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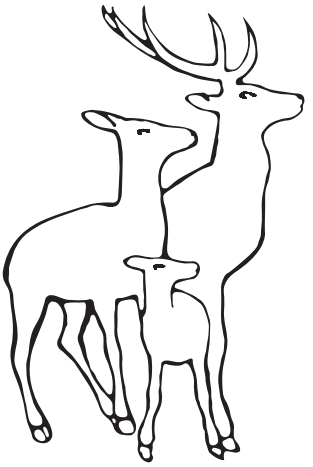

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Review

COVID-19 in perinatal period: questions and consequences

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ABSTRACT

Background. The effect of COVID-19 infection on newborn babies is not yet clear. Babies born to pregnant women with suspected or proven COVID-19 or babies who had contact with infected people are considered to be at risk. In this review, intrauterine problems that may be caused by COVID-19 infection, delivery room approach, postnatal follow-up, precautions and controversies regarding breastfeeding and vaccination are discussed.

Methods. The articles published between March 2020 and June 2021 were searched in Pubmed, Cochrane Library and Google Scholar databases using the keywords COVID-19 and newborn, perinatal period, vertical transmission, pregnancy, breast milk and vaccines. The updated information and recommendations are presented.

Conclusions. Our knowledge of the perinatal and neonatal effects of COVID-19 infection changes rapidly. Therefore, close follow-up of the mother-infant dyads is important. Larger epidemiological and clinical cohort studies are needed to better understand the possible implications and long-term outcomes of COVID-19 infection and also maternal vaccination in newborn infants.

Key words: COVID-19, newborn, pandemic, vertical transmission, breastfeeding.

In November 2019, severe cases of pneumonia started to increase in Wuhan, China, and an agent could not be isolated in the beginning. As of February 7, 2020, it was determined that this virus, which causes fever, cough and intense inflammation in the lungs, is a virus from the Coronaviridae family and named SARS-CoV-2 due to its similar genetic structure with SARS coronavirus.[1,2] The high contagiousness has caused the virus to spread rapidly to different geographical regions of the world and on March 11 2020 the World Health Organization (WHO) declared a pandemic due to COVID-19 infection (Coronavirus Infectious Disease 2019).[3]

Although we still do not have enough information about the spread, course, diagnosis, treatment and prevention of this viral disease, according to the early period data published by China, it was stated that the disease was not fatal in children. Mortality and morbidity compared to adults are still lower in the pediatric age group, especially under 1 year of age. However, we now know that the children can be seriously affected by COVID-19.[4,5]

Our knowledge on the care and treatment of newborn babies in the prenatal, natal and postnatal periods is updated in the later stages of the pandemic. In this article, current recommendations are made regarding the treatment and management of newborns in the perinatal period (Fig. 1).

Method

The articles published in English and Turkish between March 2020 and June 2021 were searched in Pubmed, Cochrane Library and Google Scholar databases using the keywords COVID-19 and newborn, perinatal period, vertical transmission, pregnancy, breastfeeding and vaccines. The updated information and

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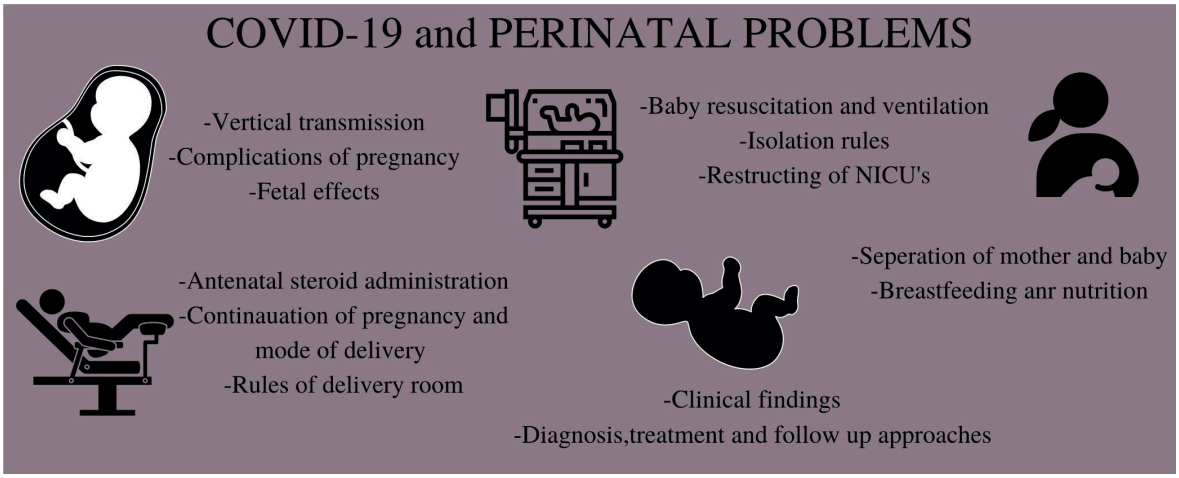

Fig. 1. COVID-19 and perinatal issues.

recommendations in the articles, reviews, guidelines, case series and case reports are presented.

Fetal Effects and Vertical Transmission

The course of the disease in pregnant women and the route of transmission are important in determining the management of newborn infants. In pregnant women infected with COVID-19, the need for intensive care, mechanical ventilation and mortality are significantly increased compared to nonpregnant infected women.[6,7]

The risk of vertical transmission and fetal-neonatal effects may differ in each stage of pregnancy. Therefore, the physiology of the placenta and the pathological evaluation of the placental samples from pregnant women positive for COVID-19 are important in understanding the effects of the virus. Amniotic fluid, placenta, cord blood during delivery and respiratory tract samples of the baby should be carefully examined in terms of viral involvement in search of intrauterine transmission mechanisms.

Theoretically, four mechanisms related to intrauterine infection are possible. These mechanisms include; 1) Direct infection of syncytiotrophoblasts with the virus propagating through the syncytial layers via the angiotensin converting enzyme (ACE_2) and Fc receptor (FcR), 2) Passage of the virus from the maternal circulation to extravillous trophoblasts or other placental cells, 3) Via maternal immune cells, 4) Vaginal infection and ascending route.[8]

COVID-19 binds to cells by ACE_2 receptors and fuses into the cell with type II transmembrane serine protease (TTSP2). Placental ACE_2 receptor expression and TTSP2 expressions have been reported to be low in the first two trimesters in fetal-placental tissues and both of them increase over time.[9] In addition, it has been shown that the number of ACE_2 receptors is higher in the endothelium of the umbilical artery in the placenta of preeclamptic pregnant women compared to those without preeclampsia. It is hypothesized that the risk of vertical transmission increases in COVID-19 positive pregnant women accompanied by preeclampsia.[8] Thus, the maternal diseases and the physiology of the placenta in different periods of pregnancy are important in terms of vertical transmission.

It has been reported that thrombosis, fibrin deposits, villous stromal karyorrhexis and vascular perfusion disorders were the most common findings in the pathological examination of the placental samples.[8,10]

At the beginning of the pandemic newborn babies of mothers who had COVID-19 infection in the third trimester were evaluated by upper respiratory tract real-time polymerase chain reaction (RT-PCR), amniotic fluid, blood PCR and blood IgM levels but there was no conclusive evidence of vertical transmission and no fetal or neonatal deaths were reported.[11-14]

More recently, in a case report from India, a pregnant woman was found to be positive for coronavirus at the 8th gestational week. Hydrops fetalis and fetal loss occurred at the 13th week of gestation when the mother tested negative for the COVID-19 virus. Coronavirus genes were detected in the placenta, amniotic fluid and fetal membrane samples. Hydrops fetalis and fetal death were associated with COVID-19 infection in the first trimester as the other etiological factors were ruled out. The authors speculated on the persistence of the coronavirus replication in the placenta even after the mother tested negative in respiratory samples.[15]

In a multicenter study COVID-19-related fetal and neonatal outcomes were evaluated. Fetal and neonatal deaths were mostly associated with prematurity and correlated with the severity of the mother's clinical condition. However, conclusive evidence of vertical transmission could not be demonstrated.[16]

Vertical transmission is still an important question to be answered. Although various mechanisms have been proposed, there is a need for high quality evidence to show intrauterine transmission of COVID-19 (Fig. 2).

Antenatal Corticosteroids

Antenatal steroids (ANS) increase lung maturation by stimulating type 2 alveolar cell development in the fetus. ACE_2 receptors are also found in these cells. Thus, it is thought that the administration of ANS may be harmful to the fetus by stimulating ACE_2 receptor expression. However, as the maturation of these receptors occurs in late childhood or even during adolescence.[17] Considering the short and long term benefits of ANS in preterm babies, it is recommend to be administered to COVID-19 infected pregnant women with preterm birth risk.[18] However the effects of steroid administration to mothers infected with COVID-19 are not yet clear, The American College of Obstetricians and Gynecologists (ACOG) recommends the administration of betamethasone (2x12mg) to pregnant women with either asymptomatic COVID-19 infection or with mild symptoms.[7] In pregnant women with severe coronavirus related disease, it is recommended to make a decision together with the family by informing them about the beneficial effects on the baby and the complications that may occur in the mother.[7,18]

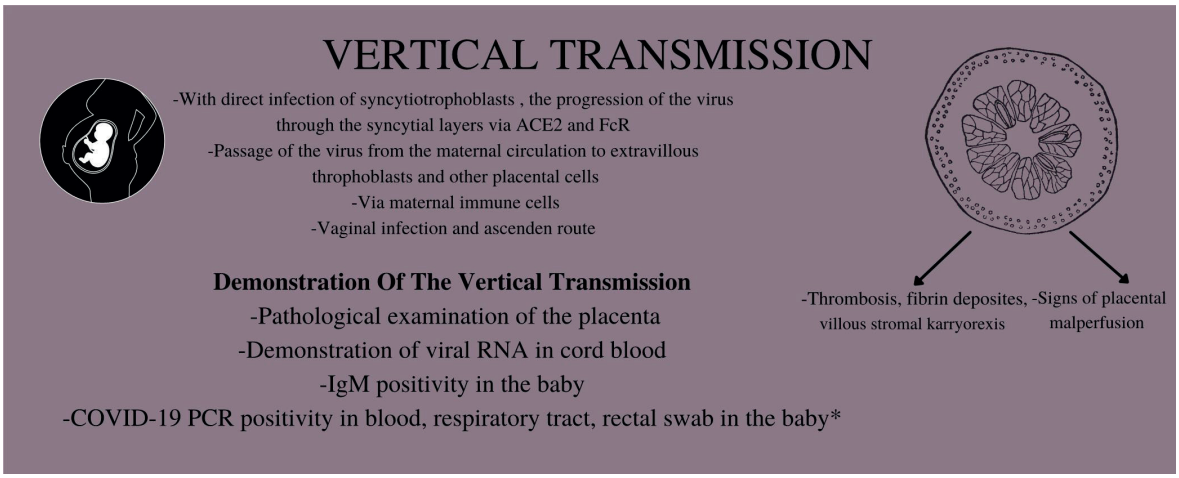

Fig. 2. COVID-19 and possible vertical tranmission mechanisms.

Delivery Room Management and Resuscitation

The timing and delivery method of COVID-19 positive pregnant women should be determined according to obstetric indications as the delivery method has no effect on neonatal infection.[7,19-22]

Pregnant women with suspected or proven COVID-19 infection should be taken to a room with negative pressure. Since the use of general anesthesia during emergency cesarean section increases the risk of aerosol transmission, the delivery team should use personal protective equipment (PPE) (protective apron, N95 mask, visor, mask, gloves). All pregnant women with a diagnosis of or suspected COVID-19 infection should wear a surgical mask during delivery. The baby may be taken to a different room preferably. If this is not possible post delivery care should be given in an area separated by a physical barrier 2 meters away from the mother.[7,18] After the first evaluation, if there is no need for resuscitation, the baby should be transferred to the neonatal intensive care unit (NICU) with a transport incubator.[18,23] If the newborn baby needs resuscitation, Neonatal Resuscitation Program guidelines should be followed.[18,24] Aeresole generating procedures like aspiration, balloon-mask ventilation, T-piece resuscitator use and intubation may be needed during resuscitation. Therefore, appropriate measures should be taken to prevent transmission of infection.[7,22]

There were controversial opinions about delayed cord clamping (DCC) and skin to skin contact after birth at the beginning of the pandemic. Updated recommendations by WHO is to follow usual the guidelines and maintain DCC and skin to skin contact in COVID-19 infected mothers after birth.[25]

Postnatal Care and Follow Up of Newborn Infants

Since there was no clear information about the course of the disease in newborn babies in the first months of the pandemic, it was recommended that the baby born to mothers with suspected or confirmed COVID-19, should be isolated and followed in the NICU.[19] In the later stages of the pandemic, this recommendation changed to keep the mother and the baby in the same room with a minimum number of people if the baby has no postnatal adaptation problems.[26,27]

Bathing the baby immediately after birth is no longer recommended as there are no proven benefits and the risk of hypothermia is high.[28]

All newborn infants born to mothers with suspected or confirmed COVID-19 infection should be tested for COVID-19 RNA with RT-PCR in the upper respiratory tract and nasopharyngeal samples twice at 24 and 48 hours after birth or once at 24 or 48 hours of age.[29] If there is any doubt of intrauterine or postnatal infection of COVID-19, additional samples from sterile sites such as blood, lower respiratory tract and cerebrospinal fluid should also be tested by PCR.[30]

Asymptomatic babies can be discharged home with a close follow up programme.[29]

Clinical Findings and Treatment in COVID-19 Infected Neonates

Symptoms and Laboratory Evaluation

There are no specific clinical findings in neonates as with other cases of sepsis. Non-specific findings like feeding intolerance, vomiting, tachypnea, respiratory distress, tachycardia, circulatory disorders make it difficult to recognize COVID-19 infection in newborns.[31] In a case series including 44 COVID-19 positive neonates reported as fever (50%), gastrointestinal symptoms (26%), hypoxia (20%) and cough (20%) were the most common symptoms.[32]

Laboratory findings of COVID-19 infection in neonates are also non-specific and include leukocytosis, lymphopenia, thrombocytopenia, elevated inflammatory markers and non-specific radiologic markers on chest radiography.[20]

Contrary to what was reported in the early days of the pandemic, it is now known that the disease can progress to serious neonatal pneumonia and multiple organ failure in newborn infants. Inflammatory clinical conditions with multisystem involvement due to COVID-19 have also been encountered in newborn babies similar to Multisystem inflammatory syndrome in children (MIS-C). This condition caused by the hyperinflammatory response in newborn babies has been defined as Neonatal MIS-C (MIS-N).[22]

Infants with MIS-N have negative for PCR tests but test positive for COVID-19 immunoglobulin G. They may have cardiac involvement (myocarditis, increased Troponin T and brain natriuretic peptide levels), respiratory distress (pulmonary infiltrates, pulmonary hypertension), gastrointestinal symptoms (feeding intolerance, vomiting, necrotizing enterocolitis like findings), hematological findings (disseminated intravascular coagulopathy, thrombocytopenia, lymphopenia, neutropenia) and renal failure. In MIS-N cases, it has been reported that intravenous immunoglobulin and steroids are used together with other supportive treatments.[22,33-35]

Medical Treatment

There is no evidence based recommendations other than supportive treatment, especially in asymptomatic cases in the neonatal period. To our knowledge, there is insufficient data on the neonatal use of antiviral agents used in adults like lopinavir, ritonavir and remdesevir. However, there are reports on the use of remdesevir, intravenous immunoglobulin, steroids and even monoclonal antibodies in severe clinical conditions such as sepsis, severe respiratory failure and multi-inflammatory response syndrome.[36-38]

Respiratory Support

Mechanical ventilation, surfactant therapy, inhaled nitric oxide and even extracorporeal membrane oxygenation may be needed in severe cases.[19] The number of newborn babies receiving respiratory support due to COVID-19 is low. It is recommended to use a HEPA filter with bag-mask ventilation, to wear a PPE during care and intervention, to use a video laryngoscope during intubation.[22] It is known that the use of a closed suction system in intubated patients reduces the transmission through droplets.[18] Although adult guidelines recommend clamping the tube during intubation and extubation to minimize the risk of contamination, there are limited data for newborns.[39]

Breastfeeding and Nutrition

In mothers who are positive for SARS-CoV2, both droplet transmission and transmission through breast milk are possible risks. However, there is currently no conclusive evidence of COVID-19 transmission through breast milk. Therefore, appropriate hygiene measures should be taken and breastfeeding should be continued considering the benefits for both the mother and the baby.[40]

In the last publication from WHO on breastfeeding and COVID-19, it was stated that the most appropriate approach for the mother is to continue breastfeading with careful hand washing and wearing a mask. If the general condition of the mother is not suitable for breastfeeding, it is emphasized that the baby can still be fed with pumped breast milk. In cases where own mother's milk is not available donor milk or formula milk can be used. It is important to continue breastfeeding counseling should be repeated after the mother's health status improves (Fig. 3).[41]

In the multicenter retrospective study of the Turkish Neonatal Society, it was reported that breastfeeding rates in babies of COVID-19 positive mothers were low (36%). This situation was explained by the isolation of the babies in the NICU, the clinical status of the mothers, the concerns of the family and healthcare workers about the possibility of transmission of the virus by breast milk.[42]

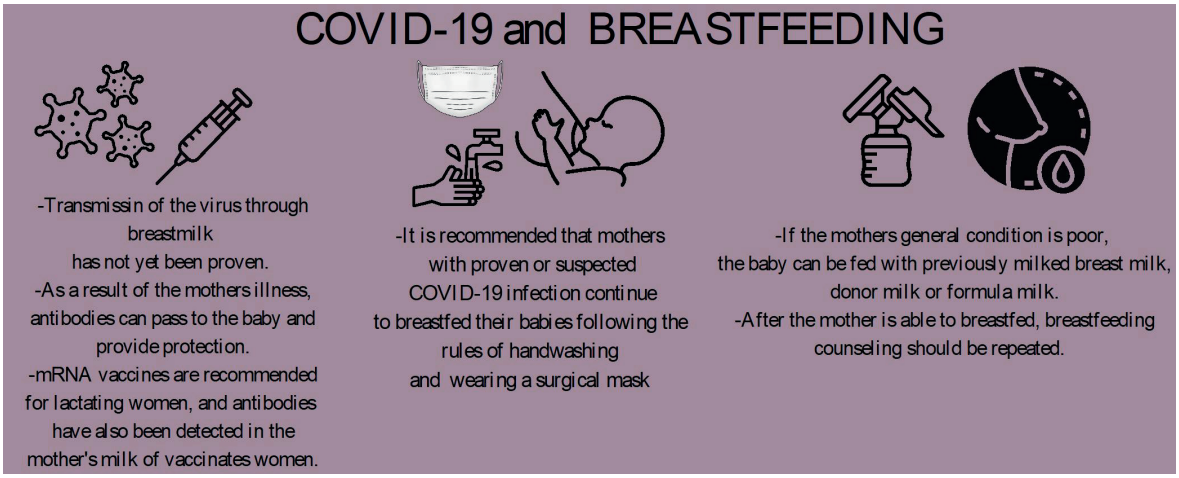

Fig. 3. COVID-19 and breastfeeding.

There is insufficient data about the effects of antiviral drugs in lactating mothers in breastfed babies. Commonly used RNA polymerase inhibitors in the treatment of COVID-19 are favipiravir and remdesevir. Remdesevir has been used in the treatment of Ebola virus infection in newborns and also in babies who developed serious illness during the COVID-19 infection in selected cases. Therefore, it has been stated that remdesevir can be used with caution in breastfeeding women, with close monitoring of side effects in the neonates.[43-45]

Favipiravir is another antiviral agent frequently used in the treatment of COVID-19. Teratogenic effects during pregnancy have been shown but we have insufficient information on the transition to breast milk and its effects on newborns. Based on animal studies, there are publications stating that it is contraindicated during breastfeeding. The Turkish Ministry of Health does not recommend the use of favipiravir either during pregnancy or lactating women.[43-46]

Vaccination in Pregnancy and Lactating Women

The rapid spread of COVID-19 and increasing mortality have caused a number of vaccines to receive emergency approval for use. However pregnant and lactating women were excluded from the COVID-19 vaccine studies which makes it difficult to recommend their use in this population.[47,48]

In a recent study comparing the immune response to two mRNA (Pfizer–BioNTech and Moderna) vaccines in pregnant, lactating and non-pregnant women, the immunogenic response was found to be equivalent in all three groups. Specific IgG was detected in the cord blood of the newborn babies and breast milk of these mothers showing immune transfer via the placenta and breast milk.[49]

In another very recent study data of 35691 pregnant women vaccinated with two mRNA COVID-19 vaccines were collected. The most common side effects were local pain at the injection site, vomiting and headache. There were 827 completed pregnancies out of which 115 (13.9%) resulted in pregnancy loss and 712 (86.1%) resulted in live birth. The incidence of prematurity was reported as 9.4% and small for gestational age as 3.2%. Prematurity was found to be higher when compared to pregnant women who were not vaccinated, and no significant difference was found in other pregnancy outcomes. Neonatal death has not been reported.[50]

To prevent COVID-19 infection in newborn infants' the vaccination of the mothers is an important issue although the available data is not sufficient to either support or refuse the use of vaccines in pregnancy. Future studies evaluating the effects of vaccine administration during pregnancy and lactating women are needed to make evidence based recommendations in this population of women.

The weak immune systems of newborn babies have been a source of concern in neonatology during the COVID-19 pandemic. Both insufficient data on the effects of the virus and the low number of patients have created limitations in the development of follow-up and treatment approaches.

Our knowledge of the perinatal and neonatal effects of COVID-19 infection changes rapidly. Therefore, close follow-up of the mother-infant dyads is important. Larger epidemiological and clinical cohort studies are needed to better understand the possible implications and the long-term outcomes of the COVID-19 infection and also maternal vaccination in newborn infants.

Also, long-term follow-up of babies of mothers who had COVID-19 infection during pregnancy and newborns infected with COVID-19 gains importance.

Author contribution

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

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Clinicoepidemiological findings of childhood brucellosis in a tertiary care center in Central Anatolia: with the emphasis of hematological findings

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ABSTRACT

Background. Human brucellosis is one of the most widespread zoonotic diseases that are presented with predominantly hematological manifestations. We aimed to evaluate the hematological findings of childhood brucellosis and to determine the predictive clinical findings and laboratory tests that might be related to hematologic involvement.

Methods. We retrospectively analyzed the medical records of children with brucellosis between 1 January 2005 and 31 December 2018. We compared predictive clinical and physical examination findings and laboratory tests in patients with and without hematological involvement.

Results. A total of 212 patients (127 boys (59.9%)) with a mean age of 9.4±4.7 years were evaluated in this study. Blood cultures were performed in 161 (75.9%) patients and *Brucella* spp were isolated in 70 (43.4%) of them. Ninety-two (43.4%) patients had hematological involvement at least in one series. Anemia was detected in 66 (31.7%) patients, leukopenia in 22 (10.6%) and thrombocytopenia in 10 (4.8%). Four patients (1.9%) had pancytopenia. Age distrubutions of the patients with and without hematological involvement were similar (p=0.6). In patients presented with fever, hepatomegaly and splenomegaly, hematologic involvement was significantly higher (p<0.05). Hematological involvement was higher in patients who had elevated aspartate aminotransferase and alanine aminotransferase concentrations (p<0.05). Hematological involvement was higher in patients with positive blood culture (p=0.005). Six patients (2.8%) were treated with intravenous immunoglobulin at 1000 mg/kg/day for two days in addition to anti-brucellosis treatment.

Conclusions. Hematological involvement in brucellosis is a common finding regardless of age, especially in febrile, bacteremic patients and in patients who had hepatosplenomegaly and elevated liver enzymes. Anemia is the most common hematological abnormality.

Key words: brucellosis, children, cytopenia, hematological findings.

Human brucellosis is one of the most widespread zoonotic diseases and a public health problem in many countries of the Mediterranean and Middle East where bovine brucellosis has not been controlled. It can affect people at any age including children. Approximately 20-30% of cases are diagnosed during childhood.[1-3] Humans can acquire infections via consumption of infected unpasteurized milk and milk products or direct contact with infected animals, secretions and carcasses. Brucellosis is a multisystemic disease which can be presented with various non-specific clinical signs. The most common clinical manifestations include arthralgia, fever, sweating, weight loss, fatigue, and anorexia.[1] Some of these

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patients can be presented with hematological manifestations such as mild anemia, leukopenia and less frequently pancytopenia and thrombocytopenia. Brucellosis may mimic many hematologic diseases, especially in patients presenting predominantly with hematological findings.[2,4]

In this study, we aimed to evaluate the hematological findings of childhood brucellosis and to determine the predicting clinical and physical examination findings and laboratory tests that might be related to hematologic involvement in the setting of a tertiary care pediatric hospital.

Material and Methods

We retrospectively analyzed the medical records of children with brucellosis who were admitted to a referral tertiary care pediatric hospital between 1 January 2005 and 31 December 2018. This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital Institutional Review Board (Number: 2019/8). Data regarding age, sex, family history of brucellosis, occupation, fresh milk product consumption history, clinical complaints, physical examination findings and laboratory results were recorded. Organomegaly was assessed with physical examination. Laboratory tests including complete blood count [total leukocyte, absolute neutrophil, absolute lymphocyte, platelet count and mean platelet volume (MPV)], C-reactive protein (CRP), erythrocyte sedimentaton rate (ESR), serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, blood culture, brucella agglutination tests, bone marrow aspiration smear results if had been applicated and treatment data were evaluated. Brucellosis was diagnosed on the basis of epidemiological and clinical findings, together with positive serum agglutination test (SAT) ≥1/160 titers and/or Brucella immune capture test with Coombs ≥1/160 titers and/or isolation of *Brucella* spp. from blood culture. Agglutination titer range was set at 1/20–1/5120 for each serum sample to avoid false negative results in consequence of prozone effect. Hematological findings were defined as follows: Thrombocytopenia, a platelet count <150.000/μL, leukopenia and anemia, a level lower than age-determined references. Pancytopenia was defined as abnormally low counts of white cells, platelets and low level of hemoglobin in the same patient.[5]

Data were entered to a database and statistical analyses were performed using IBM SPSS Statistics (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The variables were investigated using visual and analytical methods (Kolmogrov-Simirnov/Shapira-Wilk test) to determine whether or not they are normally distributed. Descriptive analyses were presented using means ± standard deviations for normally distributed variables and as medians (minimum-maximum) for the non-normally distributed and ordinal variables. Frequencies were given to summarize the categorical variables. The chi-square test or Fisher's exact test was used to compare patients with or without hematological involvement. The correlation coefficients and their significance were calculated using the Pearson test between SAT titer and mean platelet volume. A p-value less than 0.05 was considered a statistically significant result.

Results

A total of 212 patients [127 boys (59.9%)] diagnosed with brucellosis were evaluated in this study. Age distribution was from one day old neonate to 17.9 years. Median age of the patients was 9.4 years (SD 4.7 years) and 10.09 years (IQR: 5.35 years-13.3 years). Four (1.8%) patients were younger than one year of age (one patient was a preterm neonate, one patient an exclusively breast-fed young infant, two patients complementarily fed babies including

unpasteurized cheese), 45 patients were between 1-5 years, 87 patients were between 5-12 years, and 76 patients were ≥12 years. None of the patients had a known hematologic disease. One hundred and twenty eight (60.4%) patients were hospitalized for a mean duration of 11±6.7 days. A total of 183 (86.3%) patients had a history of consuming unpasteurized milk and dairy products. At least one parent of 121 patients (57.1%) were working in animal breeding and had intimate contact with sheep and/or cows. Eighty-four (39.6%) patients had positive family history for brucellosis. The symptoms and physical examination findings were shown in Table I. Median duration of fever was 15 days (range 1-365 days). The most commonly involved joint was knee in 21 (65.6%) patients, followed by ankle in seven (21.9%) patients and hip in four (12.5%) patients. Nine (75.0%) patients had cervical, two (16.7%) had inguinal, and one (8.3%) had mesenteric lymphadenopathy. Blood cultures were performed in 161 (75.9%) patients and *Brucella* spp were isolated in 70 (43.4%) of them. Serum agglutination test titers at presentation were ≥1/160 in 201 patients (94.8%). Ten (4.7%) patients with SAT titers of <1/160 were diagnosed with positive blood culture.

Table I. The clinical symptoms and physical examination findings of patients with brucellosis.

	Patient number	%
Clinical symptoms		
Arthralgia	154	72.6
Fever	119	56.1
Weight loss	46	21.7
Night sweats	30	14.2
Arthritis	36	17
Headache	17	8
Abdominal pain	17	8
Physical examination findings		
Hepatomegaly	38	17.9
Fever	35	17.5
Arthritis	32	15.1
Splenomegaly	28	13.2
Lymphadenopathy	12	5.7

Ninety-two (43.4%) patients had hematological involvement at least in one lineage. The initial complete blood count findings of patients were summarized in Table II. On admission, anemia was detected in 66 (31.7%) patients, leukopenia was detected in 22 (10.6%) patients [14 (6.7%) neutropenia, 14 (6.7%) lymphopenia], and thrombocytopenia was detected in 10 (4.8%) patients. Four patients (1.9%) had pancytopenia. The epidemiological and laboratory findings of patients presenting with pancytopenia were shown in Table III. Aspartate aminotransferase and ALT levels were ≥40 IU/L in 69 (32.5%) and in 55 (25.9%) patients, respectively.

The mean age of patients with and without hematological involvement were 9.6±4.5 years and 9.3±4.8 years, respectively (p=0.6). Hematologic involvement was higher in patients with fever (p<0.001). There was no association between duration of fever and hematologic involvement (p=0.68). In patients who had hepatomegaly and splenomegaly, hematologic involvement was statistically higher (p=0.001 and p=0.005, respectively). Hematological involvement was also higher in those who had elevated AST and ALT levels (p<0.05). The presence of hepatomegaly and splenomegaly, results of blood cultures and transaminase concentrations of patients with and without hematologic involvement were shown in Table IV. Mean platelet volume was 8.1 ± 0.95 fL, and there was no correlation between SAT titer and mean platelet volume (r = -0.02, p = 0.7). It was found that SAT titers were not correlated with the presence of hematologic findings (p=0.55). Hematological involvement was higher in patients with positive blood culture (p=0.005). Bone marrow aspiration was performed in 14 (15.2%) patients with hematological impairement. In one patient with pancytopenia, the bone marrow aspiration revealed multiple hemophagocytic histiocytes, in four patients (one patient with leukopenia and thrombocytopenia, three patients with anemia and leukopenia) revealed a few number of hemophagocytic histiocytes. Two patients with pancytopenia had normal bone marrow

Table II. The initial complete blood count findings of patients.

Parameter	All patients (mean ± SD)	Hematologic involvement present (mean ± SD)	Hematologic involvement absent (mean ± SD)
Hemoglobin (g/dL)	12 ± 1.4	10.5 ± 1.1	12.7± 0.9
Total leukocyte (/mm^3)	7450 ± 4735	3700 ± 916	7907 ± 4810
Absolut neutrophil (/ mm^3)	3267± 1965	1067± 330	3471± 1929
Absolute lymphocyte (/ mm^3)	3411± 2686	1321± 315	3605± 2727
Platelet (/mm^3)	284.509± 97.585	93.785± 37.529	297.994± 85.667

Table III. Epidemiological and laboratory findings of patients with pancytopenia.

Patient number	Age (month)	Gender	Hb g/dL	WBC /mm^3	Nuetrophil /mm^3	Lymphocyte /mm^3	Platelet /mm^3	ESR mm/h	CRP mg/L	SAT	Blood culture
1	145	F	11.4	2600	900	1410	140.000	28	101	1/320	*Brucella* spp.
2	91	M	8.5	1800	830	710	69.000	-	39	1/640	*Brucella* spp.
3	173	F	10.8	2900	1400	1000	70.000	120	77	1/5120	No growth
4	178	F	9.5	1800	770	930	62.000	30	11	1/640	*Brucella* spp.

Hb: hemoglobin, WBC: white blood cell, ESR: erythcyte sedimentaton rate, CRP: C-reactive protein, SAT: serum agglutination test

Table IV. Hepatomegaly, splenomegaly, transaminase and blood culture results in patients with and without hematologic involvement.

	Hematologic involvement present n / %	Hematologic involvement absent n / %	p value
Hepatomegaly			
Positive	26 (68.4%)	12 (31.6%)	p=0.001
Negative	66 (37.9%)	108 (62.1%)	
Splenomegaly			
Positive	19 (67.9%)	9 (32.1%)	p=0.005
Negative	72 (39.3%)	111 (60.7%)	
ALT			
Normal	58 (37.9%)	95 (62.1%)	p=0.005
>40 IU/L	33 (60%)	22 (40%)	
AST			
Normal	49 (35.3%)	90 (64.7%)	p<0.001
>40 IU/L	42 (60.9%)	27(39.1%)	
Blood culture			
Brucella spp.	41 (58.6%)	29 (41.4%)	p=0.005
No growth	33 (36.3%)	58(63.7%)	

AST: aspartate aminotransferase, ALT: alanine aminotransferase

aspiration smears. All of the patients were treated with combination regimens (doxycycline plus rifampicin for patients older than eight years and cotrimoxazole plus rifampicin for those younger than eight years). Six patients (2.8%) were treated with intravenous immunoglobulin (IVIg) (1 g/kg/day, for two days) in addition to anti-brucellosis treatment.

Discussion

In the present study, anemia was found as the most common hematological finding of brucellosis followed by leukopenia, thrombocytopenia, and pancytopenia. We found that the presence of hematological involvement was not affected by age, but patients who had fever, hepatosplenomegaly, positive blood culture for *Brucella* spp. and patients with elevated liver transaminase had higher rates of hematological involvement in brucellosis.

