atypical) HUS; however, this classification system was abandoned in 2015 as it was considered misleading. Currently, the disease is categorized according to etiological subgroups. Nevertheless, the

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terms "typical" and "atypical" are still used in daily practice. Typical HUS is mainly caused by STEC (Shiga toxin-producing *Escherichia coli*) or other verotoxin-producing *E. coli* (VTEC), and is referred to as STEC-HUS. In addition, HUS can develop secondary to infections caused by *Streptococcus pneumoniae*, underlying disease (such as malignancy), some medications (such as cyclosporine, tacrolimus and quinine), bone marrow transplantation, and pregnancy. Atypical HUS (aHUS) is a rare form of HUS with an annual incidence of 0.26-1.9 per million. It may be a devastating disease if not managed properly.[4] The kidneys are usually involved and it may progress to chronic kidney disease (CKD) stage 5, requiring sustained kidney replacement therapy (KRT). Less commonly, extrarenal organs, primarily the central nervous and cardiovascular systems, lungs, gastrointestinal (GI) tract, eyes, and skeleton, can also be affected by TMA associated with aHUS, modifying the clinical presentation of the disease.[5-10]

From the pathogenetic point of view, HUS is a clinical expression of TMA characterized by platelet aggregation and thrombus formation in small vessels that lead to luminal narrowing or occlusion resulting in end-organ ischemia and infarction. Endothelial cell damage is the primary cause that initiates TMA.[1] In aHUS, the induction of endothelial cell injury is sustained, such as dysregulation of the alternative complement pathway (complement-induced HUS), mutations that cause loss of function of the lipid kinase diacyl glycerol kinase epsilon (DGKε),[9,10] or recently identified *TSEN2* mutations that disrupt tRNA biology (Table I).[11] Complement-mediated HUS, the most common type of aHUS, occurs due to dysregulation of the alternative complement system that is caused by mutations in genes encoding complement factors or autoantibodies against some of the complement components. To date, more than 120 mutations responsible for aHUS have been found in the genes encoding regulatory proteins of the complement alternative pathway (Fig. 1).[12-14]

Complement-blocking therapies have significantly improved the prognosis of aHUS and are the first line therapy for complement mediated aHUS; however, there is still no consensus regarding long-term therapeutic management in children with aHUS. Therapeutic approaches vary geographically due to differences in healthcare policies. For instance, eculizumab is still not available in underdeveloped countries because of economical constraints. As such, currently available treatment guidelines may not be applicable in all countries, which highlights the necessity of each country developing their own national treatment protocol based on consensus. The present report aimed to summarize the most important aspects of diagnosing and treating aHUS based on the Turkish national registry and the literature, so as to improve clinical practice.

Table I. Classification of hemolytic uremic syndrome (HUS).

1. Infection-associated HUS
- Shiga toxin-associated HUS
- *Pneumococcus*-associated HUS
2. HUS with coexisting conditions
- Malignancy
- Medications (e.g., VEGF inhibitors)
- Bone marrow transplantation
3. Atypical HUS (aHUS)
- Complement mediated aHUS
• Genetics (*CFH, CFI, CD46 (MCP), C3, CFB,* CFHRs)
• Autoimmunity (Anti-CFH antibody)
- Complement independent aHUS
• Genetics (*DGKE,* cblC, *INF2*)
• Syndromic form (TRACK syndrome)

aHUS: atypical hemolytic uremic syndrome, CblC: cobalamin C, *CFB*: complement factor B, *CFH*: complement factor H, CFHR: complement factor H related proteins, *CFI*: complement factor I, *DGKE*: diacylglycerol kinase epsilon, HUS; hemolytic uremic syndrome, *INF2*: inverted formin 2, *MCP*: membrane cofactor protein, TRACK syndrome; *TSEN2* related atypical hemolytic uremic syndrome, craniofacial malformations, kidney failure, VEGF: vascular endothelial growth factor

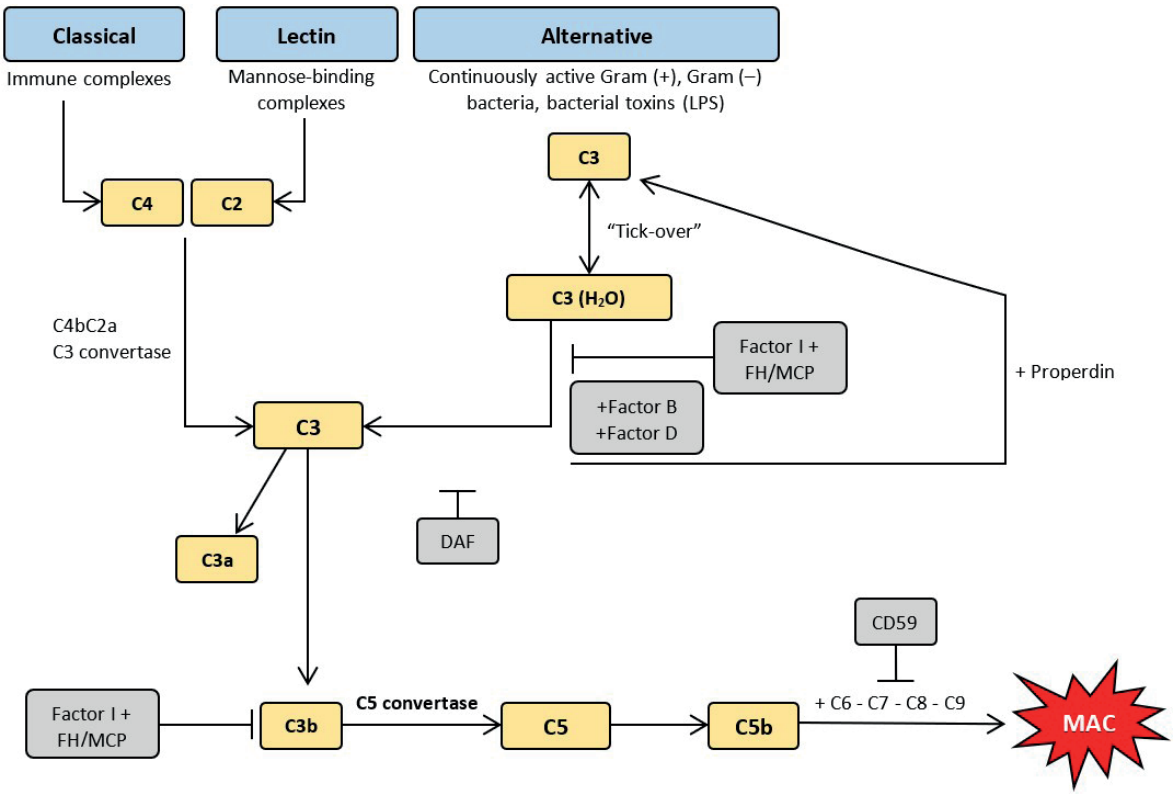

Fig. 1. The classical, lectin and alternative complement pathways and their regulatory proteins.

C: complement, DAF: decay accelerating factor, FH: factor H, LPS: lipopolysaccharide, MAC: membrane attack complex, MCP: membrane cofactor protein.

1. Classification of HUS

The current classification, which was proposed by Lemaire et al.[15] is based on etiological subgroups. STEC-HUS and pneumococcus-associated HUS (SP-HUS) are classified as infection-associated HUS. The term aHUS is generally used for cases related to the alternative pathway dysregulation or complement independent genetic abnormalities such as *DGKE*, *MMACHC* (CblC), *INF2* and *TSEN2* mutations. Coexisting conditions and/or medications with the potential to cause HUS should also be investigated (Table I). Despite all efforts, the etiology may not always be determined for a particular group of patients.

1.1. STEC-HUS

Definition and clinical characteristics

STEC-HUS occurs following acute gastroenteritis secondary to enterohemorrhagic *E. coli* or *S. dysenteriae*, and most commonly occurs in children aged 3-5 years. In patients with STEC gastroenteritis, *E. coli* O157 is usually isolated; however, infections caused by non-O157 strains of *E. coli* have increased in the last decade. The most prevalent strain in the outbreak in Germany was O104:H4.[15-17] Diarrhea typically begins 3–8 days after consuming contaminated food, direct contact with the cattle or as a result of household contact and precedes the clinical manifestation of HUS. Watery diarrhea occurs in the early stages, which may turn into bloody diarrhea. Fever, nausea, vomiting, and abdominal pain are also common symptoms. These symptoms typically appear 2-14 days following the onset of diarrhea.[18]

Diagnostic criteria

1. Microangiopathic hemolytic anemia: Hemoglobin level below the lower limit of

normal, reticulocytosis and increased lactate dehydrogenase (LDH), decreased haptoglobin level, hemolysis in red blood cells (such as schistocytes on peripheral blood smear), negative direct Coombs test (sometimes positive in SP-HUS, autoimmune diseases, or with prior transfusion of blood products).

2. Thrombocytopenia: Platelet count <150x10^9/L or >25% decrease from baseline.

3. Kidney compromise: Acute kidney injury (AKI) is defined when serum creatinine increase ≥0.3 mg/dL within 48 h or a ≥1.5-fold increase in the baseline value, which is known or presumed to have occurred within the previous 7 days[2,19,20], and oliguria/anuria.

Confirmation of STEC

The collection of material for Shiga toxin testing is very important. The probability of obtaining a positive shiga toxin test via polymerase chain reaction (PCR) or stool culture diminishes 10 days after the diarrhea. Accordingly, 90% of patients have a negative stool culture result after 15 days.[21] Immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against *E.coli* lipopolysaccharides increase in the acute phase of the disease and high levels of IgG antibodies persist in later stages of the disease when fecal specimens fail to demonstrate Shiga toxin.[2,22]

Clinical characteristics

In STEC-HUS, the kidney presentation may be different from patient to patient, ranging from severe AKI with anuria to only proteinuria, hematuria, or both. In rare cases, ischemia can lead to cortical necrosis as a pathological finding.[1] Ardissino et al.[23] reported that only 41% of patients met the 3 criteria for the standard diagnosis of HUS; therefore, a high degree of suspicion is necessary for appropriate management of patients. Multisystem involvement can occur; the most frequently involved system is the central nervous system (CNS). Findings suggesting CNS involvement include disorientation, altered mental state, convulsions and stroke. GI involvement can also be observed in some patients. In this case, intestinal ischemia, necrosis, or thrombosis, perforation, pseudomembranous colitis, rectal prolapse, elevated transaminases or hyperbilirubinemia may occur.[24] An outbreak of HUS was reported in 2013 that included 70 patients with a median age of 5.7 years; the rate of CNS complications and mortality was 21.4% and 4.2%, respectively.[25]

Management

The management of STEC-HUS is supportive. The constitution of circulatory volume is very crucial. Ake et al.[26] reported that patients with non-oliguric kidney failure had more intravenous fluid and sodium before HUS development compared to the patients with oligo-anuric HUS. Subsequent studies and meta analyses also highlight the importance of early fluid infusion, which decreases the requirement for dialysis, duration of hospitalization, and even mortality[27,28]; therefore, when HUS is associated with diarrhea, every normotensive patient with a normal cardiac silhouette on chest X-ray and no signs of fluid overload should be evaluated for early fluid infusion.[29] Intravenous furosemide can be considered in children with hypervolemia and oligo-anuria. Blood pressure should be kept within normal limits for age, sex and height; for this purpose appropriate antihypertensive medication can also be indicated. Angiotensin converting enzyme (ACE) inhibitors are generally avoided as these drugs can cause further impairment of kidney functions and severe hyperkalemia. In patients with electrolyte and/or acid-base abnormalities, or volume overload unresponsive to diuretics, KRT should be administered. The type of KRT chosen should be based on clinician experience and healthcare center infrastructure. Electrolyte and acid-base abnormalities should be corrected. For ongoing hemolysis, erythrocyte transfusion may be required.[18] A transfusion is usually necessary if the hemoglobin level is below 7 g/dL or below 7.5 g/dL with a decline of more than 2 g/dL from the level of the previous

day.[30] Because of the risks of hyperkalemia and hypervolemia, it is recommended that a decision for blood transfusion be made in consultation with the nephrologist. Routine thrombocyte infusion is not recommended unless there is active bleeding, but may be given before catheter insertion for KRT. These patients are usually in a catabolic state and therefore adequate nutritional support is also critical.[30]

For patients with CNS involvement, plasma-based therapies are employed as salvage therapies.[30,31] The benefits of plasma infusion (PI) and plasma exchange (PE) on the course of the disease are not widely accepted. Recovery after PE has been demonstrated in some studies and case reports[32], but extensive research has not been done to demonstrate the effectiveness of plasma-based therapy.[33,34] Eculizumab was administered to STEC-HUS patients with CNS involvement in the 2011 German epidemic; however, detailed analyses did not show a clear benefit.[17] Similarly, Ağbaş et al.[35] reported 21 STEC-HUS; no difference in kidney prognosis was found between patients treated with and without eculizumab. Thus, complement-blocking therapies can be considered transiently during the acute phase in selected STEC-HUS patients in whom supportive treatments fail and severe disease is present. Therefore, despite clear evidence of the therapeutic utility of eculizumab in patients with aHUS, further studies are warranted for its use in patients with STEC-HUS.[30]

1.2. S. pneumoniae-associated HUS (SP-HUS)

Invasive pneumococcal disease has become less common since the introduction of the conjugated pneumococcus vaccine; however, SP-HUS incidence has not decreased. It is estimated that invasive pneumococcal infection causes roughly 0.4% to 0.6% of HUS cases.[1,36] It typically occurs following pneumonia (especially when complicated by empyema) and meningitis. Preformed IgM antibodies against Thomsen-Friedenreich cryptantigen (T-antigen) react with red blood cells (RBCs), platelets, and endothelial cells, all of which result in the development of HUS. The onset of SP-HUS typically occurs 3–13 days (often within 7-9 days) following the start of pneumococcal illness. In comparison to individuals with STEC-HUS, children with SP-HUS are younger, typically have more severe kidney and/or hematological disease, therefore need an increased duration of hospitalization.[37,38] There is no specific laboratory test for SP-HUS nor are there validated diagnostic criteria. Furthermore, it may have some overlapping features with disseminated intravascular coagulation (DIC). Therefore, Scobell et al.[39] suggested modified criteria for SP-HUS with 3 categories. According to these criteria, evidence for HUS or invasive *S. pneumoniae* infection (in any biological fluid that should be sterile) or positive sputum culture in association with pneumonia and no clinical and laboratory evidence of DIC have been considered definite. Probable cases have been defined as having evidence for HUS or invasive *S. pneumoniae* infection in any biological fluid that should be sterile or positive sputum culture in association with pneumonia (in the presence of pneumonia) with presence of evidence of DIC and of T-activation via positive Coombs test or peanut lectin assay. Possible cases have been defined as those with evidence for HUS, toxic appearance with pneumonia, meningitis or evidence of other invasive infection without identification of a specific organism, positive Coombs test or peanut lectin assay with or without evidence of DIC.

The management also includes supportive care and pneumococcal infection treatment. Given the fact that blood products may contain antibodies against T-antigen, packed RBCs should be washed with dextran. Fresh frozen plasma infusions should be avoided due to the same fact. Complement-blocking medications may also be used in severe cases.[38]

1.3. aHUS

aHUS poses significant difficulties in the management and follow-up of pediatric nephrology patients, as well as in terms of prognosis. About 70% of pediatric patients

with aHUS experience their first episode before the age of 2 years, and 25% before the age of 6 months. The frequency does not differ between males and females. In general, aHUS comprises 5-10% of all childhood HUS cases.[7] An analysis of the Turkish National Registry System (NRS) showed that 36% of the patients had disease onset at <2 years of age. In total, 72% of the patients received KRT. After a median duration of 23 months CKD stage 5 developed in 1/53 patient. Hypertension and proteinuria persisted during the follow-up period in 44% and 37% of the patients, respectively.[40] On the other hand, in the Global aHUS Registry, 42.8% of the pediatric patients had disease onset <2 years of age.[41]

Gain-of-function mutations in complement 3 (C3), complement factor B (CFB), neutralizing antibody against CFH (anti-CFH antibodies), as well as loss-of-function mutations in the genes encoding regulatory proteins (complement factor H [CFH], complement factor I [CFI], membrane cofactor protein [MCP, CD46], and thrombomodulin) cause the alternative pathway to become overactive.[42] These genetic abnormalities lead to uncontrolled C5b-9 membrane attack complex (MAC) production on endothelial cells causing endothelial damage. It has been reported that, mutations in the genes encoding alternative pathway proteins are present in 60-70% of aHUS cases.[43] CFH mutations have been reported to be the most frequent and to be associated with the worst prognosis; however, genetic causes can differ by ethnicity.[42,43] As such, the *CD46* (*MCP*) mutation was found to be most prevalent in the Turkish pediatric aHUS population, based on data from the national aHUS registry.[44] It should be emphasized that mutation analysis is not necessary for an aHUS diagnosis; however, mutation analysis can help determine the prognosis, risk of recurrence, and duration of complement-blocking therapies. As such, it is recommended to perform genetic analysis at any time during the course of the disease.

Among patients, >50% (susceptible individuals) have a history of infection (i.e. acute gastroenteritis or upper respiratory tract infection) that over-activates the alternative complement pathway. Based on the Turkish NRS, CNS involvement is the most frequent extrarenal system involvement (27.2%), followed by cardiovascular and respiratory involvement. In other series, CNS involvement was observed in 8-48% of the cases.[45] Additionally, compared to the patients without CNS involvement, patients with CNS involvement have a higher mortality rate and a lower estimated glomerular filtration rate (eGFR).[8] Distended abdomen, bloody diarrhea, and intestinal perforation suggest GI involvement.[44] The differential diagnosis is summarized in Fig. 2.

Management

The management of aHUS is classified into two categories: namely supportive and specific treatment. Principles for supportive treatment are the same as those given above for STEC-HUS. Plasma-based therapies were the mainstay of management for many years; however, the risk of hypervolemia in oliguric patients is the primary concern associated with PE and PI. PE was administered at 60-75 mL/kg and PI was administered at 10-20 mL/kg for the treatment of aHUS. However, the effectiveness of plasma based treatments in aHUS patients has not been sufficiently supported by the existing literature data.[46,47] One study reported that plasma-based therapy caused hematological remission in 78% of pediatric patients and 53% of adult patients; however, within 3 years of follow-up, 50% of pediatric patients and 66% of adult patients developed CKD stage 5 or died.[12]

Recently, we assessed the prognosis in aHUS patients who had PE (n=3) and PI (n=4) from the Turkish NRS. In this cohort, 71.4% of the patients (n=5) had complete hematological remission and an eGFR >90 mL/min/1.73m^2.[48] Khandelwal et al.[49] evaluated the efficacy and safety of 109 aHUS patients who received PE. Anti-CFH antibody was found to be an etiologic factor in 74 (67.9%) patients. Heterozygous mutations in *CFH* (n=4), *CFI* (n=3), and both *CFB* and *CFI* (n=1) were identified in the remaining

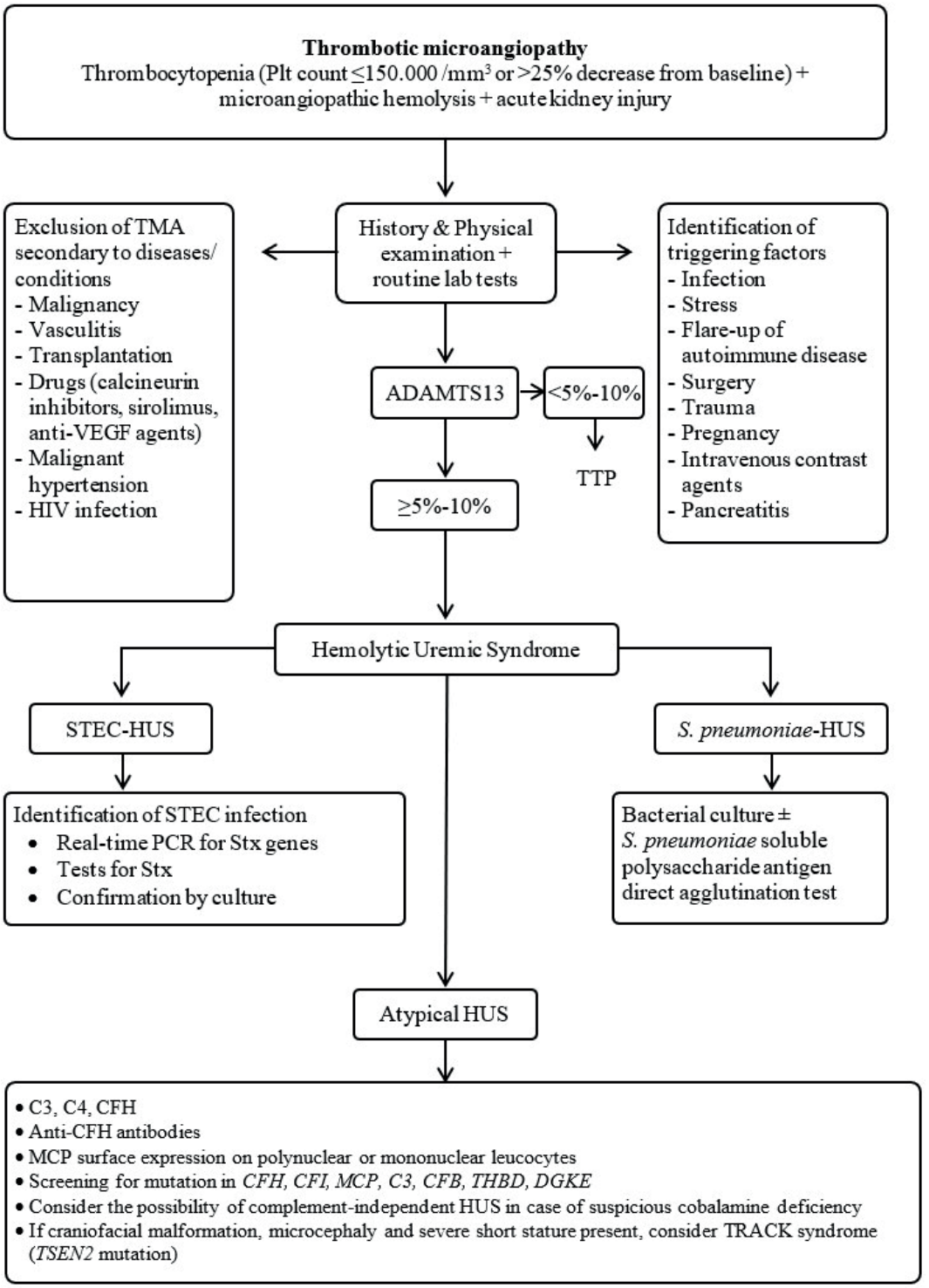

Fig. 2. Diagnostic algorithm and differential diagnosis for pediatric patients with TMA.

ADAMTS13: a disintegrin and metalloproteinase with a thrombospondin motif type 1, member 13, C3: complement 3, C4: complement 4, CFB: complement factor B, *CFH*: complement factor H, *CFI*: complement factor I, *DGKE*: diaclyglycerol kinase epsilon, HIV: human immunodeficiency virus, HUS: hemolytic uremic syndrome, *MCP*: membrane cofactor protein, PCR: polymerase chain reaction, STEC: Shiga toxin producing *Escherichia coli*, Stx: Shiga-toxin, *THBD*: thrombomodulin, TMA: thrombotic microangiopathy, TRACK syndrome: *TSEN2* related atypical hemolytic uremic syndrome, craniofacial malformations, kidney failure, TTP: thrombotic thrombocytopenic purpura, VEGF: vascular endothelial growth factor

35 patients without anti-CFH antibodies. PE with immunosuppression (n=74), PE alone (n=19) and PE followed by PI (n=16) were applied during management. In addition, 92 (84.4%) patients underwent concomitant hemodialysis. Hematological remission was achieved in 73 (98.6%) patients with anti-CFH associated disease, whereas hematological remission occurred in 32 (91.4%) patients without anti-CFH associated disease. Dialysis independence by 1-month was achieved in 77.1% of the patients. Caprioli et al.[50] evaluated the genetics and clinical characteristics of HUS and concluded that remission was achieved in 67% of plasma-treated episodes in patients with a *CFH* mutation. Based on these results, we can conclude that plasma-based therapies might still be considered in selected patients, particularly in countries in which complement-blocking therapies are not readily available or the costs are not covered by health insurance.[48] Nonetheless, it should be kept in mind that catheter-related complications may occur in up to 31% of cases.[51]

Since 2009, the use of complement-blocking therapies (primarily eculizumab) has substantially changed the prognosis of children with aHUS. Eculizumab is a recombinant, humanized, monoclonal IgG type complement 5 (C5) antibody that blocks the cleavage of C5 into fragments and, thereby, the formation of MAC (Fig. 1). The half-life of eculizumab is 11 ± 3 days; therefore, maintenance treatment is administered every two weeks.[7] When treating aHUS, complement-blocking therapies should be the first-line treatment and should be initiated within the first 24 hours of diagnosis. If none of these therapeutics are available, PE using fresh-frozen plasma (FFP) may be an option. If PE is not available, PI should be the treatment of choice until complement-blocking therapy becomes available.[48] The results of genetic tests are not necessary for initiating complement-blocking therapies.

Monitoring the efficacy of eculizumab is another important issue in aHUS patients. Complement hemolytic activity can be monitored using the CH50 test, and the results should be <10% in aHUS patients receiving eculizumab. Measurement of the trough eculizumab level is another method for drug monitoring, but it is not available in most countries. When available, its target range (C_{min}) is recommended to be kept at >100 μg/mL.[52] In cases resistant to eculizumab despite administration at a therapeutic level, variants in the C5 binding site, increased elimination of eculizumab in urine, and the presence of other genetic causes, such as *DGKE* mutations, should be investigated.[43]

Given the fact that the risk of meningococcal disease significantly increases with eculizumab, patients should be vaccinated with A, C, W, and Y meningococcal conjugate vaccine as well as the B meningococcal vaccine before starting eculizumab therapy. In some countries, long-term antibiotic prophylaxis is recommended for aHUS patients for as long as they receive eculizumab treatment. If eculizumab treatment is initiated <2 weeks after vaccination due to urgent patient management, patients should receive antibiotic prophylaxis until 2 weeks after vaccination.[52]

The duration of eculizumab treatment in dialyzed patients is another important consideration. There are anecdotal case reports that describe the effect of eculizumab in patients under prolonged dialysis (>3 months). In these reports, the duration of dialysis before initiation of eculizumab varied between 3 and 6 months. After the initiation of eculizumab, the time to dialysis discontinuation was between 1 and 6 months.[53] Based on these findings, we recommend the maintenance of eculizumab in those patients without any mutation in alternative complement pathway genes for at least 3-6 months before concluding there is no benefit.

The risk of infection, high cost, and the burden of repeated infusions all contribute to the possibility of eculizumab discontinuation. On the other hand, the risk of relapse and subsequent kidney damage are arguments for not discontinuing eculizumab. Fakhouri et al.[54]

prospectively studied the effects of eculizumab discontinuation in 55 pediatric and adult aHUS patients, of whom 28 (51%) had rare variants in complement genes, most in *MCP*. During follow-up, 13 patients (23%) developed aHUS relapse, and multivariable analysis showed that female gender and the presence of a rare variant in a complement gene were linked with an increased risk of aHUS recurrence. Recently, we investigated eculizumab discontinuation in our patients from the Turkish NRS. Eculizumab treatment was discontinued in 18 (30.7%) out of 63 patients. Four patients (22.2%) experienced a recurrence; eculizumab was initiated immediately and complete remission was achieved.[55] Based on the above findings, we recommend that eculizumab discontinuation be considered during the remission period in patients without complement gene mutations, a history of relapse, or a family history of HUS, with close clinical and laboratory monitoring. In patients with *CD46* mutation, eculizumab discontinuation can be considered after 3 months of usage. The management principles for pediatric patients with TMA are shown in Fig. 3. Eculizumab has changed the poor destiny of aHUS. However, it requires intravenous infusions every two-three weeks. Therefore, a longer-acting C5 antibody, ravulizumab, was developed with the re-engineering of eculizumab. It requires infusions every 4-8 weeks. Its efficacy was shown both in eculizumab treated and eculizumab-naïve pediatric aHUS patients and has been approved in many countries.[56-58]

Research for specific treatment of aHUS is not confined to eculizumab and ravulizumab. There are many therapeutics in the pipeline that have the potential to be used in clinical practice (Table II).[59]

1.3.1. Anti-CFH antibody-associated HUS

Dragon-Durey et al.[60] studied aHUS patients that were treated with PE only and reported CKD in 39% and CKD stage 5 in 27% of the patients. Other cohorts that were treated with early PE and additional immunosuppressive drugs had a much more favorable prognosis.[61] Sinha et al.[62] evaluated the predictors of anti-CFH antibody-associated HUS. They concluded that antibody titer ≥8000 AU/ml, low C3 and time to PE≥17 days were independent predictors of adverse outcome on long term prognosis. They also studied the effects of PE combined with induction immunosuppression, which significantly decreased the risk of severe CKD and mortality, in comparison with PE alone. Mycophenolate mofetil (MMF) can also be used for maintenance treatment. Eculizumab is also effective for acute treatment, although its effect on lowering the CFH antibody has not been ascertained.[43] In light of existing literature data, we recommend PE, steroids, and such immunosuppressives as MMF and/or eculizumab for treatment. Additionally, we also recommend serial measurement of the anti-CFH antibody level on days 7-14, day 28 and every 3-6 months. Elevated titre (>1,500 AU/ml) during the first 12–24 months suggests an increased risk of relapse.[63]

1.3.2. Complement independent hemolytic uremic syndrome

The pathophysiological mechanisms of HUS are not restricted to the alternative complement system. Recent years have witnessed the definition of many complement independent mechanisms. Cobalamin C (CblC) defect-associated HUS is one of them. CblC defect is one of the most common inborn errors of metabolism and may present with HUS. CblC defect is most commonly seen in infants and neonates, and manifests as failure to thrive, hypotonia, seizures and microcephaly, delayed development, and hypertension. Prompt diagnosis and treatment with hydroxocobalamin may allow hematological and kidney recovery.[1]

Another complement independent HUS is caused by bi-allelic recessive mutations in *DGKE* (diaclyglycerol kinase epsilon). This gene was first identified in glomerular microangiopathy mimicking membranoproliferative glomerulonephritis

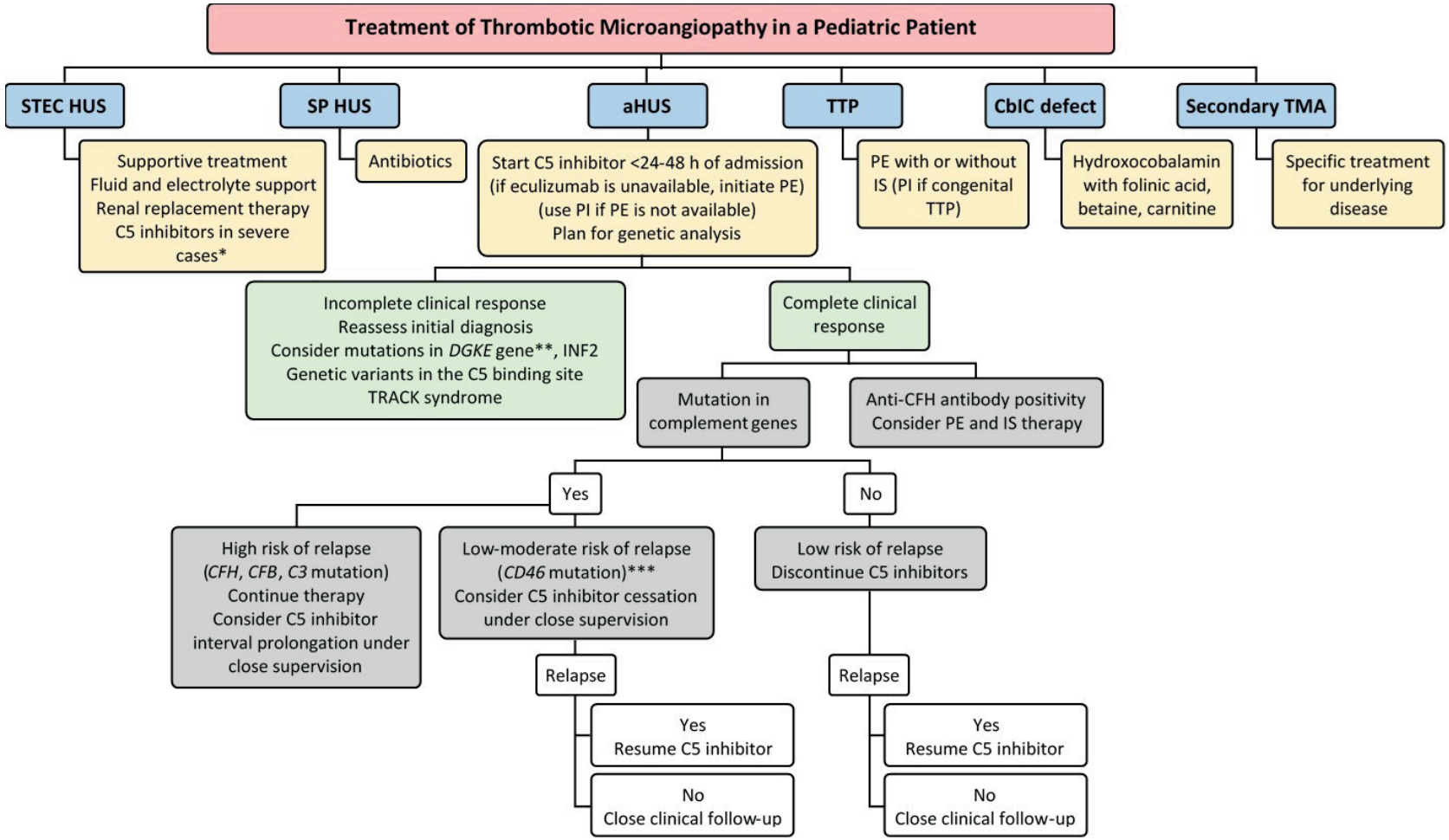

Fig. 3. Management principles for pediatric patients with different types of TMA.

*Although considered controversial, C5 inhibitors may be beneficial in cases that do not respond to supportive treatment in STEC HUS.
**If there is a *DGKE* mutation the benefit of C5 inhibitor therapy is uncertain.
***C5 inhibitor therapy can be withdrawn after 3-6 months if the clinical situation allows.
aHUS: atypical hemolytic uremic syndrome, C3: complement 3, C5: complement 5, CblC: cobalamin C, *CD46*: cluster of differentiation 46 (MCP: membrane cofactor protein), *CFB*: complement factor B, *CFH*: complement factor H, *DGKE*: diaclyglycerol kinase epsilon, HUS: hemolytic uremic syndrome, IS: immunosuppressive, PE: plasma exchange, PI: plasma infusion, PE: plasma exchange, SP HUS: *Streptococcus pneumoniae* associated HUS, STEC: Shiga toxin producing *Escherichia coli*, TMA: thrombotic microangiopathy, TRACK syndrome: *TSEN2* related atypical hemolytic uremic syndrome, craniofacial malformations, kidney failure, TTP: thrombotic thrombocytopenic purpura

Table II. Current aHUS drugs and those under development.

Therapeutics	Name of the drug	Drug class	Mechanism of action
Current therapeutics	Eculizumab	Monoclonal antibody, terminal complement inhibitor	Binds to C5 and prevents cleavage to C5a and C5b
	Ravulizumab		Prevents cleavage of C5 to C5a and C5b
	Nomacopan	C5aR1 antagonist	Inhibits C3a, C4a, and C5a protein function
	Avacopan	Recombinant protein derived from a tick C5 inhibitor	An oral antagonist of C5a receptors. Inhibits C5 and leukotriene B4
	Cemdisiran	Short sequences of interfering RNA	Matches mRNA for the C5 protein, with N-acetylgalactosamine
Biosimilars	ABP 959	Biosimilar to eculizumab	Binds to C5 and prevents cleavage to C5a and C5b
	Elizaria	Biosimilar to eculizumab	
Therapeutics under development	ALXN1720	Anti-C5 antibody	Binds to C5 protein and blocks its activation
	Avdoralimab	Anti-C5aR1 antibody	Blocks T-cell and natural killer cell activity via C5aR1suppression
	Crovalimab	Binds to a C5 epitope	Binds to C5b and prevents formation of the MAC complex. Crovalimab is a long-acting C5 inhibitor that could be administered subcutaneously.
	IFX-1	Targets C5a protein directly	Binds to C5a
	MAC Inhibitor HMR59	Promotes CD59 production	Enhances synthesis of CD59, which blocks C5b-9 formation
	Pozelimab	C5 antibody	Decreases hemolysis and the C5 level
	Tesidolumab	C5 monoclonal IgG1 antibody	Binds to C5, preventing its cleavage
	Zilucoplan	Binds to the C5b protein and the C5b part of C5	Inhibits C5b binding to C5 by binding to its C5 domain

aHUS: atypical hemolytic uremic syndrome, C3: complement 3, C4: complement 4, C5: complement 5, C5aR1: complement 5a receptor 1, IgG1: immunoglobulin 1, MAC: membrane attack complex, mRNA: messenger RNA

and was associated with aHUS thereafter.[9,10] The most important characteristics of *DGKE*-associated aHUS are significant proteinuria in addition to the classic triad of HUS and usually early onset of disease (i.e. less than 1 year of age).[9,52] INF2, a ubiquitously-expressed formin protein, regulates the actin cytoskeleton and related cell functions including secretory pathway by accelerating its polymerization and depolymerization. Challis et al.[64] identified a family in which proposita presented with aHUS unresponsive to eculizumab and her mother experienced TMA after kidney transplantation. Both patients had posttransplant Charcot-Marie-Tooth disease. Analyzing the Newcastle aHUS cohort, another family with a mutation of *INF2* was identified in which kidney transplantation has been associated with post-transplant TMA.[64]

Recently, a team led by the 2nd author of the present manuscript has identified a new complement independent, syndromic form of aHUS (*TSEN2* Related Atypical hemolytic uremic syndrome, Craniofacial malformations, Kidney Failure; TRACK syndrome) that arises from pre-tRNA splicing defect.[11] In this syndrome, an intronic mutation that disrupts normal splicing of *TSEN2* (tRNA splicing endonuclease 2) has been associated with aHUS and distinct craniofacial abnormalities including microcephaly, adenohypophysial hypoplasia and associated growth retardation,

cone-shaped and sparse teeth, deeply set eyes, and long philtrum in 6 children.[11] As expected, none of the patients responded to eculizumab treatment. Three patients died at the time of writing the original article. One patient is 11 years old now and is still being followed up with peritoneal dialysis (PD). Another patient was transplanted from a deceased donor when she was 11 years old. She received a short period of eculizumab treatment during the post-transplantation period, which was stopped after a genetic diagnosis and post-transplant aHUS was not observed. She is now 14 years old with normal graft functions (serum creatinine 0.33 mg/dL). Another patient was also transplanted when he was 7 years old. He experienced a biopsy proven acute T cell mediated rejection at post-transplant 9th month and BKV associated nephropathy without evidence of TMA. Modification of the immunosuppressive regimen resulted in rapid improvement of graft functions. He never experienced a post-transplant aHUS. He is 12.5 years old now with a normal functioning graft (serum creatinine of 0.32 mg/dl). During the identification of underlying genetic abnormalities in these children, whole exome sequencing (WES) method was applied to all individuals in the index family. A stringent filtering strategy ended up with the identification of an intronic variant, that is very close to the splice site. Subsequent studies confirmed that this intronic variant disrupted normal splicing, with the presence of 3 different transcripts: (i) the retention of a normally spliced transcript, (ii) transcript in which exon 10 and correspondingly many evolutionary conserved aminoacids are skipped, and (iii) transcript that contains two extra amino acids. Accordingly, bulk RNA sequencing also showed five abnormal tRNAs confirming impaired TSEN2 enzymatic function in all affected individuals that are absent in healthy individuals. This finding also supported the hypothesis that the tRNA inventory necessary for normal life has been changed in the cells.

Future directions

In the last twenty-year period, there have been many breathtaking advances in the treatment of aHUS. Among them, eculizumab has revolutionized the management of patients but put new questions on the table. Eculizumab discontinuation is possible in selected patients. Patients in remission may have normal laboratory values in terms of TMA, however, the extent of disease activity on the kidneys is still unknown. Certain needs are reliable biomarkers that predict early relapses and that are useful in monitoring disease activity. Besides eculizumab, there are also other C5 antibodies and biosimilars for the treatment of aHUS. The safety and efficacy of these molecules in pediatric aHUS patients will be important research subjects in the future. In addition, more individualized treatment options, such as CFH administration in those patients with CFH deficiency instead of blocking the complement system, are expected. The definition of genetic abnormalities which are unrelated to the alternative complement pathway (like DGKE, cblC, etc) will also lead to the development of personalized treatment options for this group of patients. As more genetic abnormalities are identified, more insight into TMA pathogenesis will become possible.

Conclusion

In conclusion, HUS still stands for a challenging clinical condition despite recent developments leading to a better understanding of the patho-mechanisms of the disease that result in more effective therapeutic approaches. Nevertheless, these can explain a fraction of patients, which strongly suggests that many yet unidentified mechanisms may still be present.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BG, FÖ, OS; data collection: BG, KF, ZBÖ, FÖ; analysis and interpretation of results: BG, KF, ZBÖ, FÖ, OS; draft manuscript preparation: BG, FÖ; All authors reviewed the results and approved the final version of the manuscript.

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Original Article

Early neonatal outcomes in infants of mothers with organ transplantation under immunosuppressive treatment

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ABSTRACT

Background. This study aimed to examine early clinical and laboratory findings in infants born to mothers who had organ transplants and received immunosuppressive treatment.

Methods. Between 2016 and 2023, the study examined infants of mothers who underwent organ transplantation and were receiving immunosuppressive treatment, and followed at the Department of Neonatology at Akdeniz University. Demographic, clinical, and laboratory characteristics of mothers and infants were recorded. On the first day of life, complete blood count values were examined, as well as potassium levels on the first, third, and seventh days, and creatinine levels on the third and seventh days. The tacrolimus blood level was calculated by taking the average of the tacrolimus blood values of the mother measured during the pregnancy. The infants were evaluated for any potential morbidities caused by intrauterine immunosuppressive drug exposure.

Results. The study included 21 mothers (some with multiple pregnancies) and 27 infants. According to the findings of this study, 74% of these infants were born premature, 67% had low birth weight, and all were delivered via cesarean section. Prematurity was associated with the morbidities found in the infants. In the early period, lymphopenia was detected in 37%, neutropenia in 25.9%, thrombocytopenia in 11.1%, hyperkalemia in 18.5%, and creatinine elevation in 7.4%, all of which returned to normal within a few days. There was no significant relationship between maternal tacrolimus blood levels and infant potassium and creatinine levels.

Conclusion. Apart from an increased risk of prematurity, low birth weight, and cesarean delivery, no effects were observed in these infants during the early period. However, long-term follow-up is necessary to monitor for any potential morbidities.

Key words: organ transplantation, pregnancy, immunosuppressive treatment, prematurity.

With the increasing success of organ transplantation, the number of pregnancies among women who regularly take immunosuppressive drugs after transplantation is increasing. Immunosuppressive treatments, such as calcineurin inhibitors (tacrolimus and cyclosporine), azathioprine, mTOR inhibitors, mycophenolate, and corticosteroids, help to prevent organ rejection. However, some of these medications may pose risks to a developing fetus. Female transplant recipients have successful pregnancies using these medicines.[1,2] With the use of such medications, there are potential risks associated with pregnancy in transplant patients, including an increased risk of premature birth, low-birth weight, nephrotoxicity, immune dysfunction, and birth abnormalities.[2-4]

Previous studies have found that maternal tacrolimus has more favorable results than cyclosporine in pregnant women receiving immunosuppressive drugs after organ transplantation. However, it has been linked to several adverse effects, including preterm birth, intrauterine growth restriction, reversible hyperkalemia, and renal effects.[5,6]

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Azathioprine, another often used agent, has been reported to cause myelosuppression. Effects on the neutrophil, platelet, and erythrocytic series, notably the lymphocyte series, have been observed.[2,7] Although early intrauterine azathioprine exposure has been shown to cause congenital anomalies, these are not more common than in the general population.[2,8]

This study aimed to evaluate the early clinical and laboratory findings in infants born to mothers who received organ transplantation and immunosuppressive treatment at our hospital.

Materials and Methods

This retrospective cohort study was conducted in a tertiary neonatal intensive care unit (NICU) after receiving approval from the Akdeniz University Ethics Committee (2023/269). The study included infants delivered at Akdeniz University Hospital between 2016 and 2023 to mothers who had organ transplants and were receiving immunosuppressive treatment during pregnancy. The medical records of mothers and infants were reviewed. Demographic, clinical, and laboratory characteristics were recorded. Infants with a gestational age of less than 37 weeks were classified as premature infants, and those with a birth weight less than 2,500 g were classified as low-birth weight infants. Urine output in the first 3 days of life (mL/kg/h), time to reach birth weight, and breastfeeding rates were also recorded in infants admitted to the NICU. A complete blood count was performed within the first 24 h to evaluate white blood cell and platelet counts, creatinine levels were measured on the third and seventh postnatal days, and potassium levels were measured on the first, third, and seventh postnatal days to evaluate renal effects. Leukocytosis was defined as a white blood cell count > 30,000/mm^3, whereas leukopenia was described as a white blood cell count < 6,000/mm^3. The lower limit of neutropenia was determined using gestational age reference curves.[9] Lymphopenia was defined as a lymphocyte count below the five percentiles for the week of gestation, thrombocytopenia as a platelet count below the five percentiles for the week of gestation, and eosinophilia as an eosinophil count > 1,100/mm^3.[10] Reference curves were used to evaluate neonatal blood creatinine levels.[11,12] While potassium levels between 3.5 and 6 mmol/L were considered normal, levels > 6 mmol/L were considered hyperkalemia. We calculated the average maternal tacrolimus blood level by dividing the total blood level by the number of measurements obtained to determine tacrolimus exposure during pregnancy.