Worldwide incidence of brucellosis in endemic countries varies from <0.01 to >200 per 100000 population.[6,7] In 2017, 381 confirmed cases of human brucellosis were reported in 20 European Union (EU) countries, with a rate of 0.10 cases per 100.000 population. The highest numbers of confirmed cases were reported in Greece, Italy and Spain that accounted for 67.2% of all confirmed cases in EU countries in that year.[8] In Turkey, brucellosis remains a major public health issue, because the majority of people live in rural areas and they are engaged in animal husbandry.[1] According to the Turkish Public Health Institution data, brucellosis affects 5.000 to 10.000 people each year and causes significant morbidity.[9] It has been reported that brucellosis often affects children aged 5 to 15 years of age in different pediatric studies.[1,10,11] A study conducted on 496 children with brucellosis in Eastern Turkey, showed that patients' age ranged from 1-16 years with a mean age of 10.0±3.95 years. Half of the children were over 10 years of age and the male/female ratio was 1.5.[12] In an another study including children with brucellosis living in central Blacksea region, 80.8% of patients were male and mean age was 11 years (range, 2-17 years).[11] In the present study male gender dominance and mean age of patients were similar with those reported in previous pediatric studies.

In endemic areas, it is known that consumption of raw milk and dairy products is the main source for childhood brucellosis. Direct contact with infected animal is a possible acquision route of infection in older children whose families are engaged in animal husbandry.[1,13] The majority of our patients had a history for consumption of unpasteurized milk, suggesting that this was the most common route of acquisition in this setting. Although human to human transmission is rare, cases of neonatal infection have raised the possibility of transplacental transmission. Furthermore, breastfed infants whose mothers have not been treated adequately might have been infected with *Brucella* spp. via human milk.[14,15] We thought that one of our patients who was a preterm neonate had acquired brucellosis through transplacental route. Furthermore, there another patient acquired brucellosis via breast feeding in our series.

Brusellosis in pediatric patients may have a wide variety of nonspecific clinical presentations. Patients may present with fever, sweats, malaise, anorexia, weight loss, arthralgias, myalgias, headache, and abdominal pain.[4,11,16,17] In a large study including 496 children (79.8% male), the most common symptoms were reported as arthralgia (46.2%), fever (32.1%) and abdominal pain (17.1%) and the most common physical examination findings were arthritis (10.1%), splenomegaly (2.2%) and hepatomegaly (1.8%), respectively.[12] In a study from China, 88 children and 354 adult patients with male predominance were included. The authors demonstrated that fever was the most comon symptom in both children and adults (82.9% vs 61.5%, respectively), followed by joint pain, fatigue, anorexia and low back pain.[10] In our study, as reported in previous studies, the most common symptoms were arthralgia and fever and the most common physical examination findings were hepatomegaly and arthritis.

In the course of childhood brucellosis hematological complications are well known. Mild anemia and leukopenia have been reported mostly during the course of acute brucellosis.[1,2,4,17] A comprehensive study including children living in an endemic area for brucellosis in Turkey analysed the hematologic manifestations of 622 patients. Hematologic involvement was observed in 292 patients (46.9%). The most common hematologic involvement was anemia (28.6%), followed by thrombocytopenia (16%) and leukopenia [13.9% (neutropenia 8%, lymphopenia 8.8%)]. Pancytopenia was observed in 7.7% of patients.[1] The incidence of anemia has been reported as 13.3%-55% in different pediatric series.[1,12,18-20] In the present study anemia was the most common hemeatologic finding of brucellosis. We used hemoglobin levels determined for age to be able to make an objective evaluation when describing hematological findings like most other studies. In a pediatric study evaluating the hematological findings of brucellosis, cytopenia at least in one blood cell lineage detected in 41.9% of the patients, leukopenia in 28.2%, thrombocytopenia in 14.5%, anemia in 13.3%, and pancytopenia in 13.3%. Anemia had not been found as the most common hematological finding of brucellosis in this study in contrast to other pediatric studies. Authors thought that, this difference may be a result of choosing relatively low cut-off for hemoglobin levels of 10 mg/dl in order to minimize the anemia cases misrelated to brucellosis.[18] Moderate to severe leukopenia and thrombocytopenia as well as normal leukocyte and thrombocyte counts in patients with brucellosis were reported.[1,12,18,19,21] On the contrary of leukopenia predominance in previous reports, in a prospective case-control study including 100 brucellosis patients and 100 healthy individuals, the authors found that WBC, CRP and neutrophil counts were significantly higher in the brucellosis group. As a result of this study, they concluded that the most significant laboratory findings of brucellosis were increased number of WBC, while decreased numbers of thrombocytes and lymphocytes.[22]

Pancytopenia and severe thrombocytopenia or isolated thrombocytopenia resulting in bleeding has rarely been reported in the course of brucellosis.[18,21,23] The pathogenesis of thrombocytopenia during brucellosis may be multifactorial such as; increased platelet clearance due to splenomegaly, the suppressive effect of brucellosis bacteremia on bone marrow, hemophagocytosis, and peripheral autoimmune destruction.[1,18,23] In a study that included five patients (2.6% of all) with isolated thrombocytopenia during the course of acute brucellosis, an examination of bone marrow aspirate had revealed increment of megakaryocytes in two of these patients that may also be seen in idiopathic thrombocytopenic purpura. Authors suggested that; the mechanisms of thrombocytopenia may be destruction of thrombocytes by antibodies against *Brucella* organisms which cross react with thrombocytes.[23] Whereas the pathogenesis of pancytopenia in brucellosis seems multifactorial, hypersplenism may be a possible explanation of pancytopenia in children infected with *Brucella spp*. In addition several possible mechanisms for pancytopenia such as hemophagocytosis (like that seen during the course of several infections including viral, bacterial, fungal and parasitic diseases), bone marrow hypoplasia, bone marrow granulomas, immune destruction, and the direct inhibitor effect of bacteria on bone marrow cells have been accused previously.[1,2,4,24] In a study from Turkey including children with brucellosis, pancytopenia had been found in 11 of the 52 patients (21%) as the initial manifestation of brucellosis. One of these patients had presented with the complaint of nose bleeding and two patients had presented with gingival bleeding. More than a half of patients had hepatosplenomegaly.[24] In a study including 146 children hospitalized due to brucellosis, 14 (9.6%) had presented with hematologic manifestations, nine of them had pancytopenia and five had immune thrombocytopenia. Bone marrow aspiration and biopsy of the patients with pancytopenia revealed hypercellularity or severe hemophagocytosis.[4] The present

study's findings for hematological involvement frequency was in agreement with those previous reports. The most common findings following anemia were leukopenia and thrombocytopenia while the least common finding was pancytopenia which was detected in only four patients, similar as previous reported studies. Hemophagocytosis in bone marrow aspirate was detected in one of the our patients who had pancytopenia. Other four patients with hemophagocytosis presented with hematologic abnormalities in two series.

A study evaluating 511 brucellosis episodes reported that 42% of patients had cytopenia in at least one series. The authors demonstrated that older age (10.49±4.81 vs. 9.25±4.89 years), fever (92% vs. 78%), positive blood culture (84% vs. 75%), and IgM ≥1:640 levels (50% vs. 39%) were associated with the presence of cytopenia. The authors suggested that presence of fever and positive blood culture are the component of acute disease and high rates of cytopenia during acute brucellosis is a common finding. It was postulated that *Brucella* organisms suppress cell production in the bone marrow during bacteremia, especially when the bacterial load is high.[18] Similary, a study of 69 children with brucellosis found a tendency to decrease platelet count in patients with bacteremic disease.[2] In an another study including 123 brucellosis patients aged 13-73 years (60 patients with *Brucella* spp. bacteremia and 63 patients without bacteremia) revealed that, bacteremic patients presented with fever and chills more than nonbacteremic patients. In addition a significant elevation of AST and ALT concentrations and higher leukopenia rates were detected in bacteremic patients.[25] On the contrary to previous reports[18] we did not find any correlation between hematological involvement and age of patients, however in line with other previous reports[2,18,25] we found a correlation between hematological involvement and presence of bactremia, fever, hepatosplenomegaly and elevated liver transaminase levels.

It is well known that, clinical and hematological improvement usually occurs within 2 to 3 weeks after the initiation of appropriate antimicrobial therapy.[2,4] In addition, IVIg may be reserved as an emergent treatment for patients presented with hemophagocytosis or patients presented with severe bleeding symptoms and had symptoms of idiopathic thrombocytopenic purpura.[4] We prescribed IVIg in addition to antimicrobial therapy in a total of six patients who presented with hemophagocytosis in bone marrow, severe thrombocytopenia, and hemolytic anemia.

In conclusion; we postulated that brucellosis should be considered in the differential diagnosis of patients presenting with one of the findings including fever, hepatosplenomegaly, elevated liver transaminase and hematologic abnormality at least in one series especially in endemic regions like our country. Hematological involvement in at least one cell lineage was a common finding (43.4% of patients) regardless of age, especially in febrile, bacteremic patients and in patients who had hepatosplenomegaly and elevated liver enzymes. Anemia was the most common hematological abnormality, pancytopenia, severe thrombocytopenia and neutropenia were uncommon findings.

Ethical approval

This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital Institutional Review Board (Number: 2019/8).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AK, FNÖ, AF; data collection: AK, SYD; analysis and interpretation of results: AK, AF, GT, TAT; draft manuscript preparation: AK, FNÖ, AF, TAT. 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

Chest imaging for COVID-19: a single-center comparative results in pediatric patients

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ABSTRACT

Background. Chest computed tomography (CT) appears to be an important radiological modality for the diagnosis of COVID-19 in adults. Studies comparing the findings of such children with those of other viral infections have not been reported either. The aim of this study was to present comparative imaging findings of 75 pediatric COVID-19 patients and four patients with other viral upper respiratory tract infections. We also aimed to demonstrate the possible association between the radiological and laboratory findings in the COVID group.

Methods. From 11 March 2020 to 20 June 2020, 79 children (aged < 18 years) were enrolled. COVID-19 was detected by RT-PCR or antibody testing. A plain chest X-ray was obtained from all subjects. Non-contrast chest CT was performed for symptomatic patients.

Results. Seventy-five patients had COVID-19 and 4 were infected with other pathogens i.e. adenovirus, rhinovirus, parainfluenza virus B, respiratory syncytial virus. The ages of the patients (36 M, 43 F) ranged from 7 months to 17 years old. The sensitivity of chest X-ray (as compared to RT-PCR) was 10.67% (95 CI%: 4.72 - 19.94%). From 23 chest CT's five of them were normal and nine of them had only nodules (< 5mm). The sensitivity of CT was 78.26% (95CI%: 54.30 - 92.54%), false-negative rate was 21.7%.

Conclusions. The sensitivity of chest CT was found to be low and any significant correlations could have not been depicted, between the radiological parameters and the presence of lymphopenia. Clinical follow-up combined with corresponding pathogen detection, and chest CT of the symptomatic COVID-19 patients might be a feasible/prompt protocol in children.

Key words: SARS-COV-2, children, radiology, CT, X-ray.

An outbreak of 2019 novel coronavirus (COVID-19) infection was first discovered in Wuhan City, China - turning into a global health pandemic thereafter.[1,2] Likewise, the number of patients is rapidly increasing out of China; and especially Europe and America are currently severely affected.

In the early days of the COVID-19 infection outbreak, pediatric patients were extremely rare.[3] Later on, it has been reported that COVID-19 can be spread within the whole age spectrum.[4,5] One of the recent epidemiologic studies has revealed that children of all ages appeared susceptible to COVID-19.[6] Additionally, newborns of COVID-19 infected mothers have also increased the concern further.[7]

Upper respiratory tract infections due to different viral etiologies are common in the pediatric age group. Additionally, the total positive rate of

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reverse transcription polymerase chain reaction (RT-PCR) for nasopharyngeal swab samples was reported to be 30-60% at the initial presentation of COVID-19.[8] Distinguishing COVID-19 from other common respiratory tract infections in the pediatric population is a big dilemma. In adults, chest computed tomography (CT) appears to be an important radiological modality for the diagnosis of COVID-19 showing typical imaging features like ground-glass opacities, multifocal patchy consolidation, and/or interstitial changes with a peripheral distribution.[9] Disease severity in COVID-19 is associated with blood cell alterations, C-Reactive Protein levels and inflammatory markers. The relationship between radiological and laboratory findings were displayed in a few studies in adult patients.[10,11] To this end, however, there is limited data reported for pediatric COVID-19 patients. Further, to the best of our knowledge, studies comparing the findings of such children with those of other viral infections have not been reported either. Accordingly, the purpose of this study was two-fold. First, we aimed to present comparative imaging findings of 75 pediatric COVID-19 patients and four patients with other viral upper respiratory tract infections. Second, we also aimed to demonstrate the possible association between the radiological and laboratory findings in the COVID group.

Material and Methods

Subjects and study design

This was a single-center, retrospective, observational study conducted at a tertiary hospital. From 11 March 2020 to 20 June 2020, a total of 79 children (aged < 18 years) were enrolled. Written informed consent was obtained from the patients or their parents, and the study protocol was approved by the ethics committees of the Ministry of Health and Hacettepe University (GO 20/392). Demographic data and clinical features as well as laboratory (white blood cell, absolute lymphocyte count, C-reactive protein, procalcitonin) and imaging findings were obtained for each enrolled patient. Viral respiratory panel tests were performed on all patients, to exclude the superimposed or concomitant pneumonia due to different infectious agents.

Radiological evaluations

Plain chest X-rays were obtained from all subjects. Non-contrast chest CT was performed for symptomatic patients i.e. having fever, cough, respiratory distress. Only one asymptomatic patient received a chest CT due to strong family positivity (seven people from the family were covid positive). Chest CT studies were performed using the SOMATOM Definiton AS 64 unit (Siemens Medical System; Siemens, Germany) with the following parameters: 80 kV, 45 to 70 mA, 1.2-mm collimation, and 1:5 pitch. The scanning range covered from lung apex to diaphragm on axial plane taken under free breathing with the patients in the supine position. Thin-section CT images were reconstructed with 1.25-mm collimation with a standard algorithm and then sent to the picture archiving and communication system (PACS) for analysis. CT images were evaluated using a lung window with a window level of −600 HU and window width of 1500 HU, and the soft-tissue window with a window level of 40 HU and window width of 300 HU. All the images were stored in PACS and reviewed by three expert pediatric radiologists (having, 25, 17 and 7 years of background in pediatric radiology) by consensus who were blinded to the symptoms and laboratory tests. The CT features were evaluated for: (a) ground-glass opacities (GGO), (b) nodules, (c) consolidations, (d) crazy paving, (e) atelectasis, (f) peribronchial thickening, (g) tree-in-bud sign, (h) pleural effusion, (i) lymphadenopathy (short axis dimension > 1.0 cm) and (j) white lung. The anatomic distribution (unilateral, bilateral, subpleural, central or mixed), zonal predominance (upper, middle, lower lung; central, middle, or peripheral location), and extent (focal, multifocal, and diffuse) of the lesions were also recorded. Visual quantitative assessment score (VQAS) was performed

according to the extent of opacities (including GGOs, crazy paving and consolidation) in each lobe. Scores were defined as following: 0 (none), 1 (affecting <5% of the lobe), 2 (affecting 5%–25% of the lobe), 3 (affecting 26%–49% of the lobe), 4 (affecting 50%–75% of the lobe) and 5 (affecting >75% of the lobe). A maximum CT score of 5 was possible for each lobe. Total CT score was reached by summing the scores in five lobes (range from 0 to 25).[12]

COVID-19 diagnosis

COVID-19 was detected by RT-PCR obtained from the nasopharyngeal swab and/or tracheal aspirate specimens or antibody testing (Ig M and Ig G) via COVID-19 [SARS-CoV-2] Antibody Test Kit; Colloidal Gold.[13] Multiplex Real time PCR (RT-PCR) method was used for the detection of other respiratory viral agents, as well. Nucleic acid isolation was performed by Gene All Ribospin vRD II Isolation Kit, Seoul, Korea and RT- PCR method was carried out by Seegene RV16 Detection Kit, Seoul, Korea.

Disease severity

We categorized the severity of cases based on the clinical characteristics, laboratory results and imaging findings as described elsewhere:[6] a) asymptomatic infection; no clinical and/or radiological findings, b) mild disease; acute upper respiratory tract infection without clinical and radiological pneumonia, c) moderate disease; pneumonia with the symptoms of respiratory tract infection, d) severe disease; progressive respiratory disease with dyspnea and central cyanosis, e) critically ill cases; acute respiratory distress syndrome or respiratory failure, shock, organ dysfunction including encephalopathy, myocardial injury, coagulation abnormalities, and acute kidney injury.

Statistical analysis

IBM SPSS software package version 23.0 was used for all the statistical analyses. Categorical variables are expressed as frequencies and percentages. Mean±standart deviation was given as descriptive statistics for the numeric variables. Fisher exact test was used to compare the distribution of a categorical variable. In order to evaluate the accuracy of the diagnosis, sensitivity and its confidence intervals (CI) were obtained as a test performance measures of CT.[14] The sensitivity value of CT obtained for children from this study was compared with the sensitivity value obtained from a previously published study in adults.[15] Two sample proportion test was used to determine whether the proportions of two independent groups differ. The results of X-ray and CT were compared with McNemar test. The relationship between lymphopenia and RT-PCR positivity were examined with the "Yates (Continuity Correction) Chi square" test.

Results

Demographic and clinical characteristics of patients with COVID-19 are given in Table I. Ages of the patients (36 M, 43 F) ranged from 7 months to 17 years with a mean value of 10.5 years (SD 5.2). Overall; 75 patients had COVID-19 and 4 were infected with only other pathogens i.e. adenovirus, rhinovirus, parainfluenza virus B and respiratory syncytial virus (RSV). All four patients with other infections had a chest CT, as they suffered from similar symptoms and/or suspicion pertaining to COVID-19. Of the 75 COVID-19 patients 67 received the diagnosis with RT-PCR and eight of them had positive antibody tests.

Among the COVID-19 patients, 23 had a chest CT and plain X-ray while the remaining 52 had only a chest X-ray whereby 23 of them were asymptomatic, 42 had mild, 7 had moderate, and 3 had a critically ill disease course. Since one of the critically ill patients had severe cardiac failure and was later on lost, a CT could not be performed. Extracorporeal membrane oxygenation was used in another critically ill patient with refractory acute respiratory distress syndrome secondary to Stevens-Johnson syndrome and was later on lost.

Table I. Demographic and clinical characteristics of pediatric cases with COVID-19.

Patient number	Age/Sex (yo)	Severity of COVID-19	Underlying conditions	Family history for COVID-19	Day from illness onset to diagnosis	Complications	ALS (/μL)	WBC (/μL)	CRP (mg/dl) (0-0.8)	PCT (ng/ml) (0-0.1)	Chest X-Ray	Chest CT	Outcome
1	1/M	Critical	No	Yes	5	Myocarditis	5500	8700	0.06	0.09	Positive	N/A	Died
2	7/M	Critical	No	No	19	MODS	900	9300	0.08	3.87	Positive	Yes	Died
3	4/F	Mild	No	Yes	0	No	2500	4400	1.24	N/A	Normal	Yes	Recovered
4	3/F	Asymptomatic	No	Yes	0	No	3000	4300	0.01	N/A	Normal	N/A	Recovered
5	2/F	Asymptomatic	No	Yes	0	No			0.19	0.14	Normal	N/A	Recovered
6	14/M	Asymptomatic	No	Yes	0	No	3500	7000	0.03	N/A	Normal	N/A	Recovered
7	11/F	Asymptomatic	No	Yes	0	No	2400	6800	0.08	N/A	Normal	N/A	Recovered
8	14/F	Moderate	No	Yes	7	No	1300	4500	0.08	N/A	Positive	Yes	Recovered
9	16/F	Asymptomatic	No	Yes	0	No	2100	5200	0.11	N/A	Normal	N/A	Recovered
10	15/M	Asymptomatic	No	Yes	0	No	2200	5300	0.13	N/A	Normal	N/A	Recovered
11	4/M	Asymptomatic	No	Yes	0	No	2900	4500	0.12	N/A	Normal	N/A	Recovered
12	1/F	Asymptomatic	No	Yes	0	No	10800	18700	0.14	0.04	Normal	N/A	Recovered
13	4/F	Mild	No	Yes	0	No	3000	4800	0.18	N/A	Normal	Yes	Recovered
14	13/M	Asymptomatic	No	Yes	0	No	2600	5800	0.22	N/A	Normal	Yes	Recovered
15	17/M	Mild	No	Yes	1	No	1600	4900	0.23	N/A	Positive	Yes	Recovered
16	11/M	Mild	No	Yes	1	No	1400	5000	0.29	0.08	Normal	Yes	Recovered
17	16/F	Mild	No	Yes	4	No	2000	7500	0.31	N/A	Normal	Yes	Recovered
18	17/F	Moderate	No	Yes	9	No	600	5800	0.48	.01	Normal	Yes	Recovered
19	16/F	Mild	No	Yes	8	No	1700	5100	0.58	N/A	Normal	Yes	Recovered
20	13/F	Asymptomatic	No	Yes	0	No	1400	8600	0.58	N/A	Normal	N/A	Recovered
21	7mo/M	Mild	No	No	1	No	2400	4000	0.61	0.08	Normal	N/A	Recovered
22	10/F	Mild	No	Yes	3	No	2100	8100	1.02	N/A	Normal	Yes	Recovered
23	3/F	Mild	No	Yes	1	No	7000	10300	1.35	N/A	Normal	N/A	Recovered
24	11/M	Mild	No	Yes	0	No	900	6500	1.68	N/A	Normal	Yes	Recovered
25	6/M	Asymptomatic	No	Yes	0	No	2400	7000	1.88	0.02	Positive	N/A	Recovered
26	9/M	Mild	Asthma	Yes	1	No	1300	11600	9.47	6.26	Normal	Yes	Recovered

yo: years old, mo: months old, WBC: White blood cell, ALS: Absolute lymphocyte count, CRP: C-reactive protein, PCT: Procalcitonin,
MODS: Multiple organ dysfunction syndrome, HLH: Hemophagocytic lymphohistiocytosis, N/A: Not applicable, Chest X-ray Positive: Ground glass opacities, peribronchial thickening, consolidation

Table I. Continued.

Patient number	Age/Sex (yo)	Severity of COVID-19	Underlying conditions	Family history for COVID-19	Day from illness onset to diagnosis	Complications	ALS (/μL)	WBC (/μL)	CRP (mg/dl) (0-0.8)	PCT (ng/ml) (0-0.1)	Chest X-Ray	Chest CT	Outcome
27	13/F	critical	Osteopetrosis, chronic osteomyelitis	Yes	4	HLH	500	1200	10.78	N/A	Positive	Yes	Recovered
28	11mo/M	Mild	No	Yes	2	No	6100	9900	1.56	0.14	Normal	N/A	Recovered
29	3/F	Asymptomatic	No	Yes	0	No	5490	7700	0.14	0.03	Normal	N/A	Recovered
30	5/F	Mild	No	Yes	0	No	1780	11800	4.00	0.27	Normal	N/A	Recovered
31	10/F	Mild	No	Yes	1	No	1370	6000	0.60	0.04	Normal	N/A	Recovered
32	15/M	Mild	No	Yes	2	No	1450	5500	0.23	0.05	Normal	N/A	Recovered
33	17/M	Mild	No	Yes	0	No	830	4800	0.25	0.08	Normal	Yes	Recovered
34	14/F	Moderate	Kartagener syndrome	Yes	4	No	420	7400	1.16	0.03	Positive	Yes	Recovered
35	7/F	Asymptomatic	No	Yes	0	No	3520	7800	0.04	0.03	Normal	N/A	Recovered
36	16/F	Mild	No	Yes	2	No	1820	4800	0.19	N/A	Normal	Yes	Recovered
37	14/M	Moderate	No	Yes	3	No	2000	4600	0.65	0.02	Normal	Yes	Recovered
38	16/F	Mild	No	Yes	3	No	1820	3900	1.03	0.02	Normal	N/A	Recovered
39	15/M	Mild	No	Yes	0	No	2170	6300	0.03	N/A	Normal	N/A	Recovered
40	10/M	Mild	No	Yes	0	No	1440	9000	6.40	0.08	Normal	N/A	Recovered
41	11/F	Mild	No	Yes	1	No	3270	6500	1.97	0.07	Normal	Yes	Recovered
42	14/F	Moderate	No	Yes	1	No	1800	3900	0.22	0.05	Normal	Yes	Recovered
43	10/M	Asymptomatic	No	Yes	0	No	1770	3400	0.26	0.06	Normal	N/A	Recovered
44	14/F	Mild	No	Yes	3	No	1150	4900	0.20	0.03	Normal	N/A	Recovered
45	13/M	Mild	No	Yes	3	No	2050	3600	0.01	0.02	Normal	Yes	Recovered
46	8/F	Mild	Midaortic stenosis	Yes	0	No	1080	5300	0.14	0.05	Normal	N/A	Recovered
47	2/M	Asymptomatic	Hirschsprung disease	Yes	0	No	3800	7300	0.01	0.03	Normal	N/A	Recovered
48	13/F	Mild	No	Yes	1	No	2490	5200	0.09	0.03	Normal	N/A	Recovered
49	16/F	Mild	No	Yes	4	No	2250	6000	0.34	0.03	Normal	Yes	Recovered

yo: years old, mo: months old, WBC: White blood cell, ALS: Absolute lymphocyte count, CRP: C-reactive protein, PCT: Procalcitonin,
MODS: Multiple organ dysfunction syndrome, HLH: Hemophagocytic lymphohistiocytosis, N/A: Not applicable, Chest X-ray Positive: Ground glass opacities, peribronchial thickening, consolidation

Table I. Continued.

Patient number	Age/Sex (yo)	Severity of COVID-19	Underlying conditions	Family history for COVID-19	Day from illness onset to diagnosis	Complications	ALS (/μL)	WBC (/μL)	CRP (mg/dl) (0-0.8)	PCT (ng/ml) (0-0.1)	Chest X-Ray	Chest CT	Outcome
50	17/F	Mild	No	Yes	1	No	2850	6500	0.43	0.02	Normal	N/A	Recovered
51	13/M	Asymptomatic	No	Yes	0	No	1950	6600	0.07	0.07	Normal	N/A	Recovered
52	2/M	Asymptomatic	No	Yes	0	No	3200	6400	0.06	0.02	Normal	N/A	Recovered
53	15/F	Mild	No	Yes	4	No	1990	3900	0.02	0.02	Normal	N/A	Recovered
54	8/F	Asymptomatic	No	Yes	0	No	2450	4500	0.48	0.32	Normal	N/A	Recovered
55	16/M	Moderate	Von Gierke	Yes	2	No	1680	6900	5.75	1.22	Normal	Yes	Recovered
56	14/M	Mild	No	No	0	No	1350	6700	0.11	.03	Normal	N/A	Recovered
57	15/M	Mild	No	Yes	1	No	1160	7400	1.00	.03	Normal	N/A	Recovered
58	4/M	Mild	No	Yes	1	No	2350	4100	0.10	.04	Normal	N/A	Recovered
59	16/F	Mild	No	Yes	0	No	1790	5100	1.01	.03	Normal	N/A	Recovered
60	12/F	Mild	Asthma	Yes	1	No	2820	5200	0.25	.04	Normal	N/A	Recovered
61	1/M	Mild	Asthma	Yes	2	No	5710	10500	0.94	.22	Positive	N/A	Recovered
62	3/M	Asymptomatic	No	Yes	0	No	4230	6400	0.27	.05	Normal	N/A	Recovered
63	8/F	Asymptomatic	No	Yes	0	No	2870	4700	0.07	.05	Normal	N/A	Recovered
64	11/F	Moderate	No	Yes	2	No	2010	4500	0.18	.04	Normal	N/A	Recovered
65	17/F	Mild	Pontine glioma	Yes	4	No	2480	5900	0.37	.02	Normal	N/A	Recovered
66	5/F	Mild	No	Yes	2	No	6000	10300	0.19	0.04	Normal	N/A	Recovered
67	6/M	Asymptomatic	No	Yes	0	No	3990	14300	0.00	.02	Normal	N/A	Recovered
68	14/M	Mild	No	Yes	1	No	1990	4600	0.03	0.03	Normal	N/A	Recovered
69	15/F	Mild	No	Yes	1	No	1180	6800	0.37	.04	Normal	N/A	Recovered
70	9/F	Asymptomatic	No	Yes	0	No	1840	4800	0.13	.04	Normal	N/A	Recovered
71	12/M	Mild	Obesity	Yes	1	No	1810	5900	0.47	.09	Normal	N/A	Recovered
72	17/F	Mild	No	Yes	1	No	1730	10000	N/A	.03	Normal	N/A	Recovered
73	12/F	Mild	No	Yes	0	No	1540	6500	0.02	0	Normal	N/A	Recovered
74	10/M	Mild	No	Yes	1	No	6440	8700	0.23	0	Normal	N/A	Recovered
75	15 /F	Asymptomatic	No	Yes	0	No	2960	13300	0.21	0.05	Normal	N/A	Recovered

yo: years old, mo: months old, WBC: White blood cell, ALS: Absolute lymphocyte count, CRP: C-reactive protein, PCT: Procalcitonin,
MODS: Multiple organ dysfunction syndrome, HLH: Hemophagocytic lymphohistiocytosis, N/A: Not applicable, Chest X-ray Positive: Ground glass opacities, peribronchial thickening, consolidation

Sixty-seven patients had normal chest X-ray findings. A total of eight children had remarkable abnormalities (GGO, peribronchial thickening, consolidation) on X-ray. The sensitivity of chest X-ray (as compared to RT-PCR) was 10.67% (95 CI%: 4.72 - 19.94%). More than half of the chest CTs were obtained on the same day with chest X-rays (58%), while 25% were obtained the next day. The remaining 16% percent of chest CTs were obtained between 2-6 days.

The CT findings of pediatric patients with COVID-19 infection are given in Table II. Visual quantitative assessment score (VQAS) and the comparison with the clinical severity of the COVID-19 patient are given in Table III. From 23 chest CT's five of them were normal and nine of them had only subpleural and/or intraparachymal nodules (< 5mm). The sensitivity of the CT (as compared to RT-PCR) was 78.26% (95CI%: 54.30 - 92.54%), the false negative rate was 21.7%. The most frequent chest CT findings were nodules, GGO, GGO and consolidation and peribronchial thickening (Fig. 1 and Fig. 2). Of note, most of the patients had bilateral chest CT findings.

Table II. Computed tomography findings of patients with COVID-19 (N=23).

Findings	Asymptomatic (n=1)	Mild (n=13)	Moderate (n=7)	Critically ill (n=2)
	N (%)	N (%)	N (%)	N (%)
Ground glass opacities	1 (4.54)	-	7 (30.4)	1 (4.54)
Nodule	1 (4.54)	7 (31.8)	5 (22.7)	-
Consolidation	-	-	-	-
Crazy Paving	-	-	-	1 (4.54)
Atelectasis	-	-	-	-
Ground glass opacities and consolidation	-	-	1 (4.54)	2 (9.09)
Peribronchial thickening	-	-	4 (18.1)	1 (4.54)
Tree in buds	-	-	-	-
Pleural effusion	-	-	-	-
Bronchiectasis				
Lymhadenopathy	-	-	-	-
White lung	-	-	-	1 (4.54)
Lung region distribution				
Unilateral	-	2 (9.09)	1 (4.54)	-
Bilateral	1 (4.54)	5 (22.7)	5 (22.7)	2 (9.09)
Subpleural	1 (4.54)	8 (36.3)	5 (22.7)	1 (4.54)
Central	-	-	-	-
Mixed	-	-	4 (18.1)	1 (4.54)
Lung lobe involved				
Right upper lobe	1 (4.54)	4 (18.1)	3 (13.6)	2 (9.09)
Right middle lobe	1 (4.54)	3 (13.6)	2 (9.09)	1 (4.54)
Right lower lobe	1 (4.54)	5 (22.7)	5 (22.7)	2 (9.09)
Left upper lobe	1 (4.54)	3 (13.6)	4 (18.1)	1 (4.54)
Left lower lobe	-	6 (27.2)	6 (27.2)	2 (9.09)
Distribution				
Focal	-	1 (4.54)	1 (4.54)	-
Multifocal	1 (4.54)	-	5 (22.7)	1 (4.54)
Diffuse	-	-	-	1 (4.54)
Normal CT	-	5 (22.7)	-	-

Table III. Visually calculated total CT scores of the pulmonary involvement in 23 COVID-19 patients.

Clinical severity	Calculated total CT scores								
	0	1	2	3	4	5	7	10	25
Asymptomatic (n=1)				1					
Mild (n=13)	5	2	2	2	1	1			
Moderate (n=7)		1	1			1	1	3	
Critical (n=2)									2

n: Number of patients

Significant positive correlations were found between disease severity and most common CT findings of COVID-19 (peripheral, subpleural and perilymphatic GGO) (p=0.001). One asymptomatic patient had peripheral GGO. The most common CT findings of COVID-19 were present in 7.7% of the mild group, 71.4% of the moderate group and 100% of the critical ill group. The sensitivity rate of the most common CT findings of COVID-19 (peripheral, subpleural and perilymphatic GGO) as compared to RT-PCR was 39.13% (95CI%: 19.71 - 61.46%). The time interval from symptom onset to chest CT was between 2-9 days (median 2.5 days). Chest X-ray and the most common CT findings of COVID-19 were similar (p=0.453).

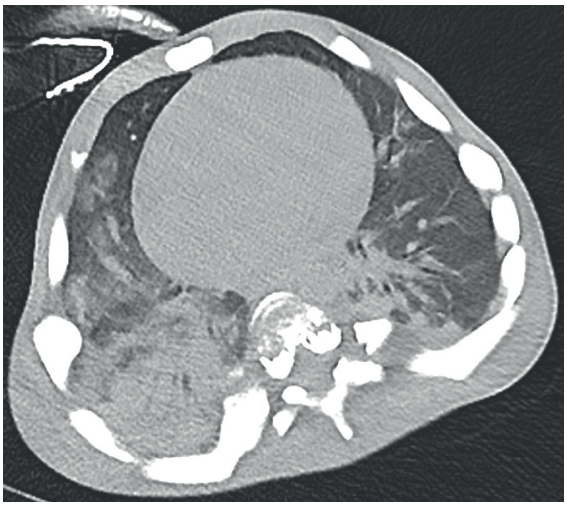

Fig. 1. A-13-year-old girl with COVID-19 pneumonia. Axial non-contrast enhanced chest CT image demonstrates diffuse consolidation in the right lower lobe, peribronchial patchy ground-glass opacities in the right middle lobe, and subpleural consolidation in the left lower lobe. Note that the diffuse osteosclerosis of all bony structures in the thoracic cage is compatible with osteopetrosis.

Five patients had a normal chest CT, and they were asymptomatic or had a mild disease course as well. Statistically significant positive correlations were found between disease severity and lymphopenia (p=0.009). Lymphopenia was present in 4.3% of the asymptomatic group, 28.6% of the mild group, 42.9% of the moderate and 66.7% of the critically ill group. No significant correlations were depicted between the radiological parameters and lymphopenia. Yet, 77.8% of patients with lymphopenia and 78.6% of those without had positive CT findings. No significant correlations were depicted between RT-PCR positivity and lymphopenia (p=0.671).

Table IV demonstrates the imaging features of patients with other infections. These

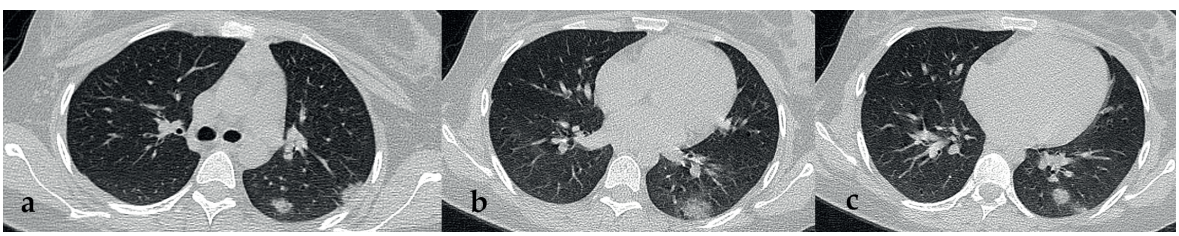

Fig. 2a-c. A-17-year-old girl with COVID-19 pneumonia. **(a-c)** Axial non-contrast enhanced chest CT images demonstrate subpleural ground-glass nodules in the left lower lobe.

Table IV. Computed tomography findings of other viral infections.

Findings	Adenovirus	Rhinovirus	RSV B	RSV A/Parainfluenza
Ground glass opacities	+	+	+	-
Nodule	+	-	-	-
Consolidation	+	-	+	+
Crazy paving	-	-	-	-
Atelectasis	-	+	+	-
Ground glass opacities and consolidation	+	-	-	+
Peribroncial thickening	-	+	+	-
Tree in buds	-	-	-	-
Pleural effusion	-	-	-	+
Bronchiectasis	+	-	-	-
Lympadenopathy	-	-	-	-
White lung	-	-	-	-
Lung region distribution				
Unilateral	-	-	-	-
Bilateral	+	+	+	+
Subpleural	+	+	+	-
Central	+	+	+	+
Mixed	+	+	+	+
Lung lobe involved				
Right upper lobe	-	-	-	+
Right middle lobe	+	+	+	+
Right lower lobe	+	+	+	+
Left upper lobe	+	+	+	+
Left lower lobe	+	+	+	+
Distribution				
Focal	-	-	-	-
Multifocal	+	+	+	+
Diffuse	-	-	-	+
Normal CT	-	-	-	-

RSV: Respiratory syncytial virus

four patients were infected with other viral respiratory viruses and RT-PCR test was negative for COVID-19. The patient with an adenovirus infection was a 10-year-old boy, who had a fever and cough, his father had Covid-19 positivity. The Rhinovirus infected patient was a 12-year-old boy and had respiratory distress. A 1-year-old girl with Respiratory syncytial virus had a fever and cough. An 8-years-old boy with Respiratory syncytial virus A/Parainfluenza had a fever. Their chest CT imaging findings were similar to those of COVID-19 patients with regards to bilateral involvement and the aforementioned most commonly seen patterns (Fig. 3 and 4).

Discussion

In this comparative imaging study, we have shown that CT findings of COVID 19 may be diverse in children. Chest CT sensitivity was found to be moderate low (78.26%) and any significant correlations could have not been depicted between the radiological parameters and the presence of lymphopenia. When compared to the relevant literature whereby

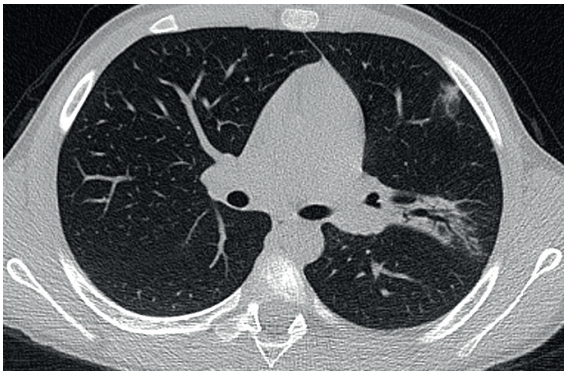

Fig. 3. A-10-year-old boy with adenovirus pneumonia. Axial non-contrast enhanced chest CT image shows subpleural ground-glass nodule in the left upper lobe with consolidation and mild bronchiectasis.

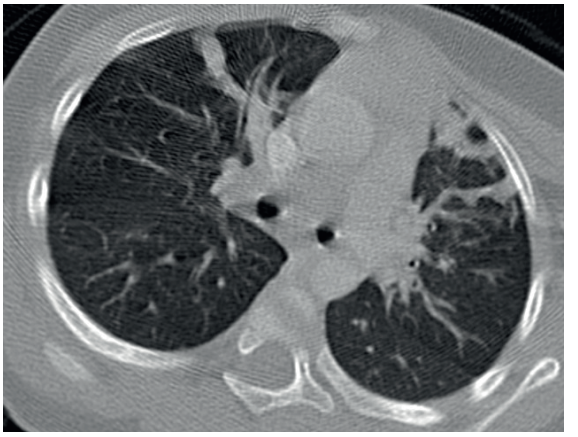

Fig. 4. A-1-year-old girl with respiratory syncytial virus-B infection. Axial non-contrast enhanced chest CT image shows subpleural atelectasis in the upper lobes.

mild pediatric patients showed normal findings on chest CT[16,17], five of our 23 cases had a normal CT and nine of them had only subpleural and parenchymal nodules. Again, GGO (the most frequently observed pattern) mainly in the peripheral, subpleural and posterior lungs was the main finding in our patients. Herein, it is noteworthy that other findings like nodules, GGO with consolidation or peribronchial thickening can also be detected in children. Significant positive correlations were found between disease severity and characteristic COVID-19 CT findings. In our study, both crazy paving pattern and consolidation were more common in critically ill patients. In COVID-19 patients, diffuse alveolar damage has been described with interstitial edema, thickening of alveolar walls and proliferation of interstitial fibroblasts.[18]

COVID-19 viral pneumonia is an acute infectious chest disease due to a novel coronavirus. The clinical spectrum of the disease ranges from asymptomatic to critically ill. Although the severity of the disease seems to be milder in pediatric patients as compared with adults, data about various clinical findings of COVID-19, particularly in children are limited.[19,20] As such, one of the challenging issues for radiologists, as well as clinicians remains to be the availability to distinguish CT findings of children with COVID-19 from those of other viral respiratory infections. Herein, CT findings of bilateral multifocal GGO with patchy consolidations and peribronchial thickening were also present in our patients who had pneumonia due to other infections. On the other hand, the characteristics of pneumonia caused by adenovirus had higher density, more consolidations and fewer subpleural lesions. Concerning both RSV and parainfluenza virus; the characteristics of pneumonia were mostly distributed along the bronchi with bronchial wall thickening. Hence, a definite diagnosis cannot be achieved on the basis of imaging features alone. Indeed, chest CT findings of pneumonia due to different pathogens seem to overlap, and COVID-19 pneumonia can be superimposed with lung involvement due to other pathogens - presenting more severe imaging findings. Therefore, radiological assessment should indisputably be coupled with clinical and laboratory examinations.

In China, chest CT was recommended for the diagnosis at the beginning of the pandemic - due to the low positivity of PCR tests as a reflex behavior against a new disease. Moreover, as chest CT was mentioned to be a more sensitive diagnostic tool rather than RT-PCR, CT has been used for screening/diagnosis for adult patients.[15] The sensitivity value of CT obtained

for children from this study was (78.26%) compared with the sensitivity value obtained from adults (97%) a previously published study of Ai et al.[15] Chest CT had higher sensitivity for COVID-19 diagnosis in adults. Most of the pediatric patients with COVID-19 in the present study had asymptomatic or mild disease course, and their radiological findings were also normal or mild. It is apparent that the sensitivity of chest radiographs (the first examination to be preferred) in showing the GGO and small consolidations is low. Therefore, according to our findings, chest CT should rather be performed for symptomatic pediatric patients. Moreover, this approach would also be crucial to prevent high levels of radiation exposure in children. Steinberger et al.[21] drew attention to the same issue, and reported that the low incidence of CT exams with positive findings and the low severity of disease in children's CTs should be noted when handling the utility and restrictions of CT for the assessment of COVID-19 in children and they suggested CT for evaluating suspected complications of COVID-19.

In a recent study, Wang et al.[22] reported that the lung abnormalities on CT progressed rapidly after the symptom onset, reached its peak around 6-11 days, and was followed by a lengthy persistence of high levels in adults. A few studies supporting this information have reported an increase in GGOs over time.[23] Shen et al.[13] reported that progressive and severe stage of CT findings were rarely seen; however, any association between the clinical and radiological course was not reported. Radiological progression of pediatric cases should not be considered as a hallmark of disease severity and clinical status of the cases should be the mainstay in the management. Again, more case examples and long-term follow-up would be necessary to better demonstrate the development of pediatric COVID-19 disease.

There are some limitations of our study. First, the number of patients infected with other viruses was small. Second, studies with a larger sample size and longer follow-up are awaited to better understand the actual disease course of pediatric COVID-19 patients during management. Lastly, we couldn't set any exclusion criteria such as preexisting diseases may mimic COVID-19 related findings, since the number of patients is small and each patient is very valuable.

In conclusion, pneumonia due to COVID-19 is generally mild in children, and chest CT can well be unremarkable. If present, characteristic imaging findings of COVID-19 infection seem to be subpleural ground-glass opacities and peribronchial thickening. The sensitivity of chest CT was found to be low and any significant correlations could have not been depicted between the radiological parameters and the presence of lymphopenia. However, it would be insufficient to diagnose COVID-19 pneumonia only by CT imaging, and it would actually also be difficult as far as its differential diagnosis from other viral infections is concerned. Therefore, clinical follow-up together with detection of the corresponding pathogens, and chest CT of the symptomatic patients might be a feasible/prompt protocol in children.

Ethical approval

Written informed consent was obtained from the patients or their parents, and the study protocol was approved by the ethics committees of the Ministry of Health and Hacettepe University (GO 20/392).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HNÖ; data collection: YÖ, PDO, SLG; analysis and interpretation of results: BO, JK, ÖT; draft manuscript preparation: HNÖ, MC, MH. 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

Prevalence of COVID-19 infection in asymptomatic school children

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ABSTRACT

Background. With the onset of the COVID-19 pandemic, discussions regarding the prevalence of COVID-19 in children and the association of this with education have started. This study aimed to determine the prevalence of COVID-19 infection in asymptomatic school children within a limited period while face-to-face education continued.

Methods. This is a descriptive and retrospective study. Screening was carried out in the schools in the three major districts of the metropolitan municipality when face-to-face education was practiced. COVID-19 RT-PCR swab samples were collected from 4,658 students from 46 schools at preschool, primary, secondary, and high school levels by using the stratified sampling method. Screening results were retrospectively analyzed by the researchers.

Results. The mean age of the children included in the study was 10.6±3.2 (5-17). Only 46 students' COVID-19 RT-PCR results were positive; the positivity rate was higher in male students than in female students ($p>0.05$); the students living in the third region had a higher positivity rate than the other students, there was a statistical difference between them ($p<0.001$); there were no positive cases in 26 (56.7%) schools, and the spreader rate of the school children was 0.98%.

Conclusions. We determined in the study that the prevalence of COVID-19 infection was not high in asymptomatic school children in the period when schools were open. This may play a role in directing the education and training during the pandemic.

Key words: COVID-19, prevalence, school children, education.

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an infectious respiratory disease that affects humans. The disease, first seen in China in the last month of 2019, rapidly spread worldwide and was declared a pandemic by the World Health Organization (WHO). Due to the pandemic, all countries around the world have taken various measures for the pandemic. These measures include a curfew, city lockdown, physical distancing, avoiding high-risk spaces like crowded indoor gatherings, staying home when ill, wearing face masks, and using contact tracing apps.[1] One of the most important of these measures was to suspend education for a while. With the prolongation of the pandemic, some countries have started the education of preschools and school children with distance education, while some countries have continued face-to-face education by taking protective measures.[2] With the onset of face-to-face education, discussions regarding the prevalence of COVID-19 in children have started.

What is known about the risks of COVID-19 for children changes the understanding of how it affects children as the information about

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COVID-19 increases.[2,3] In the early stages of the pandemic, it was thought that children had mild and asymptomatic COVID-19 and that the risk of transmission was lower.[4] However, it has been determined over time that children with COVID-19 may also require hospitalization and critical care.[5,6] Cura et al. reported a high rate (8.6%) in children assessed with the suspicion of COVID-19.[5]

On the contrary to these studies, there are also reports revealing that COVID-19 is rare among children.[7-10] It has been reported that 2.1% of the cases infected with COVID-19 are children under the age of 18. According to the current information provided by the Chinese Centers for Disease Control and Prevention, 416 (0.9%) out of 44,672 individuals were below the age of 10 and 549 (1.2%) were between the ages of 10 and 18 by the 11th of February 2020.[11]

It is important to carry out school screenings to protect and improve public health during pandemics, and the family physicians, health professionals working in the community health centers, and school health nurses take an active role and identify children in the risk group before hand.[2]

This study aimed to determine the prevalence of COVID-19 infection in asymptomatic school children within a limited period while face-to-face education continued.

Material and Methods

All the procedures performed in studies involving human participants followed the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The institutional approval was obtained from the Provincial Health Directorate (93079172-703.01) and the Ministry of Health (2020-11-18T15_37_29), and ethical approval was obtained from Nuh Naci Yazgan University Ethics Committee (2020/27).

This was a descriptive and retrospective study. In this study, the results of COVID-19 RT-PCR tests performed at the schools by the Provincial Health Directorate for screening in asymptomatic children were obtained from the records and retrospectively assessed.

Study Design and Setting

The duration of compulsory education for children in Turkey is 12 years. Before compulsory education, children receive preschool education. The first four years of compulsory education are a primary school, the second four years are a secondary school, and the third four years are high school education.

Schools were closed on March 16, 2020, when the pandemic began in Turkey. Face-to-face education started on September 21, 2020. Some precautions were taken at schools as face-to-face education started. Wearing a mask became compulsory. Social distancing rules were carefully followed in the classrooms and in common areas such as dining halls and the seating arrangements were adjusted according to these rules. The school canteens were closed. The use of sports halls, swimming pools and school clubs was suspended. Mass ceremonies were canceled. The length and number of lessons were decreased and the length of break times was increased. The classrooms were ventilated by keeping the windows open during break times. Adults except for teachers and staff in charge were not allowed in the schools. The temperature of the students and teachers was taken before attending the classes. The teachers and students with symptoms were immediately referred for PCR test analysis. Forty-five days after face-to-face education started, the Provincial Health Directorate carried out screening at schools in 3 major districts to evaluate the prevalence of COVID-19 in asymptomatic school children during the face-to-face education period. Socio-demographic characteristics of these three districts are as follows; Melikgazi: The population is 555,671; the population density is 3295; the development

index is 3.32; the literacy rate is 98.1%; the number of students per classroom is 59, and there are 1398 factories in the two organized industrial zones of Melikgazi. Kocasinan: The population is 391,661; the population density is 226; the development index is 3.32; the literacy rate is 97.1%; the number of students per classroom is 34; and it is the district where the migration from abroad (Syria, Afghanistan, etc.) is the highest. Talas: The population is 157,695; the population density is 336; the development index is 0.59; the literacy rate is 98.1%; the number of students per classroom is 36, and it is the district where the number of universities is higher. Between November 9 and 15, 2020, the students in 46 schools were screened. The cases who were positive for COVID-19 RT-PCR test were accepted as cases infected with COVID-19. The students who did not have any symptoms and signs of COVID-19 disease and those who had no history of contact were included in the screening. Throat or nasal swab samples were used for the RT-PCR test. Consent to collect swab samples were obtained from all children who were planned to be included in the screening and their parents.

Study Sample and Properties

In this process, 48 schools were randomly determined among the districts, with four schools from each level by the Provincial Health Directorate. Schools were selected using the stratified sampling method. Two schools were not included in the screening as the school management did not give consent. A power analysis was performed using the G * Power 3.1.0 analysis program to determine the sample size in our study. The number of people participating in the study with a population size of 176,000 was determined as a minimum of 3700 (α-value: 0.05, ß-value: 0.80).

In our study, the data of 4,658 students between the ages of 5 and 18 who were studying in preschool (kindergarten), primary school, secondary school, and high school and screened by the Provincial Health Directorate were evaluated. The screening results were analyzed according to the demographic characteristics and school levels. The data were obtained from the Provincial Health Directorate records and the Public Health Management System.

Real-Time Reverse Transcriptase Polymerase Chain Reaction

Real-time reverse-transcription PCR was performed using Coronex-COVID-19 (Ver.2.0) Multiplex RT-qPCR Diagnosis Kit (DS Bio and Nano Technology, Ankara, Turkey). A 20-µL of reaction mix contained 5 µL of RNA, 12.5 µL of CORONEX-COVID-19 DS Mix E [RT-qPCR master mix], and 2.5 µL of CORONEX-COVID-19 DS PP1 primer and probe mix [Orf1ab and N genes for SARS-CoV-2 detection, Rnase P gene for internal control]. Positive control for amplification control and no-template control were used to assess contamination. Thermal cycling was performed at 48 °C for 20 min for reverse transcription, followed by 95 °C for 5 min, and then 35 cycles at 95 °C for 5 s and 60 °C for 10 s in Rotor-Gene Q device (Qiagen, Hilden, Germany). Cycle threshold (Ct) values of less than 33 were defined as positive. According to the information from the user manual of CORONEX COVID-19 Multiplex qPCR Kit sensitivity: 97.06 % [95% CI : 94.92% -98.47%] and specificity: 100% [96.31%-100%].

Statistical Analysis

Data analysis of the study was performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) software program. In the data analysis, frequency, mean, median, standard deviation, and minimum and maximum values were used as descriptive data. Chi-square test was used in the comparison of categorical data; Student's T-test was used in paired groups with normal distribution in numerical data, and Mann-Whitney U test was used in groups that did not comply with a normal distribution. In comparing three or more groups that did not comply with the normal distribution, the Kruskal Wallis test was used, Post-hoc Dunn's correction was performed, and $p<0.05$ was considered statistically significant.

Results

The mean age of the students included in the study was 10.6 ± 3.2 (5-17) years. According to the results, 50.9% (n = 2370) of the students included in the study were female, 34.8% of the students were in primary school, 43.3% were in the age group of 6-10, and 49.7% were educated in the 1st region. In the study, the number of positive RT-PCR students was 46 (0.9%) (Table I). The median age of the students with positive RT-PCR test screening results was 11, and there was no statistical difference between the positivity rate and age groups (p = 0.84). Although the number of positive cases among the male students was higher than the female students, there was no statistical difference between them (p = 0.07). According to the results of the RT-PCR tests, the positivity rate of students living in the 3rd region was higher than the positivity rate of students living in the other regions, and the difference was statistically significant (p < 0.001) (Table II).

While positivity was mostly observed in the children attending secondary schools in the 1st region, positive cases were higher in the children educated in primary schools in the 3rd region (Table III). A positive result was found in 20 (43.5%) out of the 46 schools screened. In the study, positive results were found in 4, 5, and 6 students in 1 school of each region (Table IV).

Table I. Socio-demographic characteristics of students.

Characteristics	n	%
Mean (SD) age, year	10.6 (3.2)	5-17
Gender		
Male	2288	49.1
Female	2370	50.9
Age Groups		
5 years	250	5.4
6-10 years	2106	45.2
11-15 years	1892	40.6
16 years and above	410	8.8
Level of Development		
1st Region (Melikgazi)	2313	49.6
2nd Region (Kocasinan)	1009	21.7
3rd Region (Talas)	1336	28.7
RT-PCR Result		
Negative	4612	99.1
Positive	46	0.9

Discussion

All age groups are sensitive to COVID-19. The disease progresses with different clinical features from mild to severe.[12] The first COVID-19 case in children was reported from Shenzhen, China, on January 20, 2020.[13] Later, many pediatric cases and case series started to be reported.[5] In some studies, it has been reported that the rate of contracting COVID-19 is less in children than in adults, and the symptoms and signs are milder.[14,15] According to the weekly report of morbidity and mortality by the CDC, among 149,082 U.S. COVID-19 cases who had been reported as of April 2, 2020, and whose ages were known, 2,572 (1.7%) were patients under the age of 18 years and the rate was 2.4% in China.[16,17] It was reported that 3% (n=202) of 6713 daily diagnosed COVID-19 cases in Turkey on 23.11.2020 were under the age of 15.[18]

Occasional or absent clinical findings in children may cause fewer tests and fewer diagnoses. It has been reported that the rate of infected children is low, and children constitute an important group in terms of spreading the virus.[19] However, the data on this subject are insufficient and limited as children are evaluated within the scope of contact follow-up and reflect the children treated in hospitals.[20] Our study is important because to the best of our knowledge there is no study on asymptomatic school children without a history of contact in the Turkish literature.

The epidemiological data and clinical observations reveal that the prevalence of COVID-19 is lower and the possibility of mild/symptomatic COVID-19 is higher among young individuals, particularly among children.[21] The Turkish National Pandemic Plan carries out filiation practices including the infected

Table II. Evaluation of parameters according to the RT-PCR results of the students.

Characteristics	RT-PCR Result		Test/ p-value	
	Positive n(%)	Negative n(%)		
Age Groups (year)				
5 years	3 (6.5)	247 (5.4)	0.837	0.84
6-10 years	19(41.3)	2087 (45.3)		
11-15 years	21(45.7)	1871 (40.6)		
16 years and above	3 (6.5)	407 (8.8)		
Gender				
Male	28 (60.9)	2260 (49)	2.566	0.11
Female	18 (39.1)	2352 (51)		
Level of Development				
1st Region (Melikgazi)	21 (45.7)	2292 (49.7)	17.234	0.000
2nd Region (Kocasinan)	1 (2.2)	1008 (21.9)		
3rd Region (Talas)	24 (52.2)	1312(28.4)		
Total	46 (100)	4612 (100)		

Table III. Number of positive students by localization and level of schools.

	Total Number of Students	Number of RT-PCR Positive Students	Positivity Rate /p
1St Region (Melikgazi)			
Pre-school	101	1	0.99
Primary school	931	4	0.43
Secondary School	1057	11	1.04
High school	224	5	2.23
Total	2313 (49.66)	21 (45.66)	0,90
p-value	0.43	0.12	0.41
2nd Region (Kocasinan)			
Pre-school	120	0	
Primary school	552	0	0
Secondary School	278	0	0
High school	59	1	1.69
Total	1009 (21.66)	1 (2.17)	0.09
p-value	0.21	0.58	0.58
3rd Region (Talas)			
Pre-school	29	1	3.45
Primary school	623	11	1.77
Secondary School	557	9	1.62
High school	127	3	2.36
Total	1336 (28.68)	24 (52.17)	1.7
p-value	0.06	0.34	0.54
Overall total	4658 (100)	46 (0.98)	

Table IV. Number of schools according to positive RT-PCR test results of the students.

Number of positive students	Number of schools (%)
0	26 (56.5)
1	8 (17.4)
2	5 (10.8)
3	4 (8.7)
4	1 (2.2)
5	1 (2.2)
6	1 (2.2)

patients and contacts. Therefore, a higher rate of positivity can be detected in children with COVID-19 contact history or symptoms.[5]

At the time of this study, 19,273 RT-PCR tests were performed in the province and the test results of 3,785 people were positive. The rate of positive cases/tests in the province was 19.6% in the whole population. This rate was reported as 1.19% in children (under the age of 18) and 2.6% in school children (ages between 5 and 18). In our study, the positivity rate seen in asymptomatic school children was 0.98%. According to the data of the Provincial Health Directorate, the rate of positive cases in the general population is lower in children compared with adults. In addition, the positivity rate of asymptomatic school children in our study was lower than the rate of positive cases among children between the ages of 5 and 18 detected in the province in the same period. The reason why the positivity rate in our study was lower than the rate in literature and school children in the province is probably that school children with a history of contact and symptoms were not included in the study and that the asymptomatic children were randomized in the study.

In studies on infected children in China and the USA, when the infection status of children was assessed according to gender, approximately 57% of them were boys.[4] In our study, the rate of infection was higher in boys than in girls. It was reported in other studies that infected children were usually between the ages of 6 and 15.[3,15] In our study, the positivity rate among children between the ages of 6 and 10 and 11 and 15 was higher, which is consistent with the findings in the literature. It may be due to the increase in the socialization of children as they get older and their careless behaviors in obeying social distancing rules. On the other hand, it may show that preschool children obey the determined rules more.

In countries with the idea that children would not comply with social distancing, masks, and hygiene rules, it was thought that children would first transmit COVID-19 to each other in educational institutions and then to their families; therefore, schools and daycare centers were closed.[22] However, studies indicate no direct evidence on the impact of daycare and school closures on the COVID-19 outbreak.[20,22] Contrary to these studies, there are also publications stating that closing schools is an effective method to prevent the spread of the pandemic.[23-26] For this reason, it is stated that studies should aim to determine the rate of infection spreading of children in schools that have started face-to-face education.[20,22] Our study was conducted in a period of transition to face-to-face education in Turkey. Schools' rate with no cases was 56.5% (n = 26). The number of schools with no positivity was almost equal to the number of schools with positive cases. No clear view has been obtained from these results regarding the effect of the COVID-19 pandemic on preschool and school closures.

Today, epidemics and infectious diseases affect societies and people with poorer socioeconomic status.[27] Economically disadvantaged people living in crowded houses where there is no social distancing, being employed in jobs that do not provide the opportunity to work from home, and similar factors increase the possibility of being exposed to COVID-19.[28] The results of our study on localization support this. According to the RT-PCR test results, the positivity rate was higher in the students living in the 3rd region, where the level of development was the lowest ($p < 0.001$).

The most important limitation of our study was that the data of school-age children were withdrawn from the data processing system after RT-PCR screening, and the teachers and other school staff members were not included in the screening process. It is the first study performed on asymptomatic school children in Turkey, which is the strength of the study.

In conclusion, the COVID PCR positivity rate in asymptomatic school children was 0.98% in our study. Also, no positive cases were found in more than half of the schools. This study has revealed that the prevalence of COVID-19 infection in asymptomatic school children is not higher than the rates in symptomatic children and the general population. This may play a role in directing education and training during the pandemic as well as planning training about the pandemic and taking protective measures. However, the pediatric COVID-19 infectivity should not be ignored. Therefore, further studies are needed to confirm our observations.

Author contribution

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

Ethical approval

The institutional approval was obtained from the Provincial Health Directorate (93079172-703.01) and the Ministry of Health (2020-11-18T15_37_29), and ethical approval was obtained from Nuh Naci Yazgan University Ethics Committee (2020/27).

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Trend in initial presenting features of type 1 diabetes mellitus over a 24 year period in Turkey: a retrospective analysis of 814 cases

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ABSTRACT

Background. The study aim was to examine changes in trends of presenting features during the diagnosis of patients followed up with newly diagnosed Type 1 diabetes mellitus (T1DM) over the past 24 years.

Methods. The study was retrospective. Patients with a diagnosis of T1D between the years of 1996–2019 were included. Patients diagnosed in the first half of the period comprised Period I, and those from the second half comprised Period II. Patient data were extracted from medical records and included gender distribution, year of diagnosis, age at diagnosis, duration of symptoms, type of admission, frequency of diabetic ketoacidosis (DKA) and biochemical parameters. Subsequently, temporal changes in trends of these parameters were sought.

Results. For the whole cohort the gender distribution was equal; 404 (49.6%) were girls and 410 (50.4%) were boys. Mean age at diagnosis was 8.5±4.2 years and age groupings at presentation were: 23.2% (n = 189) aged 0-4; 39.2% (n = 319) aged 5-9; 27.5% (n = 224) aged 10-13; 10.1% (n= 82) aged 14-18. At presentation 72 (12.7%) had hyperglycemia, 230 (40.6%) had diabetic ketosis, and 264 (46.6%) had DKA. In those with DKA, mild DKA was found in 103 (39.0%), moderate DKA in 81 (30.6%), and severe DKA in 80 (30.3%). While the frequency of DKA was 54.9% between 1996 and 2007 (Period I), this significantly decreased to 44.4% between 2008 and 2019 (Period II). Girls and boys had a similar rate of T1DM, and this did not change over time. Three peak ages of diagnosis were evident; 5-7, 8-10, 12-14 years of age.

Conclusions. The frequency of DKA decreased and the frequency of admission with hyperglycemia and ketosis increased during the study period, which may have repercussions for mortality and morbidity rates and aid in improved treatment outcomes.

Key words: type I diabetes, diabetic ketoacidosis, childhood, trend.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that is usually diagnosed in childhood. T1DM is due to insulin deficiency caused by damage to pancreatic beta cells.[1] Insulin deficiency will severely impair normal mechanisms of cellular glucose import. This results in a change in energy metabolism to lipid catabolism that may give rise to diabetic ketoacidosis. Several international registries have documented increasing rates of T1DM including DIAMOND, EURODIAB and SEARCH, with an annual increase in incidence of 2.8%, 3.5%, and 2.72%, respectively.[2-4] The incidence of T1DM ranges from 0.7 to 40 per 100 thousand worldwide and has been shown to differ between communities and regions and within the same community in different ethnic groups.[5] T1DM accounts for about 10% of all diabetes cases at any age.[6] T1DM is more common in two periods, between the ages of 5-7 years, when school starts and the child may encounter novel infectious agents, and between

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the ages of 10-14 years when sex hormone concentrations increase during puberty which is accompanied by increased growth hormone concentrations and emotional stress is high.[7,8]

Diabetic ketoacidosis (DKA) is an acute complication of T1DM that usually occurs at the time of diagnosis, is life-threatening but rarely causes mortality. It has been reported that the frequency of DKA in patients with newly diagnosed T1DM varies widely from 15 to 80%.[9-11] Although there are Turkish national programs to decrease the frequency of DKA by increasing awareness of diabetes, it has been reported that there has been no significant decrease in the frequency of DKA at the time of diagnosis of T1DM cases in Turkey.[12-14] The aim of this study was to evaluate the clinical and laboratory features of patients with newly diagnosed T1DM presenting to Inonu University Medical Faculty Hospital and Malatya Training and Research Hospital between the years of 1996-2019, and to investigate the trend of frequency of DKA over the years.

Material and Methods

Patient data were available from hospital records, which has been a source of reliable information about all diagnoses of pediatric T1DM since 1996. In Malatya, all children and young people (up to the age of 18 years) suspected of having newly diagnosed T1DM are seen at one of the two referral centers by specialists in pediatric endocrinology and diabetes. Each confirmed new case of T1DM is subsequently added to the registry. In addition, Syrian refugees residing in this region were also included in the study. Exclusion criteria included cases with syndromic diabetes, Type 2 diabetes (T2DM), maturity onset diabetes of youth (MODY), secondary causes of diabetes such as cystic fibrosis, steroid use and lipodystrophy, or those with missing data. As a result, a total of 814 T1DM cases diagnosed according to World Health Organization (WHO) criteria were included in the study.

Patients were categorized according to age: <5, 5-9, 10-13 and 14-18 years old. Patients with a diagnosis of T1DM between the years 1996–2019 were included. Patients diagnosed in the first half of the period comprised Period I, and those from the second half comprised Period II. Hyperglycemia was defined as plasma glucose (PG) ≥200 mg/dL. Confirmation of the diagnosis of T1DM was dependent on the presence of hyperglycemia, reliance on continued insulin treatment and presence of typical symptoms of T1DM at presentation. DKA was defined as hyperglycemia concurrent with acidosis (venous blood pH 7.30 and/or serum bicarbonate 15 mmol/L), ketonemia, and/or ketonuria. In addition presenting characteristics were divided into three clinical categories: 1) hyperglycemia without ketosis or acidosis; 2), hyperglycemia with ketosis but without acidosis; and 3) ketoacidosis. For the purposes of this study, ketosis assessment was performed by measuring urinary ketone semi-quantitatively. Thus ketonuria was present when urine ketone was ≥2+. Urinary ketone measurements were performed by an automated analyzer which used sodium nitroprusside reaction as a test principle (BT URICELL 1280-1600 devices (BT products, İzmir, Turkey) in each voiding. DKA severity was classified according to the Lawson Wilkins Pediatric Endocrine Society Consensus Statement[15], as follows: severe DKA (venous pH: <7.10; serum bicarbonate <5 mmol/L), moderate DKA (venous pH: 7.10 – 7.19; serum bicarbonate between 5 and 10 mmol/L), and mild DKA (venous pH: 7.20 – 7.29; serum bicarbonate between 10 and 15 mmol/L). Demographic and clinical features at the time of diagnosis, including gender, birth date, diagnosis date, the season at presentation, duration of symptoms prior to diagnosis, and type of presentation (DKA, hyperglycemia with ketosis or hyperglycemia only) were collected. In addition, laboratory results including venous blood glucose concentration, c-peptide concentration, hemoglobin A1c (HbA1c) percentage and the presence of T1DM-associated autoantibodies such as anti-glutamic

acid decarboxyase antibody (GAD), insulin antibody (IAA) and islet cell antibody (ICA) were recorded from patient files. ICA, IAA, and GADA levels were measured using an enzyme-linked immunosorbent assays based on antigen-antibody detection with the Isletest commercial kit in the Seac Brio 410499 model instrument. The HbA1c level was measured by high performance liquid chromatography using an Agilent 1100 model instrument at our hospital. Ethics Committee Approval was obtained from the Malatya Training and Research Hospital Ethics Committee for the study (approval date: 18.11.2019; approval number: 23536505-604.02).