Statistical analysis

Patient data were analyzed using the Statistical Package for the Social Sciences for Windows 23.0 (IBM Corp., Armonk, New York) package program. Frequency (*n*), percentage (%), mean, standard deviation (SD), and median (Interquartile range-IQR) values are reported for descriptive statistics. The normality assumption was tested using the Shapiro–Wilk test, which examined the histogram, q–q plot, skewness, and kurtosis values. Pearson and Spearman correlation analyses were used to evaluate the relationships between quantitative variables. When the p value was <0.05, the results were considered statistically significant.

Results

The study included 21 mothers and 27 infants (with multiple pregnancies). The maternal and neonatal characteristics of the infants are shown in Table I. Twenty two of the infants were born to mothers who underwent kidney transplant and 5 of the infants were born to mothers who underwent liver transplant. When the medications used by the mothers were evaluated, it was shown that a combination regimen was often used, with tacrolimus being the most frequently used agent (Fig. 1). There was a mean of 6.4±2.8 yr between transplantation and first live birth. During pregnancy, the tacrolimus blood levels of all mothers were monitored. However, the blood levels of the

Table I. Characteristics of the study participants (N=27).

Maternal characteristics	
Age at delivery (years)	30 ± 4.4
Age at transplantation (years)	23.8 ± 4.5
Interval from transplantation to first live birth (years)	6.4 ± 2.8
Assisted reproductive techniques	2 (7.4%)
Number of pregnancies after transplantation	1 (1-2)
Immunosuppressive drugs during pregnancy	
Tacrolimus	25 (92.5%)
Azathioprine	18 (66.6%)
Corticosteroids	21 (77.7%)
Antihypertensive medication	10 (37%)
Characteristics of newborn	
Gestational week (GW)	32 ± 3.9
≥ 37	7 (25.9%)
32- 37	8 (29.6%)
28- 32	5 (18.6%)
≤ 28	7 (25.9%)
Birth weight (grams)	1940 (880-2760)
Low birth weight	18 (66.6%)
Male gender	15 (55.5%)
Small gestational age	5 (18.6%)
Type of delivery	
Emergency caesarean section	11 (40.7%)
Elective caesarean section	16 (59.3%)
Antenatal corticosteroids	13 (48.1%)
APGAR score (5th minute)	8 ± 1.3
Indication for caesarean section	
Preterm labor	7 (25.9%)
Preeclampsia/Eclampsia	11 (40.7%)
Premature rupture of the membranes	2 (7.4%)
Intrauterine growth restriction	2 (7.4%)
Miscellaneous	5 (18.6%)

Data are presented as n (%), mean ± standard deviation, or median (Q1-Q3).

other agents were not monitored (Table I). All infants were delivered via cesarean section, with 40.7% (n = 11) requiring an emergency cesarean section. The preterm birth rate was 74% (n = 20), and the low-birth weight rate was 66.6% (n = 18). Preterm birth occurred in 81.8% of infants born to renal transplant mothers and 40% of infants born to liver transplant mothers. The proportion of patients that required NICU monitoring was 81.4% (n = 22). The patients did not have early-onset sepsis. Culture-proven,

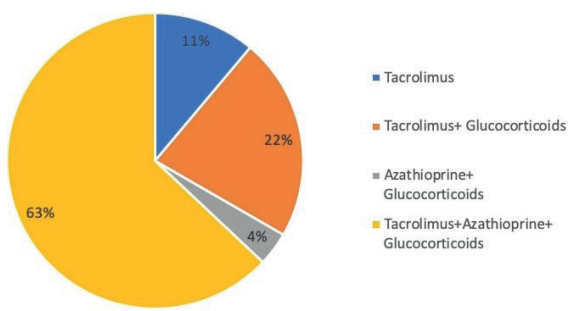

Fig. 1. Immunosuppressive regimens during pregnancy.

Table II. Clinical characteristics of patients.

Resuscitation in the delivery room	10 (37%)
Hospitalization	22 (81.4%)
Culture-proven sepsis	4 (14.8%)
Asphyxia	-
Transient tachypnea of newborn	8 (29.6%)
Respiratory distress syndrome	12 (44.4%)
Bronchopulmonary dysplasia	5 (18.5%)
Retinopathy of prematurity	3 (11.1%)
Necrotizing enterocolitis	1 (3.7%)
Intraventricular hemorrhage	-
Periventricular leukomalacia	1 (3.7%)
Patent ductus arteriosus	5 (18.5%)
Congenital malformation	1 (3.7%)
Type of nutrition	
Breastfeeding	14 (51.8%)
Breastfeeding + formula feeding	5 (18.5%)
Formula feeding	8 (29.6%)
Respiratory support	21 (77.7%)
Invasive respiratory support	9 (33.3%)
Noninvasive respiratory support	21 (77.7%)
Respiratory support (day)	4 (1-34)
Day of reached birth weight	11 ± 4.2
Urine output on first day (ml/kg/h) (n=17)	3.4 (2-4.2)
Urine output on third day (ml/kg/h) (n=17)	3.7 ± 1.0
Length of hospital stay	21 (6-75)

Data are presented as n (%), mean ± standard deviation, or median (Q1-Q3).

late-onset sepsis was found in 14.8% (*n* = 4) of infants requiring NICU support (coagulase-negative staphylococcus in three patients and *Acinetobacter baumannii* in one patient). Only one infant was found to have iris coloboma as a congenital anomaly. Fifty-two infants (*n* = 14) were exclusively breastfed, whereas 29.6% (*n* = 8) were fed solely formula. The clinical characteristics of infants who require hospitalization are shown in Table II.

At birth, 37% (*n* = 10) of the infants had lymphopenia, 25.9% (*n* = 7) had neutropenia, and 11.1% (*n* = 3) had thrombocytopenia. On the third day of life, 7.4% (n = 2) of the infants had high creatinine levels, while on the seventh day, only 3.7% (n = 1) had high levels. Notably, one of the two patients with high creatinine levels on day 3 also had high levels on day 7, but the levels returned to normal by day 10. The mothers of two patients with increased creatinine levels had undergone renal transplantation, and both mothers were receiving tacrolimus, azathioprine, and steroid combination therapy. On the first day of life, hyperkalemia was observed in 18.5% (*n* = 5) of the infants. The infants had no renal failure or mortality (Table III).

The average blood tacrolimus level of the mothers was 4.8 ± 1.1 ng/mL, and the median number of measurements was 12 (9-16). There was no significant relationship between maternal tacrolimus blood levels and infant potassium and creatinine levels.

Table III. Laboratory characteristics of patients

	Mean ± SD / median (IQR)		n (%)
White blood cell (/mm^3)	9,780 (6,255-12,295)	Leukocytosis/Leukopenia	-/5 (18.5%)
Neutrophil (/mm^3)	4,750 (3,340-8,845)	Neutropenia	7 (25.9%)
Lymphocyte (/mm^3)	2,870 (1,135-3,985)	Lymphopenia	10 (37%)
Eosinophil (/mm^3)	150 (30-255)	Eosinophilia	-
Monocyte (/mm^3)	980 (832-1,552)	Monocytosis	-
Basophil (/mm^3)	40 (20-90)	Basophilia	-
Platelet (/mm^3)	228,857 ± 85,127	Thrombocytopenia	3 (11.1%)
3rd day creatinine level (mg/dL)	0.64 ± 0.25	High creatinine (3rd day)	2 (7.4%)
7th day creatinine level (mg/dL)	0.47 ± 0.24	High creatinine (7th day)	1 (3.7%)
1st day potassium level (mmol/L)	5.3 ± 0.79	1st day hyperkalemia	5 (18.5%)
3rd day potassium level (mmol/L)	4.7 ± 0.56	3rd day hyperkalemia	-
7th day potassium level (mmol/L)	4.6 ± 0.79	7th day hyperkalemia	-

Complete blood count parameters were checked for 25 patients. On the first day, potassium level was checked for 23 patients. On the third day, creatinine and potassium levels were checked for 19 patients, and on the seventh day, potassium and creatinine levels were checked for 11 patients.

Discussion

After successful organ transplantation, the number of women taking immunosuppressive drugs and becoming pregnant have increased, along with the number of infants exposed to these drugs. However, data on the short- and long-term effects of immunosuppressives on these infants are limited. According to the findings of this study, 74% of these infants were born early, 67% had low-birth weight, and all were delivered via cesarean section. The morbidities found in these infants were related to prematurity. In the early period, lymphopenia was found in 37%, neutropenia in 25.9%, thrombocytopenia in 11.1%, hyperkalemia in 18.5%, and creatinine elevation in 3.7%, all of which returned to normal within a few days.

According to various studies, the rate of preterm birth after organ transplantation ranges from 29% to 86%.[3,13,14] Preterm birth is more common among infants born to mothers who have had a kidney transplant than in infants born to mothers who have had a liver transplant.[15] The occurrence of morbidities, such as preeclampsia, hypertension, renal failure, rejection, infection, and postpregnancy graft loss, in mothers who have undergone kidney transplantation may be the explanation for this.[3] Another study found that increasing the time between transplantation and conception (>5 yr) increased the probability of preterm birth from 55% to 85% while decreasing the average gestational week from 36 to 34 weeks.[16] In this study, we found that the premature birth rate was 81.8% in infants born to mothers who underwent kidney transplants and 40% in infants born to mothers who underwent liver transplants. The proportion of patients with an interval of more than 5 years between transplantation and first live birth was 59%. The results were similar to those reported in the literature regarding prematurity.

Immunosuppressives used during pregnancy have varying placental transfer and fetal effects. Although the pharmacokinetic parameters of corticosteroids differ depending on the agent because the placenta metabolizes 90% of the administered dose (11-beta-hydroxysteroid dehydrogenase), very low doses pass to the fetus, and fetal exposure remains relatively low.[1,2,6] Tacrolimus enters the placenta (at approximately 70% of the maternal concentration), but fetal exposure is reduced by placental expression of glycoprotein P, a transporter that returns the drug to the maternal circulation.[1,5] Tacrolimus has been reported to cause reversible neonatal hyperkalemia, renal impairment, intrauterine growth restriction, and premature delivery

as a result of hypertension, preeclampsia, and premature membrane rupture.[5] Borek-Dzieciol et al. observed that only 10% of the study group (40 infants of mothers who had undergone kidney transplantation and 40 control patients) developed hyperkalemia.[17] Creatinine levels did not increase significantly. Another study compared the infants of mothers who underwent liver transplants with a control group. The results showed no difference in blood urea nitrogen and creatinine levels between the two groups. Additionally, only 5.9% of patients who underwent liver transplantation had increased creatinine levels, which was comparable with the control group.[18] Because of the toxic effects of tacrolimus, it is critical to maintain normal levels in the blood during pregnancy. However, a case of transient acute kidney injury occurred despite the mother's blood tacrolimus level being within the normal range.[19] This study found that 7.4% and 3.7% of infants had increased creatinine levels on the third and seventh days of life, respectively. In 18.5% of infants, hyperkalemia was observed on the first day of life, but these values normalized throughout follow-up. Although the creatinine level was comparable with other studies, the higher incidence of hyperkalemia in this study could be attributed to the higher number of preterm births. During the first days of life, urine output of the patients was normal. There was no relationship between the mother's average blood tacrolimus level during pregnancy and renal parameters.

Azathioprine metabolism is a complex process that produces several metabolites. However, azathioprine and its first metabolite have a low transplacental passage, accounting for only 1–5% and 1–2% of the maternal levels, respectively. In addition, the fetal liver does not synthesize the enzyme necessary for its activation. Only 6-thioguanine nucleotides, known as toxic to the blood, can pass the placental barrier, accounting for 22–91% of the maternal concentration.[1,2,20] Azathioprine and its metabolites are unlikely to increase the prevalence of congenital anomalies, although they may cause anemia, leukopenia, and thrombocytopenia due to myelosuppression.[21,22] Another study found that the number of T and B cells at birth was lower in patients taking azathioprine than in the control group.[23] Previous studies suggest that exposure to immunosuppressive drugs, particularly azathioprine, during pregnancy may increase the incidence of infections during the first year of life.[24] According to the findings of this study, 37% of the patients had lymphopenia, 25.9% had neutropenia, and 11.1% had thrombocytopenia. Furthermore, 14.8% of individuals admitted to the hospital had culture-proven sepsis.

Prematurity increases the risks of neonatal respiratory conditions (e.g., respiratory distress syndrome and bronchopulmonary dysplasia), necrotizing enterocolitis, patent ductus arteriosus, sepsis, and neurological conditions (e.g., periventricular leukomalacia, seizures, intraventricular hemorrhage, and hypoxic-ischemic encephalopathy).[25] In this study, morbidities found in infants were mainly related to premature birth, such as respiratory distress syndrome (44.4%), bronchopulmonary dysplasia (18.5%), necrotizing enterocolitis (3.7%), and retinopathy of prematurity (11.1%).

There are few studies on the long-term effects of intrauterine exposure to immunosuppressive drugs in children. No significant medical or developmental effects were found in these studies, which focused on general developmental factors such as weight and height, immune function, renal and cardiovascular outcomes, and neurocognitive and behavioral development.[14,23,26,27] There are some limitations to the current study, such as the lack of a control group, the small sample size and the lack of long-term follow-up of the patients. Prospective studies with larger sample sizes and long-term follow-up are needed to establish the follow-up parameters and duration for these patients.

It is noteworthy that the rates of prematurity, low-birth weight, and cesarean section in infants born to mothers who underwent organ

transplants were found to be high, which is consistent with previous studies. Transient hematological and renal disorders were also found in the first few days of life. However, we believe that this situation is caused by a combination of many factors, including prematurity, low-birth weight, cesarean section, maternal morbidities, and medications.

Ethical approval

The study was approved by the Akdeniz University Ethics Committee (22.03.2023/269).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KC, SA, BA, HO; data collection: KC, ZK, NOZ; analysis and interpretation of results: KC, SA, ZK, BA, HO; draft manuscript preparation: BA, HO, KC. 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

Comparison of prematurity-related outcomes and complications in very low birth weight (VLBW) neonates fed with mother's own milk versus donor milk: a comparative study

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ABSTRACT

Background. When mother's own milk (MOM) is unavailable or insufficient, donor milk (DM) from a human milk bank serves as an alternative feeding option. Our study sought to investigate and compare the outcomes and complications of very low birth weight (VLBW) preterm infants who receive MOM versus DM.

Methods. In this retrospective cohort study conducted between 2018 and 2022, we compared 70 VLBW preterm infants exclusively fed with DM to 70 randomly selected counterparts fed with MOM. Both groups began enteral feeding within 72 hours of birth. Various clinical outcomes were investigated during a three-month follow-up. The clinical outcomes were compared via independent t-tests, Mann-Whitney U, and Fisher's exact test.

Results. The mean gestational age of the infants who were included was 29.6 ± 1.6 weeks, 84 (60%) were males, and the average birth weight was 1217 ± 151 grams. Both groups had similar baseline characteristics. The results of the study demonstrated no statistically significant differences between the groups in terms of hospital length of stay (37±16.3 days in MOM vs 40.3±16.9 days in DM group, P= 0.17), growth rate (13±4 gram/day in MOM vs 13±4 gram/day in DM group, P=0.51), growth velocity (9.8±3.0g/kg/d in MOM vs 9.5±3.2 g/kg/d in DM group), infants with in-hospital vomiting (51 cases in MOM vs 59 cases in DM group, P=0.15),vomiting frequency (1.3±1.1 times in MOM vs 1.5±1.0 times in DM group), incidence of retinopathy of prematurity (ROP) (4 cases in MOM vs 5 cases in DM group, P>0.999) and incidence of bronchopulmonary dysplasia (BPD) (7 cases in MOM vs 6 cases in DM group, P>0.999).

Conclusion. Our study findings indicate that the utilization of DM didn't have a substantial negative impact on infants' outcomes nor any complications in comparison with MOM.

Key words: mother's own milk, donor milk, very low birth weight, preterm infants.

Very low birth weight (VLBW) infants are defined as newborns with a birth weight below 1500 grams as a result of preterm delivery. It is estimated that the prevalence of this condition is approximately 15-20% of all births, or over 20 million infants annually. Low- and middle-income nations bear a disproportionate burden on this condition. Prematurity pose long-term complications for these infants, including respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), and retinopathy of prematurity (ROP). The management of premature VLBW infants involves addressing their unique nutritional needs. Premature VLBW infants require specialized feeding strategies and carefully balanced nutrition to support their growth and

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promote organ development while mitigating the risk of these complications.[1-4]

Human milk holds immense significance as the optimal source of nourishment for both term and preterm infants. It contains a wide range of essential nutrients and bioactive components, such as immunoglobulines, enzymes, growth factors, lysozyme, nucleotides, antioxidants, hormones, lactoferrin, and cellular components, which play a crucial role in regulating the immune system and supporting the development of preterm infant. Feeding infants with human milk is associated with a wide range of advantages, contributing to improved short-term and long-term health outcomes.[5,6]

When a mother's own milk (MOM) is unavailable or insufficient, donor milk (DM) from a human milk bank becomes a viable alternative feeding option for premature VLBW infants. Although the pasteurization process of DM may inactivate certain components, such as growth factors, hormones, human milk oligosaccharides, immunological factors, and beneficial microbes, it still provides documented advantages over formula feeding.[5-7]

Multiple studies have consistently shown favorable outcomes when comparing feeding with MOM or DM to formula feeding. However, there is a relative scarcity of studies directly comparing the exclusive use of MOM to DM. Our study has a large sample size to investigate and compare the outcomes and complications of VLBW preterm infants who receive exclusively MOM versus DM. The study's findings empower healthcare professionals to make informed decisions and provide appropriate guidance to parents regarding the optimal feeding choice for their infants.

Methods

Study design

Our retrospective cohort study aims to compare the impact of feeding with MOM and DM on the growth rate and clinical outcomes of preterm (born before 37 weeks of gestation) VLBW infants. We conducted a comparison between two groups of 70 VLBW preterm infants each, born in the Akbarabadi Children's Hospital Newborn Intensive Care Unit (NICU) between 2018 and 2022. One group was exclusively fed DM, while the other group, selected randomly, received MOM while enteral feeding for both groups commenced within 72 hours of birth. The exclusion criteria encompassed multiple births and infants with enteral feeding abnormalities that would hinder the use of MOM or DM after three days of birth, and infants who received a combination of MOM and DM (or did not exclusively receive MOM and DM). Additionally, infants with specific medical conditions or birth defects, and small for gestational age (SGA) infants, were also excluded.

Feeding protocol

The recruited infants were initially provided with parenteral nutrition. After three days, enteral feeding was initiated. The enteral feeding volume was gradually increased over time, aiming to reach a target of 20 mL per kilogram per day. The DM utilized in this study was obtained from a human milk bank and underwent pasteurization. The same fortifier was utilized in both groups. Once the milk volume reached 50 mL/kg/ d, human milk fortifiers (HMF) were added to the milk in both groups. The fortification process followed the manufacturers' recommendation, with 1 sachet added to 25 mL of milk.

Data collection and outcome measures

The primary outcomes of interest in this study include the rate of growth (calculated from birth to discharge), growth velocity, in-hospital vomiting occurrences, the frequency of vomiting, incidences of necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), and retinopathy of prematurity (ROP). They were investigated through a three-month follow-up. In this study, we defined NEC as stage II or stage III according to Bell's classification.[8]

The growth velocity (g/kg/d) was determined using the exponential model (EM) approach, computed as [1000 x ln (discharge weight/birth weight)] / length of hospital stay, where ln represents the natural logarithm, and weights are measured in grams, with the length of hospital stay expressed in days.[9] In our study the occurrence of vomiting in infants, the frequency of vomiting during entire hospitalization was collected from medical records . As per routine, a 24-hour period of NPO (nothing by mouth) was implemented for infants who experienced vomiting before resuming feeding. The perinatal extension component of the Score for Neonatal Acute Physiology with Perinatal Extension II (SNAPPE-II) is utilized to assess the clinical severity of newborns in the NICU. This component incorporates three key elements: birth weight, size relative to gestational age, and the Apgar score at 5 minutes.[10]

Ethics approval

The study complies with the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committees of Iran University of Medical Sciences, Tehran, Iran (IR.IUMS.FMD.REC.1401.244).

Statistical analysis

The statistical analysis was conducted using SPSS version 26. The normality of continuous variables was assessed using the Shapiro-Wilk test. For normally distributed data, independent t-tests were used for between-group comparisons, while the Mann-Whitney U test was utilized for non-normally distributed variables. Categorical variables were analyzed using Fisher's exact test. For all tests, statistical significance was set at $p \leq 0.05$.

Results

Clinical characteristics

The mean gestational age was 29.6 ± 1.6 weeks, and 84 (60%) infants were male. The average birth weight of the infants was 1217 ± 151 grams. 120 (85.7%) infants were delivered via cesarean section. Their mean Apgar scores at 1 and 5 minutes were 6 ± 1 and 8 ± 1 respectively. Umbilical cord arterial blood gas (ABG) revealed a mean base deficit of 4.0 ± 2.3. Additionally, 104 (74.3%) required invasive ventilation in the early days of their lives and the overall mean duration of oxygen therapy was found to be 11.33 ± 8.8 days. The mean SNAPPE-II score was 2.1± 5.4. Table I, provides a summary of the baseline

Table I. Baseline characteristics of the infants.

	MOM group (n=70)	DM group (n=70)	P value
Gestational age, week	29.7±1.5	29.6±1.8	0.72
Male, n (%)	45 (63.8%)	39 (56.3%)	0.39
Birth weight, gram	1218±166	1217±137	0.73
Cesarean delivery, n (%)	58 (82.6%)	62 (88.7%)	0.47
Received surfactant, n (%)	53 (75.8%)	49 (70%)	0.57
Apgar score—1 min	6±2	6±1	0.70
Apgar score—5 min	8±1	8±1	0.10
Base deficit, mEq/L	4.1±2.3	3.8±2.3	0.37
Invasive ventilation, n (%)	53 (75.8%)	51 (72.8%)	0.84
Total O_2 therapy duration, d	11.2±8.7	11.5±9.1	0.94
SNAPPE-II	2.1±5.3	2.1±5.7	0.87

Values are presented as means ± SDs (*P* value from independent t test or Mann-Whitney U), or frequencies (n) and percentages (%) (*P* value from Fisher's exact test). DM: donor human milk; MOM: mother's own milk. O_2: Oxygen.

characteristics of the infants in each group. The results indicate that there were no statistically significant differences between the two groups regarding their baseline characteristics.

Clinical outcomes

The infants who were included in the study were followed up during the study period in order to evaluate the effect of receiving different types of nutrition on various outcomes such as hospital length of stay, growth rates, growth velocity, vomiting and incidence of necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and bronchopulmonary dysplasia (BPD).

The mean hospital length of stay for the MOM group was 37.0±16.3 days, while for the DM group, it was 40.3±16.9 days. There was no statistically significant difference in hospital stay length between the two groups (P= 0.17). The mean daily weight gain, measured as growth rate, was 13.1±3.9 and 12.6±4.0 gram/day in MOM and DM groups, respectively. There was no statistically significant difference observed between the two groups (P=0.51). In the context of in-hospital vomiting episodes, 82.8% (59 cases) of infants in the DM group and 72.8% (51 cases) in the MOM group experienced vomiting. Also, when considering the frequency of vomiting, with a rate of 1.52±0.94 times in the DM group versus 1.33±1.09 times in the MOM group, no statistically significant differences were observed between the two groups (P=0.15 and P=0.17 respectively). NEC was not developed in any of the cases in study. About the incidence of ROP, 4 (5.7%) infants in the MOM group and 5 (7.1%) infants in the DM group developed ROP grade 2 or 3, suggesting a similar rate of occurrence in both groups (P>0.999). No occurrences of ROP (grade 4 or 5) were observed in either group. Similarly, the incidence of BPD was almost identical in both the MOM and DM groups, with 7 (10%) and 6(8.6%) of cases, respectively (P > 0.999). Table II summarizes the clinical outcome information in each group. There was no mortality observed in either of the groups.

Discussion

In this cohort of premature VLBW infants, we evaluated the relationships between the source of human milk (mother or donor) with postnatal growth and prematurity-related outcomes and complications. The results showed that the cohorts were matched for the demographic and baseline characteristics. Our study directly compared clinical outcomes in VLBW infants fed exclusively MOM with those fed exclusively DM in the setting of an exclusively human milk diet (without formula milk), during a three-month follow-up. Our findings indicated that there was no significant difference in the hospital length of stay between the two groups, which is consistent with previously published

Table II. Clinical outcomes of the infants.

	MOM group (n=70)	DM group (n=70)	P value
Length of hospital stay, day	37±16.3	40.3±16.9	0.17
Growth rate, gram/day	13.07±3.9	12.63±4	0.51
Growth velocity, gram/kg/day	9.84±3.01	9.47±3.22	0.34
Infants experienced vomiting, n (%)	51 (72.8%)	59 (82.8%)	0.15
Vomiting frequency during entire hospitalization	1.33±1.09	1.52±0.94	0.17
NEC, n (%)	0	0	> 0.999
ROP, n (%)	4 (5.7%)	5 (7.1%)	> 0.999
BPD, n (%)	7 (10%)	6 (8.6%)	> 0.999

Values are presented as means ± SDs, (independent t test or Mann-Whitney U) or as frequencies (n) and percentages (%) (Fisher's exact test). DM, donor human milk; MOM, mother's own milk; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia.

studies.[7,11] This suggests that feeding with DM does not prolong the hospital stay for VLBW infants.

Approximately half of pediatric mortality occurs during the neonatal stage, with low birth weight (LBW) being recognized as a primary contributing factor to neonatal mortality. Nearly 80% of deaths occur among VLBW infants. This subgroup of preterm and LBW neonates represents a vulnerable population, facing a greater likelihood of encountering physical and mental challenges in contrast to infants born at normal weights.[12,13] Weight gain is crucial to a newborn's growth and overall health. Our study examined the weight gain of VLBW infants who received DM versus those who received MOM. Although the DM group had a slightly lower weight gain than the MOM group, this difference was not statistically significant. These findings align with a study by Sun and colleagues where infants fed raw breast milk and those fed pasteurized human milk showed similar growth patterns.[11] The duration of follow-up in Sun's and our study was relatively long compared to other studies[7,14,15] that reported significantly higher growth rates in infants fed with unpasteurized mothers' own milk. These data suggest that DM-feeding infants may have a slower growth speed in the first 4 weeks of life, but longer follow-up will remove this effect and equalize the growth rate in both groups. In a study conducted by Montjaux-Régis et al. in 2011, three distinct cohorts of infants were compared. These cohorts comprised infants who received less than 20% of their required intake of MOM, infants who received between 20% and less than 80% of their MOM, and infants who received at least 80% of their MOM. The findings revealed that infants who were fed MOM demonstrated greater weight gain in comparison to infants who were fed DM. However, no discernible disparity in linear growth was observed among the three aforementioned groups.[16] In a similar study, Alizadeh et al. recently reported no statistically significant differences in weight gain velocity between DM and MOM groups.[17]

The results demonstrated no significant difference in the incidence of in hospital vomiting among infants. It is important to note that our definition of feeding intolerance focuses on the vomiting of consumed food, while the general well-being of the individual remains satisfactory, and there are no indications of abdominal distention. To manage the situation, we simply need to withhold one or two meals from the patient's diet. Ford et al. employed varying definitions of feeding intolerance in their study. Notably, no statistically significant distinctions were observed regarding the duration required to achieve the ultimate enteral feed volume or the duration of parenteral nutrition across the different definitions. However, it was found that the number of feeds withheld per day and the duration of NPO subsequent to the initiation of feeds were greater in the DM group.[7]

The incidence of complications, including NEC, ROP, and BPD were also examined in our study. We found no incidence of NEC in either the DM or MOM group. This aligns with previous research that showed no significant difference in NEC incidence when comparing unpasteurized and pasteurized human milk.[18,19] Similarly, the incidence of ROP did not differ between the two groups, consistent with other studies that compared unpasteurized and pasteurized human milk.[5] Furthermore, we observed no significant difference in the incidence of BPD between the DM and MOM groups, which is in line with previous studies.[7,15,19,20]

This study demonstrates the non-inferiority of DM compared to MOM in preventing complications such as BPD, NEC, ROP, and feeding intolerance in VLBW infants. While maintaining an adequate supply of MOM can be challenging for mothers of VLBW infants, our findings suggest that DM is a suitable alternative when MOM is unavailable or contraindicated. Previous studies have also indicated the superiority of DM over preterm formula in reducing complications.[19,20]

The strength of our study was the matched baseline characteristics and equal number of cohort groups which provided the statistical power to determine relationships between DM and the outcomes. Another important strength of this study is that the DM group includes infants exclusively fed with donor bank milk throughout their hospitalization period. However, it is also important to consider the limitations. Our study was retrospective in design. As such, we were unable to account for potential confounding variables and other sources of bias. Additionally, randomization was not possible because it is not acceptable to not give MOM when available. We also did not take stool exams to evaluate how source of human milk could be associated with gut microbiota diversity. We did not explore differences in the onset of sepsis, both early and late, between the two groups. Additionally, neurodevelopmental outcomes such as head circumference were not assessed. Lastly, our study did not evaluate the volume of gastric residue in the reported vomiting incidents. Further research is recommended to extend the follow-up period and evaluate the long-term effects of feeding type on later growth.

Our study findings show that DM is an effective alternative to MOM for feeding VLBW newborns. Both DM and MOM showed similar outcomes in terms of hospital length of stay, weight gain, vomiting, and the incidence of complications. Whenever the MOM is unavailable, the education and support for using pasteurized and appropriately fortified DM should be prioritized in the care of preterm infants. Future studies with extended follow-up periods are recommended to assess the effects of feeding type on long-term growth.

Ethical approval

The study complies with the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committees of Iran University of Medical Sciences, Tehran, Iran (IR.IUMS.FMD.REC.1401.244).

Author contribution

The authors contributed to this article as follows: NS and ZV designed the research; MK, AA, HZ, and MS conducted the research; AA and HZ analyzed the data; MK, AA, and HZ wrote the first draft of the manuscript; and all authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Comparison of the adolescent pregnancy outcomes between refugees and Turkish citizens

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ABSTRACT

Background. Adolescent pregnant women have significant risk factors in terms of preterm birth, low birth weight, gestational and neonatal complications, and neonatal and infant deaths. In many countries, living as a refugee differs from living as a local citizen regarding education level, access to health services, and lifestyle. We aimed to compare the obstetric, perinatal, and neonatal outcomes of Turkish and refugee adolescent pregnant women admitted to a tertiary maternity center.

Methods. The study was planned as a retrospective cross-sectional. We included adolescent pregnant women who delivered between February 2018 and August 2023. Adolescent pregnant women were divided into two groups, the Turkish group and the Syrian refugee group, and compared with each other.

Results. One thousand and fifty-one Turkish and 742 refugee adolescent pregnant women were included in the study. Adolescent pregnancy rates are higher in refugees than in the Turkish group ($p < 0.001$). We found that maternal age ($p < 0.001$), preeclampsia rates ($p=0.029$), gestational age at delivery ($p < 0.001$), and cesarean delivery rates ($p=0.02$) were lower in refugee adolescent pregnant women. Furthermore, we found that the anemia rates ($p < 0.001$) and low birth weight newborn rates ($p = 0.011$) were higher in refugee adolescent pregnant women.

Conclusions. Enhancing the outcomes of adolescent pregnancies among refugees necessitates a heightened focus on education regarding sexual reproduction, increased prenatal follow-ups, and enhanced training in family planning.

Key words: adolescent pregnancy, immigrants, obstetric outcome, refugees.

Each year, 12 million adolescents give birth in developing countries.[1] In 2023, the adolescent fertility rate was 4.13% worldwide, and this rate was 1.46% in Turkey.[2] Adolescent pregnant women are less likely to access early and adequate prenatal care than adults.[3] In general, adolescents seek antenatal care late due to lack of information, limited access to health services, social pressure, fear of stigma, or all of these factors.[4] Adolescent pregnancies constitute a significant risk for many adverse outcomes, including preterm birth, low birth weight, gestational and neonatal complications, and neonatal and infant deaths.[5]

Pregnancy outcomes are riskier for pregnant refugee women due to inadequate shelter and nutrition, security problems, stress, barriers to accessing health services, and difficulty in accessing prenatal care.[6]

In March 2011, when the civil war broke out in Syria, the mass migration to Turkey began. As of June 2023, Turkey hosts the world's largest refugee population, with approximately 3.7 million under temporary protection: Syrians, refugees, and asylum seekers of other nationalities.[7] About half (47.56%) of the refugees are women, and the rate of adolescent girls between the ages of 10-18 is 18.77% among

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the refugee women. Since 2014, Turkey has continued to host the most refugees worldwide. The rate of refugee babies born in Turkey has been over 750,000 since 2011. Istanbul is a metropolitan city in Turkey with a population of 16 million. The most significant number of refugees, with 532,236 individuals, live in Istanbul, and the rate of refugees living in the city is 14.37% of the refugees in the country.[8,9] Sixteen percent of the refugees in Istanbul are adolescent girls between the ages of 10-18.[10]

This study aimed to compare Turkish adolescent pregnant and refugee adolescent pregnant women in terms of obstetric, perinatal, and neonatal outcomes.

Materials and Methods

Our maternity center had 26,364 births between February 2018 and August 2023. Among these, 22,403 births belonged to the Turkish group, while 3,961 births were from the refugee group. Notably, 1,792 of these births were teenagers. Among the Turkish group, 1051 out of 22,403 births (4.69%) were from adolescents, while in the refugee group, 742 out of 3961 births (18.7%) were from adolescents. We included adolescents between 14 and 19 who gave birth in our clinic. Because there were no pregnant adolescents who gave birth younger than the age of 14 in our hospital. Adolescent pregnant women were divided into two groups, the Turkish group, and the Syrian refugee groups, and compared with each other. All 1793 adolescents (1051 Turkish adolescent pregnant women and 742 refugee adolescent pregnant women) were included in the study. We accessed the pregnant women's data from the hospital database electronic records. Pregnant women aged 20 and over, births before 20 weeks of gestation, and pregnant women of non-Turkish or Syrian nationality were not included in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. During the data collection phase, the adolescents' identification numbers were anonymized. Informed consent is not required due to the type of research. The maternal outcomes were identified as age, gravida, parity, abortion, chronic diseases, coronavirus disease (COVID-19) history, hepatitis B positivity, chronic hypertension, gestational hypertension, preeclampsia, hemolysis elevated liver enzymes low platelet (HELLP) syndrome, eclampsia, gestational diabetes mellitus (GDM) and cholestasis of pregnancy. The perinatal outcomes were designated as gestational week at delivery, preterm labor, premature rupture of membranes, intrauterine growth restriction (IUGR), small for gestational age (SGA) infant, large for gestational age (LGA), macrosomia, oligohydramnios, spontaneous vaginal delivery, instrumental delivery (only vacuum-assisted vaginal delivery), cesarean delivery and indication, perineal laceration, placental abruption, stillbirth, maternal complications, puerperal complications, maternal anemia, prepartum and postpartum hemoglobin values, postpartum hemorrhage, postpartum need for blood transfusion and postpartum intravenous iron treatment. The neonatal outcomes included birth weight, newborn first and fifth-minute APGAR scores, newborn intensive care unit (NICU) needs and indications, newborn complications, newborn congenital anomalies, and neonatal death. Approval for the study was received from the ethics committee of the Sancaktepe Sehit Prof. Dr. İlhan Varank Training and Research Hospital (approval number: 2023/166).

Statistical analysis

Data were statistically analyzed using International Business Machines (IBM, Armonk, NY, USA) Statistical Package for the Social Sciences (SPSS) Statistics for Windows v.20.0 (IBM Corp.). Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used when evaluating the study data. The normal distribution of quantitative data was checked using the Kolmogorov-Smirnov test and graphics. If the variables were parametric, a Student t-test was used. If the variables were

non-parametric, the Mann-Whitney U test was used. Pearson chi-square test and Fisher's exact test were used to compare qualitative data. A p-value < 0.05 was considered statistically significant.

Results

There were 26,364 births in our maternity center between February 2018 and August 2023. In our study, 1,051 (4.69%) of 22,403 births were of the adolescent period in the Turkish group, and 742 (18.7%) of 3,961 births were of the adolescent period in the refugee group.

In our study, with 1,793 adolescent pregnant women, the mean age of refugee pregnant women was 17.96 ± 1.07 years, and the mean age of the Turkish group was 18.36 ± 0.83 years. The mean age of the refugee adolescent group was lower and statistically significant (p < 0.001). Gravida and parity were higher in the refugee adolescent pregnant group, but abortion numbers were higher in the Turkish group. These differences are statistically significant (p < 0.001). Chronic disease (p < 0.001), gestational hypertension (p = 0.009), and preeclampsia (p = 0.029) rates were significantly higher in the Turkish group. The two groups were similar in terms of the history of COVID-19 (p = 0.482), hepatitis B positivity (p = 0.061), chronic hypertension (p = 0.414), eclampsia (p = 0.515), HELLP syndrome (p = 1.000), GDM (p = 1.000) and cholestasis of pregnancy (p = 0.655) (Table I).

The gestational age at delivery was 38.94 ± 1.80 weeks in the Turkish group and 38.44 ± 1.69 weeks in the refugee adolescent group. The gestational age at delivery was significantly lower in the refugee adolescent pregnant group (p < 0.001). The rate of spontaneous vaginal delivery (p < 0.001) was significantly higher in the refugee adolescent pregnant group, and the rates of cesarean delivery (p = 0.02) and instrumental delivery (p = 0.013) were significantly higher in the Turkish group. Oligohydramnios (p = 0.038) and postpartum intravenous iron treatment (p = 0.043) were significantly higher in the Turkish group. Episiotomy (p < 0.001) was significantly higher, and perineal tear (p < 0.001) was significantly lower in the Turkish

Table I. Maternal clinical characteristic of Turkish and refugee pregnant adolescents.

	Turkish citizens (n=1051)	Refugees (n=742)	p
Maternal age (years)	18.36 ± 0.83	17.96 ± 1.07	**<0.001**
Gravida	1 (1-5)	1 (1-6)	**<0.001**
Parity	0 (0-2)	0 (0-3)	**<0.001**
Nulliparous women	836 (79.5)	451 (60.8)	**<0.001**
Multiparous women	215 (20.5)	291 (39.2)	**<0.001**
Previous induced abortions	0.08 ± 0.31	0.04 ± 0.29	**0.009**
Chronic disease	42 (4)	7 (0.9)	**<0.001**
History of COVID 19	6 (0.6)	2 (0.3)	0.482
Hepatitis B positivity	10 (1)	1 (0.1)	0.061
Chronic hypertension	0 (0)	1 (0.1)	0.414
Gestational hypertension	13 (1.2)	1 (0.1)	**0.009**
Preeclampsia	20 (1.9)	5 (0.7)	**0.029**
Eclampsia	2 (0.2)	0 (0)	0.515
HELLP syndrome	1 (0.1)	0 (0)	1.000
Gestational diabetes mellitus	4 (0.4)	2 (0.3)	1.000
Cholestasis of pregnancy	4 (0.4)	1 (0.1)	0.655

Values are presented mean ± SD, median (range) and n (%).
COVID 19: Coronavirus disease, HELLP: hemolysis elevated liver enzymes low platelet.

group than in the refugee group. In addition, the maternal anemia rate was significantly higher in the refugee group than in the Turkish group (p < 0.001). There was no statistically significant difference between the two groups in preterm labor (p = 0.412), premature rupture of membranes (p = 0.474), placental abruption (p = 1.000), maternal complications (p = 0.057), puerperal complications (p = 0.221), pre and postpartum hemoglobin (p = 0.394, p = 0.059 respectively) postpartum hemorrhage (p = 0.858), postpartum blood transfusion (p = 0.858) (Table II).

The mean birth weight in the Turkish group was 3177.19 ± 491.25 grams, and 3101.17 ± 466.85 grams in the refugee group. Birth weight is statistically significantly lower in the refugee group (p = 0.001). The two groups were similar

Table II. Perinatal clinical characteristic of Turkish and refugee pregnant adolescents.

	Turkish citizens (n=1051)	Refugees (n=742)	p
Gestational week at delivery	38.94 ± 1.80	38.44 ± 1.69	**<0.001**
Preterm labor	71 (6.8)	43 (5.8)	0.412
Premature rupture of membranes	53 (5)	35 (4.3)	0.474
Oligohydramnios	66 (6.3)	30 (4)	**0.038**
Spontaneous vaginal delivery	850 (80.9)	647 (87.2)	**<0.001**
Instrumental delivery	12 (1.1)	1 (0.1)	**0.013**
Cesarean delivery	189 (18)	94 (12.7)	**0.02**
Placental abruption	3 (0.3)	2 (0.3)	1.000
Perineal wound*	826 (95.8)	588 (90.7)	**<0.001**
Episiotomy	636 (73.8)	405 (62.5)	**<0.001**
Perineal tears	101 (11.7)	131 (20.2)	**<0.001**
Episiotomy + Perineal tears	89 (10.3)	52 (8)	0.128
Maternal complication	12 (1.1)	17 (2.3)	0.057
Atony of the uterus	4 (0.4)	7 (0.9)	
Retained placenta	2 (0.2)	7 (0.9)	
Cervical laceration	2 (0.2)	2 (0.3)	
Vaginal hematoma	2 (0.2)	0 (0)	
OASIS	2 (0.2)	1 (0.1)	
Puerperal complication	17 (1.6)	7 (0.9)	0.221
Episiotomy dehiscence	8 (0.8)	5 (0.7)	
Hemorrhoids	4 (0.4)	0 (0)	
Endometritis	2 (0.2)	2 (0.3)	
Anal fissures	1 (0.1)	0 (0)	
Skin incision dehiscence	2 (0.2)	0 (0)	
Maternal anemia (Hemoglobin < 11g/dl)	250 (23.8)	249 (33.6)	**<0.001**
Prepartum hemoglobin (g/dl)	11.53 ± 1.31	11.47 ± 1.45	0.394
Postpartum hemoglobin (g/dl)	10.38 ± 1.40	10.53 ± 1.46	0.059
Postpartum hemorrhage	20 (1.9)	15 (2)	0.858
Postpartum intravenous iron treatment	89 (8.5)	44 (5.9)	**0.043**
Postpartum blood transfusion	20 (1.9)	15 (2)	0.858
Maternal death	0 (0)	0 (0)	-

*In women who had vaginal delivery.
Values are presented mean ± SD, and n (%).
OASIS: Obstetric anal sphincter injuries.

in terms of IUGR (p = 0.197), SGA (p = 0.148), LGA (p = 0.501), macrosomia (p = 0.135), stillbirth (p = 0.537), very low birth weight (p = 1.000), extremely low birth weight (p = 0.696), first and fifth minute APGAR score (p = 0.197, p = 0.316 respectively), need for NICU (p = 0.608), newborn complications (p = 0.466), newborn congenital anomalies (p = 0.516) (Table III).

We evaluated the indications for cesarean delivery: previous cesarean section (p < 0.001) was significantly more prevalent, whereas macrosomia (p = 0.014) and arrested labor (p = 0.04) were significantly less prevalent in the refugee group compared to the Turkish group. There was no statistically significant difference between the groups among other cesarean delivery indications (Table IV). There were no maternal or neonatal deaths during the study period.

Discussion

The adolescent birth rate was about 7% of all births in the hospital. In the refugee adolescent group, maternal anemia, perineal tears, and low birth weight were higher than in the

Table III. Fetal and newborn clinical characteristic of Turkish and refugee pregnant adolescents.

	Turkish citizens (n=1051)	Refugees (n=742)	p
Intrauterine growth restriction	45 (4.3)	23 (3.1)	0.197
Small for gestational age (3-10th percentile)	134 (12.7)	78 (10.5)	0.148
Large for gestational age (90-95th percentile)	20 (1.9)	11 (1.5)	0.501
Macrosomia (>95th percentile)	15 (1.4)	5 (0.7)	0.135
Stillbirth	7 (0.7)	3 (0.4)	0.537
Birth weight (grams)	3177.19 ± 491.25	3101.17 ± 466.85	**0.001**
Low birth weight (1500-2500 grams)	47 (4.5)	54 (7.3)	**0.011**
Very low birth weight (1000-1500 grams)	4 (0.4)	3 (0.4)	1.000
Extremely low birth weight (<1000g)	3 (0.3)	3 (0.4)	0.696
First minute APGAR	7.79 ± 0.85	7.83 ± 0.73	0.197
Fifth minute APGAR	8.87 ± 0.78	8.9 ± 0.64	0.316
Need for neonatal intensive care	100 (9.6)	66 (8.9)	0.608
Respiratory failure	63 (6)	50 (6.7)	
Sepsis	27 (2.6)	15 (2)	
Hypoglycemia	10 (1)	1 (0.1)	
Newborn complications	33 (3.2)	28 (3.8)	0.466
Clavicle fracture	5 (0.5)	2 (0.3)	
Caput succedaneum	24 (2.3)	25 (3.4)	
Cephal hematoma	2 (0.2)	1 (0.1)	
Over riding	2 (0.2)	0 (0)	
Newborn congenital anomalies	35 (3.3)	29 (3.9)	0.516
Cardiovascular	4 (0.4)	4 (0.5)	
Skin-skeleton	12 (1.1)	9 (1.2)	
Gastrointestinal	3 (0.3)	0 (0)	
Orofacial	5 (0.5)	5 (0.7)	
Genitourinary	11 (1)	10 (1.3)	
Cerebrospinal cord	0 (0)	1 (0.1)	
Neonatal death	0 (0)	0 (0)	-

Values are presented mean ± SD, and n (%).
APGAR: Activity Pulse Grimace Appearance Respiration.

Table IV. The distribution of the indications in the in the cesarean section pregnant women.

	Turkish citizens (n=189)	Refugee (n=94)	p
Previous cesarean section	37 (19.6)	39 (41.5)	**<0.001**
Fetal distress	53 (28)	25 (26.6)	0.798
Breech presentation	32 (16.9)	16 (17)	0.985
Transverse presentation	2 (1.1)	2 (2.1)	0.602
Footling presentation	0 (0)	1 (1.1)	0.332
Multiple pregnancy	6 (3.2)	2 (2.1)	1.000
Umbilical cord prolapse	1 (0.5)	1 (1.1)	1.000
Macrosomia	23 (12.2)	3 (3.2)	**0.014**
Cephalopelvic disproportion	6 (3.2)	0 (0)	0.183
Maternal diseases that prevent pushing	4 (2.1)	0 (0)	0.305
Severe preeclampsia	5 (2.6)	1 (1.1)	0.667
Placenta previa	0 (0)	2 (2.1)	0.110
Arrested labor	16 (8.5)	2 (2.1)	**0.040**
Placental abruption	3 (1.6)	0 (0)	0.553
Gastroschisis	1 (0.5)	0 (0)	1.000

Values are presented n (%).

Turkish group. In the Turkish group, the rates of gestational hypertension, macrosomia, and preeclampsia were higher than in the refugee group.

In our study, we detected 1051 (4.69%) adolescent pregnancies among Turkish pregnant women and 742 (18.7%) adolescent pregnancies among refugee pregnant women. Adolescent pregnancy rates were higher among the refugees. In the study conducted by Çelik et al.[11], the adolescent pregnancy rate in the refugees was 17%, and the pregnancy rate in Turkish adolescents was 3%; Aktoz et al.[12] the adolescent pregnancy rate in the refugees was 16.8%, and the pregnancy rate in Turkish adolescents was 4.3%; Vardar et al.[13] the adolescent pregnancy rate in refugees was 11%, and the pregnancy rate in Turkish adolescents was 3.9%. In our study, we found that the mean age of the refugee group was lower, and the gravida and parity were higher than those of the Turkish group. According to a study by Golbasi et al.[14], the mean age was lower, and the parity was higher in refugee adolescent pregnant women. In the study by Al Nuaimi et al.[15], the age of refugee pregnant women was lower, and the gravida was higher than that of Turkish pregnant women. In the literature, we see that, similar to our results, adolescent pregnancy rates, gravida, and parity are significantly higher, and maternal age is significantly lower in refugees. Families who are forced to leave their homes and leave their countries due to war experience economic and financial difficulties in the different countries as refugees. Parents encourage daughters to marry young to reduce household size and decrease the cost of living.[16] We think that this planning causes adolescent pregnancies among refugees at younger ages and higher rates. Refugee adolescent pregnant women who are affected by civil wars and conflicts have limited knowledge of family planning[17] and lack the health literacy necessary for contraceptive use.[18] These adverse conditions explain the higher gravida and parity among refugees. In this study, the mean of previous induced abortion in the refugee group was lower than in the Turkish group. In the study reported by Demirci et al.[19], previous induced abortions were lower among refugees. It is difficult for refugees to access sexual health clinics. The negative attitude and parental pressure are very high for abortions.[20] These factors explain the lower rates of previous induced abortions in refugee adolescent pregnant women.

In our study, we found that the rates of chronic disease, gestational hypertension, and preeclampsia were higher in the Turkish group. In the study conducted by Sayili et al.[21], the rate of chronic diseases in the Turkish group was higher than in the refugee group. In the study of Erenel et al.[22], preeclampsia rate in the control group of Turkish pregnant women was higher than in refugee pregnant women. In the study by Golbasi et al.[14], gestational hypertension was more common in Turkish adolescent pregnant women. Advanced maternal age and the first pregnancy are risk factors for preeclampsia.[23] In our study, the lower gravida and parity rates are predisposing factors for developing preeclampsia. Additionally, nutritional deficiency is common in the refugee adolescent group due to difficult living conditions. This condition causes refugees to be weak and have a lower body mass index. High body mass index increases the risk of preeclampsia.[24]

When we evaluated adolescent pregnant women according to delivery method, we found that the rate of spontaneous vaginal delivery was higher in the refugee group, and the rate of instrumental delivery and cesarean delivery was higher in the Turkish group. The results are similar to those of the study conducted by Kasoha et al.[25] In our study, we think that the higher rate of spontaneous vaginal delivery in refugee adolescent pregnant women depends on the genetic structure, ethnic origin, and socioeconomic factors of the Syrian society. However, our experience shows that Turkish adolescent pregnant women prefer cesarean delivery because their economic conditions are better, and they believe that a cesarean section is less painful. In addition to this, higher maternal age and higher maternal comorbidities such as chronic disease and preeclampsia in the Turkish group support the higher cesarean delivery rates. Our study found a higher rate of oligohydramnios in the Turkish group. In our country, the government covers the health care expenses of refugees. Health care in public hospitals is free for refugees. Although it is free, refugee adolescents do not routinely visit a health center during pregnancy. We especially think that the language barrier and sociocultural factors prevent routine health center visits. The rate of oligohydramnios is low in refugee adolescent pregnant women because antenatal care is not adequate. In this study, we found high rates of maternal anemia in refugee adolescent pregnant women. In the study reported by Turkay et al.[26], maternal anemia rates are high in refugee adolescent pregnant women. We attribute the higher rates of anemia in refugee adolescent pregnant women to malnutrition, a deficiency of necessary iron and vitamin supplements during pregnancy, and lack of knowledge about healthy eating. Moreover, adolescent pregnancy is a more critical form of nutrition for bone development and growth. Our study found higher postpartum intravenous iron treatment rates in the Turkish group. This finding may be the result of higher cesarean section rates in the Turkish group.

In this study, we found that the mean birth weight of newborns was lower, and the number of low-birth-weight newborns was higher in the refugee adolescent group. In the meta-analysis reported by Bollini et al.[27], the rate of low-birth-weight newborns in refugee pregnancies was higher than in native pregnancies. We think this leads to a higher number of low-birth-weight newborns in refugee adolescent pregnant women because of pregnancy at a young age, a high rate of anemia, and inadequate prenatal care. Although chronic diseases cause low birth weight, early diagnosis and treatment could avoid worse pregnancy outcomes. Therefore, the Turkish group may have had a lower low-birth-weight rate because of good antenatal care.

When we searched the indications for cesarean section in adolescent pregnancies, we detected that the indication for previous cesarean section is more common in the refugee group, and the indications for macrosomia and labor arrest are more common in the Turkish group. Golbasi et al.[14] showed that the indication for previous cesarean sections was higher among refugees, and the indication for labor arrest was higher

among Turkish. In this study, we found lower rates of oligohydramnios, chronic disease, gestational hypertension, and preeclampsia in refugee adolescent pregnant women. We can explain the positive results in refugee adolescent pregnant women with genetic and racial differences.

To the best of our knowledge our study is the only investigation in Istanbul, the city that hosts the most refugees in Turkey, which hosts the most refugees in the world. Additionally, in this study, we evaluated two risk groups, both adolescents and refugee groups and the large sample size is also another strength of our study. However, the present study also has some limitations which should be pointed out. Although we thought that refugees generally had fewer admittions to health centers, we do not have any data to document this. The retrospective nature of the of the study and its single-center design are also important limitations.

In conclusion, adolescent refugee pregnancies, a combination of two vulnerable groups, will increase as wars continue worldwide. In our study, we found that maternal age, rates of previous induced abortion, chronic disease, gestational hypertension, preeclampsia rates, gestational age at delivery, oligohydramnios rates, instrumental delivery, and cesarean delivery rates, postpartum intravenous iron treatment need, and newborn birth weight were lower in refugee adolescent pregnant women. Furthermore, we found that the gravida and parity, the number of spontaneous vaginal deliveries, anemia rates, and low birth weight newborn rates were higher in refugee adolescent pregnant women. To improve refugee adolescent pregnancy outcomes, sexual reproduction education, prenatal follow-ups, and family planning training should be increased. They should be adapted to business life to improve economic conditions. The number of interpreters in the hospital should be increased. Refugees should be given free courses to learn the host country's language.

Ethical approval

Approval for the study was received from the ethics committee of the Sancaktepe Sehit Prof. Dr. İlhan Varank Training and Research Hospital (approval number: 2023/166).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: OA, BG, NT; data collection: OA, BG, NT; analysis and interpretation of results: OA, BG, NT; draft manuscript preparation: OA, BG, NT. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare that there is no conflict of interest.

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Original Article

The impact of the prolonged COVID-19 pandemic on adolescents with eating disorders: a follow-up study from Türkiye

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ABSTRACT

Background. At the onset of the pandemic, we conducted a study on adolescents with eating disorders (EDs) and found no deterioration in ED symptoms. The objective of this subsequent study was to conduct a follow-up evaluation of the same cohort and investigate the consequences of the prolonged pandemic.

Methods. This longitudinal study was conducted one year after the first study between May 2021 and June 2021 with 37 adolescents aged 12-18 years (pre-existing EDs). The reassessment included an evaluation of sociodemographic and clinical characteristics, the impact of pandemic-related restrictions on ED behaviors, well-being, and quality of life. All the participants underwent a re-administration of the ED examination questionnaire (EDE-Q), Beck Depression Inventory, the State Anxiety Inventory for Children, and the Maudsley Obsessive Compulsive Inventory.

Results. No significant difference was observed in the EDE-Q scores or the ED examination questionnaire scores between the initial (T1) and subsequent (T2) study. The ED-related quality of life was seen to have slightly improved in the later stage. While depression (T1: 18, T2: 15, p=0.883) and obsession scores (T1: 11, T2: 14, p: 0.536) showed no disparity between the studies, anxiety scores (T1: 38, T2: 43, p:0.011) exhibited a significant increase.

Conclusions. Consistent with the early phase, no exacerbation of ED symptoms in adolescents was observed during the later stages of the pandemic. Close clinical monitoring during the pandemic might have been protective against the deteriorating effects of the pandemic. During social isolation, it is important to monitor adolescents with EDs continously for depression and anxiety.

Key words: COVID-19, late-phase, eating disorders, adolescent, anxiety.

Towards the end of 2020, a third of the general population reported symptoms of anxiety and depression.[1] Despite the decrease in general pediatric presentations during the period of COVID-19 restrictions, the number of presentations at mental health clinics increased.[2]

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The COVID-19 pandemic also negatively affected the mental health and well-being of adolescents, who are physically and mentally in transition and therefore, a vulnerable group.[3] Many studies on adolescent mental health during the COVID-19 pandemic have drawn attention to the increase in post-traumatic stress disorder, depressive and anxiety symptoms associated with the pandemic.[4,5] It has been shown that individuals with pre-existing psychopathology may have been at

higher risk for the psychological effects of the pandemic.[1] Patients with eating disorders (EDs) are reported to be among the most vulnerable groups.[1,6]

When the literature is examined; restrictions in the provision of health services, social isolation, more exposure to social media, stress due to quarantine, anxiety and increased family conflict led to an increase in the restrictive behaviors of adolescents with EDs. During the pandemic along with negative changes in mood, there was also an increase in binge-eating episodes and vomiting behaviors.[7-10] Adolescence is a dynamic transitional period in which both physical and psychosocial development continue. In this period, peer relations, social activities and appearance can be at the forefront. It is also a period in which conflicts of opinion with family members can often be seen. For these reasons, the increase in restrictions and decrease in social activities, closure of schools, spending more time at home with family members in adolescent ED patients during the pandemic may cause adolescents who are already a vulnerable group, to be affected more negatively.[11,12] In a study of adolescents with a mean age of 14.6 years, it was suggested that the COVID-19 pandemic was the main trigger for approximately half of newly diagnosed ED patients.[13] Several studies have reported the worsening effect of the pandemic on EDs in adolescents and adults during the early stages of the pandemic.[10,14,15]

During the early stages of the pandemic (May 2020-June 2020), we conducted a study to evaluate the impact of restrictions on adolescents diagnosed with EDs and identify the factors predicting ED attitudes.[16] The study included 38 adolescents with a mean age of 15.12 ± 1.56 years (range=12-18 years) who had been diagnosed with EDs according to DSM-5 diagnostic criteria, mostly (68.4%) restrictive type AN, within the year before the pandemic. The study revealed no negative effects of the restrictions on EDs. Instead, 42.1% of the participants showed an improvement in ED symptomatology, and 36.8% reported no change. The study also revealed that depression had the highest predictive value for ED behavior during the pandemic. Similarly, most other studies investigating the effect of COVID-19 on EDs were conducted in the early stages of the pandemic in adults. However, more data are needed on the impact of the prolonged pandemic on EDs, especially in the adolescent age group. The objective of this subsequent study was to conduct a follow-up evaluation of the same cohort and investigate the impact of the prolonged pandemic on EDs.

Methods

Participants and procedure

Hacettepe University İhsan Doğramacı Children's Hospital is a tertiary healthcare institution in Türkiye. Outpatient follow-up of adolescents with EDs is conducted in the adolescent medicine and the child and adolescent psychiatry clinics.

This subsequent study re-evaluated the same group of adolescents (n=38) between the ages of 12-18 years who had been diagnosed with EDs according to the DSM-5 criteria prior to the pandemic, one year after their initial evaluation. Ethics committee approval was obtained from Hacettepe University Clinical Research Ethics Committee (GO 2021/08-14). The adolescents who participated in the initial study and their parents were contacted by phone and invited to participate in the current study either in person or online. Thirty-seven (97.3%) of the adolescents volunteered to participate in the current study. Those who preferred face-to-face participation (n:13) completed the questionnaires themselves in the waiting room at the adolescent medicine clinic. Those who preferred online participation (n:24) completed the questionnaires online. Informed consent was obtained from all the participating adolescents and their families.

The time periods were defined as follows:

T0: Time of ED diagnosis

T1: Initial study- between May 2020 and June 2020, early stages of the pandemic

T2: Current study- between May 2021 and June 2021, later stages of the pandemic

Pandemic conditions in Türkiye during the study

The participants have been under medical and psychosocial follow-up with a family-based approach for EDs, starting from their diagnosis until the onset of the pandemic. During the early stages of the pandemic, at T1, there was a curfew for adolescents under the age of 20, schools were closed, students participated in online lessons and stay-at-home regulations were enacted in Türkiye.[17] As a result, while the appointments of medically stable ED patients were postponed to a later date, adolescents who were medically or mentally unstable were continued to be followed up. An adolescent medicine physician conducted patient follow-ups via telemedicine (phone calls) at regular intervals to inquire about meal plan compliance, compensatory behavior, and general health, and saw them in person if necessary. Patients who required medical assistance were given special permission to leave their homes during the curfew and were seen in the clinic. Psychiatric follow-ups were primarily conducted via telephone or online visits during the lockdown.

During the later stages of the pandemic, at T2, the isolation restrictions were lifted, enabling easier patient access to the hospital. Schools also started to provide some hours of face-to-face education, but this varied according to the number of weekly COVID-19 cases in the country.

Anthropometric and clinical data

The body height and weight measurements of the adolescents who completed the questionnaires face-to-face in the clinic were taken by the clinic nurse. Body weight was measured using a portable electronic scale and height was measured with a portable stadiometer. The body height and weight information was obtained from the parents of the adolescents participating online. The measurements were taken by the parents at home using a digital scale. The parents of 5 adolescents did not wish to provide this information, so the current height and weight data were available for 32 adolescents. Body mass index (BMI) was then calculated by dividing body weight in kilograms by the square of the height in meters. BMI z scores and target weight percentages were calculated by adjusting for CDC cards and age.[18] The clinical information of the patients was retrieved from their medical files and from the hospital's electronic records. Age, gender, family structure, ED type, age at diagnosis of ED, measurements in T0, T1, T2, height, weight and BMI, remission status, duration of disease, duration of clinical follow-up, use of psychotropic medications, and hospitalizations of medically unstable patients were recorded.

For AN, patients were considered to be in medical remission if they had achieved their target weight, were consuming sufficient calories for their age and sex, and had not engaged in compensatory behaviors for the past three months. For BN, patients were considered to be in medical remission if they had not experienced binge-eating episodes and had not engaged in compensatory behaviors for the past three months.

Measurement tools

The impact of the pandemic on ED behavior

The questions developed by the researchers in the first study were asked again to evaluate the impact of the later stages of the COVID-19 pandemic on ED symptomatology, eating behaviours, diet adherence, diet compliance, whether meal plans had caused any struggles with parents, newly developed psychiatric symptoms, and healthcare service utilization. The adolescents were asked to rate the

difficulties in dietary compliance for the last month in the categories of none/somewhat, medium, or very difficult. The responses of the adolescents in the early and later stages were compared. The effectiveness of in-person and telemedicine visits were also compared in the current study.

The impact of pandemic on daily routines

The adolescents were asked how they felt about not going to school in person, the difficulty of staying at home, their screen time, and the time they spent on hobbies. They were asked to rate the difficulty of not attending school in person and staying at home as moderately difficult or extremely difficult. This study also evaluated sleep patterns. It was asked if there was a change in sleep duration, if the use of electronic devices affected sleep, and if the quality of sleep was poorer than it was before the pandemic.

The impact of the pandemic on well-being and quality of life

This section aimed to evaluate the overall well-being of the adolescents during the pandemic, and was assessed in the same way as in the first study by asking the dimensions of the improvement criteria for ED, previously stated in a qualitative study by de Vos et al.[19] Emotional, psychological, and social well-being items with self-adherence dimensions that seem to be related to well-being were selected in both studies and were adapted to 11 questions on a three-point Likert-type scale. In addition, two questions were asked to assess quality of life (QoL) in the past month. The first aimed to assess the overall QoL "Evaluate your quality of life in the last month", while the second aimed to assess the ED-related QoL "Evaluate your health and health-related quality of life due to an eating disorder in the past month". The participants were shown a Likert scale of 1 to 5 represented by drawings of facial expressions, ranging from very bad to very good. Higher points were evaluated as a higher QoL.

The Eating Disorders Examination Questionnaire (EDEQ)

The Eating Disorders Examination Questionnaire (EDE-Q) is a self-reported ED assessment scale[20] formed of 28 items in 4 subscales of restriction, concerns related to eating, concerns related to body shape, and concerns about weight, reflecting the severity of symptoms of ED psychopathology. There are also 3 open-ended questions to support a diagnosis of binge-eating disorder. The points for the responses to the items in each subscale are totaled and divided by the number of items to give a total score for the subscale. The total points for the 4 subscales are then totaled and divided by 4 to give an overall scale score. Higher total scale points indicate an increased severity of ED. Individuals with an overall global scale score of >2.3 are evaluated as having clinical ED behaviour.[21] A score of >4 points is suggestive of severe ED behaviour.[22,23] In our study, patients were evaluated according to their overall global scale score (> 2.3). The validity and reliability studies of this scale for use with Turkish adolescents were performed by Yucel et al.[24] The number of items in the Turkish form and the subscales are the same as the original. The internal consistency coefficient has been found to be 0.93 for the whole scale, and ≥0.70 for each of the subscales.

Beck Depression Inventory (BDI)

The BDI was developed by Beck et al. in 1961 to measure the behavioural findings of depression in adolescents and adults.[25] The scale consists of 21 items, each scored between 0 and 3 points. The patients are instructed to mark the statement which best describes their status. The total points are evaluated as 0-9: minimal, 10-16: mild, 17-29: moderate, and 30-63: severe symptoms. The validity and reliability studies of the BDI in Turkish were performed by Teğin (1980) and Hisli (1988) and a cutoff value of 17 has been accepted.[25-27]

The State Anxiety Inventory for Children (SAI)

This scale was developed by Spielberger to measure individual differences when there is a predisposition to anxiety.[28] The State Anxiety Inventory (SAI) consists of 20 multiple choice questions and children are requested to evaluate how they feel "at that moment". The 20 items of this scale aim to evaluate feelings related to state anxiety such as tension, irritability, fussing, and nervousness. The presence or absence of these feelings is marked and scored from 1 (absent) to 3 (very much). The validity and reliability studies of this scale in Turkish were performed by Özusta, and the Cronbach alpha coefficient was found to be 0.82 for the SAI.[29]

The Maudsley Obsessive Compulsive Inventory (MOCI)

This scale was developed by Hodgson and Rachman[30] to investigate the type and severity of obsessive compulsive symptoms. Each item on the scale is answered as true or false, with "true" responses scored as 1 point and "false" responses as 0. The points for each item are totaled to give an overall total score. A higher total score indicates an increase in obsessive-compulsive symptoms. The validity and reliability studies of this scale in Turkish were performed by Erol and Savaşır.[31]

Evaluation of the data and statistical analysis

Data obtained in the study were analyzed statistically using IBM SPSS vn.23 software (Statistical Package for the Social Sciences). In the descriptive analyses, continuous data were stated as mean±standard (SD) values if data were normally distributed and as median (minimum-maximum), first and third quartile values if distribution was not normal. Categorical data were stated as number (n) and percentage (%). Conformity of the quantitative data to normal distribution was assessed with the Shapiro Wilk test and visual methods (histogram, box-line graph). When the assumption of normal distribution was not met, non-parametric statistical tests were used in the group comparisons. To determine whether there was a difference or not in quantitative variables, the Wilcoxon Matched Two Samples Test was used for two dependent groups and for more than two groups, the Friedman test was applied. To determine any difference between two dependent percentages, the McNemar test was used and for more than two dependent percentages, the Marginal Homogeneity test. If the comparisons of more than two groups were found to be significant ($p<0.05$), the groups that caused the difference were evaluated using post-hoc tests of Post Friedman and the Dunn-Bonferroni test. Relationships between continuous variables were examined with the Spearman correlation coefficient as distribution was not normal. As the mean age of the study participants was not correlated with the depression, anxiety, obsession, and EDEQ total points, no correction according to age was applied.

Linear regression analysis was performed to determine the factors affecting the severity of ED symptoms. By examining the assumptions (residual normality, no problems of multicollinearity), a model was obtained which was clinically appropriate and met the assumptions. A value of $p<0.05$ was accepted as statistically signficant in all the tests.

Linear regression analysis was applied to examine the predictive power of the variables for the EDEQ total scores. In this analysis, the variables with correlation coefficients >0.5 including BDI (T2), MOCI (T2), SAI (T2) scores, BMI Z score value (T1), and the 'Family conflict concerning the meal plan within the last month' (T2) were taken as predictors. Multicollinearity was determined between the BDI (T2), MOCI (T2), and SAI (T2) scores, so of these three variables, only the SAI (T2) was included in the final regression analysis model because SAI (T2) had the highest β coefficient in the regression analysis performed one by one, and only SAI had a statistically significant difference between T1 and T2 evaluations. Finally, the SAI (T2) scores, the BMI z score value (T1) and the 'Family conflict concerning the meal plan within the last month' (T2) were taken as predictive variables.

Results

Antrhopometric and clinical data

The mean age of the adolescents was 15.6±1.6 years at T0, 16.52±1.68 years at T1, and 17.9±1.68 years at T2. The majority of the participants were female (n:35, 94.6%). The most common diagnosis was Anorexia Nervosa-Restricting Type (AN-RT) (n: 25, 67.5%), followed by AN-Binging-Purging (AN-BP) (n:5, 13.5%), Atypical AN (n:3, 8.1%), Bulimia Nervosa (BN) (n:3, 8.1%), and Unspecified Feeding or Eating Disorder (UFED) (n:1, 2.7%). Due to the medical instability of the participants, the hospitalization numbers were 7 (18.9%) in T0, zero in T1, and 2 (5.4%) in T2. At T2, 31 (83.7%) adolescents were given selective serotonin reuptake inhibitor, 5 (13.5%) atypical antipsychotics, and 1 (2.7%) methylphenidate, which was similar to T1 (p>0.05).

The BMI Z score and target weight percentage values of the patients diagnosed with AN-RT and AN-BP are presented in Table I. Friedman test overall p values for BMI Z score and target goal weight percentage, respectively p=0.018, p=0.006. The BMI Z-score values at T2 were not significantly different from those at T1 and T0 (p>0.999, p=0.80, respectively) .The mean BMI Z score value was significantly higher in T1 compared to T0 (p=0.031). The target weight percentage values at T2 were found to be significantly higher than at T0 (p=0.011) and there was no significant change compared to T1 (p>0.999).

From T0 to T1, among 38 adolescents, 6 (15.7%) were discharged from ED follow up. From T1 to T2, among 37 adolescents 12 (32.4%) were discharged from ED follow up (p=0.065). A total of 25 (67.6%) adolescents were under regular clinical follow-up. The mean follow-up period of 12 adolescents who discontinued treatment was 27.7±16.8 months (0.9-65.3 months), and 7 (58.3%) of them were in medical remission when they left the treatment. Of the 25 patients who were under regular follow-up, 12 (48%) were in medical remission at T2 (p=0.255).

The impact of the pandemic on self-reported ED related behavior, psychiatric symptoms, family conflict, and healthcare utilization

The effect of the pandemic on ED symptomatology is presented in Table II, with no significant change observed in the ED behaviours from T1 to T2. There was a decrease of approximately 2.7% in compliance with the diet from T1 to T2, but the difference was not significant (p=0.869). In T1, 73.4% of the adolescents stated that they did not have newly developed psychiatric symptoms, and this rate was not statistically significant compared to T2 (54.1%) (p=0.144). Conflict with family was similar in T1 and T2; with 70.3% of adolescents

Table I. Comparisons of the weight and BMI values of the patients diagnosed with AN.

Characteristics	Time of diagnosis (T0) (n=31)	Early stage of pandemic (T1) (n=31)	Later stage of pandemic (T2) (n=26)	P
BMI Z score				
Median	-1.83	-0.92	-0.79	**T0-T1: 0.031**
1st-3rd quartile	-2.62 - -0.80	-1.90- 0.03	-1.74- -0.23	T0-T2: 0.800
Min-max	-5.14- 1.06	-3.91- 1.03	-3.90- 1.10	T1-T2: >0.999
Target goal weight percentage (%)				
Median	79	84.0	88.0	**T0-T2: 0.011**
1st-3rd quartile	75.0 -90.0	79 -90	79- 90	T1-T2: >0.999
Min-max	60.0 -110	65.0- 110.0	65.0-106	

Evaluation periods: T1 defines the period between May 2020 and June 2020, T2 defines the period between May 2021 and June 2021.
AN: Anorexia Nervosa, BMI: Body mass index, min: minimum, max: maximum, n:number, (Friedman test)

Table II. The impact of the pandemic conditions on eating disorder symptomatology.

Symptomatology		Early stage of pandemic (T1) n(%)	Later stage of pandemic (T2) n(%)	P
Were you able to comply with your meal plan during the pandemic?	Never/Rarely	17(45.9)	16(43.2)	0.869
	Sometimes	6(16.2)	9(24.3)	
	Often/Always	14(37.8)	12(32.4)	
Did the lockdown negatively affect your access to ED healthcare?	Never/Rarely	17(45.9)	27(73.0)	0.005
	Sometimes	11(29.7)	9(24.3)	
	Often/Always	9(24.3)	1(2.7)	
Have you had any new psychiatric symptoms during this period?	Never/Rarely	27(73.4)	20(54.1)	0.144
	Sometimes	6(16.2)	12(32.4)	
	Often/Always	4(10.8)	5(13.5)	
Did you experience conflict with your parents due to eating during the lockdown?	Never/Rarely	26(70.3)	23(62.2)	0.317
	Sometimes	6(16.2)	7(18.9)	
	Often/Always	5(13.5)	7(18.9)	
During the lockdown, I spent less time thinking about my weight/ appearance	Disagree	17(45.9)	20(54.1)	0.564
	Somewhat agree	10(27.0)	7(18.9)	
	Totally agree	10(27.0)	10(27.0)	
During the lockdown, I spent less time tracking my weight	Disagree	16(43.2)	16(43.2)	>0.999
	Somewhat agree	6(16.2)	6(16.2)	
	Totally agree	15(40.5)	15(40.5)	
During the lockdown, I spent less time doing things to try and control my weight	Disagree	13(35.1)	13(35.1)	0.336
	Somewhat agree	11(29.7)	6(16.2)	
	Totally agree	13(35.1)	18(48.6)	

Evaluation periods: T1 defines the period between May 2020 and June 2020, T2 defines the period between May 2021 and June 2021.
ED:Eating Disorder, (Marginal homogeneity test)

in T1 and 62.2% in T2 (p=0.317) reporting not experiencing conflict with the family. Difficulty in accessing healthcare in person increased and 73% of the participants stated that the tele-health service was more feasible than in-person clinic visits.

The impact of pandemic on daily routines

When screen time within the last month was examined at T1 and T2, an increase of 5.4% was found (p=0.819). Having to remain at home was reported as very difficult by 13 (35.1%) at T1, and by 16 (43.2%) at T2 (p=0.715). While not being able to go to school was reported as very difficult by 2 (5.4%) at T1, this number increased to 14 (37.8%) at T2 (p=0.023). A significant decrease was determined in time spent doing hobbies in the last month from T1 to T2 (45.9% and 21.6%, respectively, p=0.040). Almost half of the adolescents (n=16, 43.2%) reported that their sleep quality had deteriorated. Seventeen (45.9%) reported increased screen time before bedtime, and 14 (37.8%) stated it takes longer to fall asleep.

The impact of the pandemic conditions on well-being and quality of life

Considering the responses to the general quality of life (QoL); at T1 32.4% stated good, 32.4% neither good nor bad, and 35.1% bad, and these rates were 18.9%, 54.1% and 27%, respectively, at T2 (p=0.255). Considering the ED-related QoL; at T1, 37.8% stated good, 27% neither good nor bad, and 35.1% bad, and at T2, these rates were 51.4%, 32.4% and 16.2%, respectively (p=0.134). The mean general QoL scores were

3.00±1.20 at T1, and 3.00±0.97 at T2 (p=0.603). The mean ED-related QoL scores were 3.00±1.18 at T1, and 4.00±1.09 at T2 (p=0.056). The results of the impact of the pandemic conditions on well-being and quality of life are shown in Table III.

EDEQ, MOCI, BDI and SAI results

According to the global score cut-off value for the EDEQ (> 2.3), ED behaviour was present in 17 (45.9%) patients at T1, and in 14 (37.8%) at T2 (p>0.999). No statistically significant difference was determined between the EDEQ total and subscale points between T1 and T2 (Table IV). No statistical difference was seen between the MOCI and BDI scale scores at T1 and T2, while the SAI scores increased significantly at T2 (p=0.536, p=0.883, p=0.011, respectively). The results of the MOCI, BDI, and SAI are presented in Table IV.

Variables predicting the EDEQ total score

According to the cutoff value for the BDI, the risk of depression was determined in 20 (54.1%) patients at T1, and in 18 (48.6%) at T2 (p=0.180). The median depression score of the adolescents was 29 (IQR:7-38) for those under clinical follow-up, and 14 (IQR:6-26) for those who had discontinued the treatment (p=0.240). These values for the anxiety scores were 43 (31-48) and 42.5 (34-45.5), respectively (p=0.737).

Linear regression analysis was applied to examine the predictive power of the variables for the EDEQ total scores at T2. (Table V)

The SAI (T2) scores explained 39% of the EDEQ total score (R^2:0.397, p<0.001). When the BMI Z score value (T1) was added, this rate increased to 49.9%, and when the variable "Family conflict concerning the meal plan within the last month" was added, the rate reached to 57.5% (Table VI).

Discussion

The aim of this longitudinal follow-up study was to re-evaluate adolescents with EDs one year after the first study during the prolonged COVID-19 pandemic. To our knowledge, this is one of the few follow-up studies and the first to examine the factors predicting ED behavior and disordered eating in the same patient population.[6,32,33] Consistent with the findings in the early stages, no increase was observed in the EDEQ scores later in the pandemic. In addition, while depression remained the most significant predictor for ED behaviour both in the early and later phases, anxiety showed a significant increase in the later phase and became the second most important predictor of ED behaviour.

In this study, participants reported that their ED symptomatology did not worsen in the later stages of the pandemic. Among our participants, 67.6% were followed up regularly by adolescent medicine and psychiatry clinics, suggesting that this follow-up may have had a protective effect against the aggravating effects of the pandemic. Another study[34], evaluated the symptoms of ED patients (mean age 27.6±8.45 years) before, during, and after quarantine. In that study, more than half of the patients were treated, and a small percentage of the patients did not receive treatment. About half of the treated patients reported improvement in ED symptoms during the quarantine period. On the other hand, 29% of those who did not receive treatment showed improvement after the quarantine period. Similar to our study, it was thought that being under treatment was associated with an improvement in ED symptomatology. In contrast to our study, a large-scale longitudinal study reported that women with EDs (mean age 32.1±8.73 years) who were asymptomatic at the initial stage of the pandemic relapsed within 6 months. However, less than 50% of participants were under clinical follow-up and the follow-up lasted only 6 months. The study also linked higher ED symptoms to anxiety, which aligns with our findings.[32] Another study[35] conducted in 2020 also reported worsening of ED behaviors in both patients with ED (aged > 18 years) and the general population. However, this study lacked information on patients' clinical follow-up and higher rates of comorbid psychiatric disorders, such as depression and anxiety

Table III. The impact of the pandemic conditions on well-being and quality of life.

Impact items		Early stage of pandemic (T1) n(%)	Later stage of pandemic (T2) n(%)	P
I understand the value of being healthy	Disagree	3 (81.0)	6 (16.2)	0.617
	Somewhat agree	13 (35.1)	9 (24.3)	
	Totally agree	21 (56.8)	22 (59.5)	
My self-confidence has increased	Disagree	13(35.1)	12(32.4)	0.866
	Somewhat agree	12(32.4)	15(40.5)	
	Totally agree	12(32.4)	10(27.0)	
I accepted myself as I am	Disagree	14(37.8)	11(29.7)	**<0.001**
	Somewhat agree	14(37.8)	14(37.8)	
	Totally agree	9(24.3)	12(32.4)	
I realized I am in control	Disagree	3(81.0)	3(8.1)	0.819
	Somewhat agree	12(32.4)	13(35.1)	
	Totally agree	22(59.5)	21(56.8)	
A new era has begun in my life	Disagree	8(21.6)	8(21.6)	0.857
	Somewhat agree	14(37.8)	15(40.5)	
	Totally agree	15(40.5)	14(37.8)	
I realized I have the right to decide and choose	Disagree	11(29.7)	7(18.9)	0.384
	Somewhat agree	13(35.1)	16(43.2)	
	Totally agree	13(35.1)	14(37.8)	
I feel more mature	Disagree	11(29.7)	4(10.8)	**0.020**
	Somewhat agree	12(32.4)	13(35.1)	
	Totally agree	14(37.8)	20(54.1)	
My goals in life have changed	Disagree	9(24.3)	10(27)	0.369
	Somewhat agree	19(51.4)	12(32.4)	
	Totally agree	9(24.3)	15(40.5)	
I realized that I have things to do with the society I live in	Disagree	10(27)	11(29.7)	0.879
	Somewhat agree	12(32.4)	9(24.3)	
	Totally agree	15(40.5)	17(45.9)	
I started to understand my family better.	Disagree	11(29.7)	8(21.6)	0.286
	Somewhat agree	17(45.9)	16(43.2)	
	Totally agree	9(24.3)	13(35.1)	
My family has started to better understand me	Disagree	16(43.2)	12(32.4)	0.450
	Somewhat agree	11(29.7)	15(40.5)	
	Totally agree	10(27.0)	10(27.0)	
General quality of life (QoL)	Good	2(32.4)	7(18.9)	0.255
	Neither good nor bad	12(32.4)	20(54.1)	
	Bad	13(35.1)	10(27)	
ED-related QoL	Good	14(37.8)	19(51.4)	0.134
	Neither good nor bad	10(27)	12(32.4)	
	Bad	13(35.1)	6(16.2)	

Evaluation periods: T1 defines the period between May 2020 and June 2020, T2 defines the period between May 2021 and June 2021.
ED: Eating disorder, QoL: quality of life, (Marginal homogeneity test)

Table IV. Comparisons of the EDEQ Total Points and Subscale Points and MOCI, BDI and SAI scores in the earlier and later periods of the pandemic.

Scales	Early stage of pandemic (T1) n(%)	Later stage of pandemic (T2) n(%)	P
EDE-Q Total points			0.587
Median	1.5	1.6	
1st-3rd quartile	0.1-3.9	0.7-3.4	
min-max	0-5.2	0.1-5.4	
Restraint subscale			0.829
Median	1	1	
1st-3rd quartile	0-3	0-2.6	
min-max	0-3	0-6	
Eating concerns subscale			0.961
Median	0.8	1	
1st-3rd quartile	0.2-2.8	0-2.6	
min-max	0-4.4	0-4.6	
Shape concern subscale			0.509
Median	2.3	1.8	
1st-3rd quartile	0.38-5	1.2-4.7	
min-max	0-6	0-6	
Weight concern subscale			0.350
Median	1.8	2	
1st-3rd quartile	0.4-4.4	1.2-3.8	
min-max	0-6	0-6	
MOCI			0.536
Median	11	14	
1st-3rd quartile	9-18	10-19	
min-max	2-29	0-31	
BDI			0.883
Median	18	15	
1st-3rd quartile	9-25	6-34	
min-max	2-53	0-51	
SAI			**0.011**
Median	38	43	
1st-3rd quartile	30-43	33-47	
min-max	21-55	26-53	

Evaluation periods: T1 defines the period between May 2020 and June 2020, T2 defines the period between May 2021 and June 2021.
min: minimum, max: maximum, BDI: Beck Depression Inventory, EDEQ: Eating Disorders Evaluation Questionnaire, MOCI: Maudsley Obsessive Compulsive Inventory, SAI: State-Anxiety Inventory for Children, (Wilcoxon Test)

were reported.[36] A meta-analysis exploring the relationship between depression and EDs in adults revealed that EDs are a risk factor for depression and, thereby contributing to the result.[37]

These studies suggest that adult patients with EDs were affected more negatively than adolescents in regard to ED symptomatology while in social isolation.[38] We believe that this is because adolescents adapted better to

Table V. Simple linear regression models for predicting the total EDEQ points in the later period of the pandemic.

Model	Predictive variable	β	Standardised β	T	p	95% Confidence Interval
1	MOCI	0.09	0.408	2.64	0.012	0.023 - 0.173
2	BDI	0.06	0.64	4.94	<0.001	0.039 - 0.093
3	SAI	0.12	0.63	4.80	<0.001	0.072- 0.17

Linear regression analysis dependent variable: EDEQ total points, MOCI: Maudsley Obsessive Compulsive Inventory, BDI: Beck Depression Inventory, SAI: State-Trait Anxiety Inventory for Children. (linear regression analysis)

Table VI. Multiple linear regression models predictive of the EDEQ

Predictive variable	B	Standardised β	t	p	95% confidence interval
SAI	0.092	0.468	3.981	0.000	0.045 - 0.139
BMI Z score at T1	0.191	0.387	3.576	0.001	0.082 - 0.299
Family conflict concerning the meal plan within the last month	0.410	0.331	2.823	0.008	0.115 - 0.706

Linear regression analysis, dependent variable: EDEQ total points, SAI: State Anxiety Inventory for Children, BMI: body mass index. (linear regression analysis)

their meal plans, as they most likely ate under the supervision of their families during the pandemics. Studies have shown that eating with the family significantly influences ED recovery.[39] In our study, contrary to some literature findings[40], over half of the adolescents reported no family conflict concerning their meal plans, which likely contributed to the stable EDEQ scores observed. The increased time spent with family during the pandemic may have fostered greater support and understanding among family members, and increased parental involvement in the treatment. Family based therapy (FBT) is the most effective and evidence-based treatment for adolescents with EDs and its main components include meal support, family control and close monitoring of nutrition.[41] Furthermore, the closure of schools may have reduced peer communication, which could have prevented some deterioration in body perception due to fewer instances of visual scrutiny by others.

Although many studies have shown negative effects of the pandemic on the course of AN[9,42,43], there are also studies suggesting that AN patients experienced fewer negative effects compared to other EDs, and that the impact of quarantine varied based on ED subtypes.[44,45] In a study comparing AN and BN patients, BN patients reported experiencing more negative effects during the pandemic.[6,45] The more positive outcomes of adolescents with AN were attributed to their development of better coping strategies and having better access to healthcare services. Although the majority of our patients were AN, it was not possible to compare the EDEQ scores across different ED types due to the small number of patients.

We have shown that, depression was the most significant predictor of ED behavior in the early stages of the pandemic.[16] However, in this study we observed that, while depression remained the most important predictor, its predictive power decreased over time. Conversely, the predictive power of anxiety increased significantly during the later stages. The relationship between anxiety and ED has been demonstrated in many studies[45-47], but there is limited information on their association during the later stages of the pandemic. A study by Cascino et al.[48] found that anxiety showed the most significant increase over time during the pandemic, while binge-eating and vomiting returned to pre-pandemic levels. The effects of the pandemic on mental health seem to have become more pronounced in later periods. Factors like reduced social isolation, eased restrictions, fear of contracting the virus and changes in routine activities may have contributed to the continuation of the depression and the increase in anxiety in the

late period. In another longitudinal study of adolescents without ED, a significant increase in anxiety and depression was found in the later period compared to the early period[49], indicating that the effects on mental health are likely to persist in the prolonged COVID pandemic term, beyond its immediate effects. The increase in anxiety among adolescents with EDs may also be influenced by changing social determinants of health over time. During the later phase of the pandemic, there was more face-to-face education compared to the initial stage, along with the onset of exams, potentially contributing to increased academic anxiety. In addition, increasing inflation and the emergence of economic difficulties may have further increased anxiety levels.[39]

The strength of this study was that it was one of the few longitudinal studies evaluating the effects of the prolonged pandemic on adolescents with EDs. However, there were also some limitations, primarily the relatively small sample size and the inability to perform sub-group analyses by ED subtypes. In addition, body weight and BMI data for adolescents who prefer online participation were based on parental statements and were not available for five adolescents. The results of this study cannot be generalized to all adolescents with EDs, especially to those diagnosed during the pandemic, as all of our patients were diagnosed before the pandemic. Furthermore, while the impact of the pandemic on eating disorders in adolescents has been studied, it is important to consider various factors beyond the pandemic itself. These factors may include school-related anxiety, communication patterns with parents, family dynamics, and the severity of the eating disorder prior to the pandemic. Consequently, it is not feasible to isolate the sole effect of the pandemic.

In conclusion, the results of this study demonstrated that in the later stage of the pandemic, just as in the early stage, depression was the most powerful predictor of ED symptomatology, and anxiety showed a significant increase in the later period. In the presence of strong environmental stress factors and prolonged social isolation, it is important to closely monitor adolescents with EDs for depression and anxiety, and be aware of those in need of family support and mental healthcare services. Being under regular clinical monitoring before and during the pandemic might have been protective against the deteriorating effects of the pandemic for adolescents with EDs, in addition to the possible positive effects on FBT and closer meal support by parents.

Ethical approval

Ethics committee approval was received for this study from the Ethics Committee of Hacettepe University (Protocol number: GO 2021/08-14). Informed consent was obtained from individuals who participated in this study.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SA. Data collection: ŞET. Analysis and interpretation of results: SA, KN, MPK. Draft manuscript preparation: ŞET, 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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Original Article

Evaluation of functional gastrointestinal disorders in children aged 4-10 years with autism spectrum disorder

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ABSTRACT

Background. Gastrointestinal system disorders are known to be prevalent among children with autism spectrum disorder (ASD). Some ASD-associated comorbidities are abdominal pain, constipation, diarrhea, gastroesophageal reflux, sleep disturbances, epilepsy, and psychiatric problems. Nonetheless, there is still limited information about the presence of functional GI disorders (FGIDs) among children with ASD, especially in Türkiye. Using the Rome criteria, we aimed to investigate FGIDs in children with ASD.

Methods. The sample of the study consisted of 68 children aged 4-10 years, diagnosed with ASD according to the DSM-5 diagnostic criteria and had scores greater than 30 on the Childhood Autism Rating Scale (CARS-2) and an age-sex matched control group (n=78). The Rome III criteria were used to evaluate FGIDs.

Results. The frequency of FGIDs in the ASD group was higher (76.5%) compared to the control group ($p<0.001$). Compared to the control group, abdominal migraine frequency increased 10 times ($p=0.012$), functional constipation 7 times ($p<0.001$), and fecal incontinence 6 times ($p<0.001$) in the ASD group. Stool retention was not present in most children in the ASD group who were found to have fecal incontinence.