Statistical analysis

The data are presented as mean±standard deviation (SD) values. Data analysis was performed using SPSS for Windows statistical software, version 17.0 (SPSS, Chicago, IL, USA). Student's two-tailed t-test was used for comparisons between independent variables with a normal distribution. Mann-Whitney U-test was used for variables showing uneven distribution. Distribution was analyzed by cross-tabulation and Chi-square statistics. A p-value of < 0.05 is considered to be statistically significant.

Results

The cohort consisted of 814 patients between the ages of 0.4-18 years. Four hundred and four were female (49.6%) and 410 were male. Across the whole cohort the mean age at diagnosis was 8.5±4.2 years with a range of 0.37-17.52 years. When patients were divided into four groups by age of diagnosis there were 189 patients (23.2%) between 0-4 years old, 319 patients (39.2%) between 5-9 years old, 224 patients (27.5%) between 10-13 years old and 82 patients (10.1%) between 14-18 years old. There were three peak ages at diagnosis, between the ages of 5-7, 8-10 and 12-14 years. When seasonality of diagnosis was examined 28.4% of the patients were diagnosed in winter, 26.7% in autumn, 24.2% in spring and 20.8% in summer.

The mean duration of symptoms prior to diagnosis (polyuria, polydipsia, nocturia) was 18.7±18 days (range 2-90). At the time of diagnosis, mean blood glucose concentration was 478±187 mg/dL (range: 128-1173), mean C-peptide was 0.6±0.5 (0.1-3.8) ng/mL and mean HbA1c was 12.4±2.7% (range: 6.0-22.4%). At diagnosis autoantibodies were assessed in 513 (63%) cases. The antibody test records of the remaining 301 patients were not available. Serum autoantibody levels of 87 patients in Period I and 426 patients in Period II were evaluated. GAD (62.40%) being most common followed by ICA (45.2%) and IAA (29.7%). Although at least one of the three antibodies was positive in 438 (85.4%) of these cases, none of the three antibodies was detected in 75 (14.6%) of the cases. Frequency of autoantibody positivity changed between Period I and Period II; ICA positivity increased from 20.7 to 50.0% ($p<0.001$) and IAA decreased from 44.8 to 26.5% ($p<0.001$) in Period I and Period II, respectively (Table I).

In 566 cases, clinical presentation at diagnosis was evaluated (122 patients in Period I and 444 patients in Period II). The records of the remaining 248 patients were not available. Of the 566 patients for whom data was available, 72 cases (12.7%) presented with hyperglycemia, 230 cases (40.6%) hyperglycemia with ketosis, and 264 cases (46.6%) with DKA. Of those with DKA 103 (39.0%) had mild DKA, 81 (30.6%) had moderate DKA and 80 (30.3%) had severe DKA. Key features of presentation were compared between Period I and Period II. In Period I 54.9% of patients presented with DKA. In Period II this dropped and plateaued at 44.4% after 2007. Over the years, the rate of presentation with DKA has declined (Fig. 1). The frequency of DKA subgroups did not change between periods. Further comparisons between Period I and Period II were made (Table I). Clinical characteristics of our cases according to age groups are shown in Table II.

Table I. Comparison of clinical and laboratory findings in type 1 diabetes by year of presentation.

	Period 1 (1996-2007)	Period 2 (2008-2019)	*p* value
Number of the patients	234	580	
Age (years)	7.6±3.7	8.9±4.28	<0.05
Gender n, (%)			
Female	122 (52.1)	282 (48.6)	NS
Male	112 (47.9)	298 (51.4)	NS
Age Groups n, (%)			
Ages of 0-4 years	67 (28.6)	122 (21.0)	0.020
Ages of 5-9 years	95 (40.6)	224 (38.6)	NS
Ages of 10-13 years	63 (26.9)	161 (27.8)	NS
Ages of 14-18 years	9 (3.85)	73 (12.7)	<0.001
Duration of symptoms before diagnosis (days)	18.9±16.4	18.6±18.7	NS
Glucose (mg/dL)	465.6±193	483±185	NS
C-peptide (ng/mL)	0.6±0.4	0.6±0.5	NS
HgA1C (%)	13.3±3.5	12.0±2.4	<0.001
Main presenting feature			
Hyperglycemia n, (%)	11 (9.0)	61 (13.7)	NS
Ketosis n, (%)	44 (36.1)	186 (41.9)	NS
Ketoacidosis n, (%)	67 (54.9)	197 (44.4)	0.039
DKA			
Mild DKA n, (%)	22 (32.8)	81 (41.1)	NS
Moderate DKA n, (%)	20 (29.9)	61 (31.0)	NS
Severe DKA n, (%)	25 (37.3)	55 (27.9)	NS
Autoantibody positivity			
Anti-GAD positivity n, (%)	56 (64.4)	262 (61.5)	NS
ICA positivity n, (%)	18 (20.7)	213 (50.0)	<0.001
IAA positivity n, (%)	39 (44.8)	113 (26.5)	<0.001

GAD: Glutamic acid decarboxylase antibody, IAA: Insulin antibody, ICA: Islet cell antibody, HgA1C: Glycosylated hemoglobin, DKA: Diabetic ketoacidosis
NS: Not significant.

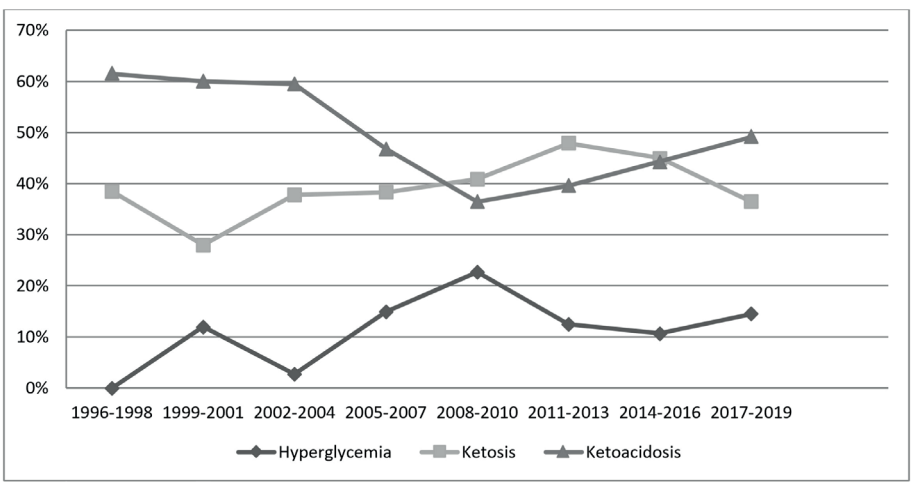

Fig. 1. Change of admission characteristics of patients with type 1 diabetes mellitus over the years.

Table II. Clinical characteristics of cases according to age groups.

Age group (years)	0-4	5-9	10-13	14-18	Total (0-18)
Main presenting feature (n=566)					
Hyperglycemia n, (%)	9 (7.0)	24 (11.7)	30 (18.4)	9 (12.9)	72 (12.7)
Ketosis n, (%)	59 (46.1)	78 (38.0)	58 (35.6)	35 (50.0)	230 (40.6)
Ketoacidosis n, (%)	60 (46.9)	103 (50.2)	75 (46.0)	26 (37.1)	264 (46.6)
Severity of DKA (n=264)					
Mild DKA n, (%)	27 (45.0)	44 (42.7)	23 (30.6)	9 (34.6)	103 (39.0)
Moderate DKA n, (%)	10 (16.6)	31 (30.0)	31 (41.4)	9 (34.6)	81 (30.6)
Severe DKA n, (%)	23 (38.3)	28 (27.3)	21 (28.0)	8 (30.7)	80 (30.3)

DKA: Diabetic ketoacidosis

Discussion

Incidence of T1D is increasing all over the world.[2-4] T1DM is one of the most common chronic endocrine diseases of childhood and adolescence and its incidence has been reported to vary with age, ethnicity, geographical region and season.[16] When the gender distribution of our diabetic patients was examined, the female/male ratio was 0.98 with no significant gender bias (p=0.64). Although autoimmune diseases tend to be more common in girls compared to boys, there is no gender difference in children and adolescent T1DM cases[17], in keeping with our findings. The mean age of diagnosis in our cohort was found to be 8.5±4.1 years. Poyrazoğlu et al.[18] in a study of T1DM patients aged <18 years reported a mean age of diagnosis of 9.2 years. Ardicli et al.[14] conducted a study between the years 1969-1991 and the average age of diagnosis was found to be 9.5 years. In our study, the average age of diagnosis between 2008 and 2019 had increased significantly to 8.9 years. The reason for this may be that the oldest age group (14-18 years) only made up 3.8% of all diagnoses in the earlier period of our study but this increased three-fold (12.7%) in the period between 2008-2019. Similarly, in the present study, diagnosis of cases in the youngest age group (0-4 years) fell from 28.6% in the earlier period to 21% between 2008 and 2019.

T1DM is generally reported to exhibit a bimodal distribution in age of diagnosis, with peaks at the age of 5-7 years and puberty. The first peak is thought to occur due to common infections, and the second peak occurring during puberty is due to stress and increased concentrations of circulating gonadal hormones.[19,20] In a multicenter study conducted by the European Diabetes Study Group[21] on 15,000 diabetics, when patients were divided into three groups (0-4 years, 5-9 years, and 10-14 years) the frequency of diabetes was 24%, 35%, and 41%, respectively, increasing with age. Demir et al.[12] reported that T1DM was seen in two separate age groups, most frequently between the ages of 6-8 and the second most frequently between 11-12. In our study group, the frequencies of diabetes in 0-4, 5-9, 10-13, 14-18 age groups were 23.2%, 39.2%, 27.5% and 10.1%, respectively. In addition, the frequency of the occurrence of T1DM in our study was found to peak in three different age groups, 5-7, 8-10 and 12-14 years old. While the first peak was detected in early childhood, the second peak was detected in early puberty and the third peak in late puberty.

First presentation with T1DM has been reported to be seasonal. Seasonal variations in onset of T1DM were reported in many population-based studies.[22,23] Similarly, in our study, it was observed that most presentations occurred in winter and the lowest number was in summer. The increasing frequency of viral infections in winter is thought to trigger the emergence of diabetes by increasing the need for insulin. The average symptom duration prior to diagnosis has been reported as 14 days from studies in Australia and Sweden[24,25] in patients with new diagnosis T1DM. In our study this was found to

be slightly longer at 17.8 days. In our opinion this stems from the relatively poorer socioeconomic level of our region, more difficult accessibility to health institutions, cultural differences and geographical factors.

In T1DM, the destruction of pancreatic islet beta cells occurs through T cell-mediated cellular mechanisms in 80-90% of patients. However, autoantibodies are present at diagnosis in a significant proportion of patients. Kong et al.[26] detected at least one of three antibodies in 71.4% of patients during the diagnosis of type 1 diabetic patients, including GAD in 66.2%, ICA in 54.1% and IAA in 35.6% in patients who were antibody positive. Demir et al.[12] found GAD (70%) antibodies to be most common, then ICA (44.4%) and IAA (42.6%), respectively, in patients with T1DM. In our study at least one of these three antibodies were positive in 85.4% of the cases, with GAD (62.40%) being most common followed by ICA (45.2%) and IAA (29.7%). When the rates of antibody positivity were compared between Period I and Period II there was no difference in GAD positivity while ICA positivity significantly increased from 20.7% to 50.0% and, at the same time, the IAA decreased significantly from 44.8% to 26.5%. The reason for this change has not been fully explained.

The first clinical presentation of patients with T1DM might range from hyperglycemia to diabetic coma. Demirbilek et al.[13] reported the frequency of DKA at first presentation to be 65.0% in their study between the years 2010-2011. Ardicli et al.[14] reported the frequency of diagnosis with DKA to be 50.5% between 1990-2000 and 51.0% between 2000-2010. Acar et al.[27] found the incidence of DKA to be 43.2% in patients with a new diagnosis of T1DM between 1999 and 2014. In our cohort the overall rate of DKA presentation was 46.6%, which is consistent with earlier Turkish reports. The frequency of DKA is lower in countries where T1DM is more frequent, such as Finland (22.0%), Sweden (14.0%), and Canada (18.6%), whereas T1DM prevalence is higher in countries with low prevalence including the Arab peninsula (80.0%), Romania (67.0%), and Taiwan (65.0%).[11] It was reported that rates of DKA ranged between 26 and 67% in a 24-center study of the European Diabetes Study Group[28], covering 1260 cases. In the same study, it was highlighted that the incidence of DKA was higher in patients aged under 5 years of age compared to previous years. In New Zealand, Jackson et al.[29] reported that the frequency of DKA, which they monitored over an eight-year period, decreased from 63 to 42%, and that the blood glucose and HbA1c values in the newly diagnosed patient group were significantly lower compared to previous years. As a result of our 24-year follow-up it was found that the frequency of DKA decreased, and the frequency of admissions with hyperglycemia and ketosis increased. In Period I the frequency of presentation with DKA was 54.9%, which decreased to 44.4% in Period II. There is a need to increase both public awareness and to ensure that physicians are alert to the possibility of T1DM, even in young patients.

The severity of DKA at presentation has been previously investigated. Acar et al.[27] found severe DKA in 22.1%, moderate in 32.8% and mild in 45.1% at the time of first presentation. In a study from Germany[30], the majority of these cases (63.8%) had no ketoacidosis, while 16.6% had mild, 12.1% moderate, and 4% severe ketoacidosis at the time of diagnosis. These rates in our cohort were 30.3%, 30.6% and 39.0% for severe, moderate and mild DKA, respectively. In addition, the frequency of severe DKA was higher in the 0-4 years-old age group. The frequency of presentation with severe DKA was higher in the earlier period compared to the later period, 34.8% vs 26.3%.

The decrease in frequency of first presentation with both DKA and severe DKA was interpreted as a positive development towards likely decreased mortality and morbidity. However, in Turkey, evidence from multiple studies have shown that the frequency of DKA at first presentation is still high and has variable outcome. We recommend multicentre studies to clearly determine the national frequency

of DKA. This will provide the foundation for development of measures, involving pediatric endocrinologists, pediatricians and family physicians, in order to increase general public awareness of the first signs and symptoms of diabetes and improve the duration between first signs and presentation to a suitable specialist for assessment. For this purpose, joint seminars, public meetings, and training days in schools can be organized. In addition, social media, which is both ubiquitous and an effective communication tool, should be used. Furthermore, improved accessibility to health centers and increased socioeconomic opportunities will encourage more rapid patient presentation.

This study has some limitations. In this retrospective study, a number of factors which may have affected our results were not assessed. These included family socio-economic status and parental education level, the status of refugees, the ease of availability of health care, the number of presentations before definite diagnosis, the type of hospital used for first presentation, the status of the pediatric endocrinology centers in the region, and the number of pediatricians involved.

The rate of first presentation with DKA in T1DM cases decreased at our city between the years 1996-2019. The proportion of patients presenting with severe DKA did not change. Interestingly and in contrast to most reports, the proportion of patients presenting in the earliest age group, 0-4 years also decreased while the proportion of patients presenting in late adolescence increased. This resulted in the average age of diagnosis increasing and, as younger patients are more likely to present with DKA, may have influenced the decreasing rate of DKA at presentation. There were three peak ages of diagnosis evident in our cohort; at the ages of 5-7, 8-10, and 12-14 years. It is suggested that the rate of DKA at presentation remains a problem in Turkey, as has previously been shown by several studies.

Ethical approval

Ethics Committee Approval was obtained from the Malatya Training and Research Hospital Ethics Committee for the study (approval date: 18.11.2019; approval number: 23536505-604.02).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: İD, NÇ, AA, EÇ; data collection: İD, NÇ; analysis and interpretation of results: İD, NÇ, AA, EÇ, LK, ÖN; draft manuscript preparation: İD, NÇ, AA, EÇ, LK, ÖN. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The potentially harmful excipients in prescribed medications in a Neonatal Intensive Care Unit in Kosovo and available safer alternatives

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ABSTRACT

Background. Medicinal products contain excipients that might be associated with toxicity in neonates. The aim of this study was to investigate the administration of medication containing potentially harmful excipients to neonates hospitalized in Kosovo and to identify the possibility of reducing neonatal exposure to these excipients through product substitution.

Methods. Data on all medication administered to hospitalized neonates from 1st of February to 1st of August 2018 along with patients' demographic data were collected from medical records for each neonate. Excipients were identified from the Summaries of Product Characteristics. Three stage criteria for product substitution were: (1) same active pharmaceutical ingredient (API) and route of administration; (2) 1 plus same dosage form; (3) 1 and 2 plus same strength.

Results. In total, 100 excipients were found in 2388 prescriptions comprising 67 medications and 60 API administered to 294 (183 preterm and 111 term) hospitalized neonates. The excipients of interest (EOI) were present in 409 (17.1%) prescriptions and were administered to 131 (71.6%) preterm and 52 (46.8%) term neonates through a relatively small number of products (n=27; 32.8%). In relation to prescription frequency, the most common EOI was polysorbate 80, found in 229 (56%) of EOI-containing prescriptions. Substitution with EOI-free products was possible for 14 (63.6%), 12 (54.5%) and 5 (22.7%) products, according to the first-, second- and third-stage criteria, respectively.

Conclusions. We have provided the first detailed description of neonatal exposure to potentially harmful excipients among neonates admitted to a neonatal intensive care unit in Kosovo. Unnecessary exposure could be reduced by using EOI-free products available in the local medicine market. Collaborative initiative is required to build up the evidence on the use of EOI in neonates and raising awareness among health care professionals on use of products without EOI where possible.

Key words: neonates, medication, excipients of interest, neonatal intensive care unit, substitution.

Pharmaceutical excipients are important chemical constituents of medications to overcome challenges such as solubility, stability and bioavailability of the active pharmaceutical ingredient (API). They also play a critical role in formulating, assuring the quality and patient acceptability of the medicine.[1-4] The strategy for the selection of excipients is a complicated task in pediatric medicine development. It requires various considerations such as acceptable taste, age, dosage forms, among others to be accounted for in order to select safe excipients.[5] Concerns

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about their safety are being more evident due to the increasing number of reports and awareness on adverse effects, especially in neonates.[6] Adverse effects are prominent in neonates and primarily preterm neonates because due to their physiological and developmental immaturity they may not be able to handle an excipient in the same way as adults.[7] In fact, tragedies have occurred after the inclusion of certain excipients in products used in neonates.[8] Vulnerability of the neonates to excipients has been addressed in the European Medicines Agency (EMA) guideline on excipients that states "excipients to be used in formulations for the pediatric population should be selected with special care, and possible sensitivities of the different age groups should be taken into consideration".[7,9] Moreover, there is a new labelling guidance that should bring quantitative data about excipients to the Summary of Product Characteristics (SPC) and patient information leaflet (PIL) for new formulations.[10] However, age-appropriate and excipient-low neonatal formulations are still scarce. Consequently, neonates may be at risk of relevant excipient exposure causing clinical harm.[7]

In order to enable researchers to more easily review important excipients, a list containing "excipients of interest" (EOI) has been proposed.[11] Unfortunately, the use of these potentially harmful excipients in medicine administered to neonates is not a rare case in practice, as demonstrated in some previous studies.[12-17] However, this issue remains largely unknown. On the other hand, according to a previous study[18], exposure of neonates to these EOI could be reduced by using EOI-free formulations.

We are not aware of any study relating to EOI exposure among neonates in a European country that is not included in two previous European studies[15,18] on these topics. Given the need to reduce the prescription of EOI containing medications in the neonatal population and the lack of such studies in Kosovo, we aimed to investigate, based on a previous study[17], the exposure to EOI among neonates admitted to a neonatal intensive care unit (NICU) in Kosovo and to identify substitution possibilities for medication containing these EOI.

Material and Methods

Subjects and study design

This retrospective study was conducted at the NICU, Department of Neonatology, University Clinical Centre of Kosovo (UCCK) from 1st of February to 1st of August 2018.

The following data were extracted from medical records for each hospitalized neonate (postnatal age ≤28 days) in the NICU during the period of this study: demographic data (gestational age, gender, birth weight, date of birth), admission and discharge days, and data concerning each prescribed medication (international non-proprietary name, trade name, pharmaceutical dosage form, strength and route of administration). Data regarding prescriptions for glucose and electrolyte solutions, vaccines and blood products were excluded from the study.

Neonates were categorized based on their gestational age (GA) as extremely preterm (<28 weeks), very preterm (28 to <32 weeks), late preterm (<37 weeks) and term neonates (≥37 weeks).[19]

Identification of excipients in prescribed medications for hospitalized neonates

The excipient content of each prescribed medicine was identified from the SPC and/or manufacturers' websites. Our research, based on previous studies[15-17], was focused on the EOI recognized as potentially harmful to neonates, namely benzalkonium chloride, benzyl alcohol, benzoic acid, sodium benzoate, ethyl-, methyl-, and propylparaben, ethanol, propylene glycol,

polysorbate 80 saccharin sodium and sorbitol.[15]

Analysis of the possibility of products' substitution

Criteria for product substitution proposed by Nellis et al.[18] and applied in our study were: stage 1: Medication could be substituted with a product with the same API and route of administration; stage 2: Stage 1 plus the requirement of the identical dosage form; stage 3: Stages 1 and 2 plus the requirement of identical strength of the API. For the analysis of the possibility of products' substitution, a search on the Kosovo Medicines Agency's (KMA) database on the list of products available locally[20] was conducted by using the name of each API. Only EOI-free products were considered for substitution in the analysis.

Statistical analysis

Data were stored in Excel for Windows version 10 and analyzed in the Statistical Package for the Social Sciences version 20.0. Patients' data were analyzed using descriptive statistical methods (percentage proportion, mean and standard deviation [SD]). Nominal data were described as the quantity (n) and percentage with a 95% confidence interval [CI]. Categorical data were analyzed with $\chi 2$ test (2×2 tables). Data with p value <0.05 were regarded as statistically significant. Categorical data were also described as the quantity and percentage proportion.

The study was approved by the Professional-Ethics Committee of the University Clinical Center of Kosovo, under Protocol No. 853/2019. As we did not record personal identifying data for the neonates and no intervention was performed on patients, we did not seek consent for participation in the study.

Results

Characteristics of the neonates and prescribed formulations

During the study period, 299 neonates were admitted to the NICU, among whom 5 (1.7%) with incomplete data were excluded. The 294 hospitalized neonates (183 (62.2%) preterm) included in the study received 2388 prescriptions for 67 different medications and 60 different API (Table I). Intravenous formulations were used most often (35/67; 52.2%), followed by oral formulations (18/67; 26.9%) of which 10 (55.6%) were oral solids such as manipulated tablets, 7 (38.9%) oral liquids and 1 (5.6%) oral gel. Inhalation (5; 7.5%), ophthalmic (4; 6.0%), topical (2; 3.0%); intramuscular (1; 1.5%), rectal (1; 1.5%) and subcutaneous (1; 1.5%) medication were scarcely used.

Table I. Demographic characteristics of neonates admitted to the NICU.

Characteristics	Extremely preterm	Very preterm	Late preterm	Term
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)
Neonates	17 (5.8) (3.1 to 8.5)	45 (15.3) (11.2 to 19.4)	121 (41.1) (35.5 to 46.8)	111 (37.7) (32.2 to 43.2)
Gestational age, weeks (mean ± SD)	26±1.5 (22 to 27)	30±0.8 (29 to 31)	34±1.4 (32 to 36)	39±1.3 (37 to 42)
Birth weight (g)	966 (450 to 1370)	1555 (980 to 4600)	2030 (1200 to 3150)	3395 (2200 to 5000)
Gender				
Female	11 (64.7)	17 (37.8)	49 (40.5)	40 (36.0)
Female receiving EOI	7 (63.6)	16 (94.1)	19 (38.8)	16 (40.0)
Male	6 (35.3)	28 (62.2)	72 (59.5)	71 (64.0)
Male receiving EOI	3 (50.0)	27 (96.4)	59 (81.9)	36 (50.7)

EOI: excipients of interest

The extent and nature of EOI administration

Information on excipients was available for all 67 medications. The total number of excipients in all the studied medications was 100. Almost three-quarters of preterm (131/183; 71.6%) and one-half of term (52/111; 46.8%) neonates were exposed to at least one EOI. In relation to GA, very preterm neonates were most frequently exposed; in this subgroup, almost all (43/45; 95.6%) neonates received at least one EOI. There was a difference between genders in the neonates' subgroups exposed or not exposed to EOI, but this difference was statistically significant only in the late preterm subgroup ($\chi 2$ test, $p<0.05$; (Table I).

EOI were present in 409 (17.1%) prescriptions. Of prescriptions, 81.6% (1949) were parenteral, however only 107 (5.5%) contained EOI. In relation to the route of administration and frequency of prescriptions containing EOI, oral prescriptions were the most common (243/409; 59.4%) (Table II).

Overall, 22 of 67 (32.8%) medications contained at least one EOI, 50% of these medications (11/22) contained more than one EOI. Intravenous formulations were most frequently used, but only 16.7% (6/36) of these formulations contained EOI. The proportion was higher in ophthalmic, oral, inhalation and topical medications: 25.0% (1/4), 55.6% (10/18), 60.0% (3/5) and 100% (2/2), respectively (Table III). Antimicrobial preservatives were found in 15 products, solvents in 8 products, sweetening agents in 7 products and solubilizing agents in 6 products. Concerning the specified excipient, methylparaben, found in 8 products, was the most common EOI, but it was present in only 16.6% (68/409) of prescriptions that contained EOI. In relation to prescription frequency, the most common EOI was polysorbate 80, found in 56.0% (229/409) of EOI-containing prescriptions. None of the formulations contained ethylparaben (Table III).

Analysis of the possibility of products' substitution

Regarding the possibility of product substitution proposed by Nellis et al.[18] when applying the first-stage criteria, the substitution of medications containing EOI with EOI-free counterparts in Kosovo was possible for 14/22

Table II. Number of prescriptions and EOI prescribed by route of administration according to gestational age category.

Characteristics	Extremely preterm	Very preterm	Late preterm	Term
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)
Prescriptions (total n)	149	506	1027	706
Prescriptions with EOI (total n)	19 (12.8)	94 (18.6)	182 (17.7)	114 (16.1)
Intravenous	120 (80.5)	339 (67.0)	712 (69.3)	483 (65.7)
Intravenous with EOI	12 (10.0)	18 (5.3)	39 (5.5)	38 (5.0)
Intramuscular	17 (11.4)	45 (8.9)	121 (11.8)	111 (15.7)
Intramuscular with EOI	0	0	0	0
Oral	7 (4.7)	100 (19.8)	162 (15.8)	68 (9.5)
Oral with EOI	4 (57.1)	64 (64.0)	123 (75.9)	52 (76.1)
Inhalation	5 (3.4)	16 (3.2)	28 (2.7)	29 (7.2)
Inhalation with EOI	3 (60.0)	11 (68.8)	18 (64.3)	20 (72.5)
Other	0	6 (1.2)	4 (0.4)	15 (2.1)
Other with EOI	0	1(16.7)	2(0.5)	4(26.7)

EOI: excipients of interest, n: number of prescriptions, 95% CI: 95% confidence interval.
Other: topical, ophtalmic, rectal and subcutaneous prescriptions.

Table III. Medications containing EOI, availability of EOI-free products in Kosovo's medicine market and prescriptions to neonates.

Medications containing EOI	Excipient (intended use[25])	Product substitution (stage)	Extremely preterm n= 19 (%)	Very preterm n=94 (%)	Late preterm n=182 (%)	Term n=114 (%)
Vitamin D+K oral drops	Polysorbate 80 (solubilizer)	Available (3)	2 (10.5)	40 (42.6)	89 (48.9)	37 (32.5)
Phenobarbital injection	Propylene glycol (solvent)	Not available	10 (52.6)	10 (10.6)	21 (11.5)	24 (21.1)
Dexamethasone injection*	Methyl- and propylparaben (preservatives), benzyl alcohol (preservative)	Available (3)	2 (10.5)	9 (9.6)	14 (7.7)	15 (13.2)
Multivitamin oral drops	Polysorbate 80 (solubilizer), sodium benzoate (preservative)	Another formulation (different strength) is available (2)	1 (5.3)	12 (12.8)	13 (7.1)	4 (3.5)
Methylprednisolone injection	Benzyl alcohol (preservative)	Available (3)	1 (5.3)	3 (3.2)	9 (4.9)	10 (8.8)
Nystatin powder for oral suspension	Propylene glycol (solvent), saccharin sodium (sweetener), polysorbate 80 (solubilizer), methyl- and propylparaben (preservatives), ethanol (preservative)	Not available	0	2 (2.1)	5 (2.7)	9 (7.9)
Vitamin C oral drops	Sorbitol (sweetener)	Two formulations (tablets and a syrup with different strengths) are available for manipulation (2)	0	5 (5.3)	6 (3.3)	0
Salbutamol aerosol	Ethanol (solvent)	Available (3)	1 (5.3)	4 (4.3)	2 (1.1)	2 (1.8)
Budesonide nebulization suspension	Polysorbate 80 (solubilizier)	EOI-free powder for inhalation is available (2)	1 (5.3)	1 (1.1)	4 (2.2)	2 (1.8)
Hydrocortisone injection	Benzyl alcohol (preservative)	Not available	0	2 (2.1)	3 (1.6)	1 (0.9)
Ursodeoxycholic acid oral suspension	Benzoic acid (preservative), propylene glycol (solubilizier)	EOI-free capsules available for manipulation (1)	0	0	5 (2.7)	0
Spironolactone tablets	Polysorbate 80 (solubilizer)	Available (3)	1 (5.3)	3 (3.2)	0	0
Hyaluronic Acid cream	Sorbitol (humectant), methyl- and propylparaben (preservatives)	Another formulation (impregnated gauze) is available (1)	0	1 (1.1)	1 (0.5)	2 (1.8)
Salbutamol nebulization solution	Benzalkonium chloride (preservative)	An EOI-free formulation (different strength) is available (2)	0	0	1(0.5)	3 (2.6)

*Dexamethasone injection was administered in two different routes of administration (intravenously and by inhalation).
EOI: excipients of interest, n: number of prescriptions containing EOI.

Table III. Continued.

Medications containing EOI	Excipient (intended use[25])	Product substitution (stage)	Extremely preterm n= 19 (%)	Very preterm n=94 (%)	Late preterm n=182 (%)	Term n=114 (%)
Ibuprofen syrup	Sorbitol (sweetener), sodium benzoate (preservative), saccharin sodium (sweetener), polysorbate 80 (solubilizier)	Syrup is not available. Granules for oral solution (different strength) are available (2)	0	2 (2.2)	1 (0.5)	0
Miconazole oral gel	Saccharin sodium (sweetener), benzoic acid (preservative), ethanol (preservative)	Not available	0	0	2 (1.1)	1 (0.9)
Tetracycline ophthalmic ointment	Methylparaben (preservative)	Not available	0	0	1 (0.5)	1 (0.9)
Sodium valproate syrup	Methyl- and propylparaben (preservatives), saccharin sodium (sweetener)	An oral solution and (different strength) are available (2)	0	0	1 (0.5)	1 (0.9)
Gentamicin injection	Methyl- and propylparaben (preservatives)	Not available	0	0	1 (0.5)	1 (0.9)
Prostaglandin E1 injection	Ethanol (solvent)	Not available	0	0	2 (1.1)	0
Heparin sodium gel	Methyl- and propylparaben (preservatives), ethanol (solvent)	Another formulation (cream) is available (2)	0	0	0	1 (0.9)
Cyproheptadine syrup	Saccharin sodium (sweetener), methyl- and propylparaben (preservatives), ethanol (preservative)	Not available	0	0	1 (0.5)	0

*Dexamethasone injection was administered in two different routes of administration (intravenously and by inhalation).
EOI: excipients of interest, n: number of prescriptions containing EOI.

(63.6%) products. By adding the second and third stage criteria, the possibility of product substitution was reduced to 12/22 (54.5%) and 5/22 (22.7%), respectively (Table III). For example, ursodeoxycholic acid oral suspension, which contains benzoic acid and propylene glycol, could be substituted with manipulated EOI-free capsules (first-stage criteria). In addition, it is possible to substitute vitamin C oral drops (contain sorbitol) with vitamin C oral solution (second-stage criteria), and methylprednisolone injection 40 mg/mL (contains benzyl alcohol) with EOI-free methylprednisolone injection 40 mg/mL (third-stage criteria) (Table III).

Discussion

Our study shows that while EOI were present in one-third of prescribed medications, approximately two-thirds of hospitalized neonates were exposed to at least one of these EOI. Generally, our data are in line with previous studies[15-17], which also reported a small number of medications containing EOI, provided that in our study the frequency of exposure was higher, suggesting that a few, commonly prescribed medications cause a high frequency of EOI exposure and that substitution of this small portion of products containing EOI with EOI-free counterparts could spare a large number of neonates from exposure to these harmful excipients.[15,16,18] Our findings on the opportunities for products' substitution make this suggestion even more reasonable; we identified a relatively high possibility of products' substitution and demonstrated that substitution of products that had alternatives with the same API as well as dosage forms would significantly reduce the exposure to EOI-containing prescriptions.