Conclusion. In this study, the most common FGIDs in the ASD group were abdominal migraine, functional constipation, and non-retentive fecal incontinence. The finding that most children with ASD who had fecal incontinence did not show stool retention implicated social, psychological, and behavioral factors as the causes of incontinence. Raising awareness of healthcare professionals about the frequency of FGIDs in children with ASD will improve many areas in the daily lives of these children.

Key words: autism spectrum disorder, functional gastrointestinal disorders, constipation, abdominal migraine, fecal incontinence, stool retention.

Gastrointestinal (GI) problems are common clinical conditions in autism spectrum disorder (ASD).[1-3] These conditions are prevalent but often overlooked.[4] It is thought that GI problems that are not treated in children with ASD are associated with behavioral, psychiatric, and sleep disorders.[5,6] GI problems were noticed in children with ASD for the first time with the demonstration of nutritional disorders.[7] Children with ASD have been found to be five times more likely to display nutritional issues such as food refusal, neurodevelopmental food selectivity, and inadequate oral intake compared to healthy peers.[8-10] In subsequent studies, GI disorders have been seen very frequently in ASD patients, in line with nutritional selection.[2,10,11]

The prevalence of children with ASD who also have GI problems have been reported as 9-91%.[1-4,11,12] It has been stated that these children are more than five times as likely to

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develop GI problems as those without ASD, and the most common problems seen in children are abdominal pain, constipation, and diarrhea.[4,11] Seizures, sleep disorders, psychiatric disorders, and GI problems are considered to be the four main comorbidities in children with ASD.[12,13] It has been shown that these disorders are also related to each other and are correlated with the severity of ASD.[14-16]

It has been found that children diagnosed with ASD who also have flatulence, diarrhea, abdominal pain, and constipation are more irritable, more likely to display social withdrawal, and more hyperactive in comparison to those who do not have GI problems.[15,16] It is known that argumentative, oppositional, defiant, and destructive behaviors are more common in children with ASD who have GI problems.[6]

Studies related to functional GI disorders (FGIDs) among children diagnosed with ASD are limited in Türkiye. We aimed to evaluate the status of FGIDs in children with ASD based on the Rome criteria and raise awareness regarding this issue among healthcare professionals interacting with ASD patients.

Methods

This cross-sectional study was performed from November 2021 to March 2022 with the participation of patients followed-up in the "Pediatric Gastroenterology" and "Child and Adolescent Psychiatry" outpatient clinics at Dokuz Eylul University Hospital.

The institutional review board approved this study (E-74660883-604.01.01-148935). Parental informed consent was obtained from all patients and controls participating in the study.

Children with a diagnosis of ASD based on the diagnostic criteria of DSM-5 in the Child and Adolescent Psychiatry outpatient clinic and who scored over 30 on the Childhood Autism Rating Scale - 2nd Edition (CARS-2) were included in the study.[17] CARS consists of 15 items, and it is an instrument of behavioral rating created to distinguish between children with intellectual disabilities and children with symptoms associated with ASD. CARS allows determining the clinical severity of ASD as mild-moderate and moderate-severe. According to scoring children who score between 15 and 29.5 do not show autistic symptoms. Children who score between 30 and 36.5 points are clinically mild to moderately autistic, and those who score between 37 and 60 points are severely autistic. In our study, children with mild and moderate autism were evaluated. The children to be included in the control group were selected from among those with non-GI complaints and normal physical examination results, who presented to the general pediatrics outpatient clinic and had similar age and sex distributions.

All cases in the autism and control groups were included in the study in our pediatric gastroenterology outpatient clinic after organic pathologies related to their complaints were excluded. The sample of the study included 68 children in the ASD group and 78 in the control group, aged between 4 and 10. Data were collected by asking questions to the parents by the pediatrician (FGIDs survey questions prepared according to the Rome III criteria, the validity and reliability of which are in Turkish).[18]

Statistical analysis

The collected data were analyzed with the IBM SPSS Statistics 2010 program. The normality of the distributions of the continuous variables was examined using the Kolmogorov-Smirnov test. The categorical variables in the study are presented with frequency (n) and percentage (%) values, and the continuous variables are presented with median (IQR: 25th-75th percentile) values since parametric test assumptions were not met. The categorical variables were analyzed with Pearson's chi-square and Fisher's exact tests, and Yates's correction was performed. Since parametric test assumptions were not provided, the Mann-Whitney U test was used for the comparison of the two groups. The level of statistical significance was accepted as $p<0.05$.

Results

In the study, the children in the ASD and control groups had similar characteristics in terms of age and sex distributions. Male sex was dominant in both groups (Table I). Some of the patients were receiving risperidone, valproic acid, and zuclopenthixol treatments due to autism-related irritability (including aggression, anger, tantrums, self-harming behaviors). Additionally, there were patients receiving melatonin treatments for autism-related insomnia disorder. The frequency of FGIDs in the ASD group was 76.5%, and this was found to be significantly higher in comparison to that of the control group (39.8%) (p<0.001) (Table I). The rate of functional constipation was approximately 7 times higher in the ASD group (38.2%) when compared to that of the control group (5.1%) (p<0.001, Table I).

In the evaluation of bowel movements, it was determined that the ASD group had a stool retention behavior rate of 61.8% and a painful defecation rate of 38.2%, which were significantly higher than the rates in the control group (p=0.001).

The rates of fecal incontinence were 29.4% in the ASD group and 5.1% in the control group (p<0.001) (Table I). The behavior of stool retention was found in 60% of the children with fecal incontinence in the ASD group, and this rate was significantly higher than that in the children with fecal incontinence in the control group (p=0.002). Moreover, when the rectal examination of the cases diagnosed with fecal incontinence was performed, there was no fecal retention in the entire autism group. In the control group, this rate was 50%, but it was not evaluated statistically because the number of cases was small.

The frequency of abdominal migraine (AM) was 13.2% in the ASD group and 1.3% in the control group (p=0.012) (Table I).

There was no significant difference in the frequencies of functional dyspepsia, functional abdominal pain, irritable bowel syndrome, cyclic vomiting disorders, or reflux between the two groups. However, postprandial distress was observed in all functional dyspepsia patients with ASD (n=9). On the other hand, in the control group, this rate was 25% (n=2) (p<0.001).

Table I. Characteristics of the autism spectrum disorder (ASD) group and the control group and comparisons based on the Rome III criteria in terms of functional GIS diseases Data presented as n (%), except when indicated otherwise.

Characteristics	Control N=78	ASD N=68	p-value
Gender			
Girl	19 (24.4)	13 (19.1)	0.532
Boy	59 (75.6)	55 (80.9)	
Age (months), median (IQR)	85 (65-99)	84 (60-108)	0.769
3-7 years old	38 (48.7)	39 (57.3)	0.335
8-10 years old	40 (51.3)	29 (42.7)	
Dyspepsia	8 (10.3)	9 (13.2)	0.785
Abdominal Pain	17 (21.8)	20 (29.4)	0.412
Irritable bowel syndrome	3 (3.9)	8 (11.8)	0.141
Constipation	4 (5.1)	26 (38.2)	**<0.001**
Abdominal migraine	1 (1.3)	9 (13.2)	**0.012**
Fecal incontinence	4 (5.1)	20 (29.4)	**<0.001**
Cyclic vomiting	1 (1.3)	3 (4.4)	0.341
Reflux	8 (10.3)	13 (19.1)	0.210
All GI Disorders	31 (39.8)	52 (76.5)	**<0.001**

Discussion

The broad range of GI symptoms (9-91%) observed in children diagnosed with ASD can be attributed to the respondent characteristics (e.g., doctor, parent) and the variations in the techniques used across research to determine GI symptomatology.[1,2] Functional GI diseases are diagnosed subjectively using the Rome criteria, which provide specific standards based on symptoms. Proven and objective indicators are not available for an accurate diagnosis. A complete anamnesis and physical examination are extremely important. When necessary, comprehensive examinations should be performed, and organic causes should be excluded. Physicians should examine patients meticulously to test the presence of any potentially concerning symptoms or signs. The Rome criteria are defined as the diagnostic criteria for FGIDs. From these criteria, questionnaires adapted to the current language were created, and these questionnaires are still the gold standard in the diagnosis of FGIDs. The Rome III criteria, which have been tested for validity and reliability in Turkish, were used in this study.[2,18]

In our study, we found the frequency of FGIDs in the children with ASD to be 76.5%, which was approximately two times the frequency in the healthy control group. This information supports studies that have found the frequency of FGIDs among children diagnosed with ASD to be 3-4 times higher than those in healthy populations.[4,19,20] The medication used in the ASD group may change the outcome, however, it should not be forgotten that the disease score was stable in our study. In our study, children with mild and moderate autism (children with a CARS score of 30-36.5 points) were evaluated.

In the literature, it has been reported that the most common disorder in children with ASD is functional constipation, and it is observed in 40% of cases.[11,16,21] In our study, in the ASD group was functional constipation at a rate of 38.2%, and it was approximately 6 times more common than the rate in the control group.

In our study, in the ASD group, we found the rate of fecal incontinence to be 29.4%, and this rate was 6 times higher than that in the control group. It was determined that stool retention behavior was observed at a rate of 61.8%, and painful defecation was observed at a rate of 38.2% in the ASD group, and these rates were higher than those in the control group. This made us think that the causes of incontinence in children with ASD may be social, psychological, and behavioral.[11,21]

Furthermore, in our study, the frequency of functional abdominal pain was 29.4%, the frequency of gastroesophageal reflux was 19.1% in the ASD group, and these rates were similar to those reported in the relevant literature.[11,21]

The prevalence of AM in children has been reported to range between 0.2% and 4.1%.[22] It is most commonly seen in children aged 4–15 years. The average age at diagnosis is between 3 and 10 years, with the peak incidence at 7 years of age. Most previous studies have demonstrated that AM is more prevalent in girls than in boys, similar to cephalic migraine and other FGIDs.[23] Equal prevalence has been reported in girls and boys in a few studies.[24] Using the Rome II and III criteria, a 2008 study examined the prevalence of various FGIDs in children diagnosed with chronic, idiopathic abdominal pain.[25] When the Rome III criteria were used, the frequency of diagnosis of AM in patients with chronic abdominal pain rose from 5% to 23%. This result showed that the Rome III criteria had a higher positive predictive value (100%) and a lower negative predictive value (7.7%). According to the authors, this difference between the diagnostic values of the two sets of criteria might have resulted in an incorrect diagnosis of other functional abdominal pain disorders, such as AM.[25] There have been no new studies reported so far that looked at the prevalence and other epidemiological characteristics of AM based on Rome IV criteria released in 2016. The frequency of AM among children in Türkiye is unknown. In the study performed by Paydaş[26] in Konya, 8.12% of patients who applied to the pediatric gastroenterology outpatient clinic

with chronic abdominal pain were diagnosed with AM. Additionally, in a study conducted in a child psychiatry clinic in our country, the frequency of AM in children with autism was found to be 1%.[27] Ninety-seven children who applied to Ankara Bilkent City Hospital Child and Adolescent Psychiatry outpatient clinic and were diagnosed with ASD due to gastrointestinal symptoms were included in the study after their organic pathologies were evaluated and excluded. In the study, FGID diagnoses were made using the data of the Pediatric Functional Diagnostic Questionnaire and Gastrointestinal Disorders Parent Report Form (prepared according to QPGS-RIII-Rome III criteria) filled out by the parents.[27]

The finding in our study that the frequency of AM in the ASD group was 13.2%, ten times higher than in the control group, was novel and had not before been reported in the literature. Recent postmortem studies in ASD, the presence of minicolumnopathy and its association with both serotonergic abnormalities and a hyperexcitable cortex were demonstrated.[28] Similarities in clinical histories and laboratory test results also suggest a presumed association between autism and migraine is also suggested by similarities in clinical histories and laboratory evidence. Some commonalities include the presence of neuroinflammation, sensory overstimulation (e.g., flickering of fluorescent lights), "food allergies," benefits from similar diets, and the role of nitric oxide.[28]

In our study, although the frequency of functional dyspepsia did not increase in children with ASD, the presence of postprandial distress syndrome in these children is noteworthy. Even though the pathophysiology of postprandial distress syndrome is not clear, gastric electrical rhythm abnormalities, delayed gastric emptying, poor gastric expansion response to feeding, and antroduodenal dysmotility are thought to be the causes. This finding suggests that these symptoms may develop due to autonomic nervous system disorders in children with ASD.

In studies, a clear and convincing link between autism and GI disorders has not yet been found. Intestinal permeability, dysbiosis, immune reactivity, GIS neurotransmitters, and genetic factors have been put forward as the cause. Changes in the gut-brain axis are thought to show a two-way interaction.[21,29,30]

It has been suggested that the absorption of poorly digested food particles or certain toxins as a result of increased intestinal permeability triggers the secretion of antibodies, resulting in inflammatory response and subsequently facilitating the development of dysbiosis by causing a drop in immunoglobulin levels.[21,29,30]

The knowledge that GI symptoms in ASD are associated with multiple pathways of the gut-brain axis has been associated with the autonomic nervous system, which affects parasympathetic activity (abnormal dynamics of neurohormones including GABA and serotonin) and sensitivity to stress, as well as the microbial and immune components.[21]

In addition, several links were found between the genetic abnormalities described in autistic cases and GI comorbidities that could explain the clinical findings. In studies, nucleotide polymorphisms of c-Met proto-oncogene encoding MET receptor tyrosine kinase, *CHD8* mutation, 5-HT transporter (SERT) gene (*SLC6A4*) variants causing serotonin elevation phenotype subtype have been reported in all cases in a subgroup of individuals diagnosed with ASD who also have additional GI disorders.[31,32]

Evidence presented by numerous studies has revealed that GI dysfunctions are particularly important and the presence of several abnormalities, including parasympathetic activity dysfunctions and elevated endocrine stress response along the nerve connections between the gut and the central nervous system. As a result, the most frequently encountered GI abnormalities, including gastroesophageal reflux, constipation, abdominal pain, diarrhea, and food selectivity are likely to be associated

with typical symptoms such as stereotypy, repetitive and ritualistic behaviors, and social withdrawal.[1,21,33,34]

It is important to note that untreated GI disorders in children with ASD have a bidirectional relationship with many problems such as sleep, behavioral, and psychiatric disorders.[1] GI problems negatively affect the quality of life, social adaptation, and treatment of children with ASD. It is thought that ASD severity and GI problems are related. It has been argued that there is a strong relationship between GI problems and psychiatric disorders such as anxiety, social withdrawal, regression in verbal abilities, and sleep problems in ASD.[1,2]

Psychiatric disorders occur in 70% of children with ASD. Anxiety is the most common diagnosed psychiatric disorder associated with ASD, while other common disorders include attention deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder.[13] Anxiety is closely linked to chronic GIS problems in children with ASD.[15] As such, functional GIS disorders in children with ASD are among the comorbidities that do not vary significantly after the age of four, along with schizophrenia, epilepsy, and sleep disorders.[20] Our study was limited in this sense; by including children with mild and moderate autism, a diagnostic correlation with FGIDs could not be made across a wide range of disease severity. In the future, the correlation between CARS scores and comorbid psychiatric disorders of children with ASD diagnosed with FGIDs can be investigated.

Consequently, in our study, the rate of functional GIS disorders in children diagnosed with ASD was 76.5%. The most frequently identified conditions were functional constipation, AM, and non-retentive fecal incontinence. The finding in this study that the majority of the children with ASD who had fecal incontinence did not show fecal retention indicated that the causes of incontinence are social, psychological, and behavioral. It should not be forgotten that increasing awareness about the recognition of functional GIS disorders in children with ASD is important for the improvement of behavioral findings as well as improving many parameters in the daily lives of these children.

Ethical approval

The local ethics committee of Dokuz Eylul University approved the study (Number: E-74660883-604.01.01-148935, Date: November 29 th 2021). Parental informed consent was obtained from all patients and controls participating in the study.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: YÖ; data collection: ÖGA, KA, GŞ, YG, SK; analysis and interpretation of results: ÖGA, YÖ; draft manuscript preparation: ÖGA, YÖ. 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

Anxiety, depression, sleep disorders and quality of life in parents of children with first unprovoked seizure and epilepsy

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ABSTRACT

Background. Parents of a child with neurological problems such as seizures and epilepsy experience significant mental distress. Little is known about the mental state of parents in such a stressful situation. This study aims to determine the prevalence of self-reported depression, anxiety, sleep disorders, and quality of life in parents of children with epilepsy and first unprovoked seizure.

Methods. This cross-sectional study was conducted among the parents of children diagnosed with first unprovoked seizure and epilepsy admitted to the Pediatric Neurology Department, Outpatient Unit of İnönü University Medical Faculty Hospital. Participants filled out a questionnaire investigating demographic variables, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Pittsburgh Sleep Quality Index (PSQI), and 36-Item Short-Form Health Survey (SF-36).

Results. 113 parents participated in the study. Depression was found in 7%, anxiety in 14%, and sleep quality disorder in 33.3% of parents of children diagnosed with epilepsy on the basis of moderate or higher severity, while depression was found in 8.9%, anxiety in 14.3%, and sleep disorder in 21.4% of parents of children diagnosed with first unprovoked seizure. There was no statistically significant difference between the groups. Mothers were at higher risk for loss of physical function and social functionality. There was a positive correlation between BAI, BDI, and PSQI scores. Quality of life sub-dimension measured by SF-36 was associated with different levels of depression, anxiety, and sleep quality.

Conclusion. Addressing parental psychiatric problems by professionals involved in the treatment of children with a history of seizures may have the potential to provide further support for the family and the care of patients.

Key words: pediatric epilepsy, parental sleep disorder, depression, anxiety, quality of life.

Epilepsy is the most common neurological problem in childhood and is observed in approximately 0.5-1% of this group.[1] Epilepsy is defined as at least two unprovoked seizures occurring at least 24 hours apart. The first unprovoked seizure occurs in 23-64.1 / 100,000 of the healthy paediatric population without epilepsy risk factors and 30-40% of these children are diagnosed with epilepsy in following periods.[2]

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The disease causes severe deterioration in the quality of life of the child and the family due to various psychiatric and cognitive disorders that are often difficult to recognize and cannot be treated.[3] Research has shown that immediately after a long or short seizure, whether febrile or non-febrile, parents are extremely frightened and suffer from anxiety and depression leading to significant impairment in familial, personal and social functions.[3,4]

In addition to these difficulties, many children with epilepsy have most seizures in their sleep and/or have sleep disorders.[5] The presence of sleep disturbance affects the quality of

life of children with epilepsy as well as their parents.[6] Although population-based data on the prevalence of sleep difficulties in children with epilepsy and their parents are limited, it has been reported that the fear of missing the seizure is frequently experienced even after a febrile seizure, and therefore sleep disturbance is frequently observed in parents due to reasons such as sleeping with the child, increased night checks or sleep problems in the child.[7,8] Although sleep disturbance is an etiological cause in the formation of fatigue, decreased functionality and psychiatric diseases, studies evaluating the effect of parents of children with first unprovoked seizure and epilepsy on mental health and sleep quality are limited in our country.

Childhood epilepsy is not only a disease but also a social problem for children and their parents.[9] Traditional and superstitious approaches such as the disease is a blood or immune system disease, that it can be transmitted to others, and that it is a punishment for previous sins often expose children and families to serious stigmatization and discrimination.[10] Therefore, more attention should be paid to the mental state of the parents during the chronic disease management of the child.

The aim of the present study is to determine anxiety, depression, sleep and quality of life and related factors in parents of children with epilepsy who have been neglected and require further research; to review the psychological changes in families after the first unprovoked seizure and to call for appropriate psychological and social intervention for these parents. In addition, since there is no similar study comparing families of children with epilepsy and families of children with first unprovoked seizure, our study may provide new findings to the literature.

Material and Methods

This cross-sectional study included parents of children aged 1 month to 17 years, with normal neurological examination and neurodevelopment diagnosed with first unprovoked seizure and epilepsy, followed at the Pediatric Neurology Department, Outpatient Clinic of Inönü University Medical Faculty Hospital between 2021-2022.

Participation in the study was voluntary, and all participants gave informed consent to participate in the study. Parents who did not reside with the child and/or were not responsible for their treatment, were illiterate, and had neurological or psychiatric diseases that could cause cognitive loss were not included in the study.

The first unprovoked seizure was defined as a seizure without a history of trauma, fever, intoxication, or acute metabolic and electrolyte disturbances.[2] Epilepsy was defined as patients who had at least two unprovoked seizures with an interval of at least 24 hours and received antiepileptic therapy.[11] Parents of children who had first unprovoked seizure were evaluated at the end of the first month after the seizure.

The İnönü University Faculty of Medicine Non-Invasive Ethics Committee approved the study design (2020/1309).

Sample Size: To compare the families of children with first unprovoked seizure and families of children previously diagnosed with epilepsy in terms of anxiety/depression rates, sleep disorder, and quality of life with 95% confidence level (α=0.05) and 80% power (β=0. If the impact power of the study was considered 0.33, the total number of participants included in the study should be at least 73.[12]

Data collection tools

Participants filled out a questionnaire with demographic variables and the child's medical history, age, gender, education, prepared by the researchers. In addition, Beck Depression Inventory (BDI) was used to evaluate depression levels, Beck Anxiety Inventory (BAI) for anxiety levels, Pittsburgh Sleep Quality Index (PSQI) to evaluate sleep quality, and the 36-Item

Short-Form Health Survey (SF-36) was used to evaluate the quality of life.

Becks Depression Inventory (BDI): This scale evaluates the level and severity of depressive symptoms in adults and the risk of depression. Beck developed it in 1961, and its Turkish validity and reliability study was done by Hisli et al. in 1989.[13,14] The scale consists of 21 questions, with 10-16 points from the scale accepted as mild depression, 17-24 points as moderate, and 25 and above points as severe depression.[15]

Beck Anxiety Inventory (BAI): It is a self-report scale that evaluates the frequency of anxiety symptoms and consists of 21 questions.[16] The Turkish validity and reliability study was done by Ulusoy et al.[17] A total of 8-15 points from the scale are accepted as mild anxiety, 16-25 points as moderate anxiety, and 26-63 points as severe anxiety.[18]

Pittsburgh Sleep Quality Index (PSQI): The PSQI is a 24-item scale that provides information on sleep quality, the type, and severity of sleep disturbances in the past month.[19] The scale evaluates 7 sub-dimensions, including subjective sleep quality, latency, duration, habitual sleep efficiency, sleep disturbance, use of sleeping pills, and daytime dysfunction. Each item on the scale scores between 0 (no distress) and -3 (severe distress). The sum of the scores of the seven sub-dimensions gives the total PSQI score. The score of each sub-dimension ranges from 0 to 3. The total PSQI score ranges from 0 to 21. The sleep quality of those with a score of 5 or less is considered "good".[20] The Turkish validity and reliability study of the scale was done by Ağargün et al.[21]

36-Item Short-Form Health Survey (SF-36): The study's participants' quality of life was evaluated by the SF-36 scale. The scale was first developed by Ware and Sherbourne in the USA, and its Turkish validity and reliability study was performed by Koçyiğit et al. in 1999.[22,23] SF-36 consists of 36 questions and measures the quality of life in eight areas of health; physical functioning (PF), general health (GH), role physical (i.e., role limitations due to the physical health problems, RP), bodily pain (BP), social functioning (SF), vitality (VT), role emotional (i.e., role limitations due to emotional problems, RE) and mental health (MH). For each domain, a score ranging from 0 to 100 was assessed with a higher score indicating better health.[24]

Statistical analysis

Statistical analysis was performed using SPSS 21.0 package program. All parameters were summarized with descriptive statistics. Participants were divided into 2 groups: parents of single-seizure children and parents of children with epilepsy. The compliance of continuous variables to normal distribution was evaluated by Kolmogorov-Smirnov test. The Student t-test or Mann-Whitney U tests were used to compare the continuous parameters between the groups and sleep quality, depression, and anxiety. The Chi-square test was used to compare categorical parameters. The relationship between sleep quality, depression, anxiety and other parameters was evaluated by Pearson correlation test. A p-value less than 0.05 was considered significant.

Results

One hundred and sixty-eight parents were invited to the study. Data from 55 participants who filled out the questionnaires incompletely were excluded from the analysis. The mean age of the 113 participants included in the study was 35.54 ± 6.7 years. Of them, 61 (54%) were mothers, and 52 (46%) were fathers. 52.1% were high school graduates, and .3.5% had a history of epilepsy. The demographic data of the participants are given in Table I.

In the evaluation of the participants' BDI scores, the total BDI score of the parents of children with epilepsy was 4.19±0.814, while the BDI score of the parents of the children presenting with the first unprovoked seizure was 3.86±0.831, and there was no statistically significant difference between them (p=0.773).

Table I. Sociodemographic characteristics of participants.

		Epilepsy	First Seizure
Age (mean±SD)		36.4 ±6.5	34.8±6.8
Parent Type	Mother	31 (27.4)	30 (26.5)
	Father	26 (23.0)	26 (23.0)
Education	Elementary	3 (2.6)	2 (1.7)
	High school	31 (27.4)	28 (24.7)
	University	23 (20.3)	26 (23)
Occupation	Employed	33 (29.2)	33 (29.2)
	Unemployed	24 (21.2)	23 (20.3)
History of chronic disease	Yes	2 (3.5)	0 (0)
History of epilepsy	Yes	2 (3.5)	2 (3.6)
Smoking history	Yes	18 (31.6)	19 (33.9)
History of psychiatric disease	Yes	2 (3.5)	1(1.8)

Data given as n (%) unless indicated otherwise.

The total BAI score of the parents of children with epilepsy was 7.82 ± 11.85, while the total BAI score of the other group was 6.71 ± 9.31 (p= 0.662).

According to the sleep quality assessment made with PSQI, sleep quality was impaired in 33.3% of parents of children with epilepsy and 21.4% of parents of children with first unprovoked seizures (p=0.156).

The comparison of SF-36 subscale scores showed no statistically significant difference between the two groups (p>0.05). The scale scores of the participants are given in Table II. Frequency of parents with moderate to severe levels of depression, anxiety symptoms and sleep disorders across all participants were shown in Table III.

The comparison between the scale scores of the parents of children with epilepsy and the parents of children with first unprovoked seizure revealed that the social functionality scores and physical function scores of mothers of children with the first unprovoked seizure were lower than the fathers (p=0.044, p=0.017; respectively) whereas the physical function scores of mothers of children with epilepsy were lower than the fathers in the same group (p=0.041). The scale scores of parent groups and the comparative data between them are given in Table IV.

In the correlation analysis conducted between the numerical parameters of the participants and the scale scores, there was a positive correlation between the BAI score and PSQI and a negative correlation with all the SF-36 subscales. A negative correlation was found between BDI score and mental health subscale score, and PUKI and bodily pain and general health subscale scores. The correlation between the scale scores of the participants is given in Table V.

Discussion

This study extends previous research by further evaluating the relationship between mood, sleep, and quality of life in parents of children with epilepsy and parents of first unprovoked seizures.

Previous studies report that parents of children with epilepsy are psychologically affected in many areas including parenting stress, and the percentage of parents who scored above the cut-off point in standardized anxiety measures was 9-58%.[25,26] Yong et al. reported that 42% of participants evaluated with The Hospital Anxiety and Depression Scale (HAD) scored in

Table II. Comparison of scale scores of participants.

Variables		Epilepsy [n (%)]	First Seizure [n (%)]	*p*
PSQI	PSQI ≤ 5	38 (66.67)	44 (78.57)	0.227[a]
	PSQI > 5	19 (33.33)	12 (21.43)	
BAI	BAI < 16	48 (85.71)	46 (88.46)	0.890[a]
	BAI ≥ 16	8 (14.29)	6 (11.54)	
BDI	BDI < 17	53 (92.98)	51 (91.07)	0.742[b]
	BDI ≥ 17	4 (7.02)	5 (8.93)	
BAI total		3(0-10.5)	3.5(0-8)	0.791*
BDI total		2(0-6)	1(0-5)	0.566*
PSQI total		4(3-6)	3(2-5)	0.380*
BP		2(1.5-3)	2.5(1.5-3)	0.791*
RE		2(1.333-2)	1.667(1.333-2)	0.879*
VT		3.41±0.58	3.53±0.81	0.380**
PF		2.8(2.6-3)	2.9(2.6-3)	0.954*
RP		2(1.25-2)	2(1.5-2)	0.703*
GH		3.05±0.56	3.1±0.4	0.616**
MH		3.85±0.48	3.8±0.66	0.702**
SF		3(2.5-3)	3(2.5-3.5)	0.231*

[a]:Yates's correction chi-square test; [b]: Fisher's exact chi square
*: Mann Whitney U test, **: Independent samples t-test. (Data are given as mean ± standard deviation or median (Q1-Q3) according to normality of distribution)
BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, BP: bodily pain, GH: General Health, MH: Mental Health, PF: Physical Functioning, PSQI: Pittsburgh Sleep Quality Index, RE: Role Emotional, RP: Role Physical, SF: Social Functioning, VT: Vitality

Table III. Frequency of parents with moderate to severe levels of depression, anxiety symptoms and sleep disorders across all participants (N=113).

	n	%
BAI ≥16	16	14.1
BDI ≥17	9	7.9
PSQI >5	31	27.4

BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, PSQI: Pittsburgh Sleep Quality Index

the clinically significant range for depression and 58% scored in the clinically significant range for anxiety, while Shariff et al. reported that 38.7% of parents scored in the clinically significant range for depression and 55% scored in the clinically significant range for anxiety.[27-29] In this study, 7.9% of all participants were found to have depression and 14.1% of all participants were found to have an anxiety disorder based on moderate and above severity levels. These rates are relatively lower than previous studies which may be due to the differences in the evaluation scales used in different studies, and may be explained by the high level of education in majority of our participants which is often not the case.[26] As a matter of fact, it has been argued in the literature that parents of children with epilepsy are exposed to more stigmatization especially in societies with low education level and this may be an important reason for the high anxiety rates in parents.[30] Similarly, a study conducted in Türkiye showed that individuals with low educational level exhibited a more stigmatizing attitude, and this was presented as a reason for parents of children with epilepsy to become more depressed and anxious over time due to a disruption in search for social support.[31] While the design of our study did not

Table IV. Comparison of scale scores of parents according to patient groups.

Variables		Epilepsy Mother	Epilepsy Father	*p*	First Seizure Mother [n (%)]	First Seizure Father [n (%)]	*p*
PSQI	PSQI ≤ 5	24 (77.42)	14 (53.85)	0.110[a]	24 (80.00)	20 (76.92)	1.0[a]
	PSQI > 5	7 (22.58)	12 (46.15)		6 (20.00)	6 (23.08)	
BAI total		4(0-11)	0(0-9)	0.168	5(0-10)	2(0-6)	0.158*
BDI total		2(0-6)	1.5(0-6)	0.755	2(0-6)	0(0-2)	0.132*
PSQI total		3(2-5)	5(3-7)	0.103*	4(2-5)	3(2-5)	0.829*
BP		2(1.5-3)	2(1.5-3)	0.556*	3(1.5-3)	1.75(1-2.5)	0.139*
RE		1.667(1.333-2)	2(1.333-2)	0.795*	1.667(1.333-2)	2(1.667-2)	0.114*
VT		3.36±0.6	3.48±0.56	0.487**	3.57±0.79	3.49±0.84	0.721**
PF		2.8(2.5-3)	3(2.7-3)	0.041*	2.7(2.3-2.9)	2.9(2.8-3)	0.014*
RP		1.875(1.25-2)	2(1.5-2)	0.711*	1.75(1.25-2)	2(1.5-2)	0.168*
GH		3.08±0.55	3.02±0.58	0.707**	3.06±0.42	3.15±0.37	0.408**
MH		3.78±0.51	3.93±0.44	0.269**	3.69±0.66	3.92±0.65	0.221**
SF		3(2.5-3)	3(3-3.5)	0.630*	3(3-3.5)	3(2.5-3)	0.017*

[a]Yates's correction chi-square test, *Mann Whitney U test, **Independent
Data are given as mean ± standard deviation or median (Q1-Q3) according to normality of distribution.
BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, BP: bodily pain, GH: general health, MH: mental health, PF: physical functioning, PSQI: Pittsburgh Sleep Quality Index, RE: role emotional, RP: role physical, SF: social functioning, VT: vitality

examine this effect, the high level of education of our participants may have played a protective role in combating anxiety caused by uncertainty through facilitating access to more accurate information about the disease and its treatment, which may explain the low rates of depression and anxiety for similar reasons. In addition, this result suggests that our clinic, which is a tertiary treatment center, may have contributed to the mental well-being of parents in its programmes for the education of families.

On the other hand, no difference was found between the levels of depression and anxiety among parents of children with both first unprovoked seizure and epilepsy in this study. This result may be an expression of the undeniable burden imposed on parents in childhood seizures and epilepsies regardless of the type of the disease, and it also supports studies showing that the mental adjustment of parents remains constant over time.[32]

Sleep studies have shown that sleep disturbance is frequently observed in parents of children with epilepsy.[33,34] It has been reported that parents, especially those who think that it is difficult to detect nocturnal seizures and that these may seriously threaten the life of the child, stay awake day and night to meet the needs of the child, wake up seven times more frequently than the healthy population and sleep an average of 4 hours/day.[34,35] Reilly et al. found that 62% of mothers and 44% of fathers of children with epilepsy had "poor quality sleep" on the PSQI.[3] Similarly, in a previous study conducted on 52 mothers of children with intractable epilepsy, 67% of the participants scored in the "poor sleep" range.[33] In this study, the rate of sleep disturbance in all parents was 27.4%. Although the rate of sleep disturbance seemed to be higher in parents of children with epilepsy (33.3%), there were no differences in sleep disturbance between groups or between parents. Sleep disturbance was also associated with increased anxiety, pain and impaired perception of general health. This finding supported the study of Cottrell et al.[36] which showed that parental sleep quality was not only

Table V. Relationship between scale scores of participants.

		Age	BDI Total	BAI Total	PSQI Total
Age	r	1	-0.115	0.000	-0.008
	p		0.257	0.996	0.940
BDI Total	r	-0.115	1	-0.105	0.032
	p	0.257		0.269	0.733
BAI Total	r	0.000	-0.105	1	0.330**
	p	0.996	0.269		**<0.001**
PSQI Total	r	-0.008	0.032	0.330**	1
	p	0.940	0.733	**<0.001**	
BP	r	0.028	0.067	-0.654**	-.0247**
	p	0.784	0.481	**<0.001**	**0.008**
RE	r	0.085	-0.002	-0.362**	-0.083
	p	0.402	0.986	**<0.001**	0.382
VT	r	0.028	0.067	-0.654**	-0.247**
	p	0.784	0.481	**<0.001**	0.008
PF	r	0.197	-0.030	-0.499**	-0.132
	p	0.050	0.756	**<0.001**	0.162
RP	r	0.057	-0.089	-0.280**	0.032
	p	0.572	0.348	**0.003**	0.733
GH	r	-0.029	-0.085	-0.560**	-0.211*
	p	0.774	0.370	**<0.001**	**0.025**
MH	r	-0.046	-0.193*	-0.616**	-0.183
	p	0.654	**0.040**	**<0.001**	0.052
SF	r	0.050	0.089	-0.461**	-0.101
	p	0.620	0.351	**<0.001**	0.200

Statistic: Spearman Correlation (r). *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed).
BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, BP: bodily pain, GH: General Health, MH: Mental Health, PF: Physical Functioning, PSQI: Pittsburgh Sleep Quality Index, RE: Role Emotional, RP: Role Physical, SF: Social Functioning, VT: Vitality

associated with the seizure frequency of the child, but also with quality of life factors such as parental depression level, physical health, pain experience, and emotional well-being. Given that sleep disturbances are associated with various unfavourable consequences in adults, including health related problems, declining quality of life and economic expenses, this result once again highlights the prevalence of sleep disturbances among parents whose children are being treated for epilepsy and the need to investigate their current sleep patterns.[37]

In our study, although there was no difference between the groups in terms of quality of life scores measured by SF-36, we observed that especially mothers were more negatively affected in the areas of physical function and social functioning. Zhang et al.[38] found that the psychological health and quality of life of mothers were affected by their children having epilepsy much more than those of fathers which is consistent with our study. One possible explanation for this is that in our society, mothers are still the primary caregivers of children, both in illness and daily life. Hence, the affinity between mothers and children is generally higher. As a result, mothers are more susceptible to the adverse effects of their children's illnesses than fathers and this may have had a greater impact on their quality of life.

One of the important findings in this study was the negative relationship between depression, anxiety, sleep disturbance and quality of life. In particular, increased anxiety negatively affected almost all subscale parameters of quality of life, while impaired sleep quality led to increased anxiety about pain and poor general health. This result is more important for children with epilepsy who need more support from their parents than their healthy peers. Because the psychological effects occurring in the parents may lead to burnout, anger, deterioration in family relations over time, this may adversely affect the treatment adaptation and development of the children.[39] Therefore, detection of current psychological symptoms in parents of children who are diagnosed with seizure as well as development of measures may provide important benefits in the prognosis of such children in addition to personal benefits.

Limitations

Our study has some limitations. This study included only patients admitted to the pediatric neurology outpatient clinic of our hospital and had a relatively small sample size. Therefore, the results obtained may not reflect the whole population. The relatively low reliability of the data obtained from self-report scales may have affected our results. In addition, the lack of a control group is an important obstacle for the comparison of mental health issues between parents with healthy children and individuals with chronic diseases. Future studies including other clinics, with a larger number of individuals and conducting one-to-one interviews will contribute to overcoming these limitations and obtain more reliable results.

Despite these limitations, our study is the first study to examine depression and anxiety symptoms, sleep and quality of life levels and the relationship between these entities among parents with a first unprovoked seizure and children with epilepsy, and may provide preliminary data for future studies.

In conclusion, our study showed that families of children with first unprovoked seizure and epilepsy diagnosis are at risk for depression and anxiety, especially sleep disorders, and their quality of life is adversely affected. Therefore, health personnel should strengthen the education of parents about their children's disease and provide psychological counselling and support when needed, which may make important contributions to both individuals and public health.

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Ethical approval

This article was approved by the Ethics Committee of İnönü University. The approval number is 2020/1309. All participants gave informed consent to participate in the study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BÖ, NC data collection: BÖ, NC analysis and interpretation of results: BÖ, NC draft manuscript preparation: BÖ, NC All authors reviewed 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

Effect of intravenous lipid therapy in critically ill pediatric patients with calcium channel blocker toxicity

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ABSTRACT

Background. Overdose with calcium-channel blockers (CCBs) still maintain their importance with a high lethality rate after exposure. We report the intravenous lipid emulsion therapy (ILE) therapy in our CCB overdose patients.

Methods. We retrospectively analyzed the records of 6 patients with CCB intoxication from Batman Training and Research Hospital PICU between March 2021 and September 2022. Patients aged 0-18 years who received ILE treatment for CCB poisoning were included.

Results. All six patients ingested CCB with the intention of committing suicide and were followed up in the pediatric intensive care unit (PICU). All patients received ILE therapy due to hemodynamic instability despite intravenous fluid boluses, calcium, glucagon, insulin-dextrose, and vasoactive agents. Vasoactive-Inotropic Score (VIS) decreased after ILE treatment. All patients were transferred from the PICU after recovery.

Conclusions. ILE therapy should be kept in mind as a salvage therapy in hemodynamically unstable CCB poisoning cases that do not respond to initial and advanced options.

Key words: calcium channel blockers, lipid emulsion, pediatrics, intoxication.

Calcium-channel blockers (CCBs) are a widely accepted class of drugs for the treatment of cardiovascular diseases. They have inhibitory effects on arterial smooth muscle and have an important potential for use in diseases other than the cardiovascular system.[1,2] It has been reported that a single tablet can be lethal, even though there are no symptoms at low levels in cases of poisoning.[3] Cardiovascular instability, bradycardia, hypotension, metabolic acidosis, hyperglycemia and seizures may be observed in poisoning.[3] CCBs still maintain their importance despite their high fatality rate following exposure.[2] intravenous lipid emulsion (ILE), which is used as a rescue therapy in lipophilic drug poisoning, has also become an option in severe cases of CCB poisoning.[4] In this case series, we report the efficacy of ILE in cases of CCB poisoning unresponsive to initial and advanced therapies.

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Material and Methods

The hospital data of 6 pediatric patients with CCB intoxication in the Pediatric Intensive Care Unit (PICU) of the Batman Training and Research Hospital between March 2021 and September 2022 was retrospectively analyzed. Inclusion criteria comprised patients aged 0-18 years who were administered ILE treatment for CCB poisoning. Patients presenting with hypotension and circulatory system disorders received fluid bolus, intravenous (IV) calcium, hyperinsulinemia-euglycemia treatment (HIET), and glucagon. In cases where stabilization was not achieved, ILE treatment was administered. ILE was given to six out of 11

patients with CCB poisoning during this period; the remaining five patients were excluded from the study as they did not receive ILE. Recorded data included age, sex, drug dosage, symptoms, vital signs, Vasoactive Inotrope Score (VIS), Pediatric Risk of Mortality III Score (PRISM III), ejection fraction percentage (EF), and laboratory data such as blood sugar and kidney function tests. All patients were consulted with cardiology before and after ILE and hemodynamic findings were recorded (Table I). A recommended dosing regimen for lipid emulsion is an infusion of 20% solution, 1 mL/kg over 1 minute, repeated every 3 to 5 minutes for a maximum of 3 mL/kg followed by 0.25 mL/kg/min.[5]

VIS was calculated as follows: dopamine dose (μg/kg/min) + dobutamine dose (μg/kg/min) + 100 × epinephrine dose (μg/kg/min) + 100 × norepinephrine dose (μg/kg/min) + 10 × milrinone dose (μg/kg/min) + 10,000 × dose of vasopressin (U/kg/min). The study adhered to the principles of the Declaration of Helsinki. The Institutional Ethics Committee of the Batman Training and Research Hospital approved the study protocol (Date: 18.09.2022/No: 319).

Results

Case 1

A 13-year-old girl was brought to the emergency department 6 hours after ingestion of 27 tablets of amlodipine (270 mg) and valsartan for suicidal purposes (Table I). She was conscious, with a pulse of 100 beats/min, blood pressure of 90/40 mm Hg, respiratory rate of 24 breaths/min, temperature of 36.5°C, and 98% pulse oxygen saturation at the arrival at the PICU. IV calcium, hyperinsulinemia euglycemia treatment (HIET; 0.1 IU/kg/h+ 0.25 gr/kg/h) was administered while closely monitoring blood glucose levels and targeting euglycemia (Table II). IV noradrenaline (up to 0.20 mcg/kg/min) and IV adrenaline (up to 0.1 mcg/kg/min) were started (Table I). ILE treatment was started during the third hour of the supportive therapy. Four hours after ILE, blood pressure increased to 110 /59 mm Hg.

Case 2

A 16-year-old girl was brought to the emergency department after receiving 20 tablets of nitrendipine (400 mg) and enalapril maleate for suicidal purposes after 1 hour. She was agitated with a pulse of 120 beats/min, blood pressure of 85/39 mm Hg, respiratory rate of 26 breaths/min, temperature of 36.5°C, and 98% pulse oxygen saturation under supplemental oxygen. IV calcium, hyperinsulinemia euglycemia treatment and IV glucagon (0.15 mg/kg bolus) were administered while closely monitoring blood glucose levels and targeting euglycemia. IV noradrenaline (up to 0.30 mcg/kg/min) and IV adrenaline (up to 0.20 mcg/kg/min) were started. ILE treatment was started during the second hour of the supportive therapy. Four hours after ILE her blood pressure increased to 110 /59 mm Hg.

Table I. Hemodynamic findings before and after ILE.

Case	Pre-ILE EF %	Post-ILE EF%*	Pre- ILE VIS	Post- ILE VIS**	Intubated	Outcome	LOS – PICU (day)
1	45	60	30	10	-	survived	8
2	40	65	50	20	-	survived	9
3	50	55	15	5	-	survived	6
4	40	55	30	10	-	survived	5
5	45	50	30	15	-	survived	6
6	35	65	40	20	+	survived	10

*72 hours after ILE treatment., **24 hours after ILE treatment.
EF: Ejection fraction, ILE: Intravenous lipid emulsion, LOS: Length of stay, VIS: Vasoactive inotrope score

Table II. Summary of patient characteristics and medical treatments applied before intravenous lipid emulsion therapy.

Case	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age/sex	13/F	16/F	14/F	17/F	16/M	15/M
Drug	Amlodipine	Nitrendipine	Amlodipine	Verapamil	Verapamil	Verapamil
Dose(mg)	270	400	20	680	800	1600
Number of tablets	27	20	4	17	20	40
Gastric lavage	-	+	-	-	+	-
Active charcoal	-	+	-	+	+	-
Fluid bolus	+	+	+	+	+	+
Bicarbonate deficit	-	-	+	+	-	+
Noradrenaline	+	+	+	+	+	+
Adrenaline	+	+	-	-	-	+
Glucagon	-	+	-	-	-	-
HIET	+	+	+	+	+	+
Time to PICU	6 hours	1 hour	24 hours	4 hours	2 hours	8 hours

HIET: Hyperinsulinemia euglycemia treatment, PICU: Pediatric intensive care unit

Case 3

A 14-year-old girl was admitted to the emergency services of another hospital 24 hours after ingesting 4 tablets of amlodipine (20 mg) and hydrochlorothiazide (14 tablets) for a suicide attempt. The patient was admitted to the PICU for further treatment after developing acute kidney injury and hypotension during an examination at the external center. She was agitated with pulse of 105 beats/min, blood pressure of 86/42 mm Hg, respiratory rate of 25 breaths/min, temperature of 36.6°C, and 99% pulse oxygen saturation under supplemental oxygen. IV calcium, hyperinsulinemia euglycemia treatment was started while closely monitoring blood glucose levels and targeting euglycemia. IV noradrenaline (up to 0.15 mcg/kg/min) was started. ILE treatment was started during the second hour of the supportive therapy. In the follow-up after the ILE her blood pressure increased to a normal level within 4 hours. Renal function tests were impaired at admission, and they regressed to normal values at the 24th hour after ILE treatment.

Case 4

A 17-year-old female patient was brought to the emergency department with nausea, 4 hours after ingesting 17 tablets of verapamil (680 mg) for a suicide attempt. The heart rate was 71 beats per minute, the blood pressure was 73/33 mm Hg, pulse oxygen saturation was 96%, the respiratory rate was 22 breaths per minute, and the body temperature was 36.9°C. IVcalcium (4x 0.5 cc/kg 10% calcium gluconate), hyperinsulinemia euglycemia treatment and bicarbonate deficit were given for refractory acidosis. IV noradrenaline as a vasopressor was started (up to 0.30 mcg/kg/min). ILE treatment was started during the first hour of the supportive therapy. During follow-up, his blood pressure increased up to 110/61 mm Hg within 4 hours after the ILE.

Case 5

A 16-year-old male patient was brought to the emergency department with nausea 1 hour after ingesting 20 tablets of verapamil (800 mg) for a suicide attempt. His heart rate was 55 beats per minute, the blood pressure was

75/35 mm Hg, pulse oxygen saturation 96% under supplemental oxygen, the respiratory rate was 20 breaths per minute, and the body temperature was 36.6°C. IV calcium (4x 0.5 cc/kg 10% calcium) and hyperinsulinemia euglycemia treatment was started while closely monitoring blood glucose levels and targeting euglycemia. IV noradrenaline as a vasopressor was started (up to 0.30 mcg/kg/min). ILE treatment was started during the third hour of the supportive therapy. In the follow-up, blood pressure increased up to 105/56 mm Hg within 4 hours after the initiation of ILE.

Case 6

A 15-year-old male patient was brought to the emergency department of another hospital with unconsciousness and respiratory distress 8 hours after ingesting 40 tablets of verapamil (1600 mg) for suicide. His heart rate was 55 beats per minute, the blood pressure was 75/35 mm Hg, pulse oxygen saturation 80% under supplemental oxygen, the respiratory rate was 40 breaths per minute, and the body temperature was 36.9°C. The patient was intubated because of the signs of respiratory failure. IV calcium (4x 0.5 cc/kg 10% calcium gluconate) and hyperinsulinemia euglycemia treatment were started while closely monitoring blood glucose levels and targeting euglycemia. IV noradrenaline (up to 0.30 mcg/kg/min) and IV adrenaline (up to 0.10 mcg/kg/min) infusion were started. ILE treatment was started during the first hour of the supportive therapy. Blood pressure increased up to 110/60 mm Hg within 4 hours after the initiation of ILE. On the third day, the patient was extubated.

Discussion

CCBs are used in the treatment of hypertension, angina pectoris and arrhythmias.[1] CCBs reduce extracellular calcium through ion-channels that span the cell wall. Several types of such channels have been identified and existing CCBs inhibit L-type channels in humans.[6] CCBs can be physiologically divided into two main categories: dihydropyridines, which block L-type calcium channels in vessels; and non-dihydropyridines such as verapamil and diltiazem, which block L-type calcium channels in the myocardium.[1] When calcium channels are blocked, vascular smooth muscle cells relax, causing vasodilation and lowering of blood pressure. Cardiac contraction decreases and atrioventricular conduction slows down.[6] Dihydropyridines only have a mild effect on decreasing myocardial contraction, while verapamil causes profound inhibition of the sinoatrial and atrioventricular nodes.[7] Non-dihydropyridine poisonings like diltiazem and verapamil may present with bradycardia or cardiogenic shock. In cases of overdose, the selectivity of calcium channel blockers may be lost.[8]

Although CCBs have a limited number of FDA-approved pediatric indications, very high CCB exposures are reported to the US Poison Control Centers. According to the 2019 report of the American Association of Poison Control Centers, calcium antagonist poisoning is prominent after antipsychotics and analgesics.[2] Although small ingestions pose little risk, deaths have been reported even after exposure to 1 or 2 tablets, and symptomatic cases can be suddenly fatal.[3]

Cardiovascular imbalance may present with hypotension, bradycardia, conduction abnormalities, and arrhythmias. Patients may present with hyperglycemia, metabolic acidosis, seizures, mental status changes, and respiratory depression.[9] Our patients exhibited symptoms such as nausea, vomiting, mental status abnormalities, and respiratory distress either alone or in combination.

There is no effective antidote and the mainstay of treatment is hemodynamic support.[10] With careful clinical evaluation, dysregulation of cardiac activity can be prevented, and symptoms can be improved with advanced life support.[8] More specifically, IV calcium, glucagon and insulin-glucose medical treatments are also used.[4] Our patients were given IV calcium,

glucagon, insulin-dextrose treatments after fluid boluses. Bicarbonate infusion was started in patients with resistant acidosis.

The fact that CCBs have a wide distribution in volume in the body and are highly bound to proteins makes it difficult to remove them by extracorporeal methods.[10] Hemodialysis and hemoperfusion can be applied to hemodynamically unstable patients despite initial treatments.[11] There are also case reports in which extracorporeal membrane oxygenation (ECMO) is activated and advanced life support is given to patients who do not respond to other extracorporeal life support.[12] Vasoactive agents, which are one of the main supports in providing hemodynamics, were titrated by frequent blood pressure monitoring in 6 of our patients. With the initiation of ILE treatment in the early period, extracorporeal methods were not needed in any of our patients.

Lipid emulsion has been used as a nutritional supplement as a component of total parenteral nutrition.[13] It has been reported that the use of ILE, known as lipid rescue, is beneficial for local anesthetic toxicity in animal models and human case.[14] Although there is no definitive mechanism of action for the treatment of ILE, there are possible mechanisms. It is thought that the main mechanism in local anesthesia poisoning because of the binding property of the lipid emulsion.[15] ILE can reduce the amount of free drug by keeping the lipophilic medicine in a separate compartment. ILE can promote drug clearance by hepatic administration of compound-laden chylomicrons and can also transition from lipid to glucose metabolism in cardiac myocytes by increasing nitric oxide and α-ketoacids.[16] These compounds increase the calcium influx from blocked cells.

ILE therapy is generally recommended for use in life-threatening poisonings due to its balance of benefits and negative effects, despite a neutral agreement on its general usage regions.[17] It is also recommended to be used for life-threatening bupivacaine and other local anesthetics, amitriptyline, bupropion poisoning where other treatments are ineffective.

Pediatric case series are not commonly found in medical literature. However, Katlan et al.[18] published a pediatric case series and literature review which demonstrated that ILE treatment can be an effective rescue treatment option for patients who do not show cardiovascular improvement despite receiving supportive treatments.

A recommended dosing regimen for lipid emulsion is an infusion of 20% solution, 1 mL/kg over 1 minute, repeated every 3 to 5 minutes for a maximum of 3 mL/kg followed by 0.25 mL/kg/min.[5]

Although further studies are needed, potential adverse events with ILE therapy include allergy, anaphylaxis, fat embolism, thrombophlebitis, and seizures.[13] After ILE treatment, none of our patients had any side effects during their stay in the PICU.

When patients with cardiovascular hemodynamic instability do not respond to initial and advanced supportive treatments, ILE treatment can be considered a rescue therapy. This treatment can help reduce VIS and avoid the need for extracorporeal life support. Therefore, it is important for clinicians to use ILE treatment without prejudice during the early period.

Ethical approval

Batman Training and Research Hospital Scientific Research Ethics Committee approved this study (approval date/no: 22.09.2022 / 319).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AA; data collection: RGS; analysis: MMK; methodology: DA; software; SY; writing – original draft and writing review & editing. 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 childhood malignancies presenting with musculoskeletal manifestations from two different divisions: a multicenter study

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ABSTRACT

Background. The aim of the study was to evaluate the approaches of pediatric rheumatologists and pediatric hematologists to patients with similar musculoskeletal (MSK) complaints and to highlight the differences that general pediatricians should consider when referring patients to these specialties.

Methods. This is a cross-sectional study involving the patients who applied to pediatric rheumatology centers with MSK complaints and were diagnosed with malignancy, as well as patients who were followed up in pediatric hematology centers with a malignancy diagnosis, and had MSK complaints at the time of admission.

Results. A total of 142 patients were enrolled in the study. Of these patients, 83 (58.4%) applied to pediatric rheumatology centers, and 59 (41.6%) applied to pediatric hematology centers. Acute lymphoblastic leukemia (ALL) was the most common diagnosis among the patients who applied to both centers, with 80 cases (56.3%). The median age of diagnosis was 87 (interquartile range, IQR: 48-140) months. The most common preliminary diagnosis in pediatric rheumatology centers was juvenile idiopathic arthritis (JIA), with 37 cases (44.5%). MSK involvement was mainly seen as arthralgia, and bone pain. While arthralgia (92.7%) was the most common complaint in rheumatology centers, bone pain (88.1%) was more common in hematology centers. The most frequently involved joints were the knee (62.9%), ankle (25.9%), hip (25%), and wrist (14%). The most common laboratory abnormalities were high lactate dehydrogenase (LDH), high C-reactive protein (CRP), anemia, and high erythrocyte sedimentation rate (ESR). Thrombocytopenia, neutropenia, and high LDH were statistically significantly more frequent in patients admitted to hematology centers than in patients admitted to rheumatology centers ($p<0.001$, $p=0.014$, $p=0.028$, respectively). Patients who applied to rheumatology clinics were found to have statistically significantly higher CRP levels ($p=0.032$).

Conclusions. Malignancies may present with only MSK system complaints in childhood. Therefore, malignancies should be included in the differential diagnosis of patients presenting with MSK complaints.

Key words: musculoskeletal complaints, malignancy, pediatric rheumatology, pediatric hematology.

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Musculoskeletal (MSK) system complaints are one of the most common symptoms in childhood. It is observed in approximately half of the school-age children.[1] In the majority of patients, the underlying cause is of a traumatic or benign origin. Nevertheless, cancer patients can also present with MSK system complaints. Some patients presenting with only MSK complaints may have cancer as the underlying cause. While most of the patients present with systemic findings such as fever, weight loss, night sweats, pallor, palpable mass, bleeding, bruising, vomiting and headache, in some cases, MSK complaints are the first and only symptom.[2,3] As a result, patients are referred to other medical specialties, such as rheumatology. Moreover, because systemic symptoms such as fever, weakness, weight loss, and skin rash can be confused with the symptoms of systemic rheumatic diseases in cancer patients, rheumatologists may experience diagnostic delays when investigating the diagnosis of rheumatic disease. Used in treating rheumatic diseases, immunosuppressive agents and corticosteroids, may also cause delays in diagnosis. Whereas, the most critical condition that determines the 5-year survival of patients is the early diagnosis and treatment of cancer.[4]

The most common MSK complaints are bone pain, arthritis, and arthralgia. Primary tumors of bone, cartilage, muscle, or fibrous tissue, leukemic infiltration of bone, or paraneoplastic conditions are the causes of these complaints.[5] The character of the pain is very important for the differential diagnosis. Severe bone pain, especially at night, suggests malignancy, while pain with morning stiffness suggests juvenile idiopathic arthritis, but it is not always easy to make this distinction.

Since the time to diagnosis has a direct impact on the prognosis of these patients, the approaches and referrals of general pediatricians who see them for the first time are directly related to their prognosis. First of all, after a wide differential diagnosis including malignancies, traumatic, infectious, and rheumatic causes, the patients should be referred to the appropriate field based on the diagnosis considered.

Our aim in this study is to evaluate the approaches of pediatric rheumatologists and pediatric hematologists to patients with similar MSK complaints and to highlight the differences that general pediatricians should consider when referring patients to these specialties.

Material and Methods

This was a cross-sectional study involving both pediatric rheumatology and pediatric hematology centers. Patients from nine pediatric rheumatology centers and three pediatric hematology centers in Türkiye were included in the study between June 2016 and October 2022. Patients who applied to pediatric rheumatology centers with MSK complaints and were diagnosed with malignancy, as well as patients who were followed up in pediatric hematology centers with a malignancy diagnosis, and had MSK complaints at the time of admission, were selected.

Clinical and laboratory features were recorded from the medical charts and electronic files of the patients retrospectively. In all cases, a definitive diagnosis was made by bone marrow examination or histological examination of surgical specimens.

C-reactive protein (CRP) > 5 mg/L and the first hour's erythrocyte sedimentation rate (ESR) > 20 mm were defined as elevated inflammatory markers. White blood cell (WBC) >10000 10^6/L was defined as leukocytosis, WBC <4000 10^6/L as leukopenia, absolute neutrophil count (ANC) <1500 10^6/L as neutropenia, absolute lymphocyte count (ALC) < 1500 10^6/L as lymphopenia, hemoglobin <12 g/dL as anemia, platelet count <150000 10^6/L as thrombocytopenia, lactate dehydrogenase (LDH) > 225 U/L as high LDH, and uric acid >5.5 mg/dL as hyperuricemia. Patients with pain intensities of 7 or higher on the visual analog scale were classified as having severe pain.

The study protocol was reviewed and approved by the Ethics Committee of the University of Health Sciences, Ümraniye Training and Research Hospital (Approval No: B.10.1.TKH.4.34.H.GP.0.01/336) with the ethical principles laid down in the Declaration of Helsinki.

Statistical analysis

The statistical analyses were made using SPSS version 25.0. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov test) to determine whether or not they were normally distributed. In the descriptive analysis, normally distributed variables were presented as mean ± standard deviation (SD), and non-normally distributed variables were presented as median and interquartile range (Q1-Q3). Categorical variables were compared with the chi square test. The Mann–Whitney *U* test was used to compare the non-normally distributed variables between independent two groups. One-way ANOVA was used to compare the parameters between the groups. An overall *p* value of less than 0.05 was considered to show a statistically significant difference.

Results

A total of 142 patients were enrolled in the study. Of these patients, 83 (58.4%) applied to pediatric rheumatology centers, and 59 (41.6%) applied to pediatric hematology centers. Among them, 67 (47%) were female and 75 (53%) were male. The median age of diagnosis was 87 (interquartile range, IQR: 14-211) months. The demographic, clinic, and laboratory characteristics of the patients are described in Table I.

The time between applying to pediatric rheumatology or pediatric hematology centers and receiving a diagnosis ranged from one day to 20 months. Acute lymphoblastic leukemia (ALL), and acute myeloblastic leukemia (AML) patients had the shortest time to diagnosis, with a median of 4 (IQR: 1-10) days, and 5 (IQR: 4-7) days. The patient with the longest diagnosis time of 600 days had recurrent arthralgia complaints, was diagnosed with familial Mediterranean fever (FMF), and was later diagnosed with Ewing sarcoma due to increased pain intensity in follow-ups. Fatigue (63.3%), fever (47.8%), hepatomegaly (45%), lymphadenopathy (44.3%), and splenomegaly (41.5%) were the most common findings accompanying MSK involvement. The duration of the fever ranged from one to 180 days. Patients with Ewing sarcoma had the shortest duration of fever, with mean of 3.5±0.7 days, while patients with neuroblastoma had the longest duration, with median of 19.5 (IQR: 11-97.5) days. The most common laboratory abnormalities were high levels of LDL (83.8%), high levels of CRP (80.9%), anemia (78.1%), and high levels of ESR (75.3%). Thrombocytopenia, neutropenia, and high levels of LDH were statistically significantly more frequent in patients admitted to hematology centers than in patients admitted to rheumatology centers ($p<0.001$, $p=0.014$, $p=0.028$, respectively). Patients who applied to rheumatology centers were found to have statistically significantly higher CRP levels ($p=0.032$) (Table I).

MSK involvement was mainly seen as arthralgia, and bone pain. While arthralgia (92.7%) was the most common complaint among patients admitted to rheumatology centers, bone pain (88.1%) was more common among patients admitted to hematology centers. The most frequently involved joints were the knee (38.7%), hip (17.6%), ankle (14%), and wrist (9.8%). The most common sites of bone pain were the lower extremity (52%), generalized bone pain (36.6%) and the lumbar region (16.9%). The details of MSK involvement are shown in Supplementary Table S1.

ALL was the most common diagnosis among patients who applied to both pediatric rheumatologists and pediatric hematologists. Eight patients were diagnosed with AML, all of whom were diagnosed at pediatric hematology centers. While non-Hodgkin lymphoma (NHL) and neuroblastoma were frequently diagnosed in pediatric rheumatology centers, other

Table I. Demographic, clinic, and laboratory characteristics of the patients in pediatric rheumatology and pediatric hematology centers.

	Rheumatology (n=83)	Hematology (n=59)	Total (n=142)	P value
Diagnosis age (months), median (IQR)	85 (47-141)	92 (48-140)	87 (48-140)	0.524
Gender (F/M)	36/47	31/28	67/75	0.284
Time to diagnosis (day), Median (IQR)	14 (3-41.2)	4 (2-10)	7 (2-30)	**0.002**
Fever days , Median (IQR)	7.5 (4-22.5)	5 (4-8)	7 (4-10)	0.056
Musculoskeletal involvement, n (%)	83 (100)	59 (100)	142 (100)	
Arthritis	16 (19.2)	11 (18.6)	27 (19)	0.552
Arthralgia	77 (92.7)	28 (47.4)	105 (73.9)	**<0.000**
Bone pain	47 (56.6)	52 (88.1)	99 (69.7)	**<0.000**
Constitutional symptoms, n (%)	57 (68.6)	44 (74.5)	101 (71.1)	0.283
Fever	39 (68.4)	29 (65.9)	68 (67.3)	0.466
Fatigue	49 (85.9)	41 (93.1)	90 (89.1)	0.107
Weight loss	21 (36.8)	15 (34)	36 (35.6)	0.569
Night sweats	10 (12)	8 (13.5)	18 (12.6)	0.491
Skin rash, n (%)	14 (16.8)	2 (3.3)	16 (11.2)	**0.010**
Lymphadenopathy, n (%)	27 (32.5)	36 (61)	63 (44.3)	**0.001**
Hepatomegaly, n (%)	26 (31.3)	38 (64.4)	64 (45)	**<0.000**
Splenomegaly, n (%)	21 (25.3)	38 (64.4)	59 (41.5)	**<0.000**
Abdominal pain, n (%)	22 (26.5)	11 (18.6)	33 (23.2)	0.187
Chest pain, n (%)	4 (4.8)	2 (3.3)	6 (4.2)	0.512
Headache, n (%)	4 (4.8)	5 (8.4)	9 (6.3)	0.294
Leukopenia, n (%)	9 (10.8)	7 (11.8)	16 (11.2)	0.527
Leukocytosis, n (%)	34 (40.9)	28 (47.4)	62 (43.6)	0.275
Neutropenia, n (%)	18 (21.6)	24 (40.6)	42 (29.5)	**0.014**
Lymphopenia, n (%)	11 (47.8)	4 (6.7)	15 (10.5)	0.190
Thrombocytopenia, n (%)	14 (16.8)	36 (61)	50 (35.2)	**<0.000**
Anemia, n (%)	65 (78.3)	46 (77.9)	111 (78.1)	0.560
High CRP, n (%)	72 (86.7)	43 (72.8)	115 (80.9)	**0.032**
High ESR, n (%)	70 (84.3)	37 (62.7)	107 (75.3)	0.512
High LDH, n (%)	65 (78.3)	54 (91.5)	119 (83.8)	**0.028**
High uric acid, n (%)	14 (16.8)	14 (23.7)	28 (19.7)	0.223

CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, LDH: lactate dehydrogenase

cancers such as Hodgkin lymphoma (HL), osteosarcoma, Langerhans cell histiocytosis (LCH), and Ewing sarcoma were diagnosed in both centers (Fig. 1).

The most common preliminary diagnosis of patients who were referred to the pediatric rheumatology centers was juvenile idiopathic arthritis (JIA), with 37 cases (44.5%). Reactive arthritis, systemic JIA (sJIA)/macrophage activation syndrome (MAS), autoinflammatory diseases (AID), connective tissue disease (CTD), and vasculitis were the other preliminary diagnoses. The majority of patients who were referred with a preliminary diagnosis of JIA, reactive arthritis, sJIA/MAS, or AID were eventually diagnosed with ALL. Two of the four patients referred with a preliminary diagnosis of CTD were diagnosed with HL and two with NHL. Two patients who were referred with a preliminary diagnosis of vasculitis were diagnosed with NHL (Table II).

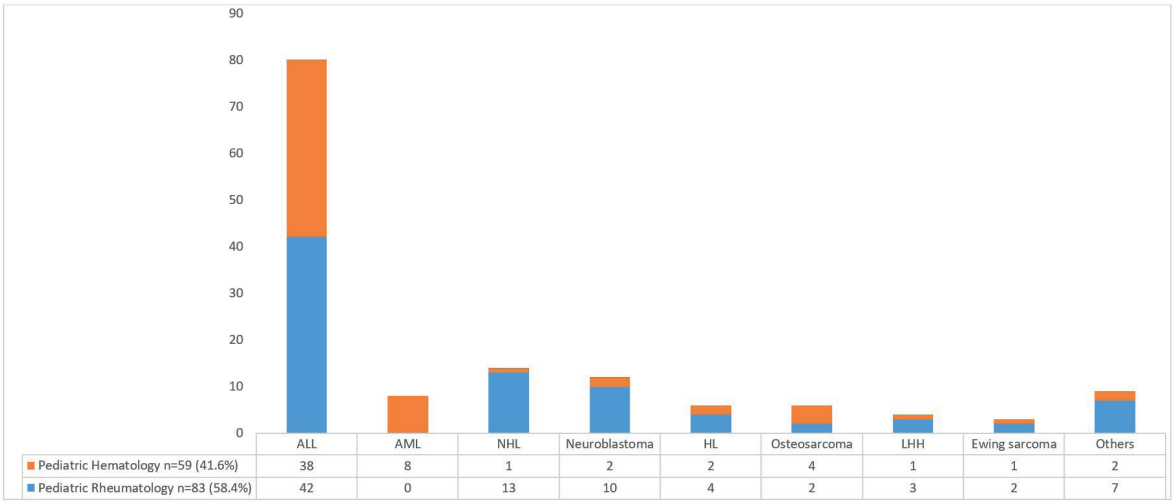

Fig. 1. The final diagnoses of the patients according to center type.

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; NHL, Non-Hodgkin lymphoma; HL, Hodgkin lymphoma; LCH, Langerhans cell histiocytosis

Table II. The preliminary and final diagnoses of the patients referred to rheumatology centers.

		Preliminary Diagnoses						
		JIA n= 37	Reactive arthritis n= 17	sJIA/MAS n=13	AID n= 9	CTD n= 4	Vasculitis n= 2	Other n= 1
Final Diagnoses	ALL, n= 42	21	10	6	5	0	0	0
	Neuroblastoma, n= 10	2	3	4	1	0	0	0
	NHL, n= 13	4	1	3	1	2	2	0
	HL, n= 4	1	0	0	1	2	0	0
	Ewing sarcoma, n= 2	0	1	0	1	0	0	0
	LCH, n= 3	3	0	0	0	0	0	0
	Osteosarcoma, n= 2	2	0	0	0	0	0	0
	Other, n= 7	4	2	0	0	0	0	1

AID, autoinflammatory diseases; ALL, acute lymphoblastic leukemia; CTD, connective tissue disease; HL, Hodgkin lymphoma; JIA, juvenile idiopathic arthritis; LCH, Langerhans cell histiocytosis; NHL, Non-Hodgkin lymphoma; sJIA/MAS, systemic juvenile idiopathic arthritis/macrophage activation syndrome

In this study, 88 patients (61.9%) were diagnosed with hematological malignancy, whereas 54 patients (38.1%) were identified with solid malignancy. Among the subset of patients admitted to pediatric rheumatology, 50.6% were diagnosed with hematological malignancies. In contrast, a higher proportion (77.9%) of patients admitted to pediatric hematology clinics were diagnosed with a hematological malignancy. Patients with hematological malignancies had a younger age at diagnosis, shorter diagnosis time, fewer days with fever, and a higher proportion of males. Bone pain, constitutional findings, hepatomegaly, and splenomegaly were observed more frequently in hematological malignancies. In hematological malignancies, leukopenia, neutropenia, thrombocytopenia, anemia, high levels of ESR, and high levels of LDH were more common (Table III).

In hematological malignancies, the most common MSK involvement was bone pain, while arthralgia was the most common in solid malignancies. Hip pain was statistically more common in patients with solid malignancies (p=0.036). Lower limb pain was

Table III. Demographic, clinic, and laboratory characteristics of the patients in hematological and solid malignancies.

	Hematological malignancies (n=88)	Solid malignancies (n=54)	P value
Diagnosis age (months), median (IQR)	83 (45-114)	126 (59-176)	**<0.000**
Gender, (F/M)	35/53	32/22	**0.018**
Time to diagnosis (day), median (IQR)	4 (2-10)	30 (11-52)	**<0.000**
Fever days, median (IQR)	5 (3-10)	10 (4-30)	**0.019**
Musculoskeletal involvement, n (%)	88 (100)	54 (100)	
Arthritis	15 (17)	12 (22.2)	0.291
Arthralgia	61 (69.3)	44 (81.4)	0.078
Bone pain	70 (79.5)	29 (53.7)	**0.001**
Constitutional symptoms, n (%)	69 (78.4)	32 (59.2)	**0.013**
Fever	44 (63.7)	24 (75)	0.319
Fatigue	65 (94.2)	25 (78.1)	**0.001**
Weight loss	19 (27.5)	17 (53.1)	0.132
Night sweats	11 (12.5)	7 (12.9)	0.565
Skin rash n (%)	7 (7.9)	9 (16.6)	0.095
Lymphadenopathy, n (%)	41 (46.6)	22 (40.7)	0.286
Hepatomegaly, n (%)	47 (53.4)	17 (31.4)	**0.008**
Splenomegaly, n (%)	46 (52.7)	13 (24.1)	**0.001**
Abdominal pain, n (%)	19 (21.6)	14 (25.9)	0.346
Chest pain, n (%)	4 (4.5)	2 (3.7)	0.586
Headache, n (%)	7 (7.9)	2 (3.7)	0.263
Leukopenia, n (%)	15 (17)	1 (1.8)	**0.003**
Leukocytosis, n (%)	36 (40.9)	26 (48.1)	0.251
Neutropenia, n (%)	40 (45.4)	2 (3.7)	**<0.000**
Lymphopenia, n (%)	7 (7.9)	8 (14.8)	0.149
Thrombocytopenia, n (%)	48 (54.5)	2 (3.7)	**<0.000**
Anemia, n (%)	76 (86.3)	35 (64.8)	**0.003**
High CRP, n (%)	72 (81.8)	43 (79.6)	0.455
High ESR, n (%)	70 (79.5)	37 (68.5)	**0.032**
High LDH, n (%)	81 (92)	38 (70.3)	**0.001**
High uric acid, n (%)	21 (23.8)	7 (12.9)	0.108

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase

statistically more common in hematological malignancies (p=0.004). MSK involvement in hematological and solid malignancies is shown in Supplementary Table S2.

Discussion

To the best of our knowledge, this is the first study to compare patients with MSK complaints who applied to pediatric rheumatology and pediatric hematology centers and were diagnosed with a malignancy. In the literature, the studies of patients who initially present with MSK involvement and are subsequently diagnosed with malignancy are generally presented as case series.[6-10] Therefore, there is limited information available on this topic. We aimed with this study to determine which patients should be referred

to the pediatric hematology centers and which patients applying to the pediatric rheumatology centers should exercise greater attention.

In our study, ALL was the most common diagnosis among both pediatric rheumatologists and pediatric hematologists. In the literature, similar to our study, ALL is the most common childhood malignancy, and MSK complaints are frequently observed in these patients.[11,12] In the studies, the frequency of MSK complaints in ALL patients was found to be between 7.1% and 62.3%[2,13,14], while the frequency of malignancy in patients presenting with MSK complaints was between 0.25-2%.[5,6] That is why it should be kept in mind that patients who apply with MSK complaints could have a cancer, and physicians should be careful during the follow-up process.

Patients with ALL and AML had the shortest time to diagnosis in our study. In some subtypes of JIA such as systemic JIA, especially in patients with atypical involvement or complicated findings, the differential diagnosis of leukemia mostly needs to be made by bone marrow aspiration, and this may explain why the diagnosis time of ALL and AML patients is shorter than other malignancies.

In the present study, patients admitted to pediatric hematology had shorter intervals between diagnoses. Due to the fact that rheumatic diseases were considered in the differential diagnosis of patients admitted to pediatric rheumatology and anti-rheumatic drugs were administered to some of these patients, the time between diagnosis was lengthened. In the study of Kang et al.[15], they compared ALL cases with and without MSK complaints and found that those with MSK involvement had a longer time to be diagnosed. In the study of Brix et al.[3], it was reported that patients with arthritis had a long time to diagnosis compared to patients with arthralgia. Kittivisuit et al.[14] reported that patients with MSK involvement had fewer hematological abnormalities and peripheral blasts compared to those without. The results of these studies support the finding that the diagnosis time of patients who apply to pediatric rheumatology centers is prolonged.

In the present study, 16.8% of patients diagnosed with a malignancy in pediatric rheumatology centers complained of a rash. Because rashes are often the first symptom of most rheumatic diseases, especially sJIA, and vasculitis, these patients are frequently referred to pediatric rheumatology centers. As a result of this study, we concluded that in these patients, special attention should be paid to the presence of hepatomegaly, splenomegaly, and lymphadenopathy, and hematological parameters should be carefully evaluated.

In the present study, we observed that, among the patients referred with MSK involvement, arthralgia was most common in those referred to pediatric rheumatology centers, while bone pain was most common in those referred to pediatric hematology centers. Patients with arthritis were rarer in both groups. Similar to our study, the most common MSK finding in the literature was arthralgia, with the most commonly involved joints being the knee, hip, and ankle.[14,16,17] In the study of Civino et al.[18], hip involvement was found to be the most frequently involved joint in malignancies and was associated with malignancy. In the present study, hip involvement was observed more frequently, especially in solid malignancies. Therefore, in patients presenting with complaints of hip pain and low back pain, patients should be evaluated for malignancies such as osteosarcoma, Ewing sarcoma, neuroblastoma, and NHL before attributing the cause of pain to spondylitis, scoliosis, or mechanical low back pain. Bone pain, especially in the lower extremities, was observed more frequently in hematological malignancies in our study. Therefore, we believe that these patients should be approached with greater attention. Although pain with morning stiffness is an expected finding of JIA, it was seen in 5% of the patients in our study. For this reason, malignancy should always be kept in mind in the follow-up of patients who are considered to have JIA.

Joint diversity was found to be higher in patients who applied to pediatric rheumatology centers. We believe this is because the joint examination is part of the rheumatological assessment.

Thrombocytopenia was observed in 61% of patients who applied to pediatric hematology centers, while this rate was only 16% in pediatric rheumatology centers. In the study by Tamashiro et al.[19] in which they compared sJIA, and ALL patients, they found that thrombocytopenia was the most important factor that differentiated ALL from sJIA. Therefore, in the presence of thrombocytopenia accompanying MSK complaints, malignancy must be excluded.

The main limitation of our study was its cross-sectional design which was based on the clinical experience of pediatric rheumatologists and pediatric hematologists. Another limitation was that we did not compare the results with patients who applied to these centers and were diagnosed with non-malignant diseases. We believe that more prospective studies are needed on this topic.

In conclusion, malignancies may present with only MSK system complaints in childhood. Therefore, malignancies should be included in the differential diagnosis of patients presenting with MSK complaints. Especially in patients with bone pain, hip joint involvement, an atypical course, and thrombocytopenia, malignancy should be considered first and these patients should be referred to pediatric hematologists.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjped.2023.446

Ethical approval

The study protocol was reviewed and approved by the Ethics Committee of the University of Health Sciences, Ü mraniye Training and Research Hospital (Approval No: B.10.1.TKH.4.34.H.GP.0.01/336) with the ethical principles laid down in the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BS and ŞÇ; data collection: ŞÇ, BŞK, ÖB, EB, BK, DGY, ACA, MÇ, GOY, KÖ, FÇ, HES, APK; analysis and interpretation of results: BS and ŞÇ draft manuscript preparation: BS and ŞÇ. All authors reviewed and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The role of proangiogenic cytokines in predicting sepsis in febrile neutropenic children with cancer

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ABSTRACT

Background. We assessed the relationship between sepsis occurrence and the serum levels of angiopoietin (Ang-1, Ang-2), vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) in pediatric patients with cancer-related febrile neutropenia.

Methods. Fifty-two children with malignant tumors who experienced 86 episodes of febrile neutropenia (FN) were examined between June 2016 and June 2018. Each FN episode was considered a separate event and the total number of FNs were recorded (86 FN episodes = FN group). The control group consisted of 21 healthy children. Ang-1, Ang-2, VEGF-A and sFlt-1 were measured at the baseline and 48th hour of each FN episode –alongside routine characterization of inflammation (C-reactive protein; white blood cell and absolute neutrophil count).

Results. Among the episodes, 29 (34.5%) developed sepsis while 57 were classified as non-complicated FN. The baseline values of patients and controls were significantly different for Ang-1, Ang-2, VEGF and sFlt-1 values (all, $p < 0.05$). In the subgroup with sepsis, Ang-2 values were higher than in the subgroup without sepsis ($p = 0.017$). In predicting sepsis, Ang-2 had 60.7% sensitivity and 66.7% specificity at the 74.6 cut-off value (AUC: 0.662 [95%CI: 0.541 – 0.783], $p = 0.022$), Ang-2 / Ang-1 ratio had 65.5% sensitivity and 60.0% specificity at the 0.405 cut-off value (AUC: 0.633 [95%CI: 0.513 – 0.753], $p = 0.046$).

Conclusions. Our results reveal that Ang-2 and Ang-2/Ang-1 were higher in the sepsis group and Ang-2 might be a biomarker to indicate the risk of sepsis in patients with FN and/or cancer.

Key words: sepsis, children, febrile neutropenia, angiopoietin, vascular endothelial growth factor, soluble fms-like tyrosine kinase.

Although there has been a significant improvement in the course of childhood cancers in recent years, infections remain as the primary cause of death and morbidity.[1] Neutropenia-associated fever develops during chemotherapy in approximately 80% of hematologic malignancies and 10-50% of solid tumors.[2] In the absence of fever, but in the presence of findings indicating focal or systemic infection, neutropenic patients are treated within the scope of febrile neutropenia (FN). FN is also associated with significant morbidity, mortality, a decrease in, and postponement of chemotherapy and the cost of treatment.[2] Several studies have aimed to make empirical therapy feasible by defining risk factors for serious infections and sepsis.[3] Although risk classifications exist for adults, there are no validated risk stratification schemas for the pediatric population.

Neutrophils, macrophages and endothelial cells play a role in early oxidative stress occurring during sepsis and fight against the pathogen as the first defense mechanism of the immune

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system.[4] In the human body, the endothelium with a surface area of about 1000 m^2 is a very dynamic organ. Endothelium forms a surface between blood and tissue and also has an important role in regulating vascular tone, coagulation and inflammation response. The endothelium, which is exposed to the direct effect of microorganisms and products during sepsis and is highly activated, becomes self-injurious after a while. Vascular endothelial growth factor (VEGF) triggers endothelial cell proliferation, migration, and differentiation. The significance of VEGF extends to its crucial role in both childhood and adulthood for the processes of vasculogenesis and angiogenesis [5]

The best-known members of angiopoietins (Ang), another family of growth factors affecting endothelial barrier function, are Ang-1 and Ang-2, which exert their influence via Tie receptors.[6] The biological process of angiogenesis is closely regulated by many factors including Ang-1, Ang-2, VEGF and soluble fms-like tyrosine kinase-1 (sFlt-1). The barrier function of endothelium is bolstered by sFlt-1 and Ang-1; whereas, VEGF-A and Ang-2 act to disrupt cellular junctions.[7] Owing to their direct involvement in the endothelium, researchers have explored these proteins for their association with the sepsis process, with promising data being reported for different groups of patients.[8-13]

We aimed to measure serum levels of Ang-1, Ang-2, VEGF, sFlt-1, and calculate Ang-2/Ang-1 ratio as a marker of capillary endothelial injury[12] in pediatric cancer patients with febrile neutropenia. These results were then used to assess whether these parameters could be utilized to predict the risk of sepsis in this population.

Material and Methods

Study design

The study has been conducted in accordance with the principles of the Helsinki Declaration and approved by the ethics committee of Ankara Oncology Hospital (Date: 03.05.2016/ No.20033663). Written informed consent was obtained from the parents or legal guardians.

We prospectively evaluated 52 cancer patients aged 0-18 years with FN treated between June 2016 and June 2018. Each FN episode was considered a separate event, and the total number of FNs was recorded (86 FN episodes). The control group comprised 21 healthy children.

Inclusion criteria

1) Fever ≥ 38°C lasting for over one-hour or 38.3° measured once, 2) Chemotherapy-induced severe neutropenia (absolute count < 500/mm^3 or anticipated to drop to this level within 24-48 hours from 500-1000/mm^3).

Febrile neutropenia protocol and sepsis definitions

Blood cultures were drawn in accordance with FN protocols (central venous catheter lumens with concurrent peripheral cultures) at fever onset. Urinalysis and urine culture were acquired with clean-catch, midstream specimens. Other laboratory tests were performed including a complete blood count, peripheral blood smear, serum C-reactive protein (CRP), liver (alanine aminotransferase, aspartate aminotransferase, bilirubin) and renal (urea, creatinine) functional tests. Standardized chest X-ray protocols were adhered to when managing patients with relevant symptomatology.[14] Empirical monotherapy was initiated promptly in all patients.

Sepsis was characterized by the manifestation of two (or more) of the following: 1) Temperature > 38.5°C or < 36°C, 2) Tachypnea (adjusted for age) unrelated to neuromuscular disease or anesthesia, 3) Tachycardia or bradycardia (adjusted for age), along with a clinically evident source of infection or a microbiologically documented infection.[15] In accordance with this classification, we categorized febrile neutropenia (FN) episodes into two groups: (i) Non-complicated FN and (ii) Sepsis.

Outcome parameters

Blood specimens were obtained at fever onset (baseline) and 48 hours later. Following routine centrifugation for serum separation, samples were preserved at -80°C until analysis. The processing of samples was performed by a single investigator who was unaware of the patients' outcomes.

Laboratory analyses

The quantification of targeted analytes were performed with enzyme-linked immunosorbent assays (ELISA). A ELx 800 microplate reader (BioTek Instruments, Vermont, USA) and ELx50 microplate strip washer (BioTek Instruments, Vermont, USA) were used. The assays were performed according to ELISA kit manufacturer's instructions. Measurements exceeding the linear range underwent repeat analysis with appropriate dilution. The RayBio® Human ANGPT1 ELISA kit (Georgia, USA) and RayBio® Human ANGPT2 ELISA kit (Georgia, USA) were used for the measurement of ANGPT-1 and ANGPT-2.

The minimum detectable concentrations for Human ANGPT1 and Human ANGPT2 were established at 30 pg/ml and 10 pg/ml, respectively. The intraassay coefficient of variation (CV) was below 10%, and the interassay CV was below 12% for both ELISA kits.

For the Human VEGF-A ELISA kit (eBioscience Thermo Fisher, California San Diego, USA), the analytical sensitivity was 7.9 pg/mL, and the assay range spanned from 15.6 to 1,000 pg/ml. The interassay CV was 4.3%, while the intraassay CV was 6.2%.

The VEGF Receptor 1 Monoclonal Antibody (Hu VEGF-A) ELISA VEGF- A ELISA (e Bioscience Thermo Fisher, California San Diego, USA) kit analytical sensitivity was 0.03 pg/mL and the assay range was between 0.16-10 pg/mL. The interassay CV was 5.1 % whereas intraassay CV was 5,5 %.

Statistical analysis

The SPSS v20 software was used for analysis. The Kolmogorov-Smirnov test was employed to assess the normal distribution suitability of the data. Continuous variables were presented as median (minimum – maximum), while categorical variables were expressed as frequency (percentage). The Mann-Whitney rank sum test was used to scrutinize differences in continuous variables between patients and healthy controls. Additionally, the Wilcoxon test was applied to compare two dependent groups (baseline vs. 48th hour). Binary logistic regression analysis was carried out to investigate independent risk factors influencing sepsis. Determination of optimal cut-off values for biomarker concentrations was conducted through receiver operator characteristics (ROC) analysis and the Youden Index. The statistical evaluation was performed at a 95% confidence level, and significance was attributed if the p-value was less than 0.05.

Results

The study encompassed 52 participants aged between 0 and 18 years, comprising 28 males and 24 females, along with 21 controls. The age and sex distribution of the groups were similar ($p > 0.05$).

The most common diagnoses were acute lymphoblastic leukemia (22%), osteosarcoma (22%), and Ewing's sarcoma (22%). The median duration of neutropenia was 6 days (min–max: 3-30), and absolute neutrophil count of the whole group was 55/mm^3 (min–max: 10-900). The groups were similar for age, sex, remission, diagnosis, neutrophil count ($p > 0.05$). Patients with sepsis had significantly longer duration of neutropenia and fever ($p < 0.001$) (Table I).

Of the 86 FN episodes, 29 (34%) were complicated with sepsis. A microbiological agent was isolated in 11 (13%) episodes (6 methicillin resistant *Staphylococcus aureus*, 3 *Escherichia coli*, 1 *Enterobacter spp.*, 1 *Candida spp*). Eight patients had pneumonia, 6 had mucositis,

Table I. Characteristics of FN episodes with and without sepsis.

Features	Sepsis (-) (n:57)	Sepsis (+) (n:29)	Total (n=86)	*P*-value
Age (years)				
Range	0.7-17	0.7-18	0.7-18	0.374
Median	13	14	13	
Sex				
Male	27 (47%)	14 (48%)	41 (48%)	0.937
Female	30 (53%)	15 (52%)	45 (52%)	
Primary diagnosis				
Ewing sarcoma	17 (30%)	2 (7%)	19 (22%)	0.102
ALL	14 (25%)	5 (17%)	19 (22%)	
Osteosarcoma	11 (19%)	8 (28%)	19 (22%)	
AML	2 (4%)	6 (21%)	8 (10%)	
RMS	7 (12%)	1 (3%)	8 (10%)	
NHL	1 (2%)	5 (17%)	6 (7%)	
Others	5 (8%)	2 (7%)	7 (7%)	
Disease status				
AD	17 (30%)	13 (45%)	30 (35%)	0.645
PR	11 (20%)	3 (10%)	14 (16%)	
CR	23 (40%)	7 (24%)	30 (35%)	
R/RD	6 (10%)	6 (21%)	12 (14%)	
Neutrophil count (cells/mm3)				
Range	10-900	10-690	10-900	0.165
Median	60	50	55	
Duration of neutropenia (days)				
Range	3-30	3-30	3-30	<0.001
Median	6	8	6	
Days with fever				
Range	1-8	2-15	1-15	<0.001
Median	2	5	3	

*Data are given as median (min- max) for continuous variables and as frequency (percentage) for categorical variables
AD: active disease, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, CR: complete remission, RMS: rhabdomyosarcoma, NHL: non-Hodgkin lymphoma, PR: partial remission, R/RD: relapse/resistant disease

3 had anal abscess, 2 had cellulitis and 1 had catheter insertion-site infection. Additionally, 7 patients had catheter-related infection, one with pneumonia and another with urinary tract infection. None of the cases were fatal. Of the 86 FN episodes, 35% were recorded during active disease, 35% in complete remission, 16% in partial remission and 14% during relapsed/resistant disease. Demographic, clinical and laboratory data were similar in patients with sepsis or non-complicated FN.

Ang-1 and VEGF were significantly higher in controls; whereas, Ang-2, Ang-2/Ang-1 and sFlt-1 were higher in the FN group ($p < 0.05$). Baseline and 48th hour Ang-1, Ang-2/Ang-1 and VEGF values were similar in patients with FN ($p > 0.05$). However, Ang-2 and sFlt-1 values demonstrated a significant change, the former was higher at 48 hours while the latter was lower ($p < 0.05$) (Table II). Baseline Ang-2 and Ang-2/Ang-1 values in the sepsis subgroup were higher compared to the non-sepsis group

Table II. Baseline and 48th hour measurements of control and FN groups.

	Control (n=21)	FN (NOE: 86) Baseline	FN (NOE: 86) 48th hour	*p*1	*p*2
Ang-1 (ng/ml)	5614.04 (1501.03-13305.35)	303.51 (11.57 – 14452,17)	370.37 (32.41 – 14913.04)	< 0.001	0.370
Ang-2 (ng/ml)	50.79 (18.11 – 2013.70)	89.41 (15.64 – 1111.11)	165.26 (22.3 – 2897.40)	0.022	< 0.001
Ang 2 / Ang 1	0.01 (0.00 – 0.35)	0.36 (0.00 – 11.28)	0.55 (0.00 – 10.48)	<0.001	0.084
VEGF (pg/mL)	211.67 (108.44 – 1000.0)	55.33 (0.00 – 1000.00)	40.35 (0.00 – 1000.0)	< 0.001	0.052
sFlt-1 (ng/ml)	940.00 (780.00 – 1000.00)	1150.00 (750.00 – 3980.00)	1010.00 (720.00 – 4210.00)	< 0.001	0.004

Data are given as median (min – max)
Ang: Angiopoetin, FN: febrile neutropenia, IQR: interquartile range, NOE: number of episodes, sFlt-1: soluble fms -like tyrosine kinase-1, p1: Control vs FN baseline, p2: FN baseline vs 48th hour, VEGF: vascular endothelial growth factor

Table III. Baseline and 48th hour measurements according to the presence of sepsis.