The proportion of prescriptions containing at least one EOI in our study was the same as that reported by Saito et al.[16] (17%), whereas studies performed by Nellis et al.[15] and Sviestina and Mozgis[17] reported higher percentages of these prescriptions (31% and 28%, respectively). We assume that differences in clinical settings protocols, as well as antibiotic prescribing practices, may account for this difference with the two latter studies. For example, gentamicin injection contains two EOI. In our study, there were only two prescriptions for gentamicin injection, while this medicine accounted for the largest number of EOI-containing prescriptions in the Latvian study.[17]

An important factor associated with EOI exposure was the route of administration. The main route of administration in hospitalized neonates is parenteral.[21] However, parenteral formulations may not require some kind of excipients, for example antimicrobials, as they can be produced in single dose vials. In our study, 84% of parenteral formulations and 77% of parenteral prescriptions did not contain EOI at all. These findings are similar to those reported by Sviestina and Mozgis[17], who reported that only 13% of parenteral products and 17% of parenteral prescriptions contained EOI. Moreover, in the multi-country study, only 15% of parenteral prescriptions contained benzoates and parabens, suggesting that these excipients could be avoided.[15]

Polysorbate 80 was the most frequently present EOI in prescribed medications, even though it was found in a smaller number of products compared to methylparaben. Our results are similar to two previous studies looking at the specified EOI present in administered medications to neonates. Souza et al.[13] reported higher exposure to polysorbate 80 compared to methylparaben (73% vs 57% of neonates). Similarly, Saito et al.[16] demonstrated that while only 0.03% of neonates received parabens, 23% were exposed to polysorbate 80. The main reason for high exposure to polysorbate 80 in our study could be its presence in vitamin D+K preparation given to 57% of neonates, which is used for daily prevention of late hemorrhagic disease and rickets to all neonates from the 8th day of life. Importantly, we demonstrated that this product could be substituted according to the "ideal" criteria with an EOI-free counterpart in the local market. Moreover, given that the use of this product continues after hospitalization,

we suggest that the overall cumulative benefit would be greater. On the other hand, we found no substitution possibility for phenobarbital, the second most commonly used EOI-containing medicine that exposed 68 neonates to propylene glycol, whereas EOI-free injectable phenobarbital formulations are available in the European market.[18]

Antimicrobial preservatives should be included in medications only if absolutely needed.[9] Not surprisingly, the pharmaceutical industry is encouraged to consider and develop novel strategies that allow commercialization of preservative-free products since mainly liquid dosage forms are used in these vulnerable patients. In our study, 68% of medications contained these EOI. However, we also demonstrated that 60 % of medications containing these excipients could be substituted with EOI-free alternatives. In our study, parenteral medications contained antimicrobials and solvents. Of the 4 parenteral formulations containing only antimicrobial preservatives, we found substitution opportunities for 2 of them, whereas substitution was not possible for parenteral prostaglandin E1 containing ethanol, suggesting that the availability of products free from EOI may be related to the role of specified excipient in a formulation.[15,18] This could be explained by the fact that is easier to avoid preservatives like parabens in the manufacturing process of single-dose parenteral medications than avoiding a solvent like ethanol. The situation is even more complex in the case of oral multi-dose liquid formulations where excipients like sweetening agents are needed to improve consistency and palatability.[18,21] Our results show that 6 oral medications (60%) contained sorbitol and saccharin sodium. However, substitution was possible for two-thirds of them, whereas in the study performed by Sviestina and Mozgis[17] no substitution possibilities for oral medications containing sweeteners were available.

As expected, the possibility of substitution was not the same for all products. For the majority of products, it would be necessary to ignore the requirement for an identical dosage form. The proportion of products containing EOI that could be substituted, according to three stage criteria (first, second and third, respectively), with EOI-free products in our study was lower than that reported by Nellis et al.[18] (88%, 66% and 31%, respectively), but higher than in the study performed by Sviestina and Mozgis[17] (31%, 24% and 14%, respectively). Regional characteristics (e.g., products marketed in each country) may have had an influence on these differences.

Excipients may be harmful not only to neonates, but also to other patient populations such as critically ill adults, and children.[22] However, considering the greater vulnerability of neonates, especially critically ill ones, to the toxicity of drugs and excipients, and little is known about this subject, this research was focused on this particularly vulnerable patient population. Almost two-thirds of our study population consisted of preterm neonates and this further increases the importance of our findings.[13,23,24] The possible difference in the number of patients exposed to at least one EOI between preterm and term neonates may be polypharmacy, comorbidity or length of hospital stay.

Considering our findings, which indicate that health care professionals (HCP) lack awareness about the safety of excipients, the focus should be on raising prescriber's awareness about the excipients present in medications used in neonates by offering information and education about EOI and integrating scientific evidence in the decision-making process. Another solution-oriented approach could be the development of alert systems that calculate the number and ratio of excipients in concomitant medications with a patient-centered approach and alert them through integrated Computerized Physician Order Entry (CPOE). In addition, when compiling a formulary for the treatment of neonates, attention should be paid to the identification of EOI in medications

and substitution of these EOI-containing medications with EOI-free counterparts available in the local market. We agree with Nellis et al.[18] that substitution may incur additional costs as different stakeholders such as financial, regulatory and hospital specialists are involved. However, considering the overall health benefit of this susceptible patient group, this should be considered a valuable approach in sparing many neonates from exposure to these potentially harmful excipients.

Some limitations should be considered. There was limited information about quantitative data on excipients (single and daily dose of the excipient). In addition, this study was conducted in a small country with a developing health care system, which can make comparison with the results of other countries more difficult.

We have provided the first detailed description of neonatal exposure to potentially harmful excipients among neonates admitted to a NICU in Kosovo. Neonates in our country are exposed to several EOI which might be associated with toxicity. This exposure could be prevented or reduced by using EOI-free products available in the local market. However, collaboration is required to build up the evidence on the use of EOI in neonates and to raise the awareness among HCP on the use of products without EOI where possible, in accordance with the best practice standards. Our findings also constitute an important contribution to the strengthening of the national regulatory environment for pediatric medications registration in Kosovo.

Further studies are required to determine a balance of risks which include potential toxicity, exposure to EOI and safe substitution of medications as well as cost considerations.

Ethical approval

The study was approved by the Professional-Ethics Committee of the University Clinical Center of Kosovo, under Protocol No. 853/2019.

Author contribution

Study conception and design: BK, AG and RR; data collection: RM, BK and AG; analysis and interpretation of results: DN and PS; draft manuscript preparation MD, MC and RR. 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

Glomerulonephritis with crescents in childhood; etiologies and significance of M2 macrophages

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ABSTRACT

Background. Crescentic glomerulonephritis (CGN) is a rapidly progressive and rare cause of glomerulonephritis in childhood. The aim of this study is to evaluate demographic data of children with crescentic glomerulonephritis, to classify the etiologies and to investigate the correlation between the severity of kidney disease and the expression of CD163+ macrophages.

Methods. Between the years 2000 and 2016 in a single center, patients under 18 years of age with kidney biopsies containing crescents were included in the study. A total of 88 children were enrolled. The expression of CD163 in kidney tissues was detected by immunohistochemistry in 61 patients. Clinical features and outcome were collected from their medical records.

Results. The most common etiology was Henoch-Schönlein purpura (HSP) nephritis/Immunglobulin A vasculitis (26.1%), followed by lupus nephritis (22.7%) and idiopathic crescentic glomerulonephritis (18.2%). CD163 positive cell counts in patients with GFR levels less and more than 60 ml/min/1.73 m^2 at their last visit were 7.6±6.6 cells vs. 2.0±3.0 cells (p=0.057) per one glomerulus and 52.2±18.2 cells/hpf vs. 33.3±10.0 cells/hpf (p <0.05) in tubulointerstitium, respectively. Tubulointerstitital CD163+ cells were also found to be higher in patients with end stage kidney disease than complete and partial responders (68 cells/hpf vs 39 cells/hpf, p<0.05).

Conclusions. CD163 positive cell counts, particularly in tubulointerstitial areas, have been associated with poor prognosis of CGN.

Key words: crescentic glomerulonephritis, CD163 macrophages, M2 macrophages.

Crescentic glomerulonephritis (CGN) is a rare entity in childhood. Membranoproliferative glomerulonephritis (MPGN), immunoglobulin A nephropathy (IgAN), Henoch-Schönlein purpura (HSP) nephritis/IgA vasculitis, post-streptococcal glomerulonephritis, lupus nephritis, polyarteritis nodosa and ANCA associated vasculitis may cause CGN.

A few clinicopathologic data are available in childhood CGN in English literature. Altough the exact mechanism of crescent formation is unclear; it has been thought of as a nonspecific response of the parietal epithelial cells of Bowman capsule against an injury of glomerular capillary wall. Polymorphonuclear neutrophils, lymphocytes ($CD4^+$ and $CD8^+$ cells) and macrophages have primary roles in the pathogenesis.[1,2] Moreover, infiltration of macrophages is a universal characteristics of glomerular injury both in human and experimental studies.[3-7] Macrophages are

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divided into two groups as M1 and M2 according to the expression of different surface molecules and transcription factors.[4] M1 macrophages are activated classically, promote T helper (Th) 1 responses and produce proinflammatory cytokines.[8,9] Whereas M2 macrophages are activated in alternative way.[5] They contribute to tissue repair and promote Th2 responses. CD163 is the marker of M2 macrophages and is a member of the cysteine-rich scavenger receptor family.[4] An experimental study with crescentic glomerulonephritis rat models demonstrated that inflammatory lesions in the first 14 days changed to chronic fibrotic damage while a classically activated proinflammatory M1 phenotype was turning to an alternatively activated M2 phenotype from 14 to 35 days.[10] In a clinical study related with proliferative glomerulonephritis, CD163 as a marker of M2 was found to be more prominent in crescentic samples, suggesting CD163 involvement in pathogenesis in CGN.[4] Zhao et al.[5] also reported that CD68 and CD163 positive cells and polymorphonuclear neutrophils were seen more in glomerulus with crescents than only focal necrotizing glomerulus in patients with pauci immune glomerulonephritis, anti-GBM glomerulonephritis and immune complex induced glomerulonephritis. Additionally, a few clinical studies of proliferative glomerulonephritis suggested a correlation between the disease severity and CD163 positive macrophages infiltration. However, the data regarding the relationship between the prognosis of the crescentic glomerulonephritis and the number of $CD163^+$ macrophages (M2) is still scarce.

In this study, we aimed to evaluate the demographic data of 88 children from our center whose kidney biopsies contained crescents, to classify etiologies and to investigate the relation of $CD163^+$ macrophage infiltration with disease severity and prognosis. This is the first study to evaluate the relationship between the severity and prognosis of the CGN in childhood and the number of $CD163^+$ macrophages.

Material and Methods

Patients

A total of 1344 pediatric kidney biopsies between the years 2000 and 2016 at Pediatric Pathology Department, Hacettepe University Faculty of Medicine were reviewed. A total of 88 patients with biopsies containing crescents were included in this study. The data of age, sex, follow-up duration, blood pressure, proteinuria, levels of creatinine, albumin, glomerular filtration rate (GFR), time of biopsy, treatment before biopsy, and outcome were collected from patients' medical records. Estimated GFR was calculated based on serum creatinine levels and height by bedside Schwartz formula.[11] All biopsy specimens in the study were reevaluated on light microscopy and percentages of crescent, crescent type and fibrosis were recorded. Patients were divided into groups according to the underlying etiology such as HSP/IgA vasculitis, lupus nephritis, postinfectious glomerulonephritis, IgA nephropathy, MPGN and pauci-immune glomerulonephritis. If no underlying cause was found, patients were included in idiopathic crescentic glomerulonephritis group.

The control group included 22 patients without crescent (six girls and sixteen boys); 8 of them with minimal change disease, 4 of them with IgA nephropathy and 10 of them with GFR less than 60 ml/min/1.73 m^2. The cases that had no crescent with GFR less than 60 ml/min/1.73m^2 included Wegener granolomatosis (n=1), tubulointerstitial nephritis (n=4), lupus nephritis (n=1), juvenile nephronophithisis (n=3) and hemolytic uremic syndrome (n=1). Their mean age was 12.4 ± 3 years (2-18 years).

Response of treatment was categorized into four groups.

Complete response: patients with normal physical examination, urinary test, albumin and GFR level at last visit,

Partial response: patients with microscopic hematuria or 24-hr proteinuria 4-40 mg/m^2/hr at last visit,

Non-responder: patients with 24-hr proteinuria above 40 mg/m^2/hr or GFR below 60 ml/min/1.73m^2 but above 15 ml/min/1.73m^2 at last visit,

End stage kidney disease: patients with kidney replacement therapy (dialysis, transplantation), or GFR 15 ml/min/1.73m^2 or below at last visit,

Histopathological analysis

Kidney biopsies of 61 patients and 22 control biopsies were available for immunohistochemical stainings since paraffin blocks of 27 patients were not sufficient for immunohistochemical staining. Sections from every paraffin block were taken by using Shandon Finesse ME (Thermo Scientific) label microtom and Feather A35 microton knife (Japan) on Histobond + (Mariefeld, German) and Objekttrager (Isotherm, German) adhesive polylysin slide. Two sections were taken from each sample. Sections were dried for one night at room temperature and were hidden until staining. Samples from paraffin blocks were stained with ready-to-use CD163 antibodies in Leica Bond-Max (England) device. For immunohistochemical analysis CD163 positive cells were shown the average number of positive cells in every ten high power glomerular and tubulointerstitial field (hpf, x40). Cells in ten fields were counted and the average was used for analysis.[4,12] Histopathological analysis was performed by the pathologist who was blinded to the clinical parameters.

Statistical analysis

The research data were uploaded to a computer and evaluated by Statistical Package for Social Sciences (SPSS) for Windows 22.0 (SPSS Inc, Chicago, IL). Descriptive statistics were presented as frequency distribution and percentage. Frequency distributions and percentages for categorical variables were given as basic descriptive statistics such as mean, standard deviation, median, minimum, maximum for quantitative variables. Repeated measures ANOVA was applied to the measurements in order to analyze the time-dependent changes of the repeated measures. LSD test was applied in order to be able to determine which measure is different when there was a statistically significant difference ($p<0.05$) between the means of the repeated measures in the result of the analysis.

Non-parametric Mann-Whitney U Test, parametric independent samples t test, Chi-square analysis, Tamhane's T test were used. For correlation analysis, Spearman correlation coefficient between the variables was given as the result of analysis.

This study was approved by the Ethical Committee of Hacettepe University (GO 15/755-29). This study was supported by the Scientific Research Unit of Hacettepe University (THD-2016-8972).

Results

Kidney biopsies of 88 patients with crescents between the years 2000-2016 and 22 controls were included in the study. There were 40 girls (45.5%) and 48 boys (54.5%) in the patients' group. The mean age of the patients was 11.49 ± 3 years at the time of biopsy. There were six girls and sixteen boys in the control group and their mean age was 12.4 ± 3 years ($p=0.363$). At the time of biopsy, proteinuria and hematuria were detected in 100% and 85% of patients, respectively. The median duration from the onset of clinical findings to kidney biopsy was 42 days (IQR 20-120).

Seventy percent of the patients received steroid, immunosuppressive or immunomodulation treatment before biopsy.

Etiology

The most common underlying etiology was HSP/IgA vasculitis (26.1%), followed by lupus nephritis (22.7%) and idiopathic CGN (18.2%) (Fig. 1). Idiopathic CGN was defined if no underlying cause was found.

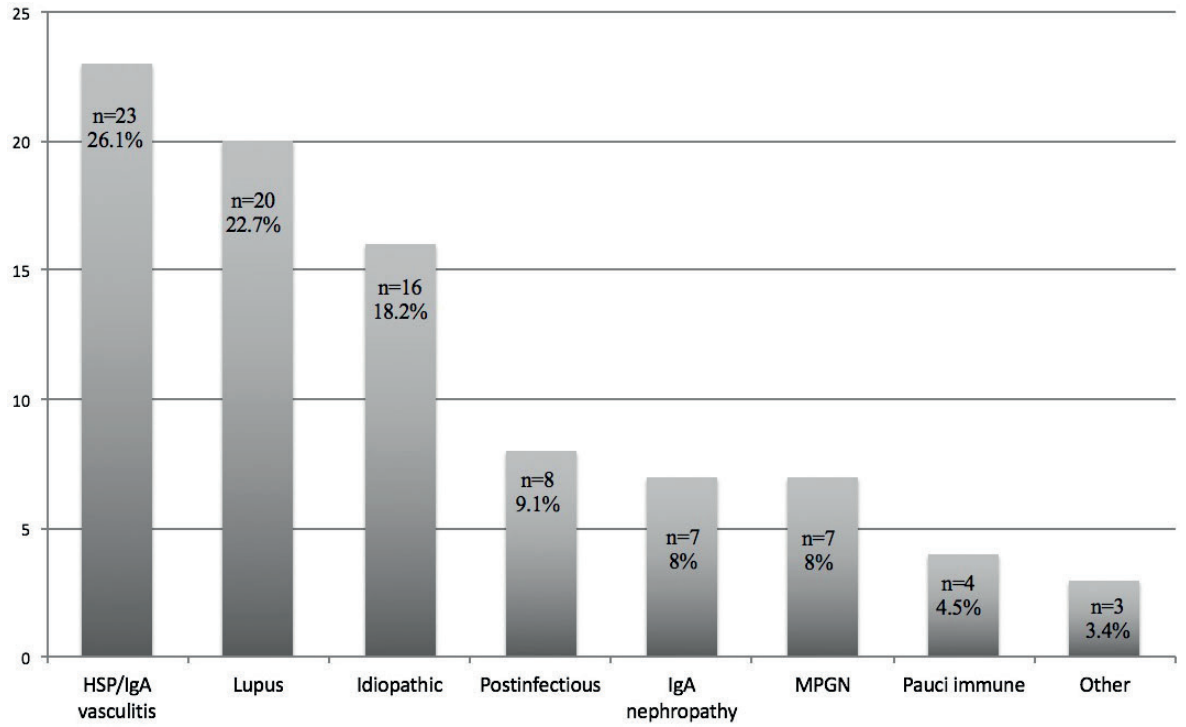

Fig. 1. Classification of cresentic glomerulonephritis according to the etiology. Others included one patient with C3 glomerulonephritis, one with chronic pyelonephritis and an Alport syndrome.

Prognosis

The outcome data of 70 patients were available. Mean follow-up duration of these 70 patients was 3.2 ± 3 years. At last visit; 52% (n=36) was complete responder, 17% (n=12) was partial responder, 14% (n=10) was non-responder and 17% (n=12) was end-stage kidney disease. During follow-up, four patients had kidney transplants and two patients who were administered hemodialysis died of sepsis. Underlying kidney diseases of four patients who underwent kidney transplants included lupus nephritis (one patient), dense deposit disease (one patient) and idiopathic crescentic glomerulonephritis (two patients). One patient with MPGN and one patient with pauci immun glomerulonephritis died of sepsis.

Crescents

All biopsy specimens in the study were reevaluated by light microscopy. There were cellular, fibrocellular and fibrous crescents in 70.5%, 27.2% and 2.3% of the patients, respectively. The mean crescent percentage was 38.9% (min. 2.8%, max. 100%) and 30.7% patients (27/88) had crescents more than 50%. Seventy patients (79.5%) had partial crescent formation and other 18 patients (20.5%) had global crescent formation.

The relationship between crescent percentage and the outcome were evaluated (Fig. 2). Patients with crescents less than 50% had more complete and partial responder rates compared to patients with crescents more than 50%. Cases with crescents more than 50% had more end-stage kidney disease compared to ones with crescents less than 50% [8/20 (40%) vs. 4/50 (8%); $p < 0.05$].

Immunohistochemical staining

Biopsy specimen of 22 controls and 61 patients were stained with CD163 (Figs 3 and 4). Patients in the control group had no crescent formation. The control group was divided

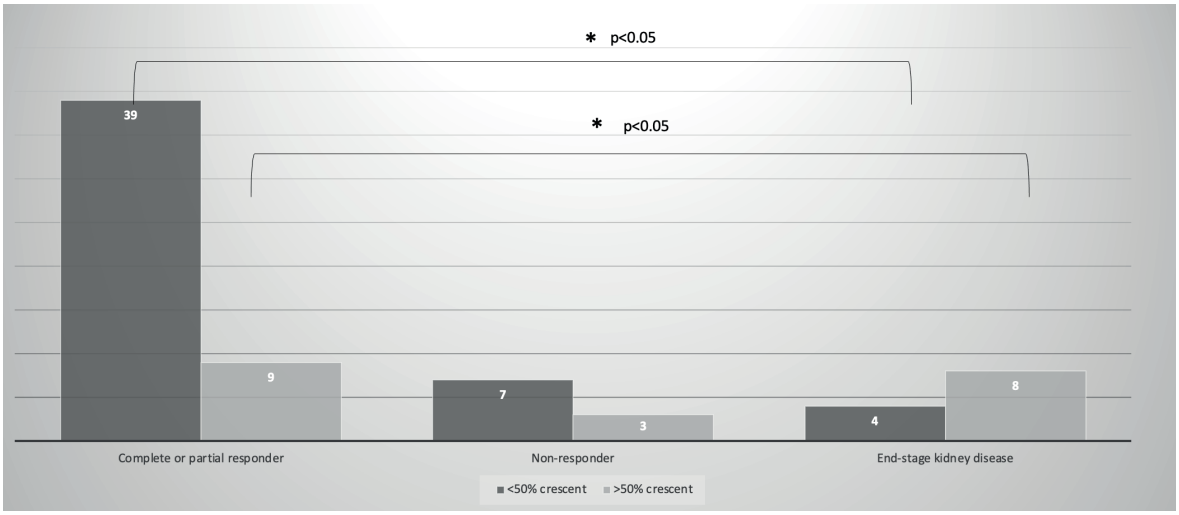

Fig. 2. Relationship between crescent percentage and the outcome. (*demonstrates statistically significant difference between two groups).

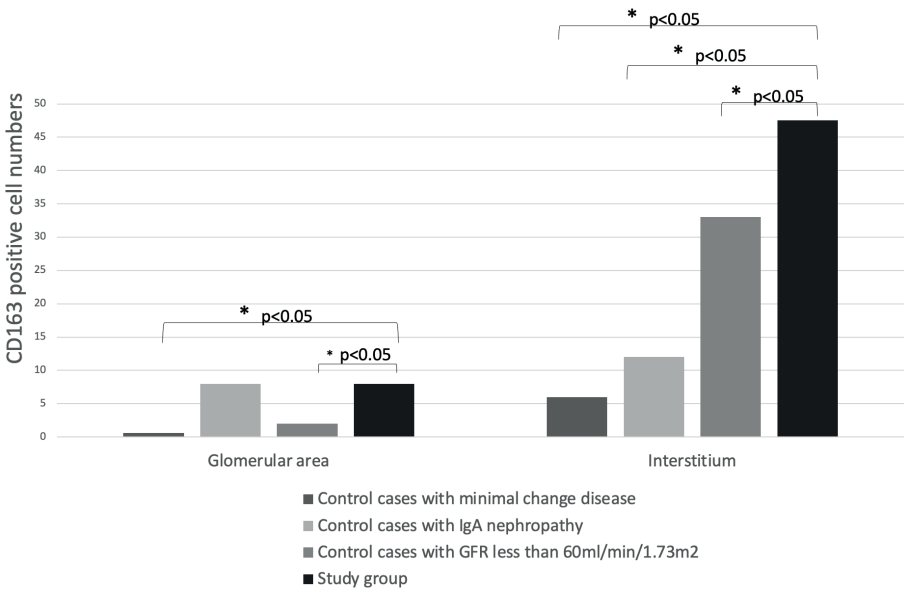

Fig. 3. CD163 + cells' counts of study and control groups. (*demonstrates statistically significant difference between two groups).

into three subgroups and each group was compared with the patients individually. While comparing the results, patients with crescentic glomerulonephritis had a higher number of CD163+ macrophages than the controls ($p<0.05$) except the glomerular area of the controls with IgA nephropathy. Controls with IgA nephropathy had the same amount of CD163+ macrophages as all patients with crescentic glomerulonephritis.

Immunohistopathological stainings of the patients with different etiologies are given in Table I. While comparing every etiologic group with each other, no difference in CD163 staining of glomerulus and tubulointerstitium was found ($p=0.26$, $p=0.12$, respectively).

The relationship between CD163 and crescent percentage (less or more than 50% crescent), GFR levels at their last visit (less or more

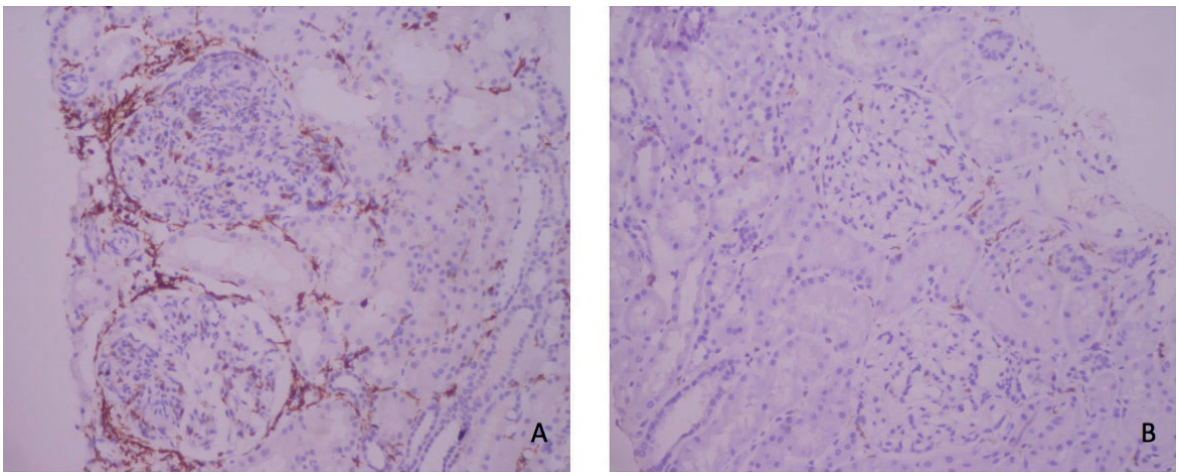

Fig. 4. A) CD163 staining in both glomerulus and tubulointerstitium in a patient with lupus nephritis. (Immunohistochemistry x200) **B)** CD163 staining of a patient in the control group (Immunohistochemistry x200).

Table I. CD163 positive cell counts in glomerulus (G) and tubulointerstitium (TI) according to the etiologies.

Marker	Tissue compartment	IC mediated (MPCC)	Pauci immune (MPCC)	Others (MPCC)	p
CD163	Glomerulus	8.38	13.5	3.2	0.26
	Tubulointerstitium	46.74	57.5	58.1	0.12

*IC: immune complex, MPCC: mean positive cell count

than 60 ml/min/1.73 m^2), and the percentage of fibrosis (<5%, 5-25%, 25-50%, >50%) were investigated (Table II). There was a statistically significant increase in tubulointerstitial CD163 positive macrophages in patients with GFR levels less than 60 ml/min/1.73 m^2 at the last visit (52.2±18.2 cells/hpf vs. 33.3±10 cells/hpf, p <0.05). Additionally, there were significantly more CD163 positive cells in tubulointerstitial areas of patients who had fibrosis higher than 50% (p <0.05).

Treatment protocols were available in 78 patients. Among these, 69.3%, 19.3% and 34.1% received pulse steroid, azathioprine and cyclophosphamide, respectively. Rituksimab (5 patients) and cyclosporine (5 patients) were also given. Dialysis was needed in 19.3% patients.

Patients were classified into four groups (complete responder, partial responder, non-responder and end-stage kidney disease) at last visit according to the outcome. Patients with end-stage kidney disease had statistically significant more CD163 positive cells in tubulointerstitium compared to the patients with complete and partial response (p<0.05) (Table II).

Discussion

Crescentic glomerulonephritis is a rare entity in childhood. In this study, 88 children with kidney biopsies with crescents were evaluated for clinical and histopathological characteristics. The relationship between the disease severity and the number of CD163 positive macrophages was investigated for 61 patients.

The mean age of the patients at the time of biopsy was 11.49 ± 3 years which is similar to other pediatric crescentic glomerulonephritis series with the mean ages ranging from 10 to 12.27 years.[13-15]

The most common etiologic cause in our cohort was HSP nephritis/IgA vasculitis followed by lupus nephritis and idiopathic CGN. The

Table II. CD163 positive cell counts in glomerulus (G) and tubulointerstitium (TI) according to crescent percentage, GFR levels and fibrosis percentage.

Parameters		Glomerulus $CD163^+$ cells/hpf	Tubulointerstitium $CD163^+$ cells/hpf
Crescent percentage	<50%	8.2	45.1
	>50%	7.8	57.5
	p	0.18	0.54
GFR levels	>60 ml/min/1.73 m^2	2	33.3
	<60 ml/min/1.73 m^2	7.6	52.2
	p	0.06	<0.05
Fibrosis percentage	<50%	8.2	42.5
	>50%	9.3	67.3
	p	0.79	<0.05
Treatment response	Complete or partial responder	8.5	39.2
	End-stage kidney disease	2.3	68.1
	p	0.16	<0.05

etiologic causes of CGN in children and adults have been known to be different. According to the study covering 528 adult patients from China, the most common etiology was pauci immune GN. It was also the most common etiology in series from USA, India, Japan and Spain (60%, 72%, 64% and 67.2%, respectively).[12,13,16-18] Jennette et al.[12] divided patients with CGN into groups according to their ages and stated that the most common etiology was immune complex mediated glomerulonephritis in patients younger than 20 years. The incidence of pauci immune glomerulonephritis was 42% and its incidence increased with age. The childhood series from other countries reported immune complex mediated glomerulonephritis as the most common etiology, as well.[14] Ozlu et al.[15] reported that the most common etiology was HSP nephritis/IgA vasculitis (24 out of 45 patients) followed by idiopathic CGN (9/45) similar to our study.

HSP nephritis/IgA vasculitis which was the leading etiology in our study, has also been the most common vasculitis in childhood in our country.[15,19] It was reported to be the underlying cause in 10-15% of childhood glomerulonephritis and 3% of the end-stage kidney disease.[20]

The mean crescent percentage was 38.9% with predominant cellular crescents (70.5%) and total 30.7% patients had crescents more than 50% in our study. Southwest Pediatric Nephrology Study Group reported the mean crescent percentage as 63% with the majority of fibrous/fibrocelular crescents.[21] Piyaphanee et al.[22] studied 67 patients with RPGN and 31.1% of them had crescents more than 50% similar to our study. As far as the relationship between crescent percentage and the outcome is concerned, the patient group with less than 50% crescent percentage had more patients with complete and partial response at the last visit compared to the patient group with crescents higher than 50%. Besides, patients with crescent precentage more than 50% had more end-stage kidney disease at last visit. This finding is compatible to the other studies in the literature.[21] It supports that higher percentage of crescents is related with worse outcome.

In the present study, complete responders were 51.4% while partial responders 17.2%, irrespective of etiology. The incidence of chronic kidney disease seemed fewer than another center in our country (17.2% vs. 42.2%).[15] However, they included only patients with crescents more than 50% while we included all

patients with crescents. It may have explained the discrepancy of this outcome, since we also found that the incidence of end-stage kidney disease was 40% in patients with crescents more than 50%. Two patients died in the end-stage kidney disease's group. One of these patients suffered pauci immune glomerulonephritis and the other had MPGN.

Patients with end-stage kidney disease had statistically significant more CD163 positive cells in tubulointerstitium than patients with complete and partial responders. $CD163^+$ macrophages were found in areas of renal fibrosis and also showed that they produce profibrotic factors in previous studies.[23,24] In this study, CD163 staining was found higher in the samples with higher fibrosis percentage, as well. This fibrotic process may be the reason for the worse outcome. A previous study supporting these findings was reported by Li et al.[4] The clinicopathologic importance of CD163 was evaluated in the study on adult patients with proliferative glomerulonephritis. They reported that CD163 staining in the tubulointerstitium was positively correlated with proteinuria and negatively correlated with albumin.[6] In their another study related with glomerulonephritis with crescents, they observed $CD163^+$ cells were correlated with the percentage of crescent in all areas, but the $CD163^+$ cells and GFR relationship was only found in the tubulointerstitial area.[25] These findings supported our data and also demonstrated the clinicopathological importance of CD163. The number and percentage of CD163 positive cells in tubulointerstitium was found to be higher in patients with crescents above 50% compared to the patients with crescents below 50%. Besides, the relationship between CD163 positive cell counts and outcome was statistically significant in the tubulointerstitial area, as well. These findings suggest that CD163 positive cells may play a role in tubulointerstitial injury in CGN patients in addition to the response to oxidative stress. Similarly, it was also shown that prominent expression of cytokines was observed in the interstitium of patients with lupus nephritis and in HSP/IgA vasculitis, the Th cytokines were stained in tubular area, as well.[26,27]

On the other hand, Li et al.[4,6] also found that CD163 staining in glomerulus was positively correlated with proteinuria and negatively correlated with GFR and albumin. In our study, CD163 positive cell counts and outcome were not found to be related in the glomerular areas. Ikezumi et al.[28] reported the histologic differences between children and adults in new-onset IgA nephropathy. They reported glomerular matrix expansion was found to be more severe in adults than children.[28] They investigated activated sialoadhesin (Sn) positive macrophages and found that Sn+ macrophages in glomerulus was correlated with glomerular matrix and kidney function in adults, but not in children. Since this finding may account for the different behavior of the local macrophages between adults and children in these cases. It can also be a reason of why there was a correlation between the $CD163^+$ cell count and prognosis in glomerular area in adults but not in our pediatric cases.

Patients with crescentic glomerulonephritis had higher number of $CD163^+$ macrophages than the controls ($p<0.05$) except the glomerular area of the controls with IgA nephropathy. However, a previous study compared IgAN without crescents and IgAN patients with crescents and demonstrated higher numbers of CD163 positive cells in both tubulointerstitial lesions and glomerulus in the crescentic disease.[6] The reason why the difference of glomerular CD163 positive cells in between controls with IgA nephropathy and the study group could not be shown may be related with the small control group size in the study.