	Baseline			48th hour		
	Sepsis (-) n:57	Sepsis (+) n:29	*p*1	Sepsis (-) n:57	Sepsis (+) n:29	*p*2
Ang-1 (ng/ml)	417.35 (11.5 – 14452.17))	197.75 (12.15 – 1983.64)	0.191	448.06 (32.41 – 15913.04)	201.04 (41.66 – 14452.17)	0.154
Ang-2 (ng/ml)	75.13 (15.64 – 831.17)	145.82 (23.05 – 1111.11)	0.017	154.39 (23.05 – 902.15)	172.21 (22.37 – 2897.40)	0.964
Ang 2/ Ang 1	0.21 (0.00 – 11.28)	0.58 (0.03 – 9.49)	0.046	0.48 (0.00 – 10.48)	0.70 (0.02 – 9.60)	0.344
VEGF (pg/ml)	58.13 (0.00 – 1000.00)	44.72 (9.88 – 1000.00)	0.504	51.27 (0.00 – 1000.00)	37.23 (0.00 – 821.28)	0.279
sFlt-1 (ng/mL)	1120.00 (750.00 – 3980.00)	1380.00 (770.00 – 3130.00)	0.294	1010.00 (720.00 – 4210.00)	1020.00 (740.00 – 3630.00)	0.631

Data are given as median (min – max)
Ang: Angiopoetin, sFlt-1: soluble fms -like tyrosine kinase-1, VEGF: vascular endothelial growth factor

($p < 0.05$). No significant difference was found in Ang-1, VEGF and sFlt-1 values ($p > 0.05$) The 48th hour values for all parameters were similar in the sepsis and non-sepsis groups ($p > 0.05$) (Table III).

In predicting sepsis, Ang-2 had 60.7% sensitivity and 66.7% specificity at the 74.6 cut-off value (AUC: 0.622 [95% CI: 0.541 – 0.783], p = 0.022). The Ang-2 / Ang-1 level had 65.5% sensitivity and 60.0% specificity at the 0.405 cut-off value (AUC: 0.633 [95% CI: 0.513 – 0.753], p = 0.046) (Fig. 1).

Discussion

Patients with hematological malignancies are prone to sepsis and sepsis complications due to intensive chemotherapy.[16] Studies have shown that in order to minimize mortality and morbidity in sepsis, it is important to distinguish those with the highest risk of complications and to initiate early and prompt treatment.[17] However, there are no reliable biomarkers that facilitate the prediction of sepsis development in patients with FN.[16,18]

VEGF-A, sFlt-1, Ang-1, and Ang-2 are crucial for angiogenesis. Although each factor has nuanced roles in the grand processes that impact angiogenesis, it is well established that VEGF-A and Ang-2 destabilize cell junctions, while sFlt-1 and Ang-1 carry out re-stabilization of the endothelial barrier. As a result, endothelial functionality retains its capability to carry out its all-important duty in vessel propagation and destruction. It is evident that, through their concerted and opposing effects, these four factors contribute to the critical process of forming, re-forming, and destroying vessels, which continues throughout life.[12]

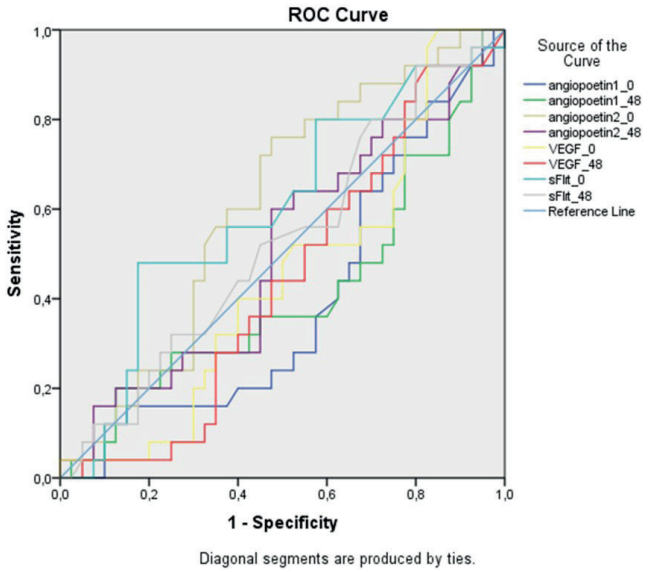

Fig. 1. Receiver operating characteristic curves of serum biomarkers at baseline (0) and at the 48th hour (48) with regards to the presence of sepsis. sFlt-1: soluble fms-like tyrosine kinase-1; VEGF: vascular endothelial growth factor.

Owing to their impact on vessels and the endothelium, we attempted to investigate whether Ang-1, Ang-2, VEGF or sFlt-1 could be utilized to predict the risk of sepsis among pediatric patients with FN. Our study showed that Ang-2 and Ang-2/ Ang-1 values were higher at baseline measurement in FN patients with sepsis. However, since the ROC analysis revealed low sensitivity and specificity, we can conclude that these parameters are weakly associated with predicting sepsis.

There are five members in the VEGF-related family of molecules, but VEGF-A has been demonstrated to be prominent in the context of sepsis.[11] Increased levels of circulating VEGF are detected in meningitis and shock.[19] It has been observed that VEGF levels increase in many conditions that cause disruption of endothelial integrity, especially sepsis.[20] In addition, VEGF level is a discriminatory factor that can predict mortality in patients admitted to the intensive care unit due to sepsis.[21,22] In an investigation involving 42 hematological patients experiencing FN, individuals with sepsis exhibited elevated VEGF compared to the non-sepsis group.[23] In a substantial clinical study by Karlsson et al., the established correlation between VEGF level and severe sepsis was once again validated. Interestingly, the authors also reported that patients progressing to shock had a substantial decrease in VEGF, suggesting a predictive capability for endothelial dysfunction.[21] We did not detect a disparity in VEGF levels between our groups (with and without sepsis) at neither baseline nor 48th hour measurement. Contrary to the literature, these results question whether VEGF is a useful biomarker for sepsis and its severity. It is of note that 37% of our cases were recently diagnosed with acute leukemia and were receiving induction therapy, potentially indicating that VEGF levels may be misleading to assess sepsis in this group of subjects. This interpretation may indeed be true, as reports have shown decreased levels of VEGF in acute leukemias, both at diagnosis and during induction therapy.[24]

sFlt-1 acts as a receptor for both VEGF and placental growth factor. In mouse models, sFlt-1 administration improves outcomes in sepsis by regulating inflammation.[19] Some studies report promising data for sFlt-1, even suggesting it

to be a reliable measure of sepsis severity.[25,26] Recombinant sFlt-1 has been shown to reduce inflammatory cytokine levels and protect mice from VEGF-A-mediated sepsis.[27] In our study, sFlt-1 levels were similar in the sepsis and non-sepsis groups, and also, there was no difference in the FN vs. control comparison. We believe that our results may be related to profound neutropenia and thrombocytopenia, as neutrophils and platelets are the main sources of these receptors. It is also known that circulating levels of proangiogenic cytokines, including VEGF, increase in both adult and pediatric malignancies. In addition, we cannot predict whether the underlying type of cancer and remission status affect basal blood levels of these mediators. For various reasons, such as these, there seems to be insufficient evidence for VEGF and sFlt-1 to be a sepsis biomarker in FN.

Ang-1 has gained notable renown as a potential measure of sepsis severity in the early phase of disease. Mankhambo et al.[28] demonstrated that decreased Ang-1 and elevated Ang-2 were linked to unfavorable outcomes in 293 children diagnosed with severe bacterial infections. In their multicenter study involving 70 patients, Ricciuto et al.[9] reported similar results, strengthening the prior interpretation. In studies focusing on other medical fields, Ang-2 has been associated with endothelial cell apoptosis, inflammation, vascular dysfunction and lung epithelial damage, all of which can be a result of sepsis.[29-31] As can be understood from most of the literature, besides vascular dysfunction in sepsis, angiopoietins can contribute to the pathophysiology of sepsis. In the present study, baseline Ang-2 values were significantly different among patients who ultimately developed or did not develop sepsis. In the subgroup with sepsis, Ang-2 values were higher than those of the subgroup without sepsis at admission. However, the baseline Ang-2 value at 74.6 cutoff points fails to predict sepsis strongly (60.7% sensitivity and 66.7% specificity). This finding was inconsistent with previous studies demonstrating that baseline Ang-2 can be utilized to assess sepsis risk.[9,32-34] In some of the mentioned studies, Ang-2 was shown to be high in the sepsis group at admission and increased gradually at the 48th hour. We did not detect any significant difference for Ang-2 in the comparison of baseline to 48th hour results among sepsis patients. Nonetheless, Ang-2 values were higher at admission and continued to demonstrate an increasing trend. Taken together, it appears that Ang-2 levels change during the acute phase of sepsis. The absence of mortality in our study may have limited the alteration, and therefore, we may have been unable to observe the previously-reported increase in Ang-2 levels. Additionally, we cannot comment on the association between Ang-2 and mortality.

In our group of patients, we detected a significant distinction between the control and FN groups with respect to baseline values of Ang-1, Ang-2, Ang-2/Ang-1 ratio, VEGF, and sFlt-1. Specifically, Ang-1 and VEGF levels were observed to be higher in the control group, whereas Ang-2 and sFlt-1 values were elevated in the patient group. Within the patient group, no significant variance was detected in baseline versus 48th-hour comparisons of Ang-1, Ang-2/Ang-1 ratio, and VEGF values. However, Ang-2 and sFlt-1 values demonstrated significant differences, with elevated Ang-2 and decreased sFlt-1 values at the 48th hour.

The small sample size was the most important limiting factor in our study. The second limitation was that the experience belonged to a single center and the third was that the patient group and the healthy groups were distinguished by more than one factor, which would ideally have been FN. The control group was comprised of healthy children without cancer, which could bias the results. Therefore, in the context of predictive performance, the inclusion of another group with cancer but without FN or sepsis could prove crucial for a comprehensive comparison of Ang-1 and Ang-2 levels in future studies.

In FN patients with sepsis, elevated levels of Ang-2 and the Ang-2/Ang-1 ratio were observed

at baseline. Notably, the baseline Ang-2 value was identified as a factor associated with an augmented risk of sepsis in individuals with cancer experiencing FN. Consequently, Ang-2 emerges as a potential biomarker indicative of the risk of sepsis in this clinical context. However, the comprehensive understanding of how these studied biomarkers interact with other inflammatory mediators, particularly additional vascular mediators, necessitates further exploration through larger-scale studies.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of Ankara Oncology Hospital (Date 03.05.2016 / No.20033663) Written informed consent was obtained from the parents or legal guardians.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SÇ, NS; data collection: SÇ, NS; analysis and interpretation of results: ÇS, SÇ, NS, İEİ; draft manuscript preparation: SÇ, NS, ÇS, İEİ. 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

Local control and toxicity outcomes following consolidative radiation therapy in patients with high-risk neuroblastoma: a 20-year experience at a single center

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ABSTRACT

Background. Intensive multimodal treatment can improve survival in patients with high-risk neuroblastoma, and consolidative radiation therapy has contributed to local control. We examined the clinical outcomes of patients who underwent consolidative radiation therapy at our institution.

Methods. We retrospectively reviewed the records of patients with high-risk neuroblastoma who underwent consolidative radiation therapy from March 2001 to March 2021 at Asan Medical Center. Patients underwent multimodal treatment including high-dose chemotherapy, surgery, stem cell transplantation, and maintenance therapy. Radiation (median, 21.0 Gy; range, 14–36) was administered to the primary site and surrounding lymph nodes.

Results. This study included 37 patients, and the median age at diagnosis was 2.8 years (range, 1.3–10.0). Four patients exhibited local failure, and 5-year free-from locoregional failure rate was 88.7%, with a median follow-up period of 5.7 years. The 5-year disease-free survival (DFS) and overall survival (OS) rates were 59.1% and 83.6%, respectively. Univariate analysis revealed that patients with neuron-specific enolase levels >100 ng/mL had significantly worse DFS and OS (P = 0.036, 0.048), and patients with no residual disease before radiation therapy showed superior OS (P = 0.029). Furthermore, patients with 11q deletion or 17q gain exhibited poor DFS and OS, respectively (P = 0.021, 0.011). Six patients experienced grade 1 acute toxicity. Late toxicity was confirmed in children with long-term survival, predominantly hypothyroidism and hypogonadism, typically < grade 3, possibly attributed to combination treatment. Four patients experienced late toxicity ≥ grade 3 with chronic kidney disease, growth hormone abnormality, ileus, premature epiphyseal closure, and secondary tumor, and recovered by hospitalization or surgical treatment.

Conclusions. In patients with high-risk neuroblastoma, consolidative radiotherapy to the primary tumor site resulted in excellent local control and a tolerable safety profile.

Key words: neuroblastoma, radiation therapy, combined modality therapy, treatment outcome, toxicity.

Neuroblastoma (NB) is one of the most common extracranial solid cancers in pediatric patients, with more than 90% of cases detected in children less than 10 years of age.[1] Patients with NB are classified into risk groups based on the Children's Oncology Group (COG) risk classification system, and approximately 55% of these patients are in the high-risk group.[2] Previously, overall survival (OS) rates of less than 15% had been reported.[3] However, patients with high-risk NB (HR-NB) currently undergo a multimodal treatment strategy, including

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induction chemotherapy, resection of the primary tumor, high-dose chemotherapy and stem cell transplantation (SCT), consolidative radiation therapy, and maintenance systemic therapy using cis-retinoic acid and/or immunotherapy.[4-6] The use of these intensive treatment courses has increased the survival rate up to 50%.[3] Among them, consolidative radiation therapy was proven to improve local control (LC) at the primary tumor site.[4,7]

Several studies on consolidative radiotherapy for patients with NB have been published and topics related to dose and toxicity are well established.[7-10] The evaluation of both acute toxicity, occurring during or within three months of treatment, and late toxicity, defined as events that occur more than 3 months or even decades after treatment, is crucial in pediatric populations, as demonstrated in the literature.[11,12] However, there are few studies on radiation therapy with domestic data, and since our institution is one of the largest centers in Korea, a review of the clinical results of these patients will add to our understanding of the role of local control with radiation for this disease.[13] Therefore, we aimed to report the clinical outcomes of patients with HR-NB who underwent consolidative radiation therapy at our clinic, with a 20-year experience.

Materials and methods

Patients

This study was approved by the Institutional Review Board of Asan Medical Center. From March 2001 to March 2021, 50 patients with NB received radiation therapy at Asan Medical Center, with 42 patients treated for consolidation and 8 patients treated with a palliative aim. Among the 42 patients to recieve consolidative radiation therapy, 39 were patients with HR-NB, defined by the following COG Neuroblastoma Risk Stratification System: (i) International Neuroblastoma Risk Group (INRG) stage L1, *MYCN* amplified disease patient <12 months with incomplete resection, (ii) INRG stage L1, *MYCN* amplified disease patient ≥12 months with incomplete resection, (iii) INRG stage L2, *MYCN* amplified disease patient, (iv) INRG stage L2, *MYCN* non-amplified, unfavorable histology (UH) disease with age 18 months to <5 years, (v) INRG stage L2, *MYCN* non-amplified, undifferentiated, or poorly differentiated histology disease with age 18 months to <5 years, (vi) INRG stage M patients, except for those with *MYCN* non-amplified disease with age <12 months or age 12 month to <18 months with UH and DNA index >1, (vii) INRG stage MS, *MYCN* amplified disease patient <12 months without MS-related symptom, (viii) INRG stage MS, *MYCN* amplified disease patient age 12 to <18 months, (ix) INRG stage MS, *MYCN* non-amplified disease patient age 12 to <18 months with any unfavorable biology.[2] Finally, we analyzed the medical records of 37 patients <18 years of age with a sufficient follow-up period of at least 3 months after treatment.

Treatments

All patients underwent induction chemotherapy, surgery, and consolidative radiation therapy. Topotecan-containing induction therapy was used, and surgery was performed after a median of 5 cycles of chemotherapy. Gross total resection (GTR) was defined as the complete removal of any visible or palpable primary tumors and lymph nodes at the regional lymph node station and was evaluated based on the operation note description and pathology reports. If GTR was not acquired and residual lesions remained at the primary site or regional lymph node station after surgery, it was evaluated as subtotal resection (STR).

The delineation of the tumor bed was done with reference to the CT or magnetic resonance imaging (MRI) taken between the period of induction chemotherapy and surgery. The primary tumor bed, residual tumor, and regional lymph nodes were targeted, and clinical target volume (CTV) was delineated with a margin of 1.0 to 1.5 cm from the initial tumor bed and lymph node, and planning target

volume was set with a margin of 5 to 7 mm from the CTV. Radiation was delivered once or twice daily (five times per week), and treatment verification was performed by weekly kV X-ray or cone beam CT imaging guidance using set-up correction based on the bony anatomy.

Follow-up and outcomes

Patients were regularly evaluated for tumors by contrast-enhanced CT, MRI, or ^{123}I-meta-iodobenzylguanidine scan at intervals of 2 to 3 months, with bone marrow biopsy and positron emission tomography CT performed if new lesions appear, or progression is suspected. After induction chemotherapy, tumor response was evaluated according to the International Neuroblastoma Response Criteria, and the presence of residual disease was evaluated right before radiation treatment.[14]

The purpose of this study is to report the LC rate, disease-free survival (DFS), OS, and toxicity of patients with HR-NB who underwent radiation therapy. Freedom-from locoregional failure (FFLRF) was defined as the time from the first day of radiation therapy to the local recurrence. Also, we reviewed whether recurrence occurred within or outside the radiation field. DFS was calculated as the period from the initiation of induction chemotherapy to any disease progression (locoregional recurrence or distant metastasis) or death, and OS was defined as the time from the initiation of induction chemotherapy to death from any cause. Three months before and after terminating radiation therapy, we reviewed treatment-related acute and late toxicity based on Common Terminology Criteria for Adverse Events version 5.0.

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). FFLRF, DFS, and OS were all calculated using the Kaplan–Meier method, and factors affecting each LC and survival were assessed using logistic regression analysis. Additionally, Fisher's exact test was used to identify factors that increase the risk of ≥ grade 3 late toxicity.

Results

Table I summarizes the patient characteristics. The median age at diagnosis was 2.8 years (range, 1.3–10.0), and 29 patients (78.4%) were diagnosed at age ≥18 months. The most common primary site was the adrenal gland, presented in 27 patients (73.0%), followed by the central abdominal compartment with six patients (16.2%) and thorax with four patients (10.8%). Six patients (16.2%) were categorized as INRG stage L2, 28 (75.7%) as stage M, and three patients (8.1%) as stage MS, and all the patients had INSS stage III or higher. At the time of initial diagnosis, 31 patients (83.8%) exhibited metastases to distant organs, and the most common sites (in order of occurrence) were bone marrow, skeletal bone, liver, lung, chest wall, and dura mater. The median levels of lactate dehydrogenase (LDH), neuron-specific enolase (NSE), and ferritin were 923.5 U/L (range, 223.0–5461.0), 170.9 ng/mL (range, 12.0–912.3), and 230.0 ng/mL (range, 19.9–941.3), respectively, and *MYCN* amplification was detected in 18 patients (48.6%).

Supplementary Table I presents the concise treatment scheme of patients with HR-NB who received multimodal treatment. Five patients (13.5%) could not receive SCT and/or maintenance therapy during the course of treatment due to intolerance to each treatment. Thirty-two patients (86.5%) received SCT, and three patients (8.1%) underwent total body irradiation (TBI) with a median of 8 Gy per four fractions (range, 5.25–10.5 Gy/ 4–7 fractions) before SCT. With radiation therapy, the median total dose for all patients was 21.0 Gy (range, 14.0–36.0) delivered by using 1.5–2.0 Gy per fraction. Patients who achieved GTR and STR received radiation doses ranging from 14.0 to 22.5 Gy and 18.0 to 36.0 Gy, respectively, with a median total dose of 21.0 Gy being the same for both groups. Twenty-nine patients (78.4%) were treated with three-dimensional conformal radiation therapy (3D-CRT) or intensity-modulated radiation therapy (IMRT), and eight (21.6%) received two-dimensional (2D) radiation therapy in the early 2000s.

Table I. Characteristics of patients with high-risk neuroblastoma having consolidative radiation therapy (n = 37).

Characteristics	No. of patients (%)
Sex	
Male	17 (45.9)
Female	20 (54.1)
Median age at diagnosis, years [range]	2.8 [1.3–10.0]
Age <18 months	8 (21.6)
Age ≥18 months	29 (78.4)
Primary site	
Adrenal gland	27 (73.0)
Central abdominal compartment	6 (16.2)
Thorax	4 (10.8)
INRG	
Stage L2	6 (16.2)
Stage M	28 (75.7)
Stage MS	3 (8.1)
INSS	
Stage III	6 (16.2)
Stage IV	31 (83.8)
Skeletal metastasis at diagnosis	
Yes	22 (59.5)
No	15 (40.5)
Bone marrow involvement at diagnosis	
Yes	23 (62.2)
No	14 (37.8)
Metastatic sites at initial diagnosis	
Bone marrow	23 (62.2)
Skeletal bone	22 (59.5)
Distant lymph node	15 (40.5)
Liver	5 (13.5)
Lung/ Chest wall	3 (8.1)
Dura mater	2 (5.4)
Median LDH at diagnosis, U/L [range]	923.5 [223.0–5461.0]
Median NSE at diagnosis, ng/mL [range]	170.9 [12.0–912.3]
Median Ferritin at diagnosis, ng/mL [range]	230.0 [19.9–941.3]
MYCN amplification	
Yes	18 (48.6)
No	17 (45.9)
Unknown	2 (5.4)
Shimada histopathology	
Favorable histology	12 (32.4)
Unfavorable histology	19 (51.4)
Unknown	6 (16.2)
Differentiation	
Well-differentiated	4 (10.8)
Poorly differentiated	18 (48.6)
Undifferentiated	3 (8.1)
Unknown	12 (32.4)
1p deletion	
Yes	6 (16.2)
No	31 (83.8)
11q deletion	
Yes	6 (16.2)
No	31 (83.8)
Trisomy 17q	
Yes	2 (5.4)
No	35 (94.6)

Abbreviations: INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; LDH, Lactate dehydrogenase, NSE, Neuron-specific enolase.
The sum of percentages may not be 100, rounded to one decimal place.

Table II summarizes the course of treatment and response or status after induction chemotherapy and surgery. Twenty-three patients (62.2%) showed a partial response on evaluation after induction chemotherapy, with no patient exhibiting a complete response or progressive disease. As a result of subsequent surgery, 24 patients (64.9%) were eligible for GTR, and the remaining 13 patients (35.1%) received STR with residual disease at the primary site which was also confirmed by postoperative imaging. In the imaging test performed before radiation therapy, 20 patients (54.1%) had no residual disease, nine (24.3%) presented with residual disease only at the primary site, four (10.8%) exhibited disease at distant sites, three (8.1%) had disease at both primary and distant sites, and one (2.7%) showed only bone marrow involvement. At the time of radiation therapy, the median age was 3.8 years (range, 2.0–11.3).

Table II. Course of treatment and response of patients with high-risk neuroblastoma having consolidative radiation therapy.

Characteristics	No. of patients (%)
Response to induction chemotherapy	
CR	0 (0.0)
VGPR	3 (8.1)
PR	23 (62.2)
MR	2 (5.4)
NR	9 (24.3)
PD	0 (0.0)
Extent of surgery	
Gross total resection	24 (64.9)
Subtotal resection	13 (35.1)
Stem cell transplant	
Yes	33 (89.2)
No	3 (10.8)
Maintenance therapy	
Yes	33 (89.2)
No	4 (10.8)
Total body irradiation	
Yes	3 (8.1)
No	34 (91.9)
Status before consolidative radiation therapy	
No residual disease	20 (54.1)
Residual at primary site	9 (24.3)
Residual at metastatic site	4 (10.8)
Residual at both primary and metastatic site	3 (8.1)
Bone marrow involvement only	1 (2.7)
Median age, at radiation therapy, years [range]	3.9 [2.0–11.3]
Total radiation dose, median, Gy [range]	21.0 [14.0–36.0]
Fraction size	
1.5 Gy/fx	21 (56.8)
1.8 Gy/fx	11 (29.7)
2.0 Gy/fx	5 (13.5)
Median BED*, Gy10, [range]	24.15 [16.80–42.48]
Radiation schedule	
Once per day (QD)	19 (51.4)
Twice per day (BID)	18 (48.6)
Radiation to metastatic site	
Yes	6 (16.2)
No	26 (70.3)
Not indicated	5 (13.5)

BED, Biologically effective dose; CR, Complete response; MR, Mixed response; NR, No response; PD, Progressive disease; PR, Partial response; VGPR, Very good partial response.
* Biologically effective doses are calculated using an α/β ratio of 10 Gy.
The sum of percentages may not be 100, rounded to one decimal place.

The median follow-up period was 69.0 months (range, 12.0–237.6) (Supplementary Table II). Among the 37 patients who received consolidative radiation therapy, four (10.8%) experienced locoregional failure and 14 (37.8%) had distant metastases; Among the four patients with locoregional failure, three patients (8.1%) also experienced distant metastases. The 1-, 3-, and 5-year FFLRF rates were 91.7%, 88.7%, and 88.7%, respectively. Table III presents the disease and treatment characteristics of four patients with locoregional failure. Two patients had INRG stage M disease, one presented stage MS, and the other had stage L2. Except for one unconfirmed patient, *MYCN* amplification was detected in all patients. All surgeries at the primary site were performed once, and all but one patient underwent STR. The total radiation dose ranged between 19.5 and 22.5 Gy. The 2D technique was performed on three patients who were treated before 2010, and one patient underwent 3D-CRT in 2014. All patients had in-field recurrence, and the median time until local failure was 2.75 months (range, 1.03–16.77). In three patients, distant metastases were noted as the first event, followed by local failure. Among the 14 patients who later developed distant metastasis during follow-up, six received RT for metastatic sites for salvage or palliative purposes. The median RT dose administered was 22.5 Gy, and detailed dose information and locations can be found in Supplementary Table III.

Also, the 1-, 3-, and 5-year DFS rates for all patients were 91.9%, 70.3%, and 59.1%, respectively. Eleven patients (29.7%) deceased during the follow-up period, with six deaths (54.5%) attributed to tumor progression, and five (45.5%) were due to sepsis during salvage or palliative chemotherapy for recurrent disease. The 1-, 3-, and 5-year OS rates were 100.0%, 83.6%, and 83.6%, respectively.

A univariate analysis was performed, and the results are shown in Supplementary Table IV. DFS and OS were both superior for patients with an NSE level <100 ng/mL (NSE ≥100 ng/mL vs. <100ng/mL; 3-year DFS, 52.5% vs.

Table III. Patients with local failure at the primary site.

Case number/Sex	1/Male	2/Male	3/Female	4/Male
Age at diagnosis, years	2.49	1.89	1.25	2.66
Disease characteristics				
INRG	M	L2	MS	M
INSS	4	3	4	4
MYCN amplification	Unknown	Yes	Yes	Yes
Primary site	Adrenal gland	Adrenal gland	Adrenal gland	Adrenal gland
Skeletal metastasis	Yes	No	No	Yes
Treatment characteristics				
Extent of surgery	GTR	STR	STR	STR
SCT	No	Yes	Yes	Yes
TBI	Yes	No	No	No
Radiation therapy				
Age, years	3.12	2.82	2.07	4.07
Total dose, Gy	19.5	22.5	21.0	21.0
Fraction size, Gy	1.5	1.5	1.5	1.5
Radiation technique	2D	2D	2D	3D
Radiation schedule	QD	BID	BID	BID
Result of treatment				
Response to initial chemotherapy	PR	PR	PR	PR
Residual disease before irradiation	Primary site	(-)	Metastatic site	(-)
Local failure	In-field	In-field	In-field	In-field
Time to local failure (months)	1.03	3.00	2.50	16.77
Distant metastasis	Yes	No	Yes	Yes
Site of distant metastasis	Mediastinal LN	(-)	Liver	Lung, Liver, Bone, BM
Survival	Death	Death	Death	Death

2D, Two-dimensional; 3D, Three-dimensional; BM, Bone marrow; GTR, Gross total resection; INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; LN, Lymph node; PR, Partial response; SCT, Stem cell transplantation; STR, Subtotal resection; TBI, Total body irradiation.

100.0%, $p = 0.036$; 3-year OS, 73.4% vs. 100.0%, $p = 0.048$). Also, patients who had no evidence of any residual disease at primary and distant sites before radiation therapy demonstrated a higher OS rate (no residual disease vs. residual disease, 3-year OS, 95.0% vs. 70.6%, $p = 0.029$), but not with FFLRF or DFS (no residual disease vs. residual disease; 3-year FFLRF, 89.5% vs. 88.2%, $p = 0.807$; 3-year DFS, 75.0% vs. 64.7%, $p = 0.496$). *MYCN* non-amplified disease seemed to provide marginally superior LC (*MYCN* amplification vs. *MYCN* un-amplification, 3-year FFLF, 81.4% vs. 100.0%, $p = 0.070$); however, no clinical factor demonstrated statistically significant results for FFLRF. Additionally, in the analysis of another important genetic or chromatin change in neuroblastoma associated with a poor prognosis, it was found that patients with 11q deletion or 17q gain exhibited poor DFS and OS, while there was little association with 1p deletion.

Treatment-related toxicity is shown in Table IV. Acute toxicity occurred in six patients (16.2%) with grade 1. Late toxicity occurred in 24 patients (64.9%). The most frequent adverse events were hypothyroidism, chronic kidney disease, and growth hormone abnormalities.

Table IV. Treatment-related toxicity in patients with high-risk neuroblastoma who received multimodality treatment.

	No. of patients (%)				
	Grade 1	Grade 2	Grade 3	Grade 4	Total
Acute toxicity	6 (16.2)	0 (0.0)	0 (0.0)	0 (0.0)	6 (16.2)
Anorexia	3 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.1)
Nausea	3 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.1)
Vomiting	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.7)
Late toxicity	3 (8.1)	17 (45.9)	3 (8.1)	1 (2.7)	24 (64.9)
Chronic kidney disease	2 (5.4)	6 (16.2)	2 (5.4)	0 (0.0)	10 (27.0)
Diabetes	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	1 (2.7)
Gait disturbance	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.7)
Growth hormone abnormality	2 (5.4)	4 (10.8)	1 (2.7)	0 (0.0)	7 (18.9)
Hypercholesterolemia	0 (0.0)	4 (10.8)	0 (0.0)	0 (0.0)	4 (10.8)
Hypertrophic hypogonadism	1 (2.7)	4 (10.8)	0 (0.0)	0 (0.0)	5 (13.5)
Hypothyroidism	0 (0.0)	17 (45.9)	0 (0.0)	0 (0.0)	17 (45.9)
Mechanical ileus	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.7)	1 (2.7)
Premature epiphyseal closure	0 (0.0)	0 (0.0)	2 (5.4)	0 (0.0)	2 (5.4)
Secondary tumor	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	1 (2.7)
Scoliosis	3 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.1)

Toxicity ≥grade 2 occurred in 21 patients (56.8%), which was detected at a median of 21.0 months (range, 4.2–201.2) from the end of radiation therapy until event occurrence. A total of four patients (10.8%) experienced ≥grade 3 adverse events, including chronic kidney disease, growth hormone abnormality, premature epiphyseal closure, secondary tumor, and mechanical ileus. One patient with grade 4 ileus recovered after laparoscopic adhesiolysis and exploration. Only one patient presented with a secondary malignant neoplasm (SMN), and papillary thyroid cancer developed, necessitating removal by thyroidectomy. The fisher's exact test was used to detect factors related to ≥grade 3 late toxicity; however, no factors with significantly increased toxicity were documented (Supplementary Table V).

Discussion

In the last decade, consolidative radiation therapy in patients with HR-NB was shown to have LC rates of 78.9–100.0% with a radiation dose of 21.0 Gy (range, 18.0–36.0 Gy).[7-10] At our center, we documented a 5-year LC rate of 88.7%, with a median follow-up period of 69.0 months in 37 patients, which was consistent with research conducted in recent years. Casey et al.[8] have reported that LDH, *MYCN* amplification, number of surgeries, and the presence of skeletal metastases could be risk factors associated with local failure. However, none of the factors, including total radiation dose and surgical extent, were significantly associated with LC in the present study. It remains unclear about the appropriate radiation dose in several studies for patients with STR.[7,8,15] Although we identified STR in three of four patients with in-field recurrence, univariate analysis revealed that surgical extent was not associated with LC. Casey et al.[16] have performed a dose escalation study for patients with HR-NB presenting gross residual disease. For 19 patients, RT doses of 21, 30, and 36 Gy with a local failure rate of 0.0% were recorded in the 30 and 36 Gy groups and 30% in the 21 Gy group, thereby showing a tendency for improved LC at higher doses (p = 0.12). In contrast, in the ALBL0532 study[17], a prescription dose of 21.6 Gy was administered to patients who had undergone complete resection, while

patients with incomplete resection received 36.0 Gy. The 5-year cumulative incidence of local progression showed a marginal difference between the two groups, with rates of 11.2% versus 7.1%, respectively (p=0.059). A study assessing only patients with GTR documented an excellent LC exceeding 90% with hyper-fractionated RT at 21 Gy after intensive systemic therapy and debulking surgery.[8] Based on the results of the present study, an RT dose of 21 Gy could be considered sufficient for LC, and efforts to reduce the dose have been recently proposed owing to concerns regarding potential late toxicity, which needs to be confirmed in future investigations. In one study[18], it has been proposed to consider de-escalation of radiation for patients with no image-defined risk factors and those with over 90% resection.

In the present study, the 3-year DFS and OS were 70.3% and 83.6% respectively, which was superior to those of other studies presented in Supplementary Table II. These favorable outcomes can be attributed to the implementation of systemic therapies both in the pre- and post-surgical phases, alongside the inclusion of SCT, with most of our patients demonstrating strong compliance.[19,20] Furthermore, while the number of patients is not large, it is believed that maintenance treatments, including interleukin-2 for 10 patients (27.0%) and anti-GD2 monoclonal antibodies for two patients (5.4%), may have contributed to longer survival to some extent.[21] Nevertheless, fourteen patients experienced distant recurrence, of which seven presented lesions at the same site as those at diagnosis, which remained invisible during the treatment course owing to intensive systemic therapy. Herein, six patients underwent palliative radiation therapy at the metastatic site after recurrence. Conversely, in a study by Chen et al.[9], 12 patients with metastatic lesions detected at initial diagnosis were treated with synchronous radiation treating the primary tumor; however, no significant difference in distant failure or survival was noted compared with those who did not receive this treatment course. No standardized treatment for distant lesions has been established, considering that most metastatic lesions are multiple and widely distributed and respond well to maintenance chemotherapy in some cases; however, radiation as a salvage or palliative strategy is recommended when warranted, rather than as an early treatment strategy.

Using univariate analysis, we found that the NSE level at diagnosis was related to poor DFS and OS. NSE is expressed in patients with NB and various cancers, including small cell lung cancer and melanoma, and has been described as a factor indicating poor prognosis. Georgantzi et al.[22] found that elevated levels of chromogranin A (CgA) and NSE were found in advanced-stage patients, with NSE correlating with outcomes and tumor size. They highlighted the clinical significance of NSE as a tumor marker in NB, while CgA merits further investigation in prospective, multicenter clinical studies. Furthermore, a study by Cangemi et al.[23], which included 505 patients with NB, has also demonstrated the prognostic value of NSE levels, in addition to ferritin, lactate dehydrogenase, catecholamine metabolites, consistent with the findings of our current study. Therefore, NSE levels at diagnosis or during treatment could be used as useful serum markers to aid in treatment-related decisions.

In addition to the biomarker changes in serum, there are several well-known genetic factors associated with poor prognosis in NB. Among them, aberrations in 11q and 17q were also confirmed to be associated with poor prognosis in our study. Regarding this, efforts are still ongoing, from the preclinical stage onwards, to understand the roles of these factors and to develop cancer treatments targeting them.[24-27]

Considering the recent development of systemic therapy and prolonged survival, late toxicity is particularly important in pediatric patients, especially as the number of long-term survivors has increased. During a 20-year follow-up period, Geurten et al.[28] reported

that 54% of patients experienced long-term adverse events, and 28% had endocrine complications. Additionally, it has been reported that 89% of patients with NB had at least one medical condition, with 50% showing abnormal endocrine function.[29,30] Furthermore, in a recently published review study[31], it was also highlighted that thyroid carcinoma and myeloid leukemia are the most commonly reported subsequent neoplasms in survivors of NB, and the risk of these neoplasms was found to be 2.8 to 10.4 times higher than in the general population. In the present study, 22 patients (59.5%) presented with endocrine dysfunction, with hypothyroidism noted most commonly, followed by growth hormone abnormality, hypercholesterolemia, hypogonadism, and diabetes. SMN occurred in one patient (2.7%) and was diagnosed as papillary thyroid cancer. The patient had a primary tumor located in the thorax with nodal metastasis involving the neck nodes, therefore including the supraclavicular lymph nodes and mediastinum within the radiation field. He received radiation therapy at the age of 3.5, with a dose of 21 Gy in 14 fractions using a 2D technique. Thyroid cancer developed approximately 10 years and 11 months after radiation therapy, and no TBI was done separately. According to the SEER data study by Applebaum et al.[32], 34 of 2,801 patients with NB developed SMN, accounting for 1.2%. Additionally, the 30-year cumulative incidence of SMN in HR patients who received intensive multimodal treatment was 10.4%. The median latency time for SMN was 38 months for all hematologic malignancies and 158 months for solid tumors. Herein, the median follow-up period for surviving children was 78.8 months (range, 16.5–240.8), which is considered insufficient time to detect SMN; hence, the incidence rate may have been low. Furthermore, since the occurrence of late toxicity in pediatric patients is presumed to be due to multifactorial attributes, special attention is required in determining the treatment for each department.

The major advantage of the present study is that we analyzed the treatment process and results of patients who received multiple treatments at a single institution with domestic data. Additionally, as seen in Supplementary Table II, the median follow-up period was 69.0 months, providing data for a markedly sufficient time to reveal treatment efficacy.

However, there are some limitations to our study. First of all, as this study is a retrospective study for a disease with a low incidence, the number of patients analyzed is small. In addition, because of the small sample size and only four patients with local failure, this study may not be sufficient to assess risk factors for local failure. Also, there were some heterogeneous approaches to radiation treatment dose or technique by setting a long recruitment period to analyze the data of as many patients as possible. For the same reason, we included patients who received radiation therapy using older techniques performed in the early 2000s. Furthermore, immunotherapy, including anti-GD2 antibodies, has recently been widely applied as maintenance therapy; however, only a small portion of patients in the present study were treated with interleukin-2 or anti-GD2 monoclonal antibodies.[33] Therefore, improved survival outcomes could have been achieved if more recent radiation technology and tailored systemic treatments were applied. Lastly, although the observation period was sufficient to observe treatment efficacy, it was deemed insufficient for late toxicity assessments. Therefore, conducting a toxicity study on the same set of patients after a longer observation period would be helpful in the future.

In conclusion, consolidative radiation therapy to the primary tumor site could provide excellent LC in patients with HR-NB. To reduce late toxicity caused by the cumulative side effects of various treatments, additional efforts are needed to reduce the total radiation dose. Additionally, controlling distant recurrence is essential for increasing the survival rate, thereby necessitating active monitoring and additional treatment, especially in patients with poor prognosis.

Supplementary Materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjped.2023.575

Ethical approval

This study was conducted in accordance with the 1964 Declaration of Helsinki. This study was approved by the Institutional Review Board of Asan Medical Center (#2022-0772), and written informed consent was waived due to the retrospective nature of this study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: JY Jang, SD Ahn, YJ Kim.; data collection: JY Jang, HU Kim, HJ Im, KN Koh, HR Kim, SH Kang.; analysis and interpretation of results: JY Jang, HU Kim, JH Park, YJ Kim.; draft manuscript preparation: JY Jang. 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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Short Communication

In light of recent discoveries: Breastfeeding is more than nutrition for term and preterm babies

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Human milk is universally recognized as the ideal source of nutrition for infants in their early stages of development. Its safety, accessibility, constant readiness, optimal temperature, and affordability contribute to its exceptional status. The American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend exclusive breastfeeding for around six months post-birth, followed by continued breastfeeding with complementary foods until two years of age.[1] Moreover, breastfeeding provides not only vital nutrition for the baby but also plays a role in fostering a strong bond between parent and child. In spite of these recommendations, the exclusive breastfeeding rate through 6 months has been shown to be 37% in low-and middle-income countries and lower than 20% in most high-income countries.[2] In this regard, as a reminder of the importance of breastfeeding, we aimed to highlight some important points based on recent insights. These insights underscore the lifelong health effects of breast milk, showing its influence on the microbiome and immune development through its prebiotic contents, stem cells, and other bioactive components.

Known advantages and distinct effects on preterm infants

Breastfeeding offers a range of protective benefits, leading to reduced incidences of infections, obesity, diabetes mellitus, inflammatory bowel disease, childhood leukemia, asthma, atopic dermatitis, dental caries, malocclusion and postneonatal mortality.[1,2] Furthermore, breastfeeding has been associated with higher levels of intelligence in children.[2-5]

Notably, for very low birth weight infants, the provision of mother's expressed milk has been linked to a decreased occurrence of necrotizing enterocolitis (NEC), late-onset sepsis, chronic lung disease, and retinopathy of prematurity.[1,6] It also shortens hospital stays and rehospitalization rates, and facilitates complete enteral feeding.

New insights

Breastmilk has unique contents with anti-inflammatory, antimicrobial, immunoregulatory agents, as well as live leukocytes and their effect on the developing immune system of the child.[1] Exploration into the human milk microbiome commenced as early as 2003,[7] uncovering a multitude of bacteria that contribute to the infant's intestinal microbiome.[7,8] This colonization process plays a pivotal role in fostering appropriate immune system development and functionality in infants.

Moreover, human milk oligosaccharides, which are composed of five fundamental monosaccharides (glucose, galactose, N-acetylglucosamine, fucose, and sialic acid), operate as prebiotics, antiadhesive antimicrobials, modulators of intestinal epithelial cell responses, and immune modulators.[5]

In addition to that, human milk contains extracellular vesicles (EVs), which are particles

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released from cells to transmit intracellular signals.[9] These vesicles are cell-derived membrane bound vesicles functioning as a means of cell to cell communication. It has been shown that human breast milk derived exosomes significantly promote intestinal stem cell proliferation and viability.[10] Human milk EVs cargo (comprising DNA, RNA, micro-RNA and proteins) can have anti-inflammatory, immunomodulatory, or neurodevelopment effects and improve the barrier function of the intestinal epithelium.[11] A recent study has shown that human milk exosomes could improve experimentally NEC-induced intestinal injury, restored intestinal regeneration, inhibited inflammation, and NEC-related complications better than amniotic fluid stem cell derived exosomes.[12,13] These findings may explain why breastfeeding regimens in neonatal intensive care units decrease the incidence of NEC. Proteomic studies have also suggested that proteins derived from breast milk EVs may play a role in the regulation of gastrointestinal tract development.[13,14]

Breastfeeding challenges

On the other hand, breastfeeding can introduce an additional layer of stress during a period characterized by rapid life changes. Parenting, baby care responsibilities, physical transformations, and feelings of isolation can collectively contribute to these potential stressors.[15] For example, a doctor described the difficulties she faced through her own breastfeeding journey as a mother.[16] Upon careful reading of this referenced article, the difficulties which she faced mostly originated from a lack of appropriate breastfeeding consultation within three days after birth. In addition to that, other factors such as using nipple guards or bottle feeding might have complicated the problem. Finally, her experience ended with her continuing to feed her baby formula. This entire process reveals how essential breastfeeding consultation and education are. Namely, in healthcare facilities with robust breastfeeding support, immediate skin-to-skin contact after birth, early initiation of breastfeeding within the first hours, active promotion of exclusive breastfeeding, avoidance of pacifier use, and timely provision of breastfeeding information are key practices.[1]

In conclusion, these newfound insights emphasize that breast milk transcends being solely a source of nourishment, warranting increased attention to its multifaceted significance. Additionally, it should be noted that during a time of significant life changes such as a child birth, difficulties with breastfeeding is not the sole cause of a mother's stress. Increasing the social support of a new mother during these periods may be a more rational solution than not recommending breastfeeding. There's a need to bolster social policies that promote and support breastfeeding practices.[17-19]

Author contribution

The authors confirm contribution to the paper as follows: study conception and design; draft manuscript preparation; G. K, S. Y. All authors reviewed the results and approved the final version of the manuscript.