The other limitations of this study were the lack of sufficient kidney tissue in some cases and information including treatments and the small size of samples in each group. Another limitation was the semiquantitative nature of immunohistochemical staining and long time period after the biopsy. After 3 years,

immunostaining of antigens may not be sufficient.

In conclusion, HSP nephritis was found to be the most common cause of crescentic glomerulonephritis in childhood in this study. Having crescents more than 50% has a negative affect on the outcome. Histopathological and immunohistochemical findings have supported that CD163 positive M2 type macrophages, especially in tubulointerstitial areas, may have a negative effect on prognosis of CGN. Experimental animal models and clinical investigations to further elucidate the effect of CD163 positive macrophages on the pathogenesis and prognosis of CGN are warranted.

Ethical approval

This study was approved by the Ethical Committee of Hacettepe University (GO 15/755-29).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: S. Gucer, G. Kayki, D. Orhan, Z. Akcaoren; data collection: G. Kayki, B. Gulhan, R. Topaloglu, A. Duzova, F. Ozaltin, S. Ozen, Y. Bilginer, S. Gucer; analysis and interpretation of results: G. Kayki, S. Gucer, B. Gulhan, R. Topaloglu; draft manuscript preparation: G. Kayki, S. Gucer, B. Gulhan, R. Topaloglu, D. Orhan. 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

Clinical and molecular findings in 6 Turkish cases with Krabbe disease

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ABSTRACT

Background. Krabbe disease is a rare lysosomal storage disorder with a neurodegenerative course that occurs because of the deficiency of the beta-galactocerebrosidase (GALC) enzyme activity. The genetic basis of Krabbe disease consists of biallelic mutations in the *GALC* gene, but the genetic spectrum in the Turkish population is poorly defined. We aimed to present a Turkish case-series with infantile-onset Krabbe disease, define the clinical and molecular findings and compare the genetic spectrum with the mutations previously reported in the literature.

Methods. Six cases, who were referred to our clinic between 2015-2019, with a definite diagnosis of infantile-onset Krabbe disease were included in the study. The family history, clinical information, biochemical and radiological examinations of the patients were screened and evaluated. All encoded exons and exon-intron regions of the *GALC* gene were sequenced using next generation sequencing technology. Multiplex ligation-dependent probe amplification analysis was used for deletion type mutations that could not be detected by sequence analysis.

Results. *GALC* gene sequence analysis revealed four known mutations including c.1394C>T (p.Thr465Ile), c.411_413delTAA (p.Lys139del), c.820G>C (p.Glu274Gln), and 30 kilobase deletion mutation among the exons 11-17 (IVS10del30kbp). Moreover, the c.1623G>A (p.Trp541Ter) variant, which was not previously reported in the literature, was detected in two cases.

Conclusions. We believe that the demonstration of the genetic spectrum of infantile-onset Krabbe disease in Turkish patients will be an important contribution to the *GALC* mutation data in our country. More importantly, two novel variants were defined. This knowledge may enable early detection and treatment with the advent of a carrier or newborn screening tests.

Key words: *GALC* gene, Krabbe disease, newborn screening tests.

Krabbe disease (KD), also known as globoid cell leukodystrophy (GCL; MIM # 245200), is a life-threatening, autosomal-recessive neurodegenerative disease with a prevalence of 1/100,000-250,000.[1] The main pathogenetic event is the progressive accumulation of galactoylceramide and galactosylsphingosine (psychosine) due to the lack of a lysosomal beta-galactocerebrosidase (GALC) activity, which causes clinical findings related to increased apoptosis rate in myelin-forming cells.[2]

Nearly 90% of the cases are "early-onset (EO)" (infantile) KD cases, which often starts before 6 months. The remaining cases are "late-onset (LO)" KD which begin in the late-infantile, juvenile, and adult period. In EOKD cases, hyperirritability, excessive crying, axial hypotonia, spasticity, gastroesophageal reflux, feeding difficulties, loss of acquired skills and peripheral neuropathy are observed. LOKD cases present with initial symptoms such as

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psychomotor regression, ataxia/gait disturbance, tremor, and febrile seizures between the ages of 7 months and 3 years. Infantile and late infantile forms progress rapidly, and cases die in the first 2-3 years of their lives. In the juvenile form, which has an age of onset between 3 and 8 years, the first finding is vision loss, which is followed by neuromotor regression and ataxia. Adult-onset cases are very heterogeneous, and the phenotype may be mild.[2]

For the definitive diagnosis of KD, it is sufficient to detect extremely low GALC activity in cases with clinical and radiological findings. In cranial magnetic resonance imaging (MRI) of EOKD, T2-weighted images show an involvement consistent with demyelination in the periventricular, deep white matter, dentate and cerebellar white matter.[3] Occasionally optic nerve/chiasma hypertrophy may be involved. GALC enzyme activity is much lower than normal (less than 0-5% of normal). There is no correlation, however, between residual enzyme activity and phenotype. The genetic basis of KD is combined heterozygous or homozygous biallelic mutations in the *GALC* gene encoding the beta-galactocerebrosidase enzyme, which is a gene consisting of 17 exons localized to 14q31 and encoding 685 amino acids. So far, 275 mutations in the *GALC* gene have been reported in the HGMD (Human Genome Mutation Database) database (Professional subscription July 2021). Although in cases with well-known mutations there is a clear correlation between the genotype and phenotype, in cases with novel mutations it is difficult to predict the phenotype. Mutations causing KD are also thought to be population specific. In European populations, 30kb deletion (IVS10del30kbp) is seen in 45% of cases, while 15% have c.1586C>T (p.Thr529Met), c.1700A>C (p.Tyr567Ser) and c.1472delA (p.Lys491Argfs*62) mutations.[4,5] Approximately 30% of Japanese Krabbe patients have c.683_694del12insCTC or c.2002C>A mutations.[6] However, the genetic spectrum in the Turkish population is poorly defined.

Early diagnosis of EOKD is crucial as it can lead to treatment with bone marrow or hematopoietic stem cell transplantation (HSCT). Since the risk of the disease being seen in the siblings of the affected patient is 25%, the existence of a definite diagnosis is important to offer prenatal diagnosis options to the family.

In this report, we reviewed the molecular and clinical findings of six Turkish cases diagnosed with KD in the infantile period and compared their genetic profile with the published cases in the literature.

Material and Methods

Six cases, who were referred to Bezmialem Vakıf University Faculty of Medicine,Department of Medical Genetics outpatient clinic between 2015-2019, with a definite diagnosis of infantile-onset KD were included in the study. The study was reviewed and approved by the Bezmialem Vakıf University, institutional review board, and written informed consent was obtained from all parents of the patients included in the study (No/Date: 54022451-050.05.04/05.02.2019). The family history, clinical information, biochemical and radiological examinations of the patients were screened and evaluated. The low activity of the GALC enzyme in patients' leukocytes was consistent with KD. Enzyme activity was determined as nmol MU/17h/mg protein by fluorometric assay (Acıbadem Labmed, Istanbul, Turkey). DNA isolation was performed from EDTA blood samples taken from six cases and their available parents of five cases. All encoded exons and exon-intron regions of the *GALC* gene were sequenced using next generation sequencing technology (Miseq, Illumina Inc., San Diego, CA, USA). Multiplex ligation-dependent probe amplification (MLPA) analysis was used for deletion type mutations that could not be detected by sequence analysis (MRC-Holland SALSA MLPA P446-GALC). The presence of novel variants in the parents of the index cases were confirmed by Sanger sequence analysis. The final diagnosis was made by the reduced GALC activity in leukocytes, characteristic clinical and radiological findings as well as molecular findings.

Results

Overall patient characteristics

Six patients from six families were included in this study. There were 4 males and 2 females. The age of onset was 2 months to 5 months. The mean age at the time of diagnosis was 6.5 months (range, 4 months to 12 months). Age of diagnosis was determined with the reporting date of GALC activity results. All cases died before 3 years of age. There was a consanguineous marriage between the parents of four cases, and the parents of the other cases were from the same or close village. The clinical, radiological, and molecular findings are summarized and compared with the previously published Turkish cases in Table I.

The initial findings observed in four out of six cases were psychomotor regression at 3 to 5 months, and hypotonia after 2 months of age in the remaining two cases. All patients had a history of hyperirritability and excessive crying, axial hypotonia and psychomotor retardation, and pathologic cranial MRI findings (Fig. 1).

A brief summary of clinical findings

Case 1, female patient, the third child of a consanguineous couple, was born at term by normal spontaneous vaginal delivery (NSVD). Family history revealed a deceased sister at 9 months and a paternal cousin with the diagnosis of KD. She had a healthy sister. The developmental milestones of her first three months were unremarkable. At that time, the

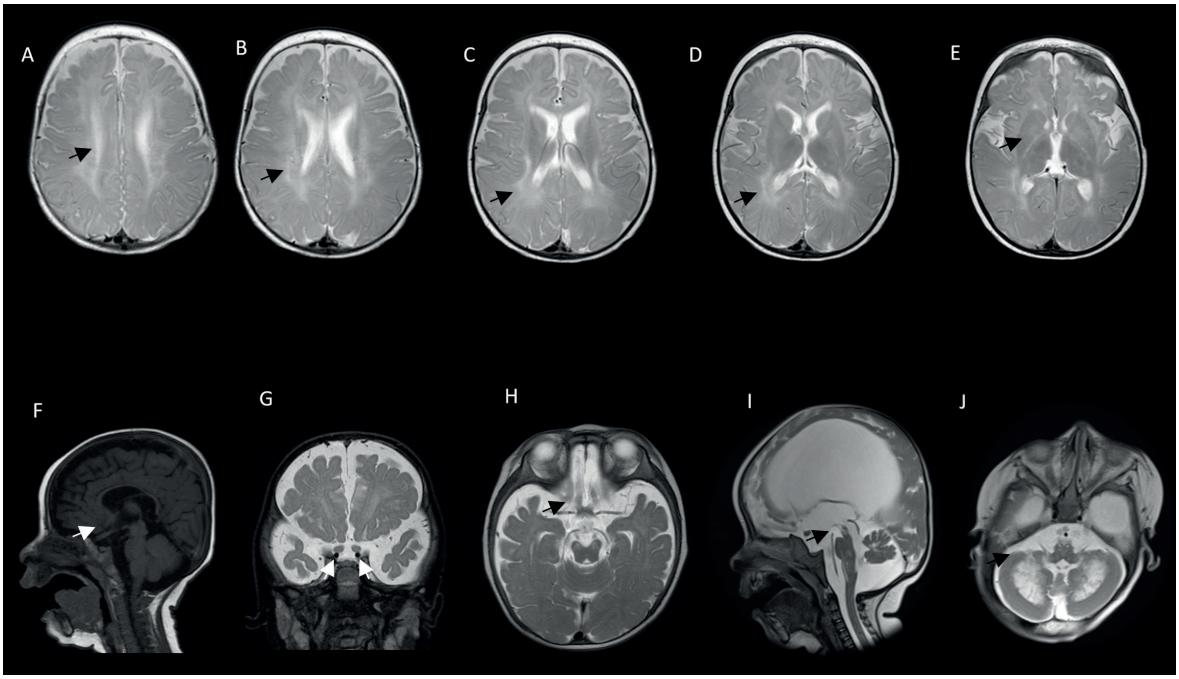

Fig. 1. Brain MRI findings in patients with Krabbe disease.

Patient #2 Sagittal T2W images: hyperintense signal changes in deep cerebral white matter (periventricular/centrum semiovale) in **A-C** and in posterior limb of internal capsule (PLIC) in **D-E** at 5 months (arrows).

Patient #5 Axial T1W and coronal T2W MR images: atrophy of corpus callosum and diffuse hypertrophy of the optic nerves in **F-G** (arrows). Sagittal T2W image: bilaterally enlarged the proximal prechiasmatic optic nerves are bilaterally in **H** (arrow) at 11 months

Patient #1 (I-J). Axial T2W image: volume loss of midbrain and severe dilation of the ventricles in **I** (arrow). Sagittal T2W MR images: hyperintense signal changes in dentate nucleus in **J** at 10 months (arrow).

Table I. Combined data of our cases along with previously published early infantile Turkish cases.

Case	CM	Clinical findings at the time of the first visit	GALC enzyme activity	MRI findings	Mutation results
Case 1 [♀] Age of OS: 3 mos Age of Dx: 4 mos DoD: 14 mos	yes	irritability, crying, feeding difficulties, neuromotor regression, hypotonia	18 nmol MU/17 h/ mg protein*	T2 hyperintense signal changes in PWM, brainstem CST and cerebellar dentate nuclei. Cerebral atrophy complicated with severe dilation of the lateral and third ventricles, diffuse brain atrophy including volume loss of midbrain.	homozygous c.1394C>T (p.Thr465Ile)[9]
Case 2 [♀] Age of OS: 3 mos Age of Dx: 6 mos DoD: 2 yrs	yes	neuromotor regression, hypotonia, spastic tetraparesis	10 nmol MU/17 h/ mg protein*	T2 hyperintense signal changes in PWM, posterior limb of internal capsule, brainstem corticospinal tract and cerebellar dentate nuclei. The enlargement of the prechiasmatic CN II and hypertrophy of the chiasmatic CN II. Atrophy of corpus callosum.	homozygous c.411_413delTAA (p.Lys139del)[10,11,12,13]
Case 3 [♂] Age of OS: 2 mos Age of Dx: 8 mos DoD: 13 mos	no	irritability, crying and hypotonia, spastic tetraparesis	12 nmol MU/17 h/ mg protein*	T2 hyperintense signal changes in PWM, PLIC, brainstem CST and cerebellar dentate nuclei, enlargement of the prechiasmatic CN II and hypertrophy of the chiasmatic CN II. Atrophy of corpus callosum.	homozygous c.820G>C (p.Glu274Gln)[14]
Case 4 [♂] Age of OS: 5 mos Age of Dx: 7 mos DoD: 10 mos	yes	irritability, incessant crying, feeding difficulties, neuromotor regression	15 nmol MU/17 h/ mg protein*	T2 hyperintense signal changes in PWM, PLIC, brainstem CST and cerebellar dentate nuclei.	homozygous exons 11-17 IVS10del30kbp[4]
Case 5 [♂] Age of OS: 5 mos Age of Dx: 12 mos DoD: 13 mos	yes	Irritability, excessive crying. feeding difficulties, neuromotor regression	10 nmol MU/17 h/ mg protein*	T2 hyperintense signal changes in PWM, PLIC, brainstem CST and cerebellar dentate nuclei. Cerebral atrophy, atrophy of corpus callosum.	homozygous c.1623G>A (p.Trp541Ter) novel present study
Case 6 [♂] Age of OS:2 mos Age of Dx: 4 mos DoD: 2 yrs	no	irritability, excessive crying, hypotonia, feeding difficulties	14 nmol MU/17 h/ mg protein*	T2 hyperintense signal changes in centrum semiovale, posterior internal capsule, dentate nucleus, brainstem, enlargement of the prechiasmatic CN II hypertrophy of the chiasmatic CN II. Atrophy of corpus callosum.	homozygous c.1623G>A (p.Trp541Ter) novel present study
Reported case #1 Kardas et al.[16] [♂] Age of OS:3 mos Age of Dx: 4 mos DoD:NA	yes	irritability, crying, poor head control, convulsions	0.01 µmol/g/h (N:0.8–4)	Bilateral, symmetrical hyperintense signal changes in cerebellar dentate nuclei and deep white matter of the cerebral hemispheres.	homozygous c.727delT (p.Leu243Serfs*8)[16]
Reported case #2 Güngör et al.[17] [♂] Age of OS:3 mos Age of Dx: 4 mos DoD:NA	NA	irritability, hypotonia, poor head control	6,5 nmol/17 h/mg protein (N:18-115)	Enlargement of the prechiasmatic CN II hypertrophy of the chiasmatic CN II, T2 hyperintense signal changes in dentate nuclei, the posterior limbs of the capsules and in the deep PVM.	homozygous c.943delG (p.Glu315Asnfs*10)[17]
Reported case #3 Isık et al.[13]	NA	NA	NA	NA	homozygous c.411_413delTAA (p.Lys139del)[13]
Reported case #4 Tuncer et al.[9] [♂] Age of OS:3 mos Age of Dx: 4 mos DoD:NA	yes	irritability, crying, hypotonia, spastic tetraparesis, convulsions	0.045 nmol/ mg/h (N:0.60–3.29)	T2 hyperintense signal changes in the PVM, the cerebellar white matter, and the dentate nucleus.	homozygous c.1394C>T (p.Thr465Ile)[9]

Dx: diagnosis, OS: Onset, DoD: Date of death, CM: Cousin marriage NA: not available
CN II: Optic Nerve, PWM: periventricular white matter, CST: corticospinal tract, PLIC (posterior limb of internal capsule)
Normal range of GALC enzyme activity in leukocytes: *25–105 nmol MU/17 h/mg protein

mother noticed that she had sucking difficulties. At three months of age, she experienced crying and irritability. She was able to control her head and smile. Loss of previously acquired head control, visual tracking and hypotonia were noted at the time of evaluation at four months of age. She required feeding via nasogastric tube at five months. She died of respiratory insufficiency due to pneumonia at 14 months of age.

Case 2, female patient, the third child born to consanguineous parents, was delivered at term by NSVD. She had a healthy sister and a history of a deceased brother at 2 years of age who had hypotonia and feeding difficulties from birth and history of aspiration pneumonia at 5 months old with a preliminary diagnosis of neurodegenerative disease. At three months of age, she was developmentally normal. Head control and smiling were present. Thereafter, her development regressed and hypotonia developed. At the time of her first evaluation at 5 months of age, she also exhibited an absence of head control and spastic tetraparesis as well as tightly fisted hands. She responded to light but abilities such as focusing on an object or visual tracking of a moving object were not noted. She underwent hematopoietic stem cell transplantation (HSCT) for early-onset KD at 10 months of age. Her clinical course worsened progressively over the next months. She died at 2 years of age.

Case 3, male patient, the second child born to non-consanguineous parents from the same village, was delivered at term via cesarean section due to breech presentation. His older sister was healthy. Family history revealed a paternal cousin with the diagnosis of KD who died at the age of 11 months. His early development was unremarkable. At 2 months, he was admitted for pneumonia and soon after increased irritability, crying and hypotonia were noted. By the time of our initial evaluation at 5 months, he did not smile or interact. Neurological examination demonstrated truncal hypotonia, spastic tetraparesis and no head control. He died of aspiration pneumonia at 13 months of age.

Case 4, male born to a first cousin marriage; the second born child was delivered at term by cesarean section due to previously history of cesarean in the first pregnancy. At five months of age, he was developmentally normal. Subsequently, irritability, incessant crying, and feeding difficulties were noted. He was admitted to the intensive care unit for aspiration pneumonia. He lost acquired head control and smiling at 6 months of age. At the time of our evaluation at 7 months of age, he had severe hypotonia. No head control and smiling were present. He passed away due to aspiration pneumonia at 10 months of age.

Case 5, the first child of a consanguineous couple, was born at term via NSVD. Developmental milestones were normal until five months of age. At that time, he developed irritability and excessive crying. At nine months, he lost previously acquired milestones. Our neurologic examination at 10 months of age revealed remarkable irritability with generalized hypertonia. He required a gastrostomy tube placement at 11 months due to feeding difficulties. His clinical course worsened progressively over the next three months. The patient died at home at 13 months of age.

Case 6, the first child of a non-consanguineous couple from the same village, was born at term via NSVD. His family history was unremarkable. His early development was normal until 2 months of age. At two months, irritability and crying were noted. Thereafter, he developed feeding difficulties and reflux disease. At the time of our evaluation at 3 months of age, he had hypotonia and hypoactive deep tendon reflexes. No head control and eye control were present. At 1.5 years of age, he had to undergo a tracheostomy operation. He died of aspiration pneumonia at 2 years of age.

Mutation results

Homozygous missense c.1394C>T (p.Thr465Ile) mutation in Case 1, homozygous 3-base c.411_413delTAA (p.Lys139del) deletion in Case 2, homozygous missense c.820G>C (p.Glu274Gln) mutation in Case 3 and homozygous nonsense c.1623G>A (p.Trp541Ter) variant in cases 5 and 6 were detected in *GALC* gene by next generation sequence analysis. 30 kilobase deletion mutation among the exons 11-17 (IVS10del30kbp) was performed with *GALC* gene specific MLPA probes in Case 4, where no mutation was detected in sequence analysis (Fig. 2).

Point mutations in all cases with available DNA were confirmed by Sanger sequencing, whereas in case 3, who had insufficient DNA sample, maternal and paternal heterozygosity was demonstrated by Sanger sequencing as parents of case 2, case 5 and case 6.

The novel c.1623G>A (p.Trp541Ter) variant has not been previously reported in the literature, but it was predicted to be pathological as it would lead to an early termination in the amino acid sequence by causing a stop codon. The parents of the cases were found to be heterozygous as expected for these mutations.

Discussion

In this study, we aimed to present the clinical, radiological, and molecular characteristics of six EOKD cases and to review the previously reported Turkish cases in the literature.

KD, which has both lethal and late-onset forms, is a relatively common storage disease in our country. It is most common in the Druze community living in northern Israel and in two Muslim-Arab villages close to Jerusalem.[7] The estimated incidence of KD in Turkey was calculated as 1/100,000 in 2004 by Ozkara and Topcu.[8] This incidence was found by proportioning 65 patients diagnosed with KD by postnatal enzyme activity test to 6.500.000 babies born alive between 1997-2002. According to this study, KD is the 3rd disease, after Metachromatic leukodystrophy (MLD) and GM2 gangliosidosis among sphingolipidoses with an incidence of 1.00. *GALC* gene molecular analysis has not been used widely in our country for many years, due to the efficient use of GALC enzyme activity for the pre- and postnatal diagnosis of KD. Unfortunately, the low galactocerebrosidase activity is unable to reliably predict the phenotype. For this reason, determining the genotypes of all cases diagnosed with low enzyme activity is especially important in terms of establishing a genotype-phenotype relationship other than genetic counseling to be given to family members under carrier risk.

As in our cases, the common initial symptoms of EOKD were excessive crying, extreme irritability, feeding difficulties complicated with gastroesophageal reflux, spasticity, developmental regression (loss of acquired milestones such as smiling, and head control), hypotonia and seizures. Previously EOKD diagnosed siblings and/or relatives (in Cases 1, 2 and 3), and the history of consanguineous marriage (in Case 1, Case 2 and Case 4) are also supportive.

The radiological findings of EOKD include in T2-weighted images of MRI commonly shows demyelination with increased T2 signaling in the deep periventricular white matter along with posterior limb of the internal capsule (PLIC), and brainstem corticospinal tract, dentate and cerebellar white matter. Occasionally optic nerve/chiasma hypertrophy and T2 hyperintensity and/or atrophy of the corpus callosum may be seen. All our cases had characteristic radiological involvement. The enlargement of the prechiasmatic cranial nerve II and hypertrophy of the chiasmatic cranial nerve II were found in Cases 2, 3 and 6. Volume loss and severe involvement in the midbrain were found in Case 1.

The mutations found in our case series were comparable to the literature in terms of genotype-phenotype correlation. The mutation of c.1394C>T (p.Thr465Ile) mutation in Case

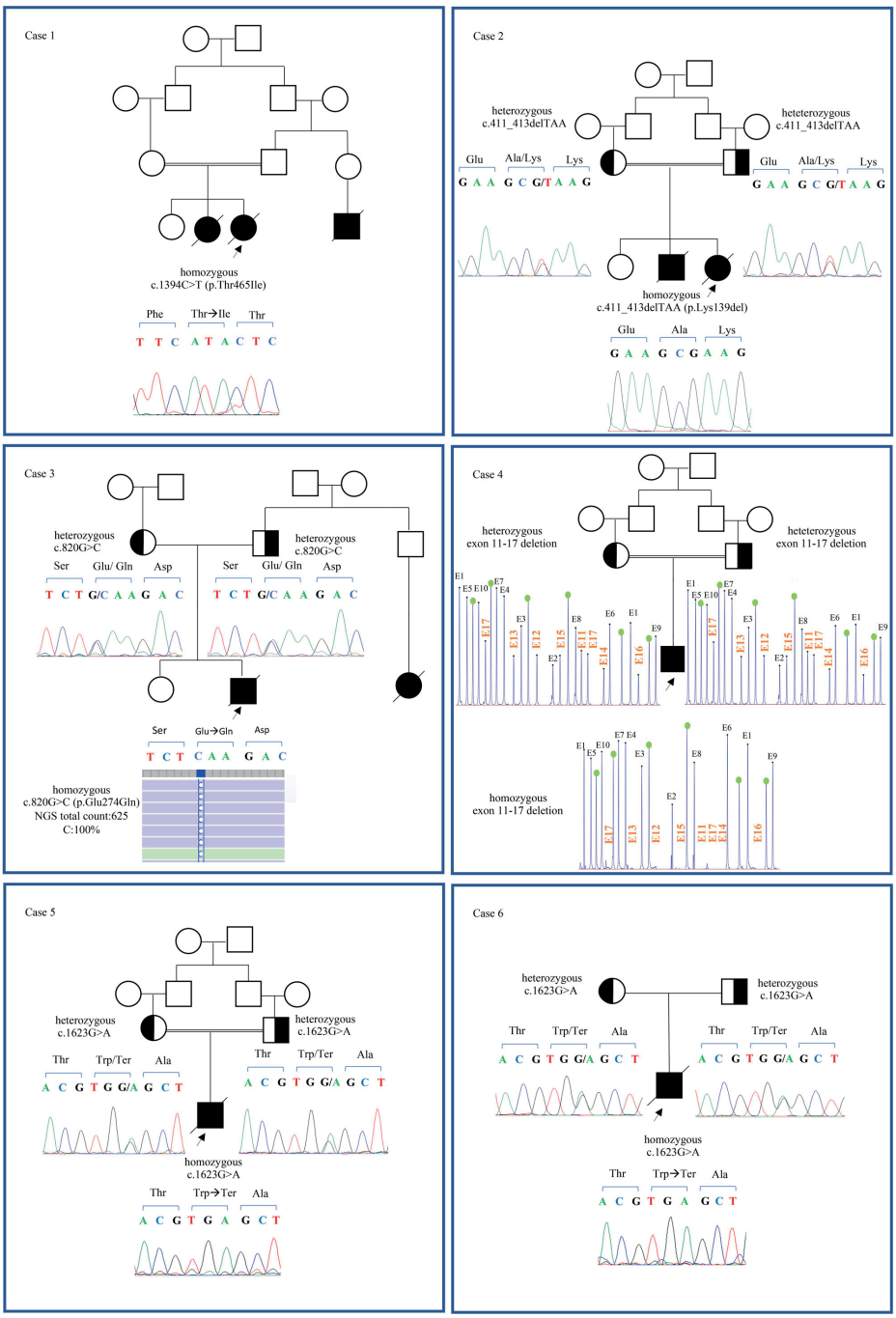

Fig. 2. Molecular findings of our cases.

Point mutations in Case 1, Case 2, Case 5 and Case 6 were confirmed by Sanger sequencing. Maternal and paternal heterozygosity of Case 2, Case 3, Case 5 and Case 6 was demonstrated by sanger sequencing. The frameshift mutation of c.411_413delTAA (p. Lys139del) in Case 2 results in one missing amino acid. Because of the two consecutive lysine amino acids in the codon reading frame, these three base deletions cause the loss of a single amino acid. Homozygous missense c.820G>C (p.Glu274Gln) mutation in Case 3 was detected by NGS. Homozygous 30 kilobase deletion mutation among the exons 11-17 (IVS10del30kbp) in case 4 and heterozygosity of his parents were performed with *GALC* gene specific MLPA probes (green dots for reference peaks).

1 was first reported as homozygous in three Turkish siblings affected by EOKD disease in 2018 by Tuncer et al.[9] Our case had classic EOKD, with a complicated course with hydrocephalus and severe midbrain involvement. Our results in addition to the previously reported cases indicate that the homozygosity of this mutation may be related to EOKD. Although there are few Turkish reported cases, the second representation of this mutation suggested that it might be a relatively common mutation in our population.

The mutation of c.411_413delTAA (p. Lys139del) in Case 2 was also reported in a Turkish patient with LOKD in 2012, as a compound heterozygous with c.200C>T (p.Thr67Ile) mutation.[10] Similarly, this mutation was reported as combined heterozygous with c.1829A> C (p.Asp610Ala) mutation in two Greek siblings with LOKD in 2015.[11] Homozygosity of this mutation has been reported in cases with three early-onset findings, two of which were Greek and the remaining one was Turkish.[11-13] In addition to the previous reports of compound heterozygous cases with late-onset KD, the homozygosity leading to infantile-onset disease in our cases demonstrates that the homozygosity of this mutation leads to more severe disease. This mutation can be seen in Turkish cases as well as in the Greek population. Moreover, the identification of optic nerve growth accompanying classical MRI findings in our case underlined the fact that the diagnosis of KD should be considered in the presence of a neurodegenerative course.

The homozygous missense c.820G>C (p.Glu274Gln) mutation found in Case 3 was first detected in a case in the EOKD screening program in the USA in 2016.[14] This is the first time that this mutation is being reported in the Turkish population. A 30 kilobase deletion mutation among the exons 11-17 (IVS10del30kbp) was found in Case 4. This was not an unexpected finding, as it is the most common mutation in the European population.

Importantly, the c.1623G>A mutation detected in Cases 5 and 6 was not previously reported in the literature and was predicted as pathogenic, because it codes a stop codon in the amino acid sequence. Consistently, the c.1622G>A mutation in the same codon was previously reported in a combined heterozygous case from Morocco.[15] It strengthens the pathological interpretation of this variant, as it also codes a stop codon (p.Trp541Ter) as in our case.

When the literature and HGMD database were scanned for Turkish cases with KD, it was seen that mutations c.727delT (p.Leu243Serfs*8) and c.943delG (p.Glu315Asnfs*10) were reported, which were not seen in our case series. The c.727delT (p.Leu243Serfs*8) mutation was reported by Kardas et al. in 2013 in a 4-month-old case affected by the EOKD.[16] The c.943delG (p.Glu315Asnfs*10) mutation was defined as previously unidentified homozygous in an infantile-onset KD patient with enlargement in the optic chiasm in 2016.[17]

All our cases died before reaching the age of three despite all the proper care and medical treatment. Unfortunately, all of the currently available treatment options are beneficial only for early diagnosed individuals.[18,19] The better identification of *GALC* genotypes is important, because emerging treatment options, such as bone marrow transplantation or HSCT, can slow the progression of KD when applied in the early phase of the disease.[18] Measuring enzyme activity on dried blood specimens (DBS) is a well-established screening method and is currently being used in some states of the USA.[18] Defective enzyme activity results obtained from neonatal screening tests of dried blood samples should be confirmed by the leukocyte enzyme assay.[19] Low GALC enzyme activity is evaluated in DBS, will detect infantile or late-onset Krabbe patients before the symptoms begin, as well as to detect healthy individuals with pseudo-deficiency alleles that

cause a decrease in GALC enzyme activity. In asymptomatic cases with positive first-line tests, the detection of toxic psychosine levels and screening of population specific common mutations or sequencing of the entire GALC gene are used for the definitive diagnosis of KD. At this stage, knowledge of which combination of mutations causes infantile or late-onset phenotype is also particularly important for the timing of treatment options. In addition, the presence of variants in the GALC gene, such as p.Arg184Cys, p.Asp248Asn and p.Ile562Thr that cause pseudo-deficiency allele in the community also makes the interpretation of this test easier.[20] The Newborn Screening Programme (NSBP) in Turkey, ongoing for over 30 years with the aim of preventing morbidities and reducing the mortality rate of manageable conditions with early treatment, has slightly expanded and recently included phenylketonuria, congenital hypothyroidism, biotinidase deficiency and cystic fibrosis. Because of the high incidence of consanguineous marriage and inherited metabolic disorders, an expanded NBSP is essential for Turkey.

There are some limitations to our study. Our study size is limited. We report only one new disease-generating mutation here, further studies are needed in order to better define the genetic spectrum of KD in Turkey. In one case (Case 3) the mutation detected by NGS could not be confirmed by Sanger analysis due to insufficient DNA sample. However, maternal and paternal heterozygosity demonstrated by Sanger sequencing indirectly supported NGS results.

In conclusion, with the prediction that newborn screening tests will expand soon, a better understanding of the disease-causing mutations specific to the Turkish population is of utmost importance. To this end, our results expand the known mutation spectrum of KD in our population. However, further studies are needed to broaden the knowledge on disease-causing mutations.

Ethical approval

The study was reviewed and approved by the Bezmialem Vakıf University, institutional review board, and written informed consent was obtained from all parents of the patients included in the study (No/Date: 54022451-050.05.04/05.02.2019).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ADA, GY; data collection: ADA, EŞ, ABK, ED; analysis and interpretation of results: ADA, AA, Aİ, GY; draft manuscript preparation: ADA, GY. 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 melatonin efficacy in prevention of bronchopulmonary dysplasia in preterm newborn infants

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ABSTRACT

Background. Excessive production of reactive oxygen species play an important role in the pathogenesis of bronchopulmonary dysplasia (BPD). Melatonin is an effective antioxidant and free radical scavenger. The aim of this study was to evaluate the efficacy of melatonin in the prevention of BPD in preterm infants with respiratory distress syndrome.

Methods. In a randomized clinical trial, 80 preterm newborn infants with respiratory distress syndrome and gestation age of 27-32 weeks were allocated randomly in two groups. Group A consisted of 40 neonates who received surfactant with the INSURE technique. Patients in group B received melatonin 5mg/kg /day per gastric tube for 3 days in addition to the surfactant. The primary outcome was on the occurrence of BPD. The secondary outcome was considered other complications of prematurity, duration of hospital stay and mortality.

Results. The mean gestational age and birth weight of studied patients were 31.3±3.8 weeks and 1189±84 grams, respectively. Thirty-five (43.8%) patients were girls and 45 (56.2%) were boys. BPD was diagnosed in 24 (60%) neonates of group A and 18 (45%) patients in group B, p=0.02. The duration of hospital stay, need for mechanical ventilation and mortality rate were significantly lower in patients in group B (p=0.02, 0.003, 0.009 respectively).

Conclusions. Our study results showed that BPD, mortality and hospital stay reduced with melatonin treatment in preterm infants. However, future studies with a larger number of patients are needed to confirm these beneficial effects.

Key words: melatonin, respiratory distress syndrome, bronchopulmonary dysplasia, preterm infants.

Melatonin is an endogenous indolamine synthesized in the pineal gland from serotonin.[1] It is an effective antioxidant and free radical scavenger.[2] Melatonin has a key role in many important physiological functions, such as regulation of circadian rhythms, stimulatory action in the immune system, visual, reproductive, cardiovascular, neuroendocrine and neuroimmunological actions.[3]

Melatonin scavenges free radicals that are oxygen and nitrogen derived metabolites and protects DNA from the damage induced by them.[4] Clinically, melatonin has been used in sleep disorders, neurodegenerative diseases, aging and cancers in adults.[5] In recent years there are few reports about its use in children and neonates.[6-8]

Preterm infants are susceptible to oxidative stress-induced injury because of oxygen use at initial resuscitation and immature fetal antioxidant system. Although oxygen therapy is essential in the management of neonatal respiratory disorders, oxygen exposure, unavoidably leads to excessive production of reactive oxygen species (ROS) in the respiratory system. Its association with peroxidant drugs, concomitent infections or extrapulmonary

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inflammation can promote the depletion of antioxidants and contribute to the development of bronchopulmonary dysplasia (BPD).[9] Gitto et al. examined melatonin treatment in preterm newborns diagnosed with respiratory distress syndrome (RDS) to determine whether melatonin may delay the development of BPD.[10] They found newborns with BPD and without melatonin treatment had higher levels of proinflammatory cytokines than those who received melatonin.

The etiology of BPD is multifactorial and factors including mechanical ventilation induced barotrauma, volutrauma, oxygen toxicity, inadequate nutrition, infection and inflammation may contribute to impaired alveolar and vascular development in vulnerable premature lungs.[11,12] Excess generation of reactive oxygen and nitrogen species multiate molecules and are important mediators of cell and tissue damage.[13] Various pharmacologic and non pharmacologic approaches have been investigated for prevention and treatment of preterm lung injury and BPD.[14-17] Full-term neonates do not produce melatonin for 3-5 months leading to transient melatonin deficiency and this deficiency is more prolonged in premature neonates.[18] Evidence from several studies suggests a protective role for melatonin in perinatal diseases including RDS, asphyxia and sepsis.[6,10,19,20] In this study, we aimed to evaluate the effectiveness of melatonin for the prevention of BPD in preterm infants with RDS.

Material and Methods

This randomized clinical trial was conducted in AL Zahra hospital which is a tertiary, university referral center in the North West of Iran from March 2019-April 2020. The study was approved by the Ethics committee of Tabriz University of Medical Sciences by code IR.TBZMED.REC. 1398.574 at 2019.08.19 and registered in the Iranian Registry of Clinical Trials (IRCT) by the number IRCT 20190518043629N21. Parental informed written consent was obtained before patient enrollment. Based on the study by Gitto et al.[10] considering reducing the rate of mortality or BPD from 66% to 42% in the intervention group with power of 80% and alpha 0.05 we estimate that 40 cases were needed for each group. Inborn preterm infants with RDS who needed surfactant replacement therapy and had a gestational age of 27-32 weeks and a birth weight less than 1250 grams were eligible for the study. Exclusion criteria were major congenital anomalies, birth asphyxia (Apgar score ≤3 at the first minute of birth), major cardiac diseases (not including patent ductus arteriosus), chromosomal anomalies, abdominal distention or evidence for necrotising enterocolitis at first hours of birth, and parental refuse. Infants who need endotracheal intubation at birth due to ineffective respiratory drive were excluded from the study. Our center policy is stabilization of the infant by using continuous positive airway pressure (CPAP) for preterm neonates in the delivery room and saving intubation only for those who have apnea or ineffective respiration.

CPAP was administered through short bilateral nasal prongs, intermittently with a nasal mask. Distending pressure was generated by a variable flow nasal CPAP device and positive end-expiratory pressure (PEEP) 5-6 cm H2O and flow 6-7 litter/min (Fisher& Paykel Health Care limited, New Zealand). Patients with evidence of respiratory distress (tachypnea, retractions, and/or nasal flaring) shortly after delivery, a persistent oxygen requirement with FiO2 more than 0.3 and radiographic findings of RDS received surfactant within 6 hours of birth. Enrolled patients were randomly allocated in two groups by random number list generated by random number generator in sequentially numbered, opaque, sealed and stapled envelopes. Curosurf® (Poractant alpha, Chiesi Farmaceutici, Italy) 200mg/kg/dose (2.5 ml/kg /dose intra-tracheally) with the INSURE technique was used in group A. Patients in group B received melatonin (Nature Made, Pharmavit, USA) 5mg/kg /day per gastric tube for 3 days in addition to Curosurf®. After surfactant was administered, when the spontaneous respirations resumed, and adequate heart rate

and oxygen saturation was established, the endotracheal tube was removed and the infants were weaned to nasal-CPAP. Arterial blood gas parameters were recorded at admission and at 6 hour intervals after surfactant administration. The primary outcome was the development of BPD. The secondary outcome was considered other complications of prematurity, such as the duration of hospital stay and mortality. BPD was defined as the need for supplemental oxygen for at least 28 days and its severity determined at 36 weeks of gestation age based on the fraction of inspired oxygen.[11] Cranial ultrasound examination was performed on days 5 to 7 of birth for the diagnosis of intraventricular hemorrhage (IVH) by an experienced pediatric radiologist. Patent ductus arteriosus (PDA) was diagnosed based on clinical signs and confirmed by echocardiography performed by an expert pediatric cardiologist. Pneumothorax was determined by the presence of air in pleural space on chest X-ray. Necrotizing enterocolitis was suspected in neonates with abdominal distention and feeding intolerance. This diagnosis was confirmed by the presence of gas or air bubbles in the wall of the intestine on an abdominal X-ray and its severity was determined by Bell's staging system.[21]

Eye examination for the diagnosis of retinopathy of prematurity (ROP) was done through indirect ophthalmoscopy by an expert opthalmologist who was blind about patients groups.

Another independent researcher who was blind about patients groups completed a detailed questionnaire.

Statistical analyses were performed using the statistical package for social sciences (SPSS) version 17.0. Quantitative data were presented as mean ±standard deviation (SD) and qualitative data as frequency and percent. Independent t test were used for testing continuous normally distributed data. Categorical data were compared between groups using Chi- square or Fisher exact test. Two tailed tests were used and a p. value less than 0.05 was considered statistically significant.

Results

A total of 120 preterm infants with RDS were admitted to the neonatal intensive care unit (NICU) between March 2019 and April 2020. Ninty four infants met inclusion criteria with respect to their gestation age and birth weight. Five neonates were excluded from the study because of major congenital anomalies, 5 cases were intubated before arrival to the NICU and 4 patients because parents refused to consent. A total of 80 neonates were enrolled in the study including forty neonates in the surfactant group (group A) and 40 in the surfactant and melatonin group (group B). The mean gestation age and birth weight of studied patients were 31.3±3.8 wks and 1189±84 grams, respectively. Thirty-five (43.8%) patients were girls and 45 (56.2%) were boys. Demographic characteristics of patients are shown in Table I.

The number of patients to develop BPD was statistically significantly less in the group that received both surfactant and melatonin (n=24, (60%) in group A, n= 18 (%45) p=0.02).

The duration of hospital stay, need for mechanical ventilation and mortality were significantly lower in patients in group B. The complications and outcomes of patients in both groups are shown in Table II.

Maximal FiO_2 at the first hour and 4 hrs after surfactant replacement therapy was 0.37 and 0.35 in group A and 0.35 and 0.34 in group B respectively, without significant difference.

No adverse drug reaction was observed in patients receiving melatonin.

Discussion

In this study, administration of melatonin in addition to surfactant in comparison to surfactant replacement therapy alone decreased the duration of mechanical ventilation and hospital stay significantly. The incidence of BPD, PDA, IVH, air leak syndrome and mortality were reduced in neonates who received melatonin.

Table I. Demographic characteristics of study groups.

	Group A N= 40	Group B N=40	P value
Gestation age, weeks	29.3±2.9	27.9±3.9	NS
Birth weight, gr	1154±98	1112±124	NS
Maternal age, years	30.1±3.1	31.8±4.2	NS
Gender			NS
Male, n(%)	22(55)	23(47.5)	
Previous preterm labor n(%)	3 (7.5)	4 (10)	NS
Apgar score			
1st minute	6.1±1.7	5.9±1.7	NS
5th minute	8.3±1.02	8.4±1.02	NS

NS= not significant

Table II. Comparision of complications in both groups.

	Group A N=40	Group B N=40	P value	RR (95% CI)
Duration of MV, d	7.6±2.1	5.3±1.6	0.003	(0.1-3.8) 2
Duration of hospital stay, days	10.7±2.7	6.4±1.5	0.02	(1.5-2.1)1.5
BPD, n(%)	24(60)	18 (45%)	0.02	(0.1-6.23)2
IVH, n(%)	16(40)	12 (30)	0.04	(0.1-9.3) 1.5
PDA, n(%)	9(22.5)	5 (15)	0.03	(0.1-76.7)1.5
Air leak syndroms, n(%)	4 (10)	2 (5)	0.01	
Pulmonary hemorrhage, n (%)	3 (7.5)	3 (7.5)	1	
Mortality , n (%)	6 (15)	3 (7.5)	0.009	(0.1-8.6)2

MV= mechanical ventilation, BPD=bronchopulmonary dysplasia, IVH= intraventricular hemmorhagea, PDA= patent ductus arteriosus

Lung injury in the neonatal period is mediated by several etiologic factors such as genetic, hemodynamic, metabolic, nutritional, mechanical, and infectious or inflammatory mechanisms. All these events lead to an increased synthesis of free radicals which, in turn, induces oxidative stress-mediated tissue damage that is involved in the development of BPD.[18]

Numerous reports have demonstrated that melatonin is a broad-spectrum antioxidant.[22] Pan and et al.[23] demonstrated that the nocturnal administration of melatonin reduced interstitial fibrosis and the total number of alveoli caused by BPD. There are reports showing inflammatory cytokines are elevated in preterm newborns who develop BPD.[24,25] Gitto and et al.[26] showed melatonin treatment reduced the proinflammatory cytokines and improved clinical outcome. Melatonin stimulates several antioxidant enzymes in addition to its direct scavenging actions without pro-oxidant effects.

We did not observe any side effects in our patients due to melatonin. Melatonin appears safe and no side effects have been reported when100mg/kg was administered in 54 hours or 10 mg/kg once daily for 5 days.[27,28] Carloni et al.[29] used three different oral doses of melatonin: 0.1, 0.5 and 5 mg/kg in neonates born before 37 weeks of gestation. They found that a single intragastric administration of these doses resulted in a high peak plasma concentration that indicates that it is well absorbed after intragastric bolus. These high concentrations

appear not to be dose-dependent and are higher than what was found after oral administration in adults.

The limitations of this study was the small sample size, lack of long-term follow-up of studied patients and the lack of paraclinical data about proinflammatory cytokines.

In conclusion, the present study showed that melatonin administration as adjuvant therapy in preterm infants with RDS is associated with better outcomes with respect to duration of hospital stay, mortality, need for mechanical ventilation and complications of prematurity such as BPD, air leak syndromes, IVH and PDA. Future studies are recommended to establish the appropriate melatonin dose and timing of administration to achieve the best clinical outcome.

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Ethical approval

The study was approved by the Ethic committee of Tabriz University of Medical Sciences by code IR.TBZMED.REC. 1398.574 at 2019.08.19 and registered in Iranian Registry of Clinical Trials (IRCT) by number IRCT 20190518043629N21.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MMG, ES; data collection: MF; analysis and interpretation of results: MMG, SY; draft manuscript preparation: MMG, MF. 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

Correlation and prediction of arterial partial pressure of carbon dioxide from venous umbilical blood gases

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ABSTRACT

Background. Arterial partial pressure of carbon dioxide (pCO_2) samples are lower in children and higher in fetuses when compared with venous samples. The correlation and prediction of pCO_2 from umbilical venous (UVBG) to umbilical arterial blood gas (UABG) dyad in neonates are identified.

Methods. A prospective study was performed from July 2018 to December 2019. Two dependent tests and a multivariate regression model were used to analyze the comparison and correlation tests.

Results. A total of 116 paired UABG and UVBG samples were obtained. The medians (interquartile ranges, IQR) were as follows: gestational age of 34 (29-37) weeks, birth weight of 2122 (1146-2839) g, and postnatal age of 2.3 (1.4-10.8) h. The median (IQR) $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ measurements were 40.2 (33.5-45.8) and 40.4 (34.7-46.8) mmHg, respectively (rho = 0.75, $p < 0.001$). The median of the differences (IQR) in $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ was −0.9 (−4.7 to 2.3) mmHg, ($p = 0.06$). The equation to predict $pCO_{2(UABG)}$ was $0.9 \times pCO_{2(UVBG)} + 4$, as derived from simple linear regression. The best model for predicting $pCO_{2(UABG)}$ was $0.9 \times pCO_{2(UVBG)}$ - 0.7 × venous base excess + 0.6 × 5-min Apgar score + 6.1 × meconium aspiration syndrome - 7.7 × patent ductus arteriosus - 6.5 (adjusted $r^2 = 0.74$).

Conclusions. $pCO_{2(UVBG)}$ correlates with and can predict $pCO_{2(UABG)}$. Therefore, $pCO_{2(UVBG)}$ can be applied to $pCO_{2(UABG)}$ in neonates for whom UAC insertion is unsuccessful or to avoid an arterial puncture.

Key words: blood gas analysis, carbon dioxide, newborn, umbilical arteries, umbilical vein.

Partial pressure of carbon dioxide (pCO_2) from arterial blood gases (ABG) is the gold standard in assessing ventilation. Hypercarbia and hypocarbia are associated with respiratory and neurologic complications.[1] Moreover, the ventilator setting for respiratory acidosis or alkalosis needs to be adjusted. This usually should improve ventilatory treatment by optimizing tidal volumes, therefore reducing acute lung injury from volutrauma.[2]

Umbilical arterial catheter (UAC), intermittent peripheral arterial punctures, or arterialized capillary blood samples can be used to directly measure ABG values in neonates. However, blood gases (BG) obtained by arterial or heel puncture are associated with the future development of cellulitis, abscess, necrotizing chondritis of the calcaneus cartilage, or calcaneal osteomyelitis and can cause severe pain in fragile neonates. A UAC can be used up to 5 days, whereas an umbilical venous catheter (UVC) can be used up to 14 days.[3]

To reduce the harmful effect of ventilatory support and promote gentle care in the neonatal intensive care unit (NICU), continuous noninvasive monitoring of ventilation (CO_2) is

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recommended. However, to date there have been few studies examining the agreement and correlation of both end-tidal carbon dioxide ($EtCO_2$) and transcutaneous carbon dioxide ($TcCO_2$) methods with arterial pCO_2.[1]

Compared with fetuses and children, neonates have a different anatomy and physiology. Between fetal and child circulation, neonatal circulation is intermediate. pCO_2 levels from arterial samples in umbilical cord (fetal),[4-8] neonates,[9] and children/adults[10-17] are physiologically higher, similar, and lower than venous samples, respectively. However, few studies have compared BGs in the neonatal period. The purpose of this study was to examine the correlation and prediction of pCO_2 from UVBG to UABG ($pCO_{2(UVBG)}$ to $pCO_{2(UABG)}$) dyads.

Material and Methods

Settings and study design

The STARD guidelines were followed in a prospective study conducted at a neonatal intensive care unit (NICU) in Thailand from July 1, 2018 to December 31, 2019. The study was approved by the Ethics Committee Board of the Faculty of Medicine, Prince of Songkla University (REC 60-383-01-1) and registered in the Thai Clinical Trials Registry (TCTR20180216001).

Neonates with both UAC and UVC readings available were the main inclusion criterion. The exclusion criteria were neonates with unstable vital signs, congenital heart disease, or the parents' decision not to participate. Umbilical blood was sampled by clinical indications. For BG analysis, after informed consent was provided, 0.2 mL each of UAB and UVB was drawn as simultaneously as possible (within 1 min) from each catheter. No repeat samples were drawn from the same neonate (one paired sample per one neonate). An ABL800 BASIC (Radiometer Medical ApS™, Denmark), a BG and electrolytes analyzer, was used to analyze all blood samples within 3 min after the blood was drawn.

Statistical analysis

To develop a categorical and continuous variable database, the R program (version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria) was used. Categorical variables are presented as frequency and percentage. Parametric continuous variables are presented as mean (standard deviation, SD) and paired *t*-test was used to compare paired samples. Nonparametric continuous variables are presented as median (interquartile range, IQR) and the Wilcoxon signed rank test with continuity correction was used to compare paired samples. Pearson (parametric variables; r) and Spearman's rank (nonparametric variables; ϱ) tests were used to analyze correlations. The cutoff points of postnatal age (for comparison) and pCO_2 level (for correlation) for the subgroup analysis were 24 h and 35-45 mmHg (normocarbia), respectively. Patent ductus arteriosus (PDA) is functionally closed by 24 h after birth.

For $pCO_{2(UABG)}$ prediction, simple and multivariate linear regression were used. Significant variables from previous studies for $pCO_{2(UVBG)}$, venous base excess (VBE), gestational age,[4,18] postnatal age, 5-min Apgar score,[9] and respiratory problems (binary variables including respiratory distress syndrome (RDS), meconium aspiration syndrome (MAS), pneumonia, and PDA)[19] were entered to adjust the outcome. The most parsimonious model was determined by the multivariate analysis model with the lowest Akaike information criteria (AIC). Sample size was calculated as a minimum of 30 neonates based on a previous study, but we increased the number of participants to 116 neonates to increase the power of the study. G*Power version 3.1.9.2 was used to calculate post hoc power analysis. All p-values were two-tailed, and values less than 0.05 indicated statistical significance.

Results

One hundred sixteen paired UABG and UVBG samples were tested in the study. The medians (IQRs) of gestational age, birth weight, and time of performing the blood gas analyses were 34 (29-37) weeks, 2122 (1146-2839) g, and 2.3 (1.4-10.8) h, respectively. BG measurements of 96 neonates (83%) were obtained within 24 h of birth. Apgar 1-min and 5-min median (IQR) scores were 7 (4-8) and 8 (6-9), respectively. The enrolled neonates had incidences of RDS of 50%, MAS of 8%, pneumonia of 7%, and PDA 7%. During blood gas collection, the numbers (percentage) of neonates on respiratory or oxygen support with high-frequency oscillation, assist-control, synchronized intermittent mandatory ventilation, bilevel positive airway pressure, and high flow nasal cannula were 64 (55.2%), 44 (37.9%), 6 (5.2%), 1 (0.9%), and 1 (0.9%), respectively.

Figure 1 shows the scatterplot between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$. $pCO_{2(UABG)}$ had a median (IQR) of 40.2 (33.5-45.8) mmHg and $pCO_{2(UVBG)}$ had 40.4 (34.7-46.8) mmHg ($\varrho = 0.75$, $p < 0.001$). The median of the differences (IQR) between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ was −0.9 (−4.7 to 2.3) mmHg ($p = 0.06$; post hoc power = 100). The box plots of the differences between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ for each gestational age are shown in Figure 2. In addition, the mean ± SD of $pCO_{2(UABG)}$ was 40.9 ± 13.6 and $pCO_{2(UVBG)}$ was 41.6 ± 12.6 mmHg ($r = 0.82$). The mean of the differences (95% confidence interval) between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ was −0.7 (−2.2 to 0.7) mmHg ($p = 0.33$). All other parameters of the blood gases are shown in Table I.

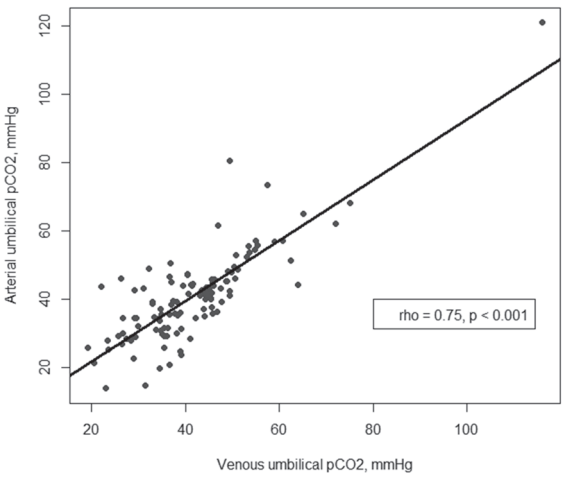

Fig. 1. The scatterplot between arterial and venous umbilical pCO_2.

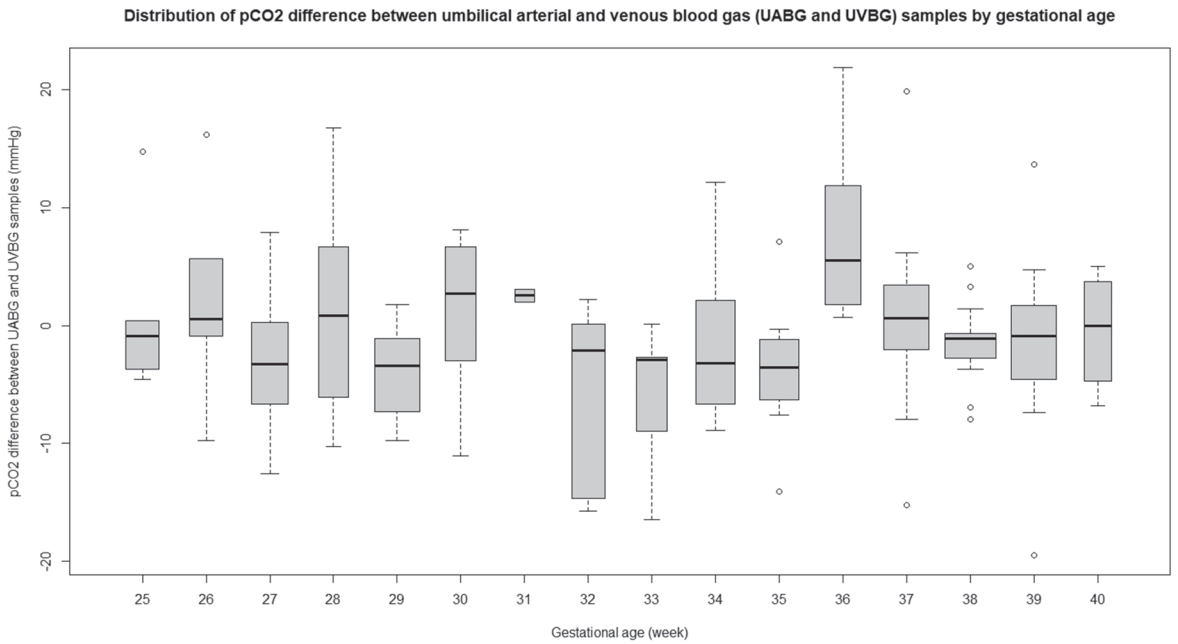

Fig. 2. The box plots of the differences between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ for each gestational age.

Table I. Comparison between umbilical arterial and venous blood gas (UABG and UVBG) of pH, pCO_2, pO_2, HCO_3, and base excess values.

	UABG*	UVBG*	UABG-UVBG*	p-value
pH	7.30 (7.26, 7.36)	7.30 (7.24, 7.35)	0.01 (−0.01, 0.02)	0.05
pCO_2, mmHg	40.2 (33.5, 45.8)	40.4 (34.7, 46.8)	−0.9 (−4.7, 2.3)	0.06
pO_2, mmHg	71.3 (58.7, 101.0)	51.0 (40.5, 61.7)	19.9 (9.5, 43.0)	<0.001
HCO_3, mEq/L	19.1 (17.4, 20.7)	19.2 (16.7, 20.4)	−0.1 (−0.8, 1.0)	0.78
Base excess	−6.60 (−8.75, −4.38)	−6.45 (−10.00, −4.70)	−0.30 (−1.10, 1.30)	0.69

*median (interquartile range)

The median of the differences (IQR) for the subgroup analysis between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ within 24 h of birth was −0.8 (−4.5 to 2.6) mmHg (ϱ = 0.73, p = 0.19). The mean of the differences ± SD between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ after 24 h of life was −2.5 ± −5.6 mmHg, (r = 0.89; p = 0.06). As shown in Table II, a pCO_2 level of 35-45 mmHg had 87% sensitivity, 94% specificity, 15.05 positive likelihood ratio, 0.14 negative likelihood ratio, and 91% accuracy of correlation.

The equation $pCO_{2(UABG)} = 0.9 \times pCO_{2(UVBG)} + 4$, from the simple linear regression, was used to predict $pCO_{2(UABG)}$ (r^2 = 0.68). The final factors to predict $pCO_{2(UABG)}$ in the parsimonious model (AIC = 786.6) were $pCO_{2(UVBG)}$, VBE, 5-min Apgar score, MAS, and PDA; all of these variables were statistically significantly different in the multivariate linear regression analysis, as shown in Table III. The equation $pCO_{2(UABG)} = 0.9 \times pCO_{2(UVBG)} - 0.7 \times VBE + 0.6 \times$ 5-min Apgar score + 6.1 × MAS - 7.7 × PDA - 6.5 (adjusted r^2 = 0.74) was the best model for predicting arterial pCO_2 values.

Discussion

The study has some clinical implications. Previous studies indicated that the pCO_2 mean differences ranges between venous and arterial samples were 3.9-4.4 in adults,[10,11] 3.5-7.3 in children,[12-16] 0.9 in neonates (one study published more than 50 y ago),[9] and −10 to

Table II. Correlation between arterial and venous pCO_2 values.

		$pCO_{2(UABG)}$, mmHg		
		<35	35-45	>45
$pCO_{2(UVBG)}$, mmHg	<35	32	0	0
	35-45	4	41	0
	>45	0	6	33

$pCO_{2(UABG)}$: partial pressure of carbon dioxide from umbilical arterial blood gas, $pCO_{2(UVBG)}$: partial pressure of carbon dioxide from umbilical venous blood gas

Table III. Multivariate linear regression for prediction of pCO_2 in umbilical arterial blood gas.

Variable	Coefficient	Standard error	t-value	p-value
Intercept	–6.5	3.84	–1.70	0.09
$pCO_{2(UVBG)}$	0.9	0.05	17.48	<0.001
Venous base excess	–0.7	0.14	–4.76	<0.001
5-min Apgar score	0.6	0.29	2.01	0.046
Meconium aspiration syndrome	6.1	2.43	2.51	0.01
Patent ductus arteriosus	–7.7	2.58	–2.98	0.003

$pCO_{2(UVBG)}$: partial pressure of carbon dioxide from umbilical venous blood gas

−14 mmHg from umbilical cord (fetal)[4-8] blood samples. Most studies were based on umbilical cord (fetal) sampling. To increase the statistical power, this study in neonates used a larger sample size (116 neonates) than the previous study (18 neonates).[9] In this study, the difference in pCO_2 between venous and arterial blood gas (0.7 mmHg) was consistent with the previous study (0.9 mmHg).[9]

In our study, 17% of the blood samples were drawn after 24 hours of birth, whereas none of the blood samples were acquired within this time in the previous study.[9] $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ showed a strong correlation and no differences in blood measurements obtained more than 24 h after birth. Based on our findings, a cut-off point of pCO_2 was established, at which a high correlation for pCO_2 (35-45 mmHg) levels was observed between the arterial and venous samples, and moderate to strong correlation in postnatal ages within and after 24 h after birth.

There were neither significant clinical (0.9 mmHg) nor statistical (p = 0.06; post hoc power = 100%) differences between paired $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$. The equations $pCO_{2(UABG)} = 0.9 \times pCO_{2(UVBG)} + 4$ (simple) and $pCO_{2(UABG)} = 0.9 \times pCO_{2(UVBG)} - 0.7 \times VBE + 0.6 \times$ 5-min Apgar score $+ 6.1 \times MAS - 7.7 \times PDA - 6.5$ (regression) were used to predict $pCO_{2(UABG)}$. A UVC can be used longer than a UAC insertion. Therefore, in neonates in whom UAC insertion is unsuccessful or to avoid an arterial puncture, $pCO_{2(UVBG)}$ can be applied to $pCO_{2(UABG)}$.

The trend and real-time assessment of arterial pCO_2 can be monitored continuously and noninvasively. In prospective studies between $EtCO_2$ and pCO_2, the average mean difference was 7 (range 2-11) and the correlation coefficient was 0.7.[1] Between $TcCO_2$ and pCO_2, the average mean difference was 2 and the correlation coefficient was 0.9.[1] In this study, the mean difference and correlation coefficient were less than 1 and 0.82, respectively. Moreover, clinical implications for both methods has limitations. The $EtCO_2$ analysis can be influenced by ventilation-perfusion mismatches, or kinks or secretion obstructions in the endotracheal tube, and cannot be used currently during noninvasive or high-frequency ventilation (not accurate due to small tidal volume and higher respiratory rate).[1] The $TcCO_2$ analysis influences heat-induced skin damage from the electrodes, which affects reliability due to technical limitations (skin edema, poor tissue perfusion, acidosis sensor preparation, positioning, and repeated changes of location), initial measurement takes time and response time is slower when compared with $EtCO_2$.[1]

This study had some limitations. First, some confounders of pCO_2 levels in previous studies are as follows: VBE, gestational age, postnatal age, Apgar score, and respiratory problems (RDS, MAS, pneumonia, and PDA) from the previous studies; however, analysis was adjusted by multivariate regression. Second, UVBG and UABG in a previous[9] and this study were compared from post-ductal samples. Most ductus arteriosus close within 24 h after birth, which affects circulation. Echocardiography was performed only on patients with suspected PDA. Information bias may have occurred because during the study period, echocardiography was not normally performed while obtaining BG. Finally, we are curious when the arteriovenous pCO_2 difference in neonates (−0.9 mmHg) becomes similar to children and adults (4-6 mmHg).

This study found a strong correlation and no significant difference between $pCO_{2(UABG)}$ and $pCO_{2(UVBG)}$ as well as within and after 24 h after birth. Thus, we suggest that $pCO_{2(UVBG)}$ values can be substituted for $pCO_{2(UABG)}$. Further studies are needed to determine the time after birth neonatal differences in pCO_2 between ABG and VBG become equal to children and adults.

Ethical approval

The study was approved by the Ethical Committee Board of Faculty of Medicine, Prince of Songkla University (REC 60-383-01-1).

Author contribution

AT, WJ, SD, GM and MP designed the study. AT and KC collected and analyzed the data. AT, KC, WJ, SD, GM and MP drafted the manuscript. AT and NA analyzed, interpreted of data, and critically revised the manuscript for important intellectual content. All authors have read, and approved the final manuscript. AT will act as Guarantor for this paper.

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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 immature granulocyte percentage in predicting acute chest syndrome and the severity of the vaso-occlusive crisis in sickle cell disease

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ABSTRACT

Background. Sickle cell disease (SCD) is an inflammatory disease that can result in both chronic and acute inflammation. Immature granulocytes (IG) are not-yet-mature white blood cells that can be easily detected in complete blood count (CBC) tests. In recent studies it has been suggested that IG may play a role in determining the prognosis of inflammatory diseases. The aim of our study was to investigate the role of IG percentage on predicting acute chest syndrome (ACS) and the severity of vaso-occlusive crisis (VOC) in patients with SCD.

Methods. The study cohort consisted of 49 SCD patients admitted to the emergency department for VOC. If symptoms did not regress despite appropriate treatment including hydration and analgesia, they were hospitalized. Patients whose symptoms regressed were discharged from the emergency department within 24 hours. Blood samples, including CBC and C-reactive protein (CRP), a marker of inflammation, were taken within the first hour of admission. Steady state laboratory parameters from the previous visit in the last three months were collected from patient files.

Results. The mean age was 18±4 (range 8-25) years. Most were hospitalized (41/49; 83.7%) and 8 of 49 were discharged from the emergency department after their treatment for VOC. ACS developed in 13 of 49 (26.5%). White blood cell, neutrophil and nucleated red blood cell counts, percentage of IG (IG%) and CRP levels were significantly increased in patients with VOC. IG% of patients with ACS was significantly higher than patients without ACS. However, ROC analysis showed that IG% was not associated with the development of ACS or hospitalization for VOC.

Conclusions. Despite a small SCD cohort, the significant increase in the IG% in patients with VOC compared to their baseline values has suggested a role for IG% in predicting VOC. Although IG% was higher in ACS, its utility in predicting ACS was poor.

Key words: sickle cell disease, inflammation, immature granulocyte.

Sickle cell disease (SCD) arises due to a point mutation in the gene encoding the beta globin subunit. Hypoxia, acidosis and dehydration induce the polymerization of sickle hemoglobin (HbS) that results in decreased deformability of red blood cells (RBC). This results in vaso-occlusive crisis (VOC), ischemia-reperfusion injury, and endothelial dysfunction.[1]

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Sickle cell vasculopathy is characterized by sterile inflammation and neutrophil counts are higher in SCD patients, both when the disease is stable, the steady-state, and during exacerbation such as VOC, than in healthy controls. It has been reported that high neutrophil counts were associated with acute chest syndrome (ACS), silent brain infarcts, hemorrhagic strokes, and early death in SCD patients.[2,3] In SCD, neutrophils have increased adhesive properties and adhesion of neutrophils to the inflammation-induced endothelium and to

sickle erythrocytes is particularly important in VOC pathogenesis.[3-5]

Immature granulocytes (IG) are a not-yet-mature subset of white blood cells that can be easily detected in complete blood count tests. IG contain granules in their cytoplasm and include metamyelocytes, myelocytes and promyelocytes. The IG count is an indicator of bone marrow activation, particularly leukopoiesis, and raised IG counts are found in response to inflammatory conditions, as well as in the neonatal period or in pregnancy. Recent studies have suggested a role for the IG percentage (%) in peripheral blood in determining the prognosis of inflammatory diseases.[6-10]

While the role of neutrophils in the pathogenesis of VOC is known, there is no data about the relation of IG% with VOC. The aim of this study was to investigate IG% in the steady state and during VOC in SCD, and to evaluate its potential role in predicting VOC. We also aimed to determine the relationship between IG% and other inflammatory parameters in SCD.