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The earthquake disaster in Türkiye: a perspective on newborn evacuation and an ophthalmological approach

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On February 6, 2023, two catastrophic earthquakes with magnitudes of 7.7 and 7.6 rocked Pazarcık and Elbistan in Kahramanmaraş, Türkiye, nine hours apart. The southeast of the country has not had an earthquake of this magnitude in hundreds of years, making it the largest to ever affect Türkiye. The severe devastating effects of the disaster were experienced in 11 provinces where a state of emergency was declared: Adıyaman, Gaziantep, Kilis, Hatay, Malatya, Diyarbakır, Adana, Osmaniye, Kahramanmaraş, Şanlıurfa, and Elazığ; it was reported that the most affected ones were Hatay, Kahramanmaraş, and Gaziantep. Two weeks after the disaster, two more powerful earthquakes with magnitudes of 6.4 and 5.8 hit Türkiye. The earthquakes and aftershocks caused catastrophic damage; 9.1 million people have been directly impacted, and almost 14 million people are affected. According to the Disaster and Emergency Management Presidency, there were hundreds of thousands of injuries and over 45,000 reported deaths in Türkiye. Our province of Adana has experienced over 400 fatalities, close to 10,000 injuries, and numerous structures that have been completely or severely damaged.[1-3] Moreover, it was decided to evacuate our hospital, Çukurova University Balcalı Hospital, immediately due to the damage caused by the aftershocks that occurred on February 21.[4]

Çukurova University Balcalı Hospital is a tertiary reference and referral center for the southern and southeastern regions of the country. During this disaster, many earthquake victims and trauma patients who required surgical intervention due to blunt and penetrating eye injuries were treated in our hospital. Hatay, which has hosted many ethnic origins throughout history and has a distinguished historical richness, now hosts many Syrian refugees. In Hatay, one of the three provinces most affected by the earthquake, two hospitals were severely damaged and destroyed. Our hospital transferred 17 newborns who were treated in the neonatal intensive care units (NICU) of these two heavily damaged hospitals. While some of the babies were pulled out of the rubble, it was possible that some of them had suffered major physical traumas, such as falling out of their incubators during the earthquake, even though they were still inside. After a thorough evaluation and testing for potential fall and crush injuries, all neonates admitted to the NICU had a screening examination for premature retinopathy (ROP) because we did not have demographic information for these newborns, such as birth dates, weeks of gestation, birth weights, treatments, or indications for hospitalization.

While certain newborns, identity information was limited to their surname, others were assigned names that included the name of the hospital and the corresponding number. It has been estimated that nine of the infants whose surnames are the only information available were born to Syrian immigrant families. An additional challenge encountered during the period of the disaster was the inability to communicate with the parents of the infants, as their identity details were unknown. One possible explanation was that these babies no

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longer had a family. A further concern was the absence of family approval for any kind of intervention, including the examination. During the disaster's acute phase, DNA testing could not be undertaken quickly to confirm the relationship of the people claiming to be the baby's family. Consequently, medical interventions and surgical procedures that were considered vital and required in circumstances posing a threat to the baby's health were carried out without obtaining consent. Following discharge from the NICU, infants were placed under the temporary protection of social services.

Out of the 17 infants referred, six were identified with ROP during the screening examinations. One newborn was diagnosed with vitreous hemorrhage in both eyes, while another case had cataracts affecting both eyes. Two patients with ROP underwent emergency laser photocoagulation. Consequently, a vitrectomy was scheduled for one eye with ROP. A lensectomy was planned for the patient with bilateral cataracts after possible infectious and metabolic causes were ruled out. However, due to our hospital's emergency evacuation as a result of the aftershock on February 21, we had to transfer all patients and preterm newborns treated at our hospital to referral centers in other provinces that were not in the area affected by the disaster. As a result, we were unable to complete the newborns' follow-up and treatment.

During this period, we painfully realized that, as a country, we were required to make certain changes. Since our country is located in an earthquake zone, all buildings, particularly hospitals, should be assessed by authorized professional organizations and built on suitable land using advanced technology, such as rail systems, to provide earthquake resistance. Hospitals, especially tertiary reference centers, which serve as the most basic shelter in case of disaster, should be constructed in an earthquake-resistant manner, and existing old structures should be renewed or strengthened. In hospital planning, floor plans for the NICU that can be easily evacuated in the event of a disaster should be designed. In NICUs, incubators must be fixed to the ground and produced in a more protective design to prevent them from moving or opening during an earthquake. Furthermore, we recognized the importance of having wristbands with identification and birth information, especially for pediatric patients, in the event of a disaster. Additionally, in the event of a crisis, a well-organized rescue team should be established, and a strategy should be developed to first evacuate newborns by a predetermined team in accordance with the hospital's floor plans. Doctors, and therefore hospitals, must continue to welcome patients and provide healthcare even in disaster situations. That means that every province, including hospitals, should have a disaster plan, with doctors, nurses, and other healthcare professionals included in the plan in a certain order. Since protecting children is our primary responsibility, we should have a separate, comprehensive plan in place. An organization that includes state institutions should be in charge of providing a safe environment and meeting their needs.

The natural disaster of the century has resulted in catastrophic losses. The loss of loved ones and the rebuilding of their lives will undoubtedly affect hundreds of thousands of people throughout the years. This short report was written as a letter to history, in memory of people who died and the babies who lost their families, so that this tragic event is never forgotten.

Author contribution

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

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Case Report

Concurrent pyoderma gangrenosum and Takayasu arteritis in an infant: diagnostic challenges and treatment considerations

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ABSTRACT

Background. Takayasu arteritis (TA) is an uncommon chronic inflammatory and autoimmune disease primarily affecting large vessels, particularly the aorta and its branches. Skin manifestations have been documented in association with TA. Pyoderma gangrenosum (PG) is a chronic neutrophilic dermatosis characterized by destructive, necrotizing, and painful ulcers, predominantly found on the lower extremities. The coexistence of PG and TA is extremely rare, with most reported cases involving adult patients. Interestingly, the association between PG and TA appears to be more common in Japan compared to North American and European populations. Childhood TA (c-TA) accompanied by PG is exceptionally rare, with only 10 cases reported in the literature thus far.

Case Report. We present the case of a 7-month-old patient initially diagnosed with PG. Despite aggressive immunosuppressive therapy, the patient's high acute phase reactants remained elevated. Although the abdominal ultrasound was normal, advanced imaging was performed due to severe abdominal pain. Contrast-enhanced computerized tomography angiography of the aorta and its branches revealed extensive vascular involvement consistent with TA.

Conclusion. In this report, we highlight an infantile case of PG that was subsequently diagnosed as infantile TA. Recognizing the rare association between PG and TA is important. Thorough evaluation and prompt diagnosis of TA in infants with PG can guide further investigations and prevent vascular complications.

Key words: Takayasu arteritis, pyoderma gangrenosum, vasculitis.

Pyoderma gangrenosum (PG) is a neutrophilic dermatosis. It is characterized by destructive, necrotizing, and noninfectious ulceration of the skin and is most often located on the lower extremities. A total of 18 patients with PG-related Takayasu Arteritis (TA) have been reported in the literature, and its association with childhood Takayasu Arteritis (c-TA) has been reported in only 10 cases.[1,2]

TA is a rare form of vasculitis involving large vessels. It is a chronic, autoimmune, granulomatous, and inflammatory disease that can cause dilatation, occlusion, stenosis and/or aneurysm by affecting the aorta and its main branches. Only approximately 30% of all cases are children. In the early stages of the disease, fever, loss of appetite, night sweats, joint pain and rash are observed, while signs of vascular insufficiency are observed in the late stages.[1]

The incidence of TA and PG shows racial differences. The highest incidence rate is in Japan, while it is rare in North America and Europe.[3,4]

Herein, we present a case of infantile PG that was later diagnosed as infantile TA and review the literature.

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Case presentation

A 7-month-old girl was admitted to the pediatric rheumatology inpatient clinic for skin lesions that had been present since she was 3 months old. She had papulopustular skin lesions, some of which were large necrotic ulcers, in many parts of the body. The lesions were mostly located on the upper and lower extremities and the gluteal region as indicated in Fig. 1 and 2. Apart from extensive skin lesions, her physical examination was normal with normal vital signs. Acute phase reactants were elevated (erythrocyte sedimentation rate [ESR]: 100 mm/hr, C-reactive protein [CRP]: 225 mg/L). Complete blood count and blood biochemistry were normal. In the etiological evaluation, investigations were conducted for immunodeficiency, infectious, autoinflammatory, autoimmune rheumatological diseases, and malignancy. Therefore, the following tests were performed: serum immunoglobulins, immunoglobulin subclasses, lymphocyte subtypes, nitroblue tetrazolium test, and tests for leukocyte adhesion deficiency, all of which yielded normal results. Blood and skin swab cultures were also negative. Additionally, bone marrow examination and imaging studies were performed to rule out a paraneoplastic reaction, and they were normal. An autoinflammatory disease gene panel (17 genes, including the *PSTPIP1* gene) was studied. Upper and lower gastrointestinal endoscopies were normal, excluding inflammatory bowel disease (IBD). Abdominal USG, echocardiography, and blood pressure were normal.

A skin biopsy was performed. Hyperkeratosis on the surface, thinning of the epidermis, scar tissue in the dermis, mixed-type inflammation, vascular proliferation, and necrosis were observed. IgM, IgG, and C3 accumulation were not observed. Elastic fiber loss was noted in the scar tissue with elastic Van Geisen stain. Ki-67 was positive in the epithelial basement. Multiple bacterial cultures from pustules and abscesses showed no evidence of bacterial infection. It was stated that these findings were consistent with the late period of PG.

The patient was ultimately diagnosed with idiopathic PG, and treatment was initiated with intravenous immunoglobulin (IVIG) at a dose of 1 g/kg, along with high-dose pulse methylprednisolone administered at a dose of 30 mg/kg/dose for a duration of 3 days. Despite the administration of conventional treatment, the patient did not experience regression of new

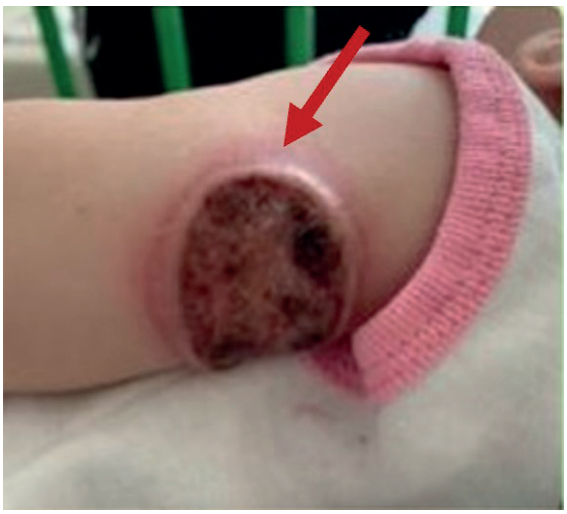

Fig. 1. Pyoderma gangrenosum lesion with necrotizing vasculitis and hyperkeratosis over the triceps muscle of the left arm.

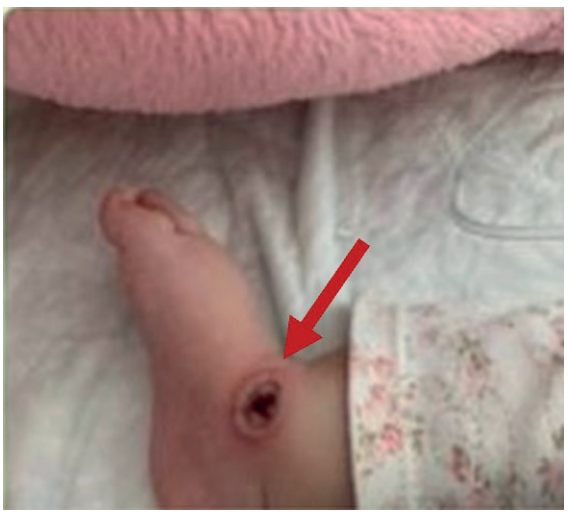

Fig. 2. Pyoderma gangrenosum lesion with necrotizing vasculitis and hyperkeratosis over the medial malleolus of the right ankle.

lesions or signs of inflammation. In light of the suspicion of an underlying autoinflammatory disease, anakinra was introduced as part of the treatment plan. The initial dose of anakinra was started, and it was gradually increased up to 8 mg/kg/day to achieve an optimal therapeutic effect.

Considering the patient's persistent symptoms and the absence of a detected mutation in a specific gene associated with autoinflammatory diseases, the decision to transition from anakinra to tocilizumab is a reasonable approach. In addition to daily steroid treatment, tocilizumab was initiated to target the underlying inflammatory process. Tocilizumab is expected to provide a different mechanism of action and potentially better control of the patient's symptoms.

After two months of treatment with tocilizumab, the patient experienced regression of old lesions, and no new lesions appeared. However, in the third month, severe PG lesions recurred. To address this, the decision was made to initiate infliximab treatment at a dose of 5 mg/kg every 4 weeks. This intervention resulted in the resolution of PG lesions and the normalization of acute phase reactants, providing positive outcomes for the patient for the first time.

At the age of 2.5 years, the patient, who had been receiving infliximab treatment for one year without developing new lesions, was admitted to the hospital with fever. On examination, it was noted that new PG lesions had recurred. Additionally, laboratory tests revealed elevated acute phase reactants, with an ESR of 90 mm/h and CRP level of 189 mg/L. Furthermore, thrombocytosis was observed, with a platelet count of 1,143,000/mm^3. These findings suggest a flare-up of the patient's PG and indicate ongoing inflammatory activity. Further evaluation and adjustment of the treatment approach may be necessary to effectively manage the condition and control the symptoms.

Despite the absence of diarrhea episodes or bloody stools, the patient was re-evaluated for IBD due to the association between PG and occult IBD. Fecal calprotectin, a marker of intestinal inflammation, was slightly elevated at 96 µg/g (normal <80). To further assess intestinal involvement, magnetic resonance (MR) enteroclysis was performed, which reported normal findings.

During the evaluation for vasculitides associated with the patient's condition, thoracoabdominal computed tomography angiography (CTA) revealed several findings. There was evidence of vasculitis and aneurysmal dilatation in the ascending aorta and its main branches. Specifically, concentric wall thickening, reaching a thickness of 3.5 mm, was observed in a segment of approximately 5 cm length at the suprarenal level of the aorta as shown in Fig. 3. This suggests active inflammation and structural abnormalities in that region. Furthermore, slight luminal narrowing was observed in a segment approximately 7 mm long at the origin of the superior mesenteric artery, which may impede blood flow to the intestine as shown in Fig. 3. Color Doppler ultrasonography revealed additional findings related to the patient's arterial system. Thickening of the arterial walls

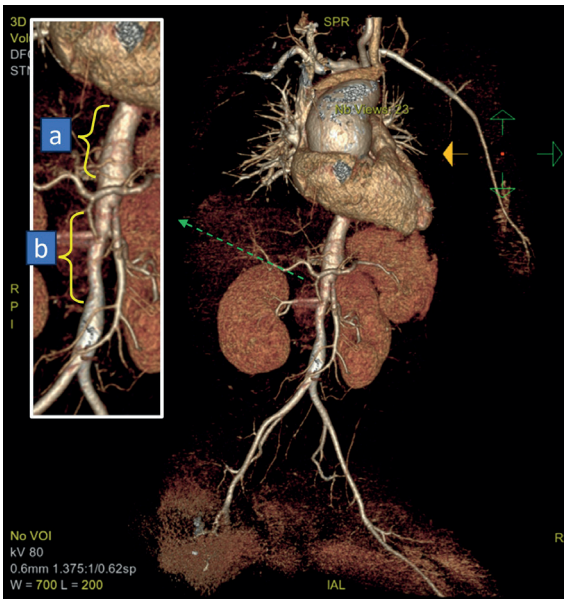

Fig. 3. Thoracoabdominal vascular mapping through computerized tomography angiography.
a. Aneurysmal dilatation at the suprarenal level of the aorta. **b.** Narrowing at the origin of the superior mesenteric artery along the abdominal aorta.

of the common carotid arteries was observed, indicating involvement of these arteries in the vasculitic process. Additionally, there were narrowing of the lumen, suggesting reduced blood flow through the affected carotid arteries.

According to the American College of Rheumatology (ACR) 1990 criteria[5] and the European League Against Rheumatism/ Pediatric Rheumatology International Trials Organization/Pediatric Rheumatology European Society (EULAR/PRINTO/PRES) criteria[6], the vascular findings observed in the patient are consistent with the diagnosis of c-TA. Considering this diagnosis, the patient was started on cyclophosphamide treatment at a dosage of 500 mg/m^2 monthly. The patient has received three infusions of cyclophosphamide thus far. It is encouraging to note that treatment with cyclophosphamide resulted in the normalization of acute phase reactants, indicating a reduction in the inflammatory response. Additionally, there were no new occurrences of PG lesions, suggesting a positive response to the treatment in terms of disease activity.

Discussion

The association between TA and PG-like vasculitic lesions is indeed rare. The first description of this association was reported in 1966.[3] The coexistence of these two conditions poses diagnostic and therapeutic challenges, as the management of TA and PG requires different treatment approaches.

When conducting a literature search, we found that there have been reports of 18 cases, including adults, with the coexistence of TA and PG-like vasculitic lesions.[1,4,7-10] Among these cases, only 10 were reported in children. In rare cases, TA has been reported to coexist with other conditions, such as ulcerative colitis, Crohn's disease, rheumatoid arthritis, and autoimmune hemolytic anemia.[11] These associations highlight the complex nature of autoimmune and inflammatory diseases and the potential overlap between different conditions. Further research is needed to understand the underlying mechanisms and the clinical management of these rare coexisting conditions.

Studies have provided support for the notion that PG-like vasculitic lesions can serve as the initial skin manifestation of TA. In our patient, at the time of the initial diagnosis of PG, there were no evident clinical or laboratory findings suggestive of TA. It is important to note that the association of PG-like vasculitic lesions with TA has been reported in only a few cases, highlighting its rare occurrence.[1,4,7-9]

In the adult population, the average age of diagnosis for PG is approximately 22.5 years, while for TA, it is approximately 26.0 years.[1] During our literature review, we identified 10 cases of concurrent TA and PG, as listed in Table I. The median age at onset was 10 years for PG and 9 years for TA, with a male predominance observed (M/F ratio of 3/2). Notably, PG and TA can manifest at any stage of the disease process. In 50% of the cases (5 out of 10), PG was diagnosed an average of 5 years earlier (ranging from 1.5 to 9 years) than c-TA. In 40% of the cases (4 out of 10), PG and c-TA were diagnosed simultaneously, and in 1 case, PG was diagnosed within 2 years after the c-TA diagnosis.[2]

In the case of our patient, TA was diagnosed 21 months after the initial diagnosis of PG, which is within the range reported in the literature. This highlights the variable temporal relationship between the two conditions and the importance of considering TA in the diagnostic workup of patients with PG, even if there is a considerable time gap between the two diagnoses.

Based on the available literature, information regarding the prediction of c-TA development in patients with PG-like vasculitic lesions is limited. Among the 10 reported patients, common findings associated with c-TA were observed in the following order of frequency: fever (70% or 7/10), bruises (60% or 6/10),

Table I. Review of cases with Takayasu arteritis and pyoderma gangrenosum.

Authors, years & reference		Sex	Age at onset of the systemic manifestation	Age at onset of the PG manifestation	Age at onset of the c-TA manifestation	Systemic symptoms	Vascular involvement; location	PG lesion location and histopathologic manifestations	Laboratory tests
Shen et al. 2022[2]	Case 1	M	6	6	7,5	Fever and chest pain	Aneurysms of AAO, AOA and its branches, DAO, and AA	Neutrophilic infiltration and necrotizing vasculitis	Elevated WBC, ESR, and CRP
	Case 2	M	5	5	5	Fever and harsh systolic murmurs over the bilateral CA	BCT and bilateral ICA were narrowed with local dilatation; AOA, the proximal end of the BCT, CCA, VA, SBC, and AA were thickened, stenosis of the SMA	Neutrophils and lymphocyte infiltration in vessel walls and vasculitis	Elevated WBC, ESR, and CRP
	Case 3	M	10	10	10	Fever, cough, wheezing, shortness of breath, and chest pain	Thickening of the bilateral CCA and SMA	Infiltration of neutrophils and lymphocytes and vasculitis	Elevated WBC, ESR, and CRP
Zhang et al. 2019[1]		F	N/A	17	24	Left arm weakness, dizziness, discrepancy in systolic BP, spray-like noise on the left SBC and bilateral FA	The left CA was narrowed and blocked; the left SBC, FA, SFA, and left POA were thickened; the AAO, AOA, DAO, and AA were thickened; the CT and SMA were occluded; and stenosis in the lumen of the HA and SA	A large number of neutrophils, plasma cells, lymphocytes, and multinucleated giant cells had infiltrated the dermis and subcutaneous tissue; no vasculitis described	Elevated ESR and CRP
Vettiyil et al. 2017[10]		F	8	10	8	Pain and claudication in the legs and back; an absence of pulses in the carotid, brachial, radial, popliteal, and dorsalis pedis on the left side and right femoral, popliteal, and dorsalis pedis; right-sided carotid bruits	Dilation of the AAO and narrowing of the left CCA; the bilateral SBC and AXA were narrowed at the origin and proximal portion	Suggestive of PG; the presence of vasculitis was not described	Elevated ESR and CRP
Barrera-Vargas et al. 2015[4]		M	25	17	26	Fever, chest pain, cough, dyspnea, lightheadedness and syncope, discrepancy in systolic BP, diminished pulses and bruits over the left CA	AO dilatation, narrowing and wall thickening of the left CCA, and CT stenosis, as well as thickening at the origin of the SMA and left ReA	No skin biopsy record	Elevated ESR and CRP

AA, abdominal aorta; AAO, ascending aorta; AO, aorta; AOA, aortic arch; AXA, axillary artery; BCT, brachiocephalic trunk; BP, blood pressure; CA, carotid artery; CCA, common carotid artery; CRP: C-reactive protein; CT, celiac trunk; DAO, descending aorta; ESR: erythrocyte sedimentation rate; F, female; FA, femoral arteries; HA, hepatic artery; ICA, internal carotid artery; M, male; NA, not available; PG: pyoderma gangrenosum; POA, popliteal artery; ReA, renal artery; SA, splenic artery; SBC, subclavian artery; SFA, superficial artery; SMA, superior mesenteric artery; ThA, thoracic aorta, WBC: white blood cells.

Table I. Continued.

Authors, years & reference	Sex	Age at onset of the systemic manifestation	Age at onset of the PG manifestation	Age at onset of the c-TA manifestation	Systemic symptoms	Vascular involvement; location	PG lesion location and histopathologic manifestations	Laboratory tests
Minagawa et al. 2009[14]	F	21	16	21	Low-grade fever, discrepancy in systolic BP, dysesthesia in the arms, fatigue, diminished left radial pulse and carotid bruits	Stenosis and thickened walls in the SBC, left carotid and right ReA	Necrotizing vasculitis with lobular panniculitis in the deep dermis	No record
Ghosn et al. 2008[15]	F	7	7	7	Acute onset right wrist drop	Marked aneurysmal dilatation of the AAO; significant enlargement of the BCT, right CCA, and right AXA; occlusion of left and right SBC and collateral vessel formation	Epidermal necrosis and ulceration, and underlying large neutrophilic collections reaching the deep reticular dermis; no evidence of vasculitis	Elevated ESR and CRP
Dagan et al. 1995[16]	M	4	0,75	4	Restlessness, fatigue, fever, heart murmurs, an absence of both radial pulses	Severe aneurysmatic dilation of the AAO, severe stenosis of the left SBC	Granulomatous dermatitis and panniculitis, with no evidence of vasculitis	Elevated ESR
Perniciaro et al. 1987[11]	M	17,5	19	19	Intermittent fever, multiple vascular bruits	NA	Necrotizing vasculitis with polymorphonuclear leukocytes and fibrinoid changes in vessel walls	Elevated ESR
Present case	F	3	0,58	3	Fever and abdominal pain	Aneurysmal dilatation of the AAO; Diffuse concentric wall thickening in the ThA and AA; slight luminal narrowing at the origin of the SMA	Hyperkeratosis, mixed-type inflammation, vascular proliferation, and necrosis. Elastic fiber loss in the scar tissue with elastic Van Geisen stain. Positive KI-67 in the epithelial basement.	Elevated ESR and CRP

AA, abdominal aorta; AAO, ascending aorta; AO, aorta; AOA, aortic arch; AXA, axillary artery; BCT, brachiocephalic trunk; BP, blood pressure; CA, carotid artery; CCA, common carotid artery; CRP: C-reactive protein; CT, celiac trunk; DAO, descending aorta; ESR: erythrocyte sedimentation rate; F, female; FA, femoral arteries; HA, hepatic artery; ICA, internal carotid artery; M, male; NA, not available; PG: pyoderma gangrenosum; POA, popliteal artery; ReA, renal artery; SA, splenic artery; SBC, subclavian artery; SFA, superficial artery; SMA, superior mesenteric artery; ThA, thoracic aorta, WBC: white blood cells.

and pulselessness (40% or 4/10). In terms of laboratory findings, an elevated ESR was observed in 90% of cases, while CRP elevation was observed in 70% of cases. Skin biopsy was performed in almost all patients (90%), with vasculitis being observed in 55.6% (5/9) of the patients, not detected in 22.2% (2/9), and with no available records in 22.2% (2/9) of cases. The most commonly involved vessels were the aorta and its branches (7/9), followed by the common carotid arteries (66.7% or 6/9), subclavian arteries (6/9), mesenteric arteries (44.4% or 4/9), and abdominal aorta (3/9). It is important to note that these findings are based on a small number of reported cases, and further research is needed to better understand the characteristics and predictive factors of c-TA in patients with PG-like vasculitic lesions.

Various hypotheses have been proposed to explain the association between PG and TA. One hypothesis is that both diseases involve an increase in proinflammatory cytokines, such as interleukin (IL)-6, IL-8, IL-18, IL-23, and tumor necrosis factor-α (TNF-α). These cytokines have been found to be elevated in both the TA and PG, suggesting similar underlying mechanisms in the two conditions.[1,2] Establishing a temporal connection between PG and TA can be challenging due to the lack of specific immunoglobulin markers and the delayed onset of vascular symptoms in TA. Additionally, the nonspecific findings during the early "prepulseless" stage of TA may not be noticeable, especially if PG does not show signs at the time of diagnosis. There are also hypotheses that suggest the treatment of PG may delay the onset of TA symptoms.[2,12] Ujiie et al.[13] reported that PG associated with TA tends to have more extensive skin involvement than PG without TA.

In our case, the patient presented with extensive papulopustular lesions, including some necrotic ulcerated lesions, predominantly on the upper extremities, ankle, gluteal region, and lower extremities. The distribution of lesions in the lower extremities aligns with the findings reported in the literature.[5] It is worth noting that the presence of skin involvement in patients with TA does not necessarily indicate a worse prognosis for the disease.[10]

The extent and distribution of skin involvement can vary among individuals with TA, and it is important to evaluate and monitor the patient's overall clinical presentation, including systemic symptoms and vascular involvement, to determine the appropriate management and treatment approach.[14]

Based on the information obtained from the literature and the case we presented, it is important to raise suspicion and consider the possibility of an underlying diagnosis of TA in cases of PG with unclear etiology, particularly in young female patients. The association between PG and TA, although rare, has been reported in the literature, and recognizing this potential association can guide further investigations and appropriate management.[15] When encountering a patient with PG, especially in an infantile age group, thorough evaluation and investigation should be conducted to identify any underlying systemic conditions, including vasculitis such as TA. Prompt recognition and diagnosis of TA in such cases are crucial for initiating appropriate treatment and preventing potential complications associated with vascular involvement.

Ethical approval

Informed consent was obtained from the parents of the child.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: GÖB, BS; data collection: GÖB; analysis and interpretation of results: GÖB, BS; draft manuscript preparation: GÖB. 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 child with intravascular fasciitis mimicking deep vein thrombosis: a case report

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ABSTRACT

Background. Intravascular fasciitis (IF) is a benign, reactive, myofibroblastic proliferation that originates from the superficial or deep fascia of small / medium-sized arteries and veins.

Case Report. An 8-year-old male patient was admitted to a health center with the complaint of swelling in the inguinal region. Lower extremity venous Doppler ultrasonography showed deep vein thrombosis (DVT) of the femoral vein and anticoagulation with low-molecular weight heparin (LMWH) was initiated. The patient was referred to our center for follow-up. The D-dimer level was detected within normal limits. Doppler ultrasonography was repeated and showed an intraluminal expanding mass lesion with increasing vascularity, without distinct borders and LMWH was discontinued. This lesion at the sapheno-femoral junction was excised surgically and the histopathological examination revealed intravascular fasciitis.

Conclusion. Clinicians should be aware that the clinical findings of IF may mimic sarcoma and thrombosis.

Key words: fasciitis, intravascular fasciitis, thrombus.

Intravascular fasciitis (IF) is a benign, reactive, myofibroblastic proliferation affecting small/ medium-sized veins and arteries. It is accepted to be a rare variant of nodular fasciitis and it may easily be mistaken for sarcoma and thrombosis. Seventeen IF cases were initially defined by Patchefsky and Enzinger in 1981.[1] Although only 3 adult patients with large vessel IF have been reported to date, no pediatric cases have been described.[2-4] Herein, we report a rare case of an 8-year-old boy initially diagnosed with deep vein thrombosis (DVT), and a mass lesion in the main femoral vein was noticed afterward, leading to a diagnosis of IF.

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Case presentation

An eight-year-old boy was admitted to a health center due to inguinal swelling, which was identified two weeks prior to admission. There were no additional symptoms, such as pain or tenderness. There was no family history of a similar disease or consanguinity between the parents of the patient. Right lower extremity venous doppler ultrasonography showed a thrombus with a length of 3 centimeters in the right femoral vein lumen, initiating from the saphenofemoral junction, and the patient was referred to our center with a diagnosis of DVT. Physical examination showed extensive superficial collateral veins and swelling in the inguinal region. The right lower extremity circumference was 1 cm wider than the left. There was no pain, tenderness, or immobility in the inguinal region. He had no history of trauma or infection. Routine laboratory parameters were within normal limits. So, low-molecular weight heparin treatment was initiated due to

the diagnosis of thrombosis. But the patient had findings of chronic thrombosis (collateral superficial veins) rather than acute symptoms and his D-dimer level was within normal limits (383 ng/ml). The patient was re-evaluated with a venous doppler ultrasonography on the first day of treatment in our center. An intraluminal low-echogenicity expansion lesion with internal vascularization within the right femoral vein was detected, extending to the proximal saphena magna. Venous lumens at the inferior and superior of the mass lesions were intact (Fig. 1-2). Due to the ultrasonographic findings, anticoagulant therapy was discontinued. Magnetic resonance imaging of the right femoral vein was performed to exclude angiosarcoma and vascular tumors and showed soft tissue with internal vascularization. The mass lesion did not show extravascular extension (Fig. 2). The 15x10x7 mm mass lesion was surgically extracted, and histopathological examination revealed the lesion to be IF (Fig. 3). The patient had complications after the surgery and was discharged in 1 week. He has been followed up at three-month intervals for nearly 1 year after surgery without complications.

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

Discussion

IF was initially defined by Patchefsky and Enzinger in 1981, as a rare variant of nodular fasciitis.[1] IF is a non-tender nodule that is clinically benign, and simple surgical excision is usually sufficient to cure the lesion. Nevertheless, the intravascular expansion ability of the nodules may cause them to be mistaken for a vascular invasion of malignancies such as myofibroblastoma, fibroblastoma, fibrosarcoma, liposarcoma, or leiomyosarcoma.[5,6] For this reason, some authors have used the term "pseudo sarcoma" to define those lesions.[7] *USP6* gene rearrangement is used as a confirmation tool, which has been shown in nodular fasciitis recently.[8] The underlying etiology of IF is not known. The myofibroblasts within the vessels are assumed to lead to local proliferative changes without any underlying vascular changes.[1] Although trauma is included among the etiological factors, a scarce number of patients have a history of trauma. Some studies have suggested viral infections and previous thromboses to be possible causes of IF.[7-10] Although our patient did not have any history of trauma, infection, or previous thrombosis, the clinical findings at the time of admission were initially thought to be suggestive of

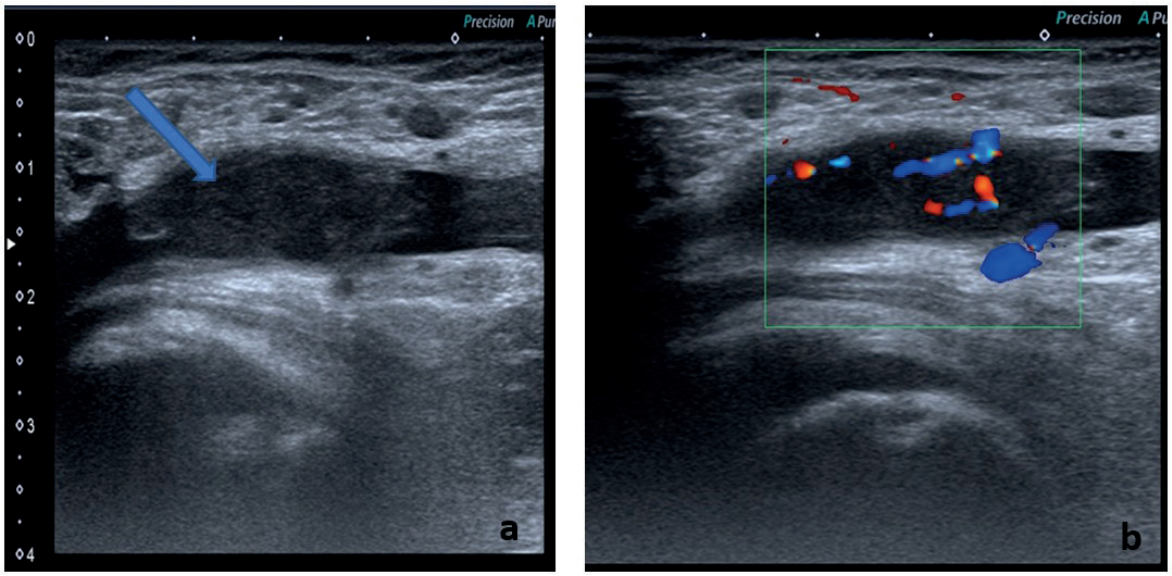

Fig. 1. Ultrasonography and Doppler ultrasonography findings of the patient. **a-** Mass located in the femoral vein on ultrasonography presented with arrow, **b-** Internal vascularization on Doppler ultrasonography

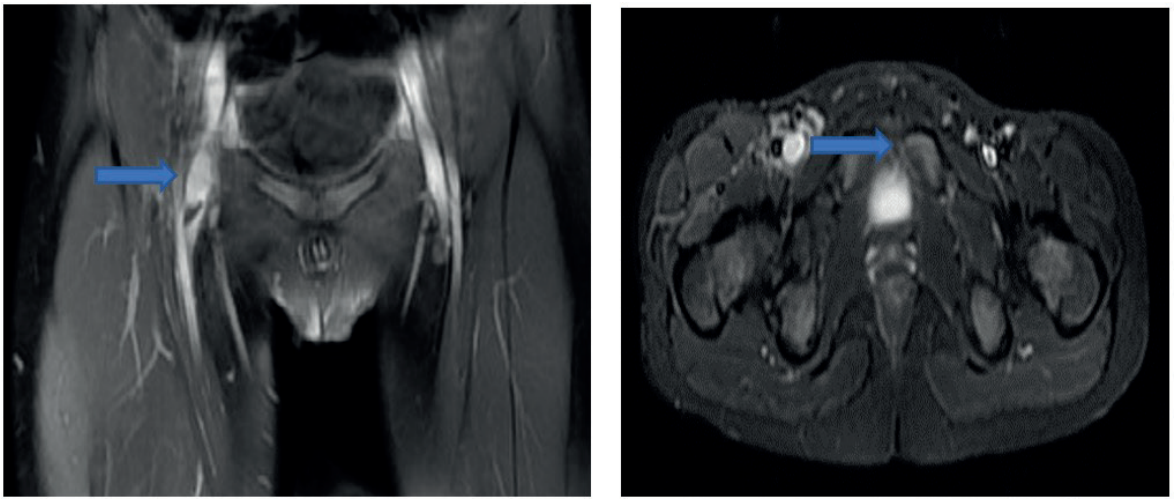

Fig. 2. Coronal (left) and axial (right) magnetic resonance images of the patient. Arrow indicates the mass of the patient

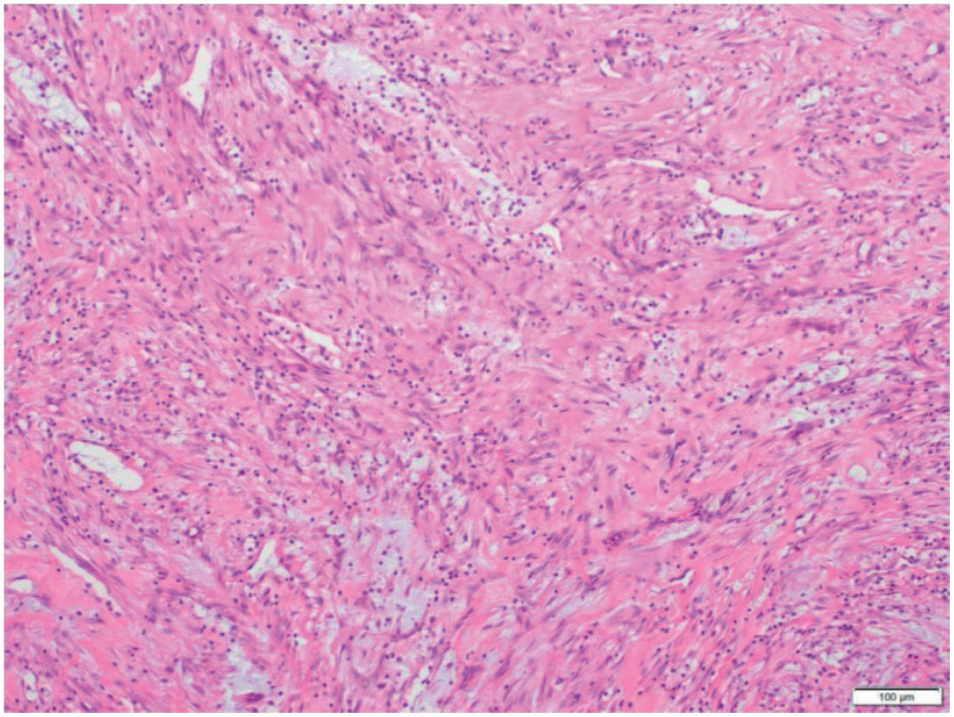

Fig. 3. Histopathologic features of the specimen. Sections show proliferation of benign spindle cells arranged in intersecting fascicles accompanying loose stroma and vascular areas. Scattered lymphocytes are also noted. (Hematoxylene-Eosin staining)

DVT. In this case, the laboratory analyses and ultrasonographic findings excluded DVT. A careful radiological evaluation is mandatory for IF to exclude DVT and other diagnoses.

The most common regions for IF are the upper extremities, head, and neck.[1] Among the 17 cases defined by Patchefsky and Enzinger, the most common location of the lesions was reported

as upper extremities (n:7, 41%), followed by head-neck (n:5, %29), lower extremities (n:3, 12%), and trunk (n:2, 12%).[1] In our case, IF was located at the lower extremity, which is a rare location. IF is mostly known to affect small/medium-sized arteries and veins and is rarely localized in large arteries. There are very few adult patients with IF involving large vessels that have been reported in the literature.[2-4,11] To the best of our knowledge, our case is the first reported pediatric patient with IF originating from a large vessel (right femoral vein).

The clinical findings of IF may mimic DVT due to the narrowing of the lumen of large vessels. Clinicians should be aware of symptoms suggestive of IF, including non-tenderness, the development of superficial collateral veins that are indicative of a chronic process, and normal D-dimer levels.

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Ethical approval

Authors declare that they have written consent from the family.

Author contribution

Conception and design: YY; data collection: OE, ATY; analysis and interpretation of results: EÖ, HG; draft manuscript preparation: YY. All authors reviewed and approved the final version of the manuscript.

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Conflict of interest

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Case Report

A case of crescentic glomerulonephritis with exacerbation of pre-existing IgA nephropathy after COVID-19

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ABSTRACT

Background. Relapses or new-onset IgA nephropathy (IgAN) have been documented in patients after vaccination against SARS-CoV-2; however, only one adult patient has been reported in whom pre-existing IgAN worsened during coronavirus disease 2019 (COVID-19).

Case. We present the first pediatric case with biopsy-proven IgAN and genetically confirmed Alport syndrome, who developed end-stage kidney disease after an exacerbation of IgAN associated with COVID-19. The patient's basal serum creatinine was 0.7-0.9 mg/dL before infection. He had not been vaccinated against COVID-19. He was admitted to the hospital with edema, hypertension, an elevated serum creatinine of 4.7 mg/dL, and massive proteinuria. Three months before admission, he had been admitted to another hospital with COVID -19 and an elevated serum creatinine (1.9 mg/dL), but no biopsy had been performed at that time. The kidney biopsy revealed IgAN with 50% fibrocellular crescents with sclerosed glomeruli, tubular atrophy, and interstitial fibrosis. His serum creatinine did not decrease even after five administrations of pulse steroids, and hemodialysis was initiated.

Conclusion. In conclusion, COVID -19 may pose a high risk for exacerbation of pre-existing glomerular disease. It is therefore necessary to closely monitor the kidney function of patients with underlying glomerulonephritis during and after COVID-19 and consider an early biopsy if serum creatinine does not return to baseline levels. In addition, this case report highlights the clinical importance of the co-occurence of IgAN and Alport syndrome.

Key words: Alport syndrome, children, crescentic glomerulonephritis, COVID-19, IgA nephropathy.

Since the first description of coronavirus disease 2019 (COVID-19) in December 2019, it has been well known that kidney involvement is the second leading cause of morbidity and mortality in patients with COVID-19.[1] There are numerous mechanisms that may contribute to kidney injury in patients with COVID-19, including virus-mediated damage, cytokine storm, activation of the Angiotensin II pathway, complement activation, hypercoagulation, and thrombotic microangiopathy.[2-4] Kidney involvement manifests as both glomerular and tubular diseases and varies from acute kidney injury to glomerulonephritis.[4] IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis (GN) and is usually triggered by infections.[5-7] Several cases of IgAN have been reported following administration of the SARS-CoV-2 vaccine[8,9], but there is only one documented instance of an adult patient experiencing an exacerbation of pre-existing IgAN during COVID-19 infection.[10]

To date, a few cases have been reported in which IgAN and Alport syndrome (AS) occurred together.[11-13] Familial cases of IgAN suggest

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that this may not be a coincidence.[11] The case presented here was previously published in the context of biopsy-proven IgAN and genetically confirmed autosomal dominant AS[14,15], but experienced a severe exacerbation of IgAN after COVID-19.

In the present report, we have attempted to highlight the following aspects: 1) presentation of the first pediatric case with an exacerbation of IgAN following COVID-19, which rapidly progressed to end-stage kidney disease (ESKD), 2) highlight the co-occurrence of IgAN and AS with the clinical significance of this association, and 3) emphasize the importance of timely kidney biopsy in such cases of management.

Case report

A 16-year-old boy, who was diagnosed with IgAN and AS at the age of 8 years, was hospitalized with complaints of an increase in serum creatinine. He was born to consanguineous parents, and his parents were first degree cousins. Within his family history, three members had hematuria, one had hematuria and proteinuria, and his father had chronic kidney disease (CKD). Furthermore, hematuria was identified in two additional family members (paternal uncle and cousin) and proteinuria was detected in two other relatives (paternal cousins). As previously reported in detail[14,15], he had concomitant glomerular pathologies, IgAN and AS. The light microscopy and immunofluorescence findings of the kidney biopsy were consistent with those of IgAN, but electron microscopy revealed a partially thinner and thickened glomerular basement membrane with a basket-weave appearance, suggesting AS. Targeted next-generation sequencing revealed a homozygous frameshift mutation in the COL4A4 gene; c.2438delG (p.Gly813AspfsTer56). In addition, he had sensorineural hearing loss and anterior lenticonus. He was subsequently treated with the maximum dose of ramipril; his basal serum creatinine was 0.7-0.9 mg/dL, and his urine protein was 1.5 g/day. He had not been vaccinated against COVID-19.

Three months prior to the current admission, he had a history of COVID-19 and had been hospitalized in another hospital with fever and elevated serum creatinine (1.9 mg/dL). During COVID-19 hospitalization, angiotensin-converting enzyme inhibitor had been discontinued, no additional treatment had been given, and no biopsy had been performed. On admission to our hospital, physical examination revealed edema and hypertension (blood pressure 140/90 mmHg, >95th percentile). No oliguria or macroscopic hematuria was noted. Laboratory examination revealed a serum urea of 142 mg/dL, a creatinine of 4.7 mg/dL, an albumin of 2.9 g/dL, and a 24-hour urinary protein excretion of 6 g. Other laboratory tests showed hyperkalemia, hyperphosphatemia and increased parathormone levels. Complete blood count was normal, serum complement levels were within the normal range. Antinuclear antibodies, anti-dsDNA antibodies, and anti-neutrophil cytoplasmic antibodies were all negative (Table I). Ultrasonography revealed bilaterally increased echogenicity of the kidney parenchyma with normal size of both kidneys.