Material and Methods

This retrospective study included SCD patients that were under follow-up between November 2020 and February 2021 by the department of Pediatric Hematology and were admitted to the emergency department for VOC. If the symptoms did not regress, despite appropriate treatments such as hydration and analgesics, they were hospitalized. Patients whose symptoms regressed were discharged from the emergency department within 24 hours. The patients who had another inflammatory disease and infection were excluded from this study. ACS was diagnosed by the presence of fever and/or respiratory symptoms and a new pulmonary infiltrate on chest X-Ray.

Blood samples were taken from all patients within the first hour of admission. Complete blood count (CBC) was performed on an automated hematology analyzer, XN-1000 (Sysmex Corp., Kobe, JAPAN). IG% was calculated from the white cell differential channel based on granularity and nucleic acid content. Steady state laboratory parameters from the previous visit and within the three months prior to the emergency admission were collected from patient records.

The study was approved by Mersin University Ethical Board (number: 03.03.2021; 2021/220).

Statistical analyses were performed using SPSS Statistics for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean± standard deviation (SD) while frequency and percentage (%) were used to define categorical data. Categorical variables were analyzed using the Chi-square test and Fischer's exact test. Normality of distribution was examined using the Kolmogorov-Smirnov test. Mann-Whitney U test was used for comparisons between two groups.

Receiver Operating Curve (ROC) analysis was performed to determine the effect of IG% on having ACS or hospitalization for VOC. The areas under the curve (AUC) of the IG% parameter, statistical significance level, cut-off sensitivity and specificity values were calculated.

Results

During the study period a total of 49 SCD patients (43 HbSS and 6 HbSß) were admitted to the emergency department for VOC. The mean±SD age was 18±4 (range 8-25) years. Thirty-two patients (67%) were male and 17 (33%) were female. Twenty-three patients had ACS, 15 patients had avascular necrosis (AVN) and three patients had a history of stroke. The mean annual VOC frequency of the patients was 1.7±0.88 (range 1-5). Eleven patients (22%) were transfused and three patients were on the chronic transfusion program in the last year. Of the 49 patients, 46 (93.9%) were on hydroxyurea treatment.

Forty-one of 49 (83.7%) patients were hospitalized and the remaining eight were

discharged from the emergency department after their treatment for VOC. ACS developed in 13 of 49 (26.5%) patients.

All inflammatory markers, including leucocyte (WBC) and neutrophil counts, C-reactive protein (CRP) levels, and IG%, were significantly different during VOC compared with the previous steady state values (Table I). Platelet counts and hemoglobin (Hb) concentrations were significantly decreased and WBC and neutrophil counts, nucleated red blood cell (NRBC) %, IG% and CRP levels were significantly increased in VOC.

Hb levels were significantly lower (p=0.027) and CRP levels were significantly higher (p=0.030) in patients who were hospitalized compared to patients discharged from the emergency department (Table II). There was no correlation between IG% and length of stay in the patients who were hospitalized.

The IG% of patients with ACS was significantly higher than patients without ACS (Table III). Using ROC analysis to investigate the relationship of IG% and ACS, the cut-off value for IG% was >1%, specificity 60.53%, sensitivity 57.14%, and the AUC was 0.52. Thus IG% was not discriminatory for ACS (p=0.82). Similarly IG% was not significant in identifying which patients would be hospitalized for VOC (p=0.829; specificity 41.7%; sensitivity 75%; and AUC=0.524).

When SCD patients were grouped according to genotype, IG% was significantly higher in SS than SB patients during VOC (p=0.016). All

Table I. Laboratory parameters of the patients (n=49) during steady state and VOC.

	Steady state	VOC	p value
WBC ((μL)	11.261 ± 3.391	17.215 ± 5967	0.001
Hb (g/dL)	9.3 ± 1.1	9.0 ± 1.5	0.037
Platelet (μL)	471.204 ± 192.504	387.122 ± 198.561	0.006
Neutrophil (μL)	5.302 ± 1.952	9.431 ±4.083	0.001
IG%	0.37 ± 0.18	1.35 ± 1.46	0.001
NRBC %	1.38 ± 2.37	3.59 ± 7.62	0.017
MPV (fl)	8.17 ± 3.45	8.42 ± 3.26	0.374
CRP(mg/dL)	12.32 ± 33.00	14.85 ± 15.90	0.001

VOC: Vaso-occlusive crisis, WBC: White blood cell, Hb: Hemoglobin, IG%: immature granulocyte percentage, NRBC: Nucleated red blood cell, MPV: Mean platelet volume, CRP: C-reactive protein

Table II. Comparison of laboratory parameters in the SCD patients who were hospitalized or non hospitalized for VOC.

	Non-hospitalized patients (n=8)	Hospitalized patients (n=41)	P value
WBC(μL)	18.030±3.947	17.055±6.098	0.438
Hb (g/dL)	9.9±1.0	8.8±1.6	0.027
Platelet (μL)	463.500±138.558	372.220±202.294	0.193
Neutrophil (μL)	9.219±4.963	9.472±3.961	0.895
IG%	1.20±0.86	1.38±1.60	0.559
NRBC %	1.57±1.20	4.02±8.47	0.391
MPV (fl)	8.23±3.36	8.46±3.28	0.523
CRP (mg/dL)	8.42±10.64	16.10±10.64	0.030

SCD: sickle cell disease, VOC: vaso-occlusive crisis, WBC: white blood cell, Hb: hemoglobin, IG%: immature granulocyte percentage, NRBC: nucleated red blood cell, MPV: mean platelet volume, CRP: C-reactive protein

Table III. SCD patients with or without acute chest syndrome (ACS).

	ACS (n=13)	Without ACS (n=36)	p value
WBC (μL)	17.816 ±8.532	16.998±8.532	0.526
Hb (g/dL)	9.4 ±2.0	8.8±1.3	0.196
Platelet (μL)	365.838±235.301	394.808±186.704	0.571
Neutrophil (μL)	8.562±3.659	9.744±4.230	0.428
IG%	2.48±2.18	1.00±0.8	0.009
NRBC %	2.95±7.0	3.86±8.15	0.545
MPV (fl)	8.22±3.71	8.49±3.13	0.856
CRP (mg/dL)	15.39±12.78	14.65±17.0	0.556

SCD: sickle cell disease, ACS: acute chest syndrome, WBC: white blood cell, Hb: hemoglobin, IG%: immature granulocyte percentage, NRBC: nucleated red blood cell, MPV: mean platelet volume, CRP: C-reactive protein

other CBC and inflammatory parameters were similar. There was no correlation between IG% and the history of the type of sickle cell disease, AVN, ACS, stroke and annual frequency of painful crisis. In addition, no correlation was found between chronic transfusion and IG%.

Discussion

Immature granulocytes are produced and differentiated in bone marrow, and their presence in the circulation indicates greatly increased bone marrow activation due to infectious or inflammatory conditions.[11] Additionally, IG% may be elevated in other conditions, like cancer and during pregnancy. To our knowledge, the relationship of IG% with clinical and laboratory findings in SCD has not been investigated to date. In our study, IG% level was found to be significantly higher during VOC than when the disease is stable, the steady state. Similarly, patients with ACS had significantly higher IG% than the patients without ACS.

CRP is recognized as a good marker of acute and chronic inflammation and is frequently used in SCD patients. High levels of CRP at steady state are associated with an increased frequency of acute pain and increased levels during VOC have been reported to be valuable in predicting the development of ACS in SCD patients.[12] We found high CRP levels in patients with SCD both during steady state and VOC. CRP levels were significantly higher in the patients who were hospitalized than the patients discharged from the emergency department after their treatment for VOC (p=0.03).

It is known that neutrophil counts are high in patients with SCD, both during steady state and VOC, and is also related with increased mortality risk.[3] In our study, both WBC and neutrophil counts were increased during VOC compared to steady state levels (p<0.001). However, there was no difference in WBC and neutrophil counts between patients with VOC who were hospitalized and those discharged from the emergency department. In addition there was no correlation between WBC and neutrophil counts were not determinants of the length of hospital stay.

IG level was used as an early biomarker to show infection and inflammatory status in some studies. IG% was found to be increased in a study of patients with peripheral enthesitis and correlated with CRP elevation and clinical activity.[13] Narcı et al.[8] investigated IG% in patients with upper gastrointestinal bleeding and reported that IG% was significantly higher in patients who died compared with patients who were discharged. The IG% was specific (93.8%) and sensitive (100%) in predicting in-hospital mortality. Unal et al.[9] suggested that increased IG% is a simple, fast, and effective marker in the early prediction of acute necrotizing pancreatitis. Additionally, Güngör et al.[10] reported that IG% had higher

sensitivity and specificity in predicting systemic inflammatory response syndrome in patients with acute pancreatitis. However, Park et al.[7] suggested that the diagnostic ability of IG% was insufficient in patients with acute and complicated appendicitis, and was of no additional benefit in investigating appendicitis compared with other inflammatory markers.

In our study, IG % was found to be significantly higher during VOC than the steady state ($p<0.001$). However, there was no difference in IG% between the patients who were hospitalized and the patients discharged from the emergency department. In addition, there was no correlation between the IG% and length of stay in the patients who were hospitalized. Also, ROC analysis indicated that IG% was not discriminatory for hospitalization for VOC.

ACS is an important cause of morbidity and mortality in children and adults with SCD. Several risk factors have been associated with the development of ACS, such as young age, low HbF, high baseline hemoglobin, steady-state leukocytosis, and airway hypersensitivity. Since two-thirds of ACS episodes occur in patients hospitalized for VOC, identifying biomarkers to predict ACS occurrence would be of clinical benefit.[14] In our study, ACS developed in 26.5% of patients who were admitted to the emergency department because of VOC. The IG% of patients with ACS was significantly higher than patients without ACS. Despite this, ROC analysis showed that the IG% parameter did not have discriminatory power for the occurrence of ACS.

Biomarker investigations are ongoing to predict VOC in SCD. While the role of IG%, an easily calculated parameter in CBC, has been investigated in determining the severity of diseases in many infectious or inflammatory diseases, it has been evaluated for the first time in SCD in this study. Despite the limited number of patients, the significant increase in IG% in patients with VOC compared to the steady state has suggested a role for IG% in predicting VOC in SCD. Although IG% was higher in patients with ACS, analysis showed that this was insufficiently discriminatory in predicting ACS.

Ethical approval

The study was approved by Mersin University Ethical Board (number: 03.03.2021; 2021/220).

Author contribution

All authors confirm their contribution with data input and interpretation, drafting the manuscript. FK; draft manuscript preparation. SÜ; study conception and design. DBT; analysis of biochemical data. YÖ; analysis and interpretation of results. GB; data collection. 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

Soluble urokinase plasminogen activator receptor: a novel biomarker of pediatric community-acquired and hospital-acquired pneumonia

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ABSTRACT

Background. Soluble urokinase plasminogen activator receptor (suPAR) is an emerging biomarker in different clinical disorders but data in pediatric pneumonia is scarce. Our objective was to assess utility of suPAR in pediatric community-acquired and hospital-acquired pneumonia.

Methods. A prospective observational study including 120 hospitalized pneumonia patients and 55 healthy controls. Patients fell into two groups: community-acquired pneumonia (CAP) group (75 patients) and hospital-acquired pneumonia (HAP) group (45 patients). CAP severity scores were calculated, including Predisposition, Insult, Response, Organ dysfunction modified (PIROm) score and Pediatric Respiratory Severity (PRESS) Score. suPAR was measured to CAP patients on admission and to HAP patients on the day of pneumonia diagnosis. suPAR was also measured to controls.

Results. suPAR was higher among the whole patient cohort compared with controls ($p<0.001$) and higher among CAP group compared with both controls ($p<0.001$) and HAP group ($p<0.001$). No significant difference was found between HAP and control groups. suPAR was higher among CAP patients with shock, PICU admission, mechanical ventilation, and death ($p=0.013$, 0.044, 0.019, 0.049 respectively). Among CAP patients, suPAR correlated with oxygen saturation, pulse rate, respiratory rate, PRESS, and PIROm. suPAR had area under Receiver Operating Characteristic Curve=0.68 for prediction of severe CAP. Among HAP group, suPAR was negatively correlated with oxygen saturation ($rs=-0.31$; $p=0.048$) and was higher among patients with shock ($p=0.005$) and among those with increased pediatric Sequential Organ Failure Assessment (pSOFA) score ($p=0.034$).

Conclusions. suPAR is promising for diagnosing pediatric CAP but not HAP. suPAR predicted illness severity in both CAP and HAP but performed better in the former.

Key words: urokinase plasminogen activator receptors, pediatrics, biomarkers, pneumonia, ventilator-associated pneumonia, healthcare-associated pneumonia.

Pneumonia is an infection in the lower respiratory tract in which the inflammatory process leads to accumulation of fluid in the airspaces which interferes with gas exchange, leading to the typical symptoms of tachypnoea, increased work of breathing, hypoxia, and cough.[1]

Pediatric pneumonia is associated with significant morbidity and mortality worldwide, accounting for around 15% of deaths in children under the age of five years.[2]

Pneumonia is classified into community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP). HAP is defined as pneumonia which is not incubating at the

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time of hospital admission and occurs more than 48 hours after admission.[3] Usage of the term HAP is inconsistent: some use it to refer to any pneumonia that develops in the hospital, including ventilator-associated pneumonia (VAP)[4], while others use the term to refer specifically to pneumonia that develops in the hospital without an association with mechanical ventilation.[3] According to the latter view, HAP and VAP are mutually exclusive entities.

Although both CAP and HAP represent infections of the lung parenchyma, major differences exist between them in terms of the causative pathogens, risk factors, diagnostic criteria, treatment options, and prognosis.

Physicians need objective tools for diagnosing pneumonia, assessing its severity, and predicting its outcome. Soluble urokinase plasminogen activator receptor (suPAR) has recently emerged as a promising candidate.

suPAR is produced by cleavage of urokinase plasminogen activator receptor (uPAR) from cell surface.[5] uPAR is a membrane receptor expressed on monocytes, macrophages, activated T-lymphocytes, and natural killer cells. It binds urokinase plasminogen activator (uPA) and its precursor (pro-uPA). After association with uPAR, pro-uPA is converted to the active enzyme uPA which subsequently activates plasminogen to generate plasmin, producing broad-spectrum proteolytic activity.[6]

uPAR was found to play a role in cell adhesion, chemotaxis, and migration. It can form complexes with the β2-integrin CD11b/ CD18, thereby modulating the migration-promoting activity. suPAR, similarly, has chemotactic properties.[5,7]

suPAR was found to possess diagnostic and prognostic roles in various clinical disorders associated with immune system activation like liver diseases, renal diseases, systemic lupus erythematosus, psoriasis, and malignancy.[8] Furthermore, suPAR proved to be a useful biomarker in sepsis, tuberculosis, malaria, and human immunodeficiency virus.[5] Nevertheless, studies investigating the role of suPAR in pediatric pneumonia are rare, particularly in pediatric HAP, where the topic has not been studied before. The aim of the present study was to assess the diagnostic and prognostic values of suPAR in pediatric pneumonia.

Material and Methods

This was a prospective observational study conducted on 120 patients admitted into the Pediatric Intensive Care Unit (PICU) and the pediatric inpatient ward of a university hospital. The study protocol was approved by the local ethical committee and a written informed consent was obtained from parents.

The study was conducted on children with pneumonia from the age of one month to 15 years. Two patient groups were recruited: the first included children hospitalized with a diagnosis of CAP while the second included children with HAP. Besides, 55 healthy children served as a control group.

CAP was diagnosed in the presence of signs and symptoms of lower respiratory tract infection in a previously healthy child, in association with pulmonary infiltrate on chest radiograph, provided that the infection was acquired outside the hospital. Patients with CAP were hospitalized and admitted into the PICU according to specific criteria.[9]

For the CAP group, exclusion criteria were age <1 month or >15 years; co-existence of another infection with CAP; cough for >14 days; acute bronchiolitis; suspected tuberculosis; chronic respiratory disorders (e.g., persistent asthma, cystic fibrosis, foreign body aspiration, congenital lung anomalies, chronic aspiration; or immune deficiency disorders). Patients who reported history of previous episodes of CAP were not excluded from the study if the number of these episodes was ≤ two and the patient had been symptom-free for> 14 days after the last episode.

CAP severity was assessed on admission according to revised World Health Organization (WHO) criteria for children aged 2 months to 5 years[10]; Respiratory Index of Severity in Children (RISC) score for children <2 years[11]; Pediatric Respiratory Severity Score (PRESS) for patients up to 15 years[12]; and Predisposition, Infection, Response and Organ failure (PIROm) score for patients up to 15 years.[13]

Patients with HAP included 2 subgroups: (1) "VAP": defined as pneumonia that occurred >48 hours after endotracheal intubation. (2) "Non-ventilator HAP": defined as pneumonia that developed >48 hours after hospital admission in the absence of endotracheal intubation.

Patients initially hospitalized for non-respiratory infections (like meningitis), then developed HAP, were not included in the study unless signs of active infection had disappeared, and their C-reactive protein (CRP) levels had become negative. Co-existence of other hospital-acquired infections with HAP was another exclusion criterion. Patients initially hospitalized for pneumonia, then improved but later developed HAP, were not included in the study except after 14 days of admission, which is the "repeat infection timeframe" necessary of an infection of the same type to be considered a new event.[14]

For patients who developed several episodes of VAP, only the first one was included in the study.

Diagnosis of HAP in the present study was made on clinical grounds, according to the Center for Disease Control (CDC) criteria for clinically defined pneumonia (PNU1).[15] Specific microbiological diagnosis of pneumonia was not thoroughly sought due to limited resources. Only blood and pleural fluid cultures were taken. Quantitative cultures from lower respiratory tract samples (e.g., bronchoalveolar lavage and tracheal aspirate) were not performed.

The diagnostic work up for patients included chest x ray; complete blood count (CBC); CRP; blood gas analysis; and serum electrolytes. Chest computed tomography was ordered in specific conditions i.e., for children with poor response to treatment; for evaluation of pleural, mediastinal, or very small parenchymal lesions; and if pneumonia is suspected despite negative or equivocal chest radiographs.

Pediatric Sequential Organ Failure Assessment (pSOFA) score was calculated for patients admitted into PICU.[16] Patients were closely monitored to assess the accuracy of suPAR in diagnosis of pneumonia and its association with hospital mortality and morbidity.

Hospital stay was considered "prolonged" if it was greater than the "median" and was considered "short" if it was≤ the "median".

Laboratory method

For CAP patients, 2 mL blood sample for serum suPAR measurement was withdrawn within 24 hours of hospital admission. For HAP patients, samples were withdrawn on the day when pneumonia was diagnosed. suPAR was also measured in children in the control group. Blood samples were withdrawn in plain vacutainer tubes, incubated for 15 minutes, centrifuged, separated into aliquots, and stored until the test was performed. Serum suPAR levels were measured by Human soluble urokinase type plasminogen activator receptor, suPAR ELISA kit (Chongqing Biospes Co., Ltd, China) which uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol.

Statistical method

Qualitative data was expressed as numbers and percents. Non-normally distributed continuous variables were presented as median and range (minimum–maximum). Chi-square test or Fisher exact test were used to assess the association between qualitative variables. Mann-Whitney U test was used for comparing non-normally distributed continuous variables. Correlations between continuous variables were determined by Spearman correlation coefficient (r_s). Receiver Operating Characteristic (ROC) curve analysis

was used to evaluate the performance of suPAR in discriminating patients from controls and discriminating severe pneumonia from non-severe pneumonia. Two-tailed p-value<0.05 was considered statistically significant. Data was analyzed using SPSS version 23 (Statistical Package for Social Science) (Chicago, Inc, Illinose).

Results

Characteristics of the study population

120 patients were recruited along with 55 age and sex-matched controls. The control group consisted of 30 males and 25 females; their median age was 36 months (range 2–144 months) which was not significantly different from that of the patient group (P=0.11). The characteristics of patients are shown in Table I in which baseline clinical and laboratory data of CAP patients were recorded on admission, while baseline data of HAP patients were recorded on the day of pneumonia diagnosis.

Among the CAP patients, 16 (21.3%) reported having had previous episodes:15 patients had one previous CAP episode and one patient had two previous CAP episodes that were treated at home.

The primary reasons for hospitalization of HAP patients included, neurological (40%), gastrointestinal (17.8%), cardiac (11.1%), traumatic (8.9%), respiratory (6.7%), metabolic (2.2%), renal (2.2%), surgical (2.2%), and toxicological (snake envenomation: 2.2%) disorders. Additionally, three patients (6.7%) were admitted for sepsis without focus.

Overall, 17 patients (37.7%) from the HAP group were initially admitted for various infections which improved, but the patients later developed HAP and were included in the study. These infections included central nervous system infections (7 patients), gastroenteritis (4 patients), and sepsis without focus (3 patients). In addition, three patients had been initially hospitalized for pneumonia that improved but HAP developed after 14 days of admission, so they were deemed to have new pneumonia episodes, and were included in the HAP group.

Most HAP patients (75.6%) had VAP. None of the remaining HAP patients needed mechanical ventilation while 13.3% of CAP patients needed mechanical ventilation. HAP patients had significantly higher pulse rate and CRP levels; longer hospital stay; and higher frequency of hypoxia, shock, lobar consolidation, and mortality compared with CAP patients.

Pathogenic bacteria were isolated from 4 patients with CAP: *Staphylococcus aureus* (2 patients, both from pleural fluid), *Streptococcus pneumonia* (one patient, from blood), and group A *Streptococci* (one patient, from blood). As for the HAP group, pathogenic bacteria were isolated from 8 patients: *Pseudomonas aeruginosa* (3 patients, from blood), *Staphylococcus aureus* (2 patient, one from pleural fluid and one from blood), *Acinetobacter* (2 patients, from blood), and *Klebsiella pneumoniae* (one patient, from blood).

Diagnostic value of suPAR

Figure 1 shows that suPAR levels were higher in the whole patient cohort compared with controls (*p<0.001*). suPAR levels were higher among CAP group compared with controls (*p<0.001*), but no significant difference was found between HAP group and controls (p=0.065). suPAR levels were higher among CAP group compared with HAP group (p<0.001). No significant difference in suPAR levels were found between patients with VAP and those with non-VAP HAP (*p=0.31*).

ROC curve analysis revealed that suPAR had an AUC of 0.76 for discriminating the whole patient group from controls [a cutoff level of ≥594.4 pg/ml had a sensitivity of 64.2% and a specificity of 89.1%; p<0.001].

suPAR had an AUC of 0.98 for discriminating CAP patients from controls [a cutoff level of ≥ 504.3 pg/ml had a sensitivity of 92% and a specificity of 90.9%; p<0.001].

Table I. Demographic, clinical, and laboratory characteristics of patients.

Variable	All patients (n=120)	CAP††† (n=75)	HAP‡‡‡ (n=45)	P value
Age, months	18 (1.5 – 168)	18 (1.5 – 108)	12 (2 – 168)	0.012*
Male sex	65 (54.2%)	48 (64%)	17 (37.8%)	0.77
Weight/age z-score				
- 2 to +2 SD	95 (79.2%)	69 (92%)	26 (57.8%)	
< -2 to -3 SD	13 (10.8%)	6 (8%)	7 (15.6%)	<0.001*
< -3SD	12 (10%)	0 (0%)	12 (26.7%)	
Temperature, °C	38.5 (35 – 41)	38.5 (36.5 – 39.5)	38.8 (35 – 41)	0.078
Respiratory rate/minute	53.5 (30 – 93)	48 (33 – 78)	62 (30 – 93)	0.068
Pulse rate/minute	140 (86 –186)	127 (86 – 178)	160 (90 – 186)	<0.001*
Minimum SPO2†, %	92.5 (51 – 99)	95 (65 – 99)	75 (51 – 88)	<0.001*
Hypoxia (SPO2<94%)	30 (25%)	29 (38.7%)	34 (75.6%)	<0.001*
Type of consolidation				
Lobar consolidation	70 (58.3%)	42 (56%)	28 (62.2%)	
Patchy	31 (25.8%)	14 (18.7%)	17 (37.8%)	<0.001*
Interstitial	19 (15.8%)	19 (25.3%)	0 (0%)	
Pleural effusion	11 (9.2%)	7 (9.3%)	4 (8.8%)	0.93
Shock	15 (12.5%)	8 (10.7%)	7 (15.6%)	<0.001*
Invasive MV‡	44 (36.7%)	10 (13.3%)	34 (75.6%)	<0.001*
PICU patients§	56 (46.7%)	20 (26.7%)	36 (80%)	<0.001*
Length of hospital stay, days	8 (5 – 90)	7 (5 – 13)	29.5 (6 – 90)	<0.001*
PIROm¶	NA	1 (0 – 5)	NA	NA
PRESS††	NA	3 (2 – 5)	NA	NA
RISC‡‡	NA	3 (1 – 6)	NA	NA
Hospital mortality	27 (22.5%)	5 (6.7%)	22 (48.9%)	<0.001*
Serum sodium, mEq/L	136 (119 – 167)	134 (119 – 141)	137 (128 – 167)	0.002*
WBC§§ (1000/μL)	9 (1.8 – 41.1)	8 (3 – 41)	12.5 (1.8 – 34.9)	0.39
Hemoglobin, g/dL	10.7 (7.4 – 16)	10.7 (7.4 – 13.5)	10 (7.4 – 16)	0.55
Platelets (1000/μL)	300 (8 – 731)	300 (48 – 731)	254.5 (8 – 661)	0.95
CRP¶¶, mg/dL	26 (0 – 385.4)	24 (0 – 385.4)	48 (12 – 160)	<0.001*

Data is expressed as median (minimum - maximum) and number (percentage); *statistically significant
†Saturation of peripheral Oxygen; ‡Mechanical ventilation; §Pediatric intensive care unit; ¶Predisposition, Insult, Response, Organ dysfunction modified score; ††Pediatric Respiratory Severity Score; ‡‡Respiratory Index of Severity Score; §§White blood cell count; ¶¶C-reactive protein; †††Community-acquired pneumonia; ‡‡‡Hospital-acquired pneumonia, NA: Non-applicable

Association of suPAR with CAP severity

suPAR levels increased significantly in parallel with higher PRESS and PIROm scores (Table II). suPAR levels were significantly higher among CAP patients who died as well as among those who had shock; required PICU admission; or required mechanical ventilation (Table III).

suPAR was positively correlated with pulse rate, respiratory rate, PRESS, and PIROm but negatively correlated with peripheral oxygen saturation (SPO2) [Table IV].

ROC curve analysis revealed that suPAR had an AUC of 0.68 for prediction of severe CAP (as classified by PRESS score) but this was smaller than that of White Blood cell Count (WBC) (Table V). When CAP severity was alternatively defined according to WHO, PRIROm, and RISC; suPAR had an AUC of 0.65 (p=0.043), 0.74 (p=0.11), and 0.69 (p=0.018) respectively.

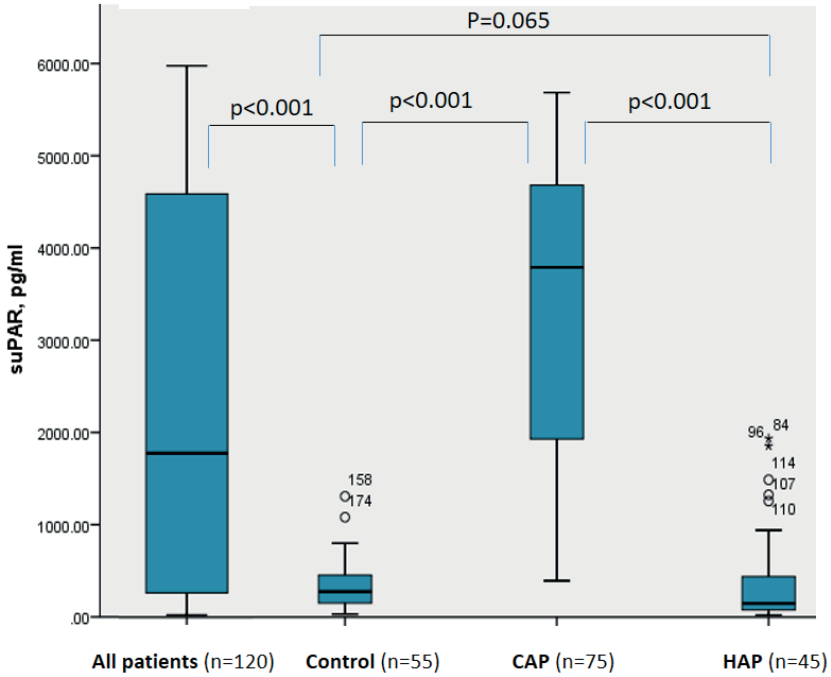

Fig. 1. Serum suPAR levels in patients and controls.

The median and range of suPAR level (pg/mL) in the whole patient cohort, CAP patients, HAP patients, and controls was 1693.7 (22.4–5694.9), 3798 (395–5694.9), 148 (22.4–1939.7), 276.1 (31.6–1311.8), respectively.
In this boxplot, the bold black lines corresponds to the median in each group. The top and bottom of the box represent the 75th and 25th percentiles respectively. The lower whisker corresponds to the 25th percentile minus 1.5 times the interquartile range while the upper whisker corresponds to the 75th percentile plus 1.5 times the interquartile range. Open circles represent "outliers". Asterisks represent "extreme outliers" i.e. data points higher than the 75th percentile plus 3 times the interquartile range.
CAP: Community-acquired pneumonia; HAP: Hospital-acquired pneumonia; suPAR: Soluble urokinase plasminogen activator receptor

Table II. The relation of suPAR levels to different community-acquired pneumonia classification systems.

Pneumonia severity classification	suPAR††, pg/ml	P value
WHO† classification		
Pneumonia (n=52)	3617.4 (395 – 5694.9)	0.11
Severe pneumonia (n=20)	4338 (586.7 – 5540.3)	
RISC‡ score		
< 4 points (n=24)	4393 (489.9 – 5694.9)	0.19
≥ 4 points (n=17)	4433 (586.7 – 5540.3)	
PRESS score§		
Mild: 0-1 points (n=0)	NA‡‡	
Moderate: 2-3 points (n=56)	3518.1 (395 – 5694)	0.02*
Severe: 4-5 points (n=19)	4430 (586.7 – 5540.2)	
PIROm score¶		
Mild: 0-2 points (n=55)	3338.6 (395 – 5694.9)	0.017*
Moderate/severe:3-6 points (n= 20)	4430 (586.7 – 5540.3)	

*Statistically significant
Data is expressed as the median (minimum - maximum)
†World health organization; ‡Respiratory Index of Severity Score; §Pediatric Respiratory Severity Score; ¶Predisposition, Insult, Response, Organ dysfunction modified score; ††Soluble urokinase plasminogen activator receptor; ‡‡Non-applicable

Table III. Soluble urokinase plasminogen activator receptor level in different patient subgroups.

Patient subgroup	CAP‡		HAP¶	
	suPAR§, pg/ml	P value	suPAR, pg/ml	P value
Hypoxia	4246 (586.7 – 5540.3)	0.053	190.3 (22.4 – 1939.7)	0.31
No hypoxia	2899.5 (395 – 5694.9)		138.7 (23.7 – 944.9)	
PICU† patients	4338 (586.7 – 5540.3)	0.044*	160.4 (22.4 – 1939.7)	0.55
Ward patients	3518 (395 – 5694.9)		145.4 (23.8 – 944.9)	
Shock	4808.6 (3246.7 – 5540.3)	0.013*	1260 (96.4 – 1939.7)	0.005*
No shock	3716.6 (395 – 5694.9)		138.9 (23.7 – 728.8)	
Mechanical ventilation	4609.5 (2240 – 5540.3)	0.019*	190.3 (22.4 – 1939.7)	0.31
No mechanical ventilation	3518 (395 – 5694.9)		138.9 (23.7 – 944.9)	
Lobar consolidation	4146.8 (489.7 – 5540.3)		160.4 (28.5 – 1939.7)	
Patchy consolidation	3757.7 (395 – 4975)	0.41	145.4 (22.4 – 1491.5)	0.85
Interstitial	2346.7 (651.9 – 5694.9)		NA	
Prolonged Hospital stay††	4265.5 (586.7 – 5540.3)	0.13	168.6 (22.4 – 1939.7)	0.37
Short hospital stay	3518.1 (395.1 – 5694.9)		148 (23.7 – 1857.4)	
Pleural effusion	3742.3 (727.4 – 4736.4)	0.76	78 (28.5 – 1327.9)	0.39
No effusion	3916.3 (395 – 5694.9)		160.4 (22.4 – 1939.7)	
Non-survivors	4828.3 (3773.5 – 5540.3)	0.049*	138.9 (22.4 – 1939.7)	0.31
Survivors	3729.4 (395 – 5694.9)		206.1 (28.5 – 1857.4)	

*Statistically significant
Data is expressed as the median (minimum - maximum).

†Pediatric intensive care unit; ‡Community-acquired pneumonia; §Soluble urokinase plasminogen activator receptor; ¶Hospital-acquired pneumonia.
††Hospital stay was considered "prolonged" if it was greater than the "median" (>7 days for CAP and > 29.5 days for HAP) and was considered "short" if it was≤ the median.

When CAP patients admitted into PICU were subgrouped according to the median pSOFA score, no significant difference in suPAR levels were found (*p=0.24*)

Association of suPAR with HAP