Because of the increase in serum creatinine, a kidney biopsy was performed, which revealed IgAN (2+ granular mesangial staining for IgA) with 50% fibrocellular crescents (6 of 12 glomeruli). In addition, of the total 12 glomeruli, 5 glomeruli were globally sclerosed, and one showed segmental sclerosis, tubular atrophy and interstitial fibrosis, interstitial nonspecific mononuclear cell infiltration (Fig. 1). Arterioles showed hyaline sclerosis and hyperplastic changes. Unfortunately, we were unable to perform an immunohistochemical examination for SARS-CoV-2. The ultrastructural analysis of renal tissue did not reveal any evidence of the presence of SARS-CoV-2. Five doses of pulse methylprednisolone (1g per dose) were administered for the treatment of crescentic GN. Despite this intervention, there was no improvement in the serum creatinine levels. Consequently, hemodialysis was initiated, and the decision was made to discontinue immunosuppressive treatment due to the

Table I. Laboratory findings of the patient before, during, and after COVID-19 infection.

	Before COVID-19	During COVID-19	After COVID-19
TLC (cells/μL)	2300	2200	1700
TNC (cells/μL)	8900	2600	9600
Urea (mg/dL)	93	94	142
Creatinine, mg/dL	0.9	1.9	4.7
eGFR (ml/min/1.73m²)	80	38	10
Serum albumin (gr/dL)	3.6	3.1	2.9
C-reactive protein (< 5 mg/dL)	<0.5	0.28	0.94
C3 (0.9-1.8 g/L)			0.97
C4 (0.1-0.4 g/L)			0.21
ANA			Negative
Anti-dsDNA (IgG) (<12 IU/mL)			2.1
PR3 ANCA (<12 IU/mL)			Negative
MPO ANCA (IgG) (< 12 IU/mL)			Negative
Urinary protein excretion (gr/day)	1.5	2.2	6

ANA: anti-nuclear antibody, anti-dsDNA: anti-double stranded DNA, C3: complement 3, C4: complement 4, MPO ANCA: anti-myeloperoxidase antineutrophil cytoplasmic antibody, PR3 ANCA: anti-proteinase 3 antineutrophil cytoplasmic antibody, TLC: total lymphocyte count, TNC: total neutrophil count

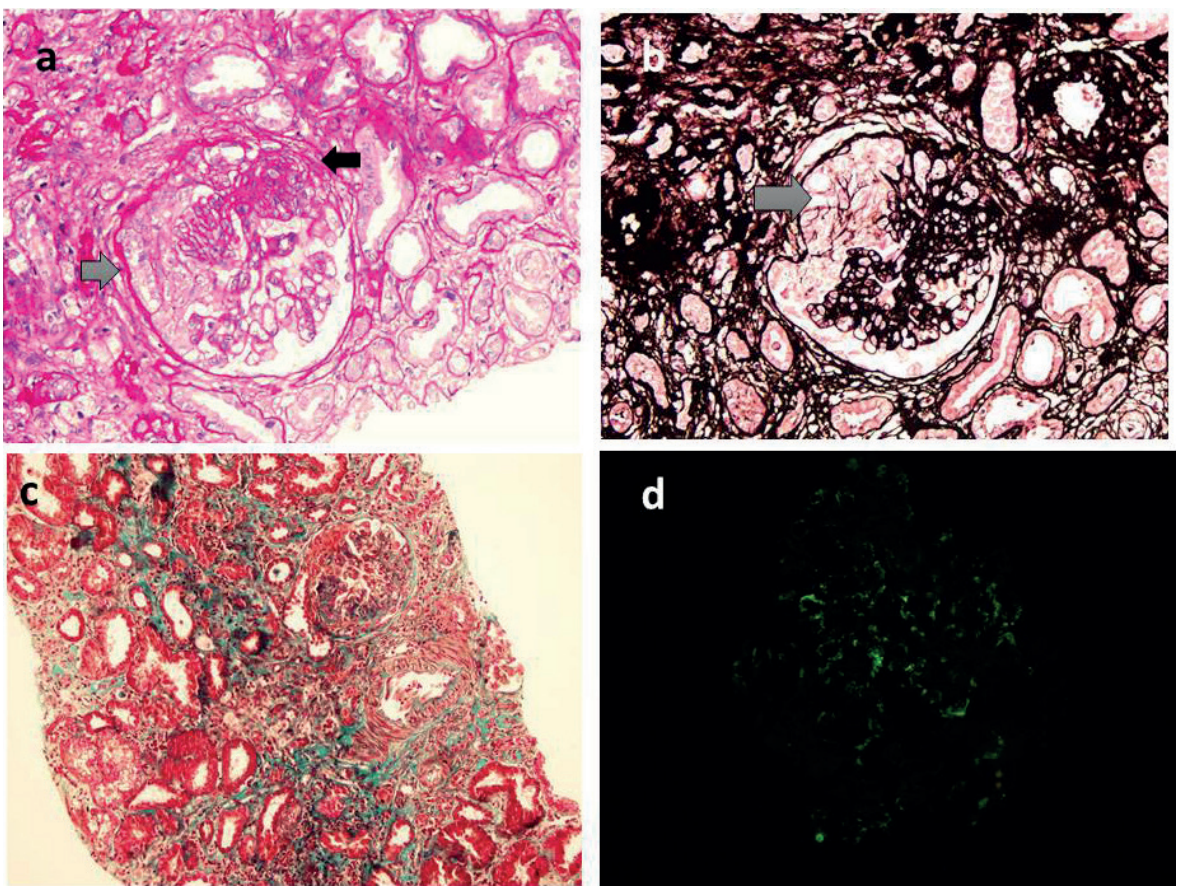

Fig. 1. Biopsy shows segmental sclerosis (black arrow) and fibrocellular crescent (gray arrows).
(a: PAS, periodic acid schiff; b: PAMS, periodic acid methenamine silver), focal tubular atrophy and interstitial fibrosis (c: MT, Masson's Trichrome) and mesangial IgA deposits by immunofluorescence (d: FITC, fluorescein isothiocyanate).

chronic findings in the biopsy specimen. Three months after the initiation of hemodialysis (HD), he remained on HD three times a week for four hours. The patient was still doing well on chronic HD and was on a waiting list due to no available living donor in the family.

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

Discussion

Although the development of new-onset or relapses of IgAN has been reported after COVID-19 vaccination, only one adult patient has been reported to have experienced an exacerbation of pre-existing IgAN during COVID-19.[10] Here, we presented the first pediatric case who rapidly progressed to ESKD with an exacerbation of pre-existing IgAN after COVID-19.

IgA nephropathy is the most common primary glomerular disease and viral infections may exacerbate IgAN. The increase in IL-6 production during mucosal infections stimulates poor glycosylation/galactosylation of IgA1. This leads to the formation of Gd-IgA1 and contributes to the development of IgA-related diseases, such as IgAN and IgA vasculitis nephritis (IgAVN).[16] It has been suggested that COVID-19 as a mucosal infection may also trigger IgAN and IgAVN through a similar mechanism.[9] Other cytokines that are elevated in COVID-19 can also lead to IgAN through the proliferation and maturation of these IgA1-producing B cells.[9] It has previously been reported that an excessive production of IgA1 monomers after influenza vaccination led to IgAN or the disease exacerbation[17]; therefore, it has been suggested that a similar process may occur after COVID-19 vaccination.[9] More cases have been reported of new-onset IgAN or exacerbation of pre-existing IgAN after SARS-CoV2 vaccination than cases of IgAN following a natural COVID-19 infection.[8]

Corticosteroid treatment is used in patients at high risk for the progression of IgAV or IgAN.[18] New-onset or relapse of IgAN has been reported following SARS-CoV-2 infection and vaccination, and all patients reported so far recovered spontaneously or with systemic steroids, and none have progressed to ESKD.[8,9] Our case had two distinct primary kidney diseases: biopsy-proven IgAN and molecularly proven AS (*COL4A4* homozygous), which we have previously reported[14,15], and had stage 2 CKD. This patient was admitted to our hospital three months after COVID-19. It was noted that the patient had acute kidney injury with an increase in serum creatinine during COVID-19 without fever, dehydration, or severe respiratory disease and no kidney biopsy was performed at the time. The kidney biopsy performed on admission to our hospital revealed a crescentic IgAN with chronic changes. Treatment with pulse steroids did not improve kidney function, and he progressed to ESKD requiring hemodialysis. It is difficult to say whether the outcome would have been different if the patient had been treated with corticosteroid earlier. This is because the patient had concomitant glomerular disease i.e. AS, that might have led to the rapid progression to ESKD. However, it seems more reasonable to assume that the deterioration in kidney function was mainly related to the exacerbation of IgAN.

Finally, some cases of co-occurrence of IgAN and AS have been reported.[12,13,19] In a large family with familial IgAN, a susceptibility locus for IgAN was detected at locus 2q36, which is in the neighborhood of the *COL4A3* and *COL4A4* genes (2q36.3)[11], suggesting that the co-occurrence of these distinct kidney pathologies in familial cases is not a coincidence. Alport syndrome and IgAN may have similar clinical and laboratory findings, but require different management strategies, including genetic counseling, treatment, and risk of recurrence after transplantation. Therefore, it is important to be aware of this coexistence. Our case demonstrated a severe exacerbation

of IgAN as a cresentic glomerulonephritis following COVID-19. This indicates that the IgA deposition in the kidneys was not an incidental finding. Therefore, this case also demonstrates that a kidney biopsy will be required in a patient with the co-occurrence of AS and IgAN if any clinical or laboratory finding that deviates from the natural course of AS.

In summary, we report an adolescent who was diagnosed with both IgAN and AS and who progressed to ESKD treated with dialysis with a relapse of IgAN after COVID-19. It is important to note that COVID-19 may also pose a high risk for exacerbation of pre-existing glomerular disease, leading to worsening of the kidney disease. This underscores the potential impact of viral infections on kidney and disease progression and highlights the need for careful monitoring and treatment of patients with IgAN and AS, particularly in the context of viral infections. In addition, it highlights that IgAN and AS together can significantly affect disease progression, and that early biopsy should be performed if the kidney function deteriorates unexpectedly.

Ethical approval

Written informed consent has been obtained from the parents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NC; data collection: EKY, GM, SS, YÖ; interpretation of results: EKY, RG, NC; draft manuscript preparation: EKY; critically review of the manuscript: SS, AA, NC. All authors reviewed the manuscript 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

Camptodactyly-arthropathy-coxa vara-pericarditis syndrome and an unusual association with mitral stenosis

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ABSTRACT

Background. Campotodactyly-artrhropathy-coxa vara-pericarditis (CACP) syndrome is a very rare autosomal recessive genetic disorder. It is characterized by flexion contracture of the fifth finger (camptodactyly); non-inflammatory arthropathy; decreased angle between the shaft and the head of the femur (coxa vara) and pericarditis. Its association with mitral stenosis has not yet been reported. Hereby we report this unique association with CACP syndrome.

Case. An eleven-year-old girl presented with non-productive cough, dyspnea, and orthopnea. She was diagnosed CACP syndrome at the age of seven and a biallelic frameshift mutation in the *PRG4* gene was determined. The physical examination revealed pectus excavatum, camptodactyly, genu valgum, tachypnea and orthopnea. The functional capacity was NYHA III-IV. She had 2/6 soft pansystolic murmur at 4th left intercostal space and a rumbling diastolic murmur at apex. Echocardiography revealed an enlarged left atrium, severe stenotic mitral valve with a mean diastolic transmitral gradient of 22.5 mmHg, mild mitral regurgitation and mild apical pericardial effusion. The patient had mitral comissurotomy and partial pericardiectomy operation. Her post-operative transmitral gradient decreased to 6.9 mmHg and the pulmonary pressure was 30 mmHg. Her functional capacity increased to NYHA I-II.

Conclusions. The main defect is the proteoglycan 4 protein which acts like a lubricant in articular and visceral surfaces. Therefore, the leading clinical feature is arthropathy. Cardiac involvement other than clinically mild pericarditis is not usually expected. Three types of proteoglycans (decorin, biglycan, and versican) are present in the mitral valve. This could be the reason of mitral valve involvement in rare cases as like ours. It is important that these patients undergo echocardiographic examination regularly.

Key words: arthropathy, camptodactyly, coxa vara, *PRG4* gene, mitral stenosis.

Campotodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome was defined by Dr. Matthew Warman in 1999. It is a rare genetic disorder with an incidence of less than 1/1.000.000. It is characterized by deformities like camptodactyly and progressive coxa vara beginning during childhood. Arthropathy is non-inflammatory and secondary to synovial hyperplasia. Pericardial effusion is infrequent and has a non-inflammatory origin, rarely causing constrictive pericarditis. The disease is inherited autosomal recessively due to a mutation in the PRG4 gene.[1-4] The PRG4 gene is located in the long arm of the first chromosome at the 1q25-31 locus and codes for a mucine like glycoprotein, namely proteoglycan 4, which is a lubricating material of the synovial fluid in the articular joints and visceral cavities like pleura and pericardium.[5,6]

The cardiological component of the disease is usually manifested as a clinically mild pericarditis. Mitral valve prolapsus and mitral

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regurgitation is rarely reported, only in a few cases.[7,8] To the best of our knowledge, this is the first case of CACP syndrome associated with significant mitral stenosis.

Case report

A written informed consent was received from the family for publication of this case and the photographs.

An eleven-year-old female patient presented with dyspnea and non-productive cough which had progressed to exercise intolerance and orthopnea in the last 3 months. She was born at term to consanguineous parents (3rd degree consanguinity) after an uneventful pregnancy. She had no notable medical problems in infancy and grew normally until three years of age. At the age of three years she started experiencing arthritis which led to deformities. The initial diagnosis by pediatric rheumatology was "juvenile idiopathic arthritis". She was given various anti-inflammatory treatment regimens without a significant clinical benefit. She was diagnosed with coxa vara deformity at the age of seven years. Thereafter she was referred to a genetics department and the whole exome sequencing (WES) analysis revealed a homozygous pathological p.Thr399Profs*513 frameshift in the *PRG4* gene.[2] Genetic counseling noted that no other family member had a similar finding.

The patient was recently referred to pediatric cardiology department for having exertional dyspnea, orthopnea and non-productive cough. The physical examination revealed pectus excavatum deformity (Fig. 1), camptodactyly (Fig. 2), genu valgum deformity (Fig. 3), tachypnea and orthopnea. The functional capacity was NYHA III-IV. Her lungs were clear with auscultation. She had 2/6 soft pansystolic murmur at 4th left intercostal space and a rumbling diastolic murmur at apex. In the echocardiographic examination, enlarged left atrium, (diameter: 47X41 mm, volume 42 ml); thickened and restricted movement of the posterolateral mitral leaflet, shortened posterolateral chorda tendinea were noted (Fig. 4). Mean diastolic transmitral gradient was 22.5 mmHg, indicating severe stenosis and there was mild mitral regurgitation (pressure half time: 83 msec; valve area 2.7 cm^2). The systolic velocity of the tricuspid regurgitation was 4m/sec, therefore the systolic pulmonary arterial pressure was estimated as 75 mmHg. There was also 9 mm pericardial effusion at the apical region.

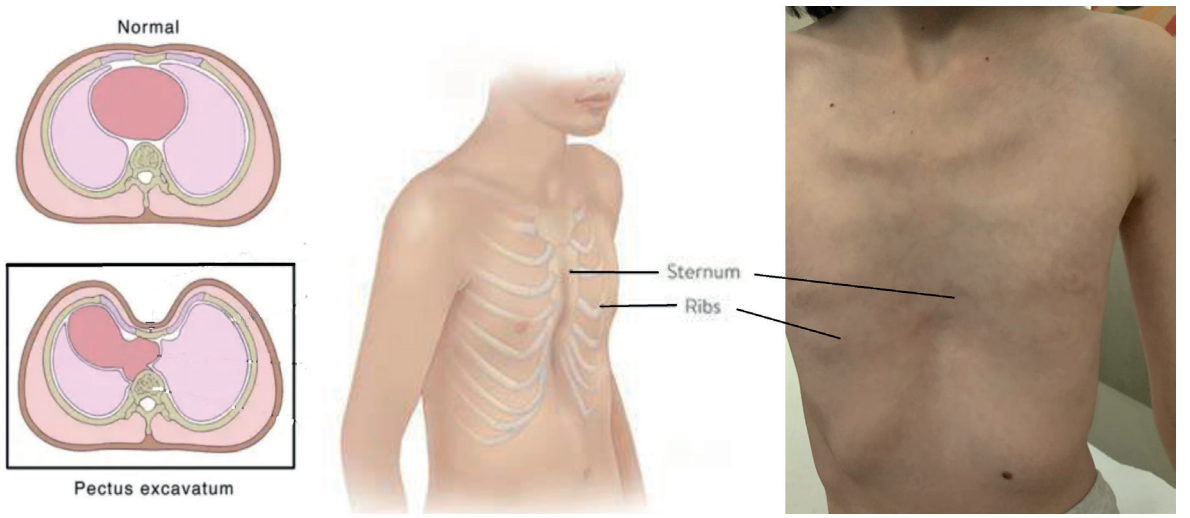

Fig. 1. Pectus excavatum deformity.
The caved-in appearance of the thorax in symmetric / central pectus excavatum deformity.

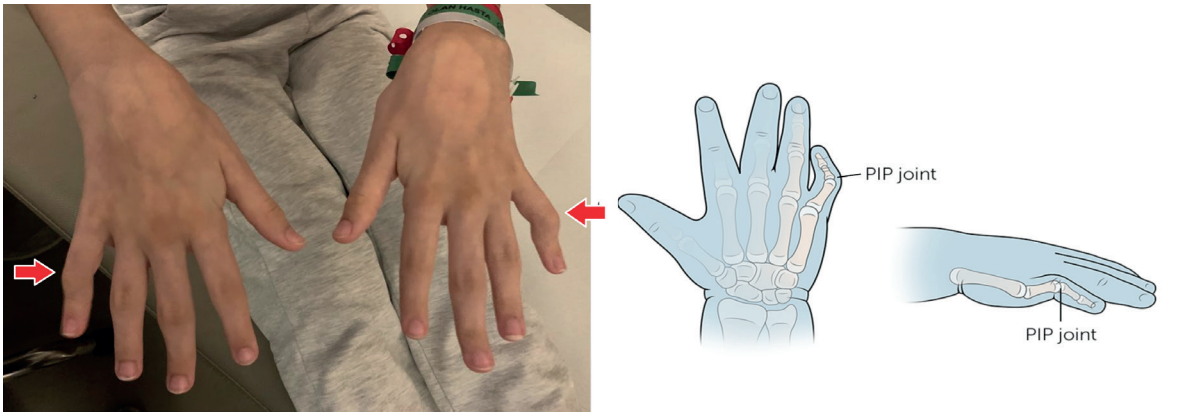

Fig. 2. Camptodactyly.
The fifth fingers are typically bent at the proximal interphalangeal joints (PIP) bilaterally.

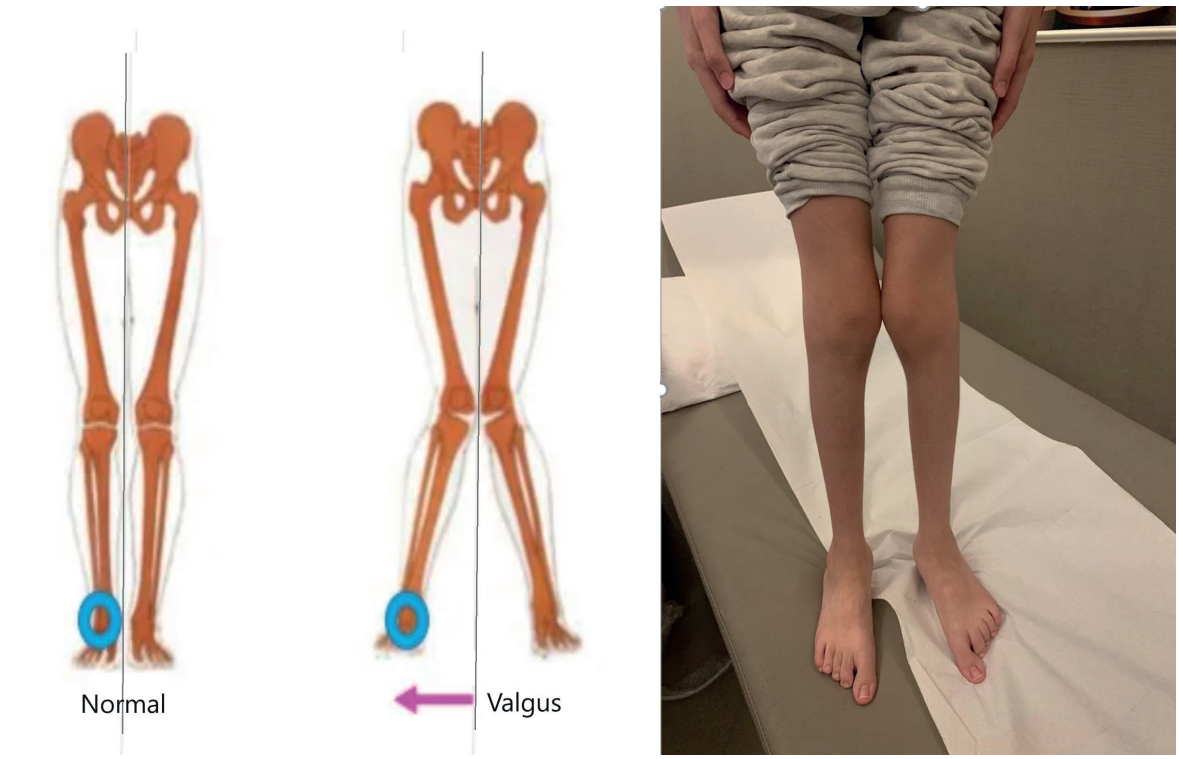

Fig. 3. Genu valgum deformity.
The distal end of the tibia deviates away from the midline, laterally. This is seen because of the coxa vara deformity.

The patient underwent a mitral comissurotomy operation and partial pericardiectomy was performed to prevent a possible constrictive pericarditis. The pericardial biopsy revealed acute fibrinous pericarditis with no elements of inflammation or infection. In the post-operative echocardiography, the diastolic mean transmitral gradient decreased to 6.9 mmHg. Mitral regurgitation was mild-moderate and the estimated systolic pulmonary arterial pressure was 30 mmHg. Her symptoms of orthopnea and dyspnea decreased and functional capacity increased to NYHA I-II.

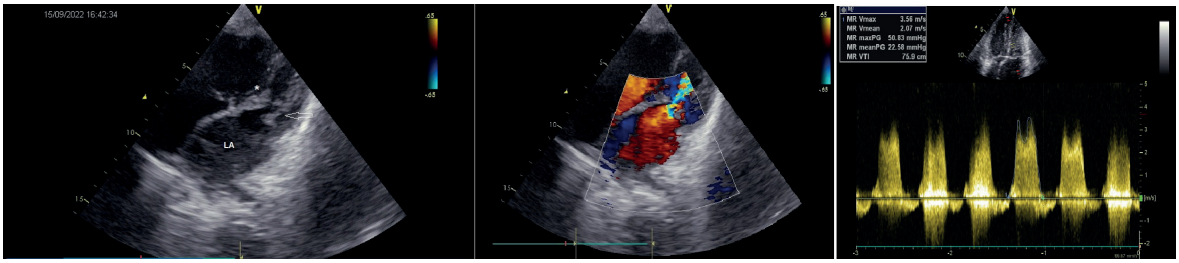

Fig. 4. Echocardiographic evaluation.

Left: Thickened mitral valve leaflet (star), thickened and shortened chorda tendinea are shown (arrow). Middle: Color Doppler diastolic flow pattern of the stenotic mitral valve. Right: CW Doppler diastolic flow pattern of the mitral valve.

Discussion

CACP syndrome is a rare single gene mutation disease with autosomal recessive inheritance. Its incidence may be relatively high in populations where parent consanguinity is frequent. The main defect is the proteoglycan 4 protein which acts like a lubricant in articular and visceral surfaces. Therefore the leading clinical feature is arthropathy with deformities. The polyarthropathy seen in early childhood has systemic involvement. The joints mostly affected are knee, wrist, elbow, and hip joints. Many patients complain about morning stiffness. Therefore it is frequently misdiagnosed as juvenile idiopathic arthritis, however the acute phase reactants and inflammatory parameters remain normal in CACP. In patients with childhood polyarthropathy resistant to anti-inflammatory treatment and with early deformities, CACP syndrome should be in the differential diagnosis list.

Other than clinically mild pericarditis, mitral valve prolapse and mitral regurgitation have rarely been reported. It is known that three particular proteoglycans (decorin, biglycan, and versican) are present in the mitral valve.[9] Extracellular matrix changes are seen in many heart valve pathologies. Myxomatous degeneration of the mitral valve is the leading cause of mitral valve prolapse and regurgitation. Myxomatous mitral valves are reported to contain excess proteoglycans and hyaluronan.[10] This could shed light on the unexpected mitral valve involvement in a patient with *PRG4* mutation, and classifying the variations in the mutation of PRG4 gene may help identify the possible associated anomalies in CACP syndrome. Our patient also indicates that other valvular anomalies like mitral stenosis may also be associated with this disease. Therefore it is important that in patients with CACP syndrome, cardiologic evaluation and echocardiographic examination should be performed regularly.

Ethical approval

We confirm that a written informed consent was received from the family for the publication of this case and the photographs.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: YKY, AA; data collection: AO, VM; analysis and interpretation of results: CA; draft manuscript preparation: DŞ, CA. All authors reviewed the results and approved the final version of the manuscript. They take public responsibility for it.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Isolated mitral valve aneurysm in a 9-year old boy

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ABSTRACT

Background. Isolated mitral valve aneurysm is rarely reported in children. In most cases it is associated with an underlying disease such as infective endocarditis. MVA can lead to severe complications that needs surgical intervention.

Case. In this report, we present a 9-year old asymptomatic male patient with anterior mitral valve aneurysm and rhythm disturbance diagnosed incidentally during pre-operative evaluation.

Conclusions. Being rare in children, isolated MVA should be kept in mind in the differential diagnosis of mass lesions seen on the atrial side of the mitral valve. A 24-hour electrocardiogram may define subtle rhythm disturbances in these patients.

Key words: mitral valve aneurysm, arrhythmia, children.

Mitral valve aneurysms (MVA) are localised saccular bulging on the mitral valve leaflets towards the atrium with systolic expansion and diastolic collapse.[1] Aortic valve infective endocarditis with aortic regurgitation is the leading cause in the literature.[2] Other associated etiologies have been reported such as; rheumatic diseases, iatrogenic diseases, connective tissue diseases (Marfan syndrome, Ehler-Danlos syndrome, osteogenesis imperfecta and pseudoxanthoma), severe aortic regurgitation and mitral valve prolapse.[2-4] Isolated mitral valve aneurysm excluding these etiologies is rarely reported in the literature.[5,6] In this report, a case of isolated MVA in an 9-year-old boy is presented with echocardiographic and ECG findings.

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Case report

A 9-year old boy consulted our clinic because of suspected arrhythmia during auscultation before general anesthesia for a dental procedure. The patient was asymptomatic and had no previous complaints suggestive of arrhythmia. His physical examination was normal except a grade 1/6 soft systolic murmur at left sternal border and possible extrasystole during oscultation. A 12-lead ECG was normal. Transthoracic echocardiography revealed a saccular aneurysm appearance on anterior mitral valve with a diameter of 15.4x12.7 mm and color flow was detected into the aneurysm (Fig. 1, Supplementary Materials 1 and 2). Doppler echocardiogram showed no regurgitation flow to left atrium. Subvalvular apparatus was normal. A 24-hour Holter ECG showed intermittent ectopic atrial rhythm, supraventricular extrasystole (2% of total heart beats) conducted with aberration in some and no tachycardia (Fig. 2). His laboratory examination was normal including blood culture and inflammatory markers. Due to the patient's uneventful past history, a normal physical examination except for a murmur, normal laboratory examination, absence of Duke criteria, aortic regurgitation and findings

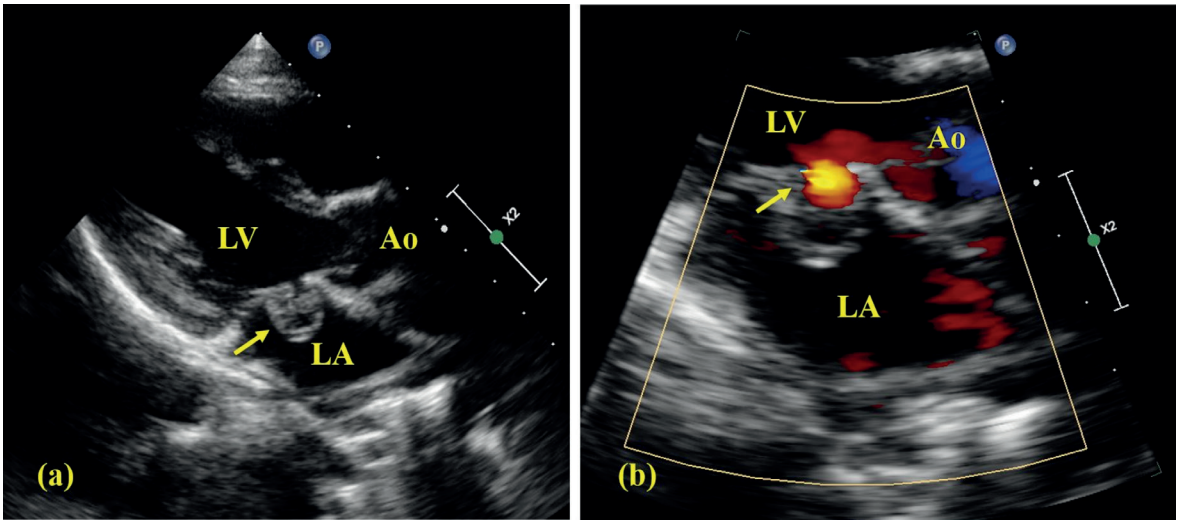

Fig. 1. (a). Echocardiographic appearance of mitral valve aneurysm from long axis view; aneurysm on anterior leaflet (arrow). **(b)** Color flow into the aneurysm without regurgitation (arrow). Ao: aortic valve, LA: left atrium, LV: left ventricle.

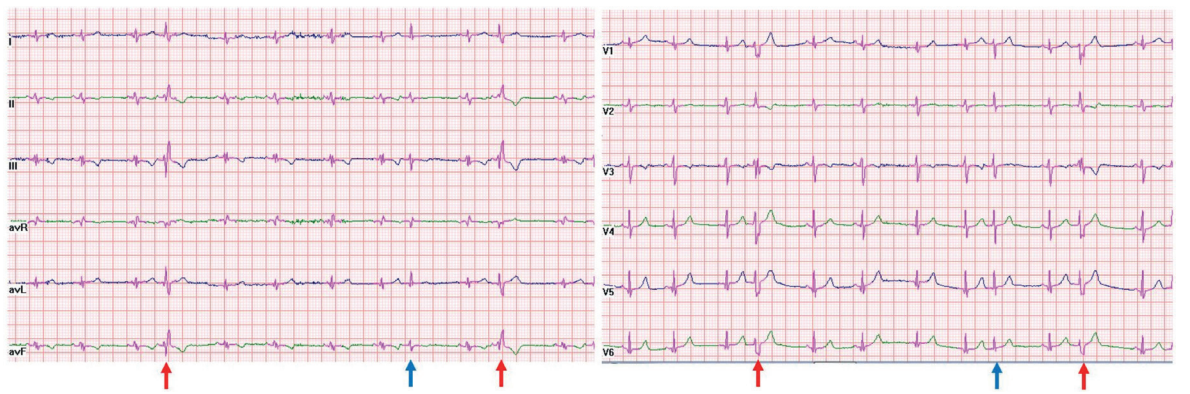

Fig. 2. Supraventricular extrasystoles with (red arrow) and without (blue arrow) aberration detected during 24-hour ECG.

suggestive of connective tissue disease, a diagnosis of isolated mitral valve aneurysm (MVA) was considered in the patient. The patient was asymptomatic and we decided on close clinical follow-up without any medication. During the 10-month follow up period the patient had no complaints and no complications were observed. The patient was planned to be followed up with echocardiography every 6 months and annual 24-hour Holter ECG. Written informed consent was received from the parents of the patient.

Discussion

Mitral valve aneurysms are rare findings, most commonly associated with infective endocarditis (IE).[2] Aortic valve infective endocarditis with aortic regurgitation is the leading cause in the literature. Pena et al.[2] evaluated 18 patients with MVA and found that 83% meet the Duke criteria for definite endocarditis. The associated diseases were mitral valve prolapse in 8, rheumatic in 6, bicuspid aortic valve in 3 and degenerative valve in 1 patient. This

study includes 2 patients under 18 years of age and rheumatic disease was the underlying pathology in both (one had endocarditis and one had aortic and mitral regurgitation). Two patients had myxomatous degeneration in the mitral leaflets with prolapse and no history of IE. In our patient, the aneurysmal lesion was on the anterior mitral leaflet without any clinical evidence of infective endocarditis or positive findings for other possible etiologies. The patient was accepted as isolated MVA after detailed evaluation. Isolated MVA is reported rarely. Nagaoka et al.[6] reported a 8-month old infant with severe mitral regurgitation due to perforated posterior leaflet MVA. They speculate the possible causes were congenital isolated mitral aneurysm that subsequently complicated with perforation or previous small mitral perforation inducing MVA formation secondary to a jet lesion. Mitral regurgitation or perforation were absent in our patient.

A 70 year old patient with perforated posterior leaflet aneurysm without concurrent IE, connective tissue (CT) disease or rheumatic disease was reported by Kim et al.[5] The patient had moderate aortic insufficiency, which was expected to affect the anterior valve more, but the aneurysm was located in the posterior valve without any evidence of an acute inflammatory process in histopathologic examination.

Its mechanism is unclear. In cases with endocarditis it is thought to be through spread of infection to mitral valve via different mechanisms.[1,7] For isolated lesions it is also speculated that a minor valve perforation progresses into severe regurgitation and complicating to aneurysm formation.[6] In our patient perforation and regurgitation were not present. As in our case, it is more common on the anterior mitral leaflet. Pena et al.[2] reported that 16 of the 18 patients had MVA on anterior leaflet. It can be explained by anatomical neighborhood with aortic valve.

Echocardiography is the primary diagnostic tool to detect these lesions, however it can be misinterpreted as vegetation, mitral valve abscess, cystic atrial myxoma, blood cyst or mass.[1,4,7] Color flow demonstrates the direct communication between aneurysm and helps in distinguishing from these lesions.

Possible complications include perforation of the aneurysm leading to severe mitral regurgitation, thrombus formation, embolism and recurrent infection.[1,2,5,6] Pena et al.[2] reported that perforation and thrombus formation in 94% and 16% of the patients respectively. Rhythm disturbances like AV block, atrial fibrillation are reported in literature. The ECG was normal in our patient, however on 24-hour holter ECG, 2% of the total beats were supraventricular with intermittent ectopic atrial rhythm without any symptoms. As in our case small, uncomplicated or unperforated aneurysms may be managed conservatively, surgery is generally indicated in others.

In conclusion, being rare in children, isolated MVA should be kept in mind in the differential diagnosis of mass lesions seen on the atrial side of the mitral valve. A 24-hour electrocardiogram may define subtle rhythm disturbances in these patients.

Supplementary Materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjped.2023.314

Supplementary Material 1. Echocardiogram videos, showing the apical four chamber and long axis views.

Supplementary Material 2. Echocardiogram videos, showing the modified long axis 2D and long axis color flow images of the aneurysm.

Ethical approval

Written informed consent was obtained from parents of the patient.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: VD, data collection and literature review: KS, draft manuscript preparation; VD. All authors reviewed the results and approved the final version of the manuscript.

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Rehospitalization indications of children hospitalized for COVID-19 infections and long COVID

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Dear Editor, we would like to share ideas on the publication "Rehospitalization indications of children hospitalized for COVID-19 infections after discharge: Should we suspect long COVID?".[1] The study was carried out in a Turkish children's hospital to investigate the rehospitalization rates and reasons among children infected with COVID-19. COVID-19 hospitalizations totaled 777 children, with 98 (12.6%) instances requiring rehospitalization. The bulk of rehospitalizations were caused by unrelated diseases, infections, or surgical procedures. Approximately one-third of the rehospitalized patients had symptoms consistent with extended COVID syndrome.

The study was carried out in a single tertiary children's hospital in Turkey, which may restrict the findings' generalizability to other settings or populations. There was no comparison group of children with COVID-19 who were not rehospitalized in the research. This makes determining the importance of rehospitalization rates and the precise influence of COVID-19 on rehospitalization problematic. Only children who were rehospitalized in the research center after being discharged were included in the study. This may add selection bias since it eliminates children who were rehospitalized elsewhere or did not request rehospitalization.

The study did not go into detail on the exact underlying disorders that resulted in rehospitalization. This limits our understanding of how these variables affect COVID-19 outcomes.

The study did not account for any confounding factors that can alter the reported symptoms and long-term outcomes, such as underlying medical conditions, socioeconomic status, or access to healthcare. These factors might have an impact on the results and make it more difficult to draw conclusive conclusions. Long-COVID-19 needs specific care due to a medical condition. There are a few significant difficulties that should be noted in addition to the general problems covered in the text. The patient's prior apparent clinical diagnosis was validated by COVID-19, notwithstanding the likelihood of unidentified co-morbid disorders. The patient may furthermore be infected with COVID-19 a second time.[2] The most recent injection must cover all earlier vaccines. To draw a conclusion regarding how the condition affects health issues, there must be sufficient data.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HP and VW; draft manuscript preparation: HP and VW; supervision: VW All authors reviewed the results and approved the final version of the manuscript.

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Response to "Rehospitalization indications of children hospitalized for COVID-19 infections and long COVID"

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We are grateful for your interest in our research and would like to respond to the letter entitled ''Rehospitalization indications of children hospitalized for COVID-19 infections and long COVID''.[1]

The research was conducted in a tertiary care children's hospital, where a significant burden of pediatric patients is met, and we believe that the number of patients is adequate for the conduction of this research. Indeed, the study included 777 patients, and 98 of them were rehospitalized. Among these 98 patients, 76 (77.6%) were rehospitalized due to their underlying disease, nonspecific infectious diseases unrelated to COVID-19, and the need for certain surgical procedures. The remaining 22 (22.4%) patients presented with ongoing symptoms such as fatigue, fever, abdominal pain, and myalgia following the SARS-CoV-2 infection. No other underlying cause was detected in approximately one-third of the patients whose manifestations were consistent with long COVID syndrome. Each patient was tested for COVID-19 at the time of re-admission, and the test results were given in Table III.[2] Three of the 22 patients had positive RT-PCR tests for the second time, therefore they were followed up in consideration of COVID-19 reinfection.

Children with COVID-19 who were not rehospitalized were not included in the comparative analyzes. The study aimed to determine the percentage of rehospitalizations that might be attributed to long COVID-19 syndrome. In the 'Method' section of the research, we emphasized that details of the underlying primary diseases other than COVID-19-associated conditions were not discussed in the text. Long COVID syndrome, which was a new diagnosis at the time of the research, was characterized by "signs and symptoms developed during or following a disease consistent with COVID-19 that have persisted for more than four weeks and whose presence cannot be explained by other alternative diagnoses".[3]

The study did not account for any confounding factors that could alter the long-term outcomes, such as underlying medical conditions, socioeconomic status, or accessibility to healthcare. The criticisms stated in the 'Editor's Letter Section'1 are emphasized by us in the 'Limitations' section of our article with this statement: "Some considerations should be noted when interpreting the results of our study. Firstly, this was a retrospective study with inherent limitations compared to randomized trials. Secondly, only hospitalized patients were evaluated, and outpatients were excluded from the study."

Ethical approval

Ethics approval was obtained from the Institutional Review Board of Dr. Behcet Uz Children's Training and Research Hospital (decision no: 2021/15-08).

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Author contribution

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The importance of transient hypothyroxinemia of prematurity and its controversial management

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We read the paper on transient hypothyroxinemia of prematurity (THOP) by Maneenil et al.[1] with great interest. They reported the incidence and risk factors of THOP in infants born before 37 weeks of gestation. They found that the incidences of THOP were 39.5%, 8.4% and 4.8% among hospitalized preterm infants born before 28, 34 and 37 weeks of gestation, respectively. According to the study conducted with preterm infants hospitalized in the neonatal intensive care unit (NICU), the risk factors for THOP were being born before the 28th gestational week, having a low Apgar score at 5 minutes, and using aminophylline, dobutamine and morphine treatments.[1] Although the authors did not present treatment data for infants with THOP in the results section, they stated in the discussion section that none of the newborns with THOP required L-thyroxine treatment. There is an ongoing debate as to whether THOP harms the brain development of preterm infants when left untreated. We recently had the chance to manage a case with THOP in our NICU.

Our case with THOP was born via vaginal delivery as a 1330 g male infant at $30^{0/7}$ weeks of gestation to a 28-year-old mother. Antenatal steroids and tocolytic therapy were administered to the mother at 28 weeks of gestation due to uterine contractions. The infant received intratracheal surfactant due to respiratory distress syndrome (RDS) on the first day of life. He also received ampicillin, gentamicin, fluconazole, parenteral nutrition and caffeine citrate treatments during the first week of NICU stay. There was no iodine exposure. The free thyroxine (fT4) and thyroid stimulating hormone (TSH) levels of the infant on postnatal day 7 were low, 0.73 ng/dL (0.84-1.76 ng/dL) and 0.685 uIU/mL (0.7-7.9 uIU/mL) respectively. Trends in fT4 and TSH levels between the first and fifth week of life are presented in Fig. 1. Although the TSH level did not reach the normal range yet, fT4 reached the normal range at the postnatal 2nd week of life. In our patient, TSH levels increased in the third week of life, confirming the diagnosis of THOP. Our patient did not have the risk factors mentioned above, identified in the study by Maneenil et al.[1] According to recent data[2], being born under 31 weeks of gestation and having a diagnosis of RDS were risk factors for our case.

Similar to the cases with THOP in the study by Maneenil et al.[1], our case did not require levothyroxine treatment for THOP. THOP is a condition characterized by low T4 and non-elevated (normal or low) TSH levels in preterm infants.[1-4] Although several factors contribute to the occurrence of THOP as described above, the main factor is the immaturity of the hypothalamic-pituitary-thyroid axis due to the low gestational age. Indeed, the combination of high TSH and low fT4 values in a newborn is linked to poor neurodevelopmental outcomes.[3,4] There is still debate on whether THOP harms the developing brain. According to the results of studies involving mainly preterm infants with a gestational age over 30 weeks, THOP has no adverse effect on the developing brain and improves within postnatal 2 weeks.[3-5] Current studies, including extremely preterm infants (under 28 weeks gestation), have shown that

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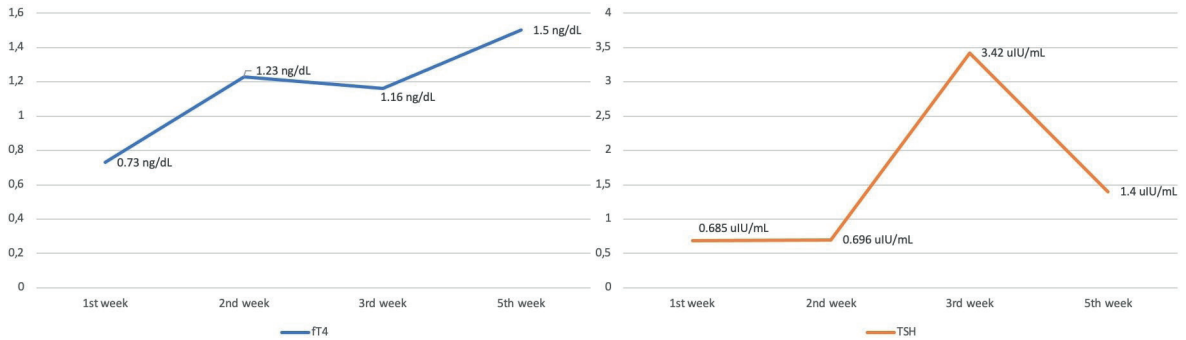

Fig. 1. Thyroid function test screening. fT4: free thyroxine, TSH: thyroid stimulating hormone.

THOP harms the developing brain.[5] According to our knowledge, prospective randomized trials for treating THOP are lacking. Therefore, there is controversy among clinicians about the indication for treating THOP. More recently, Yoon et al.[3] described that the outcomes of THOP were dependent on the severity of the THOP (fT4 <0.5 ng/dL), with infants having more severe THOP showing significantly more adverse short- or long-term outcomes whether they received levothyroxine treatment or not. Our patient did not have severe THOP according to the description of Yoon et al.[3] Maneenil et al.[1] had infants with severe THOP in their study cases (the mean fT4 level was 0.63±0.18 ng/dL). A recent retrospective study in our country suggested that clinicians tended to give levothyroxine treatment to the group with a median gestational age of 28 weeks, while levothyroxine treatment was not necessary for older preterm infants (median 31 weeks).[4] Maneenil et al.[1] did not present any data on the prognosis of untreated THOP infants born below 28 weeks of gestation (n: 17). Our patient, who did not develop any problems related to prematurity, is now two months old and neurodevelopmental follow-up is ongoing in our outpatient department.

If Maneenil et al.[1] could present the short and/or long-term results of THOP cases that did not require treatment, they would have made a valuable contribution to the controversial treatment literature of THOP.

Author contribution

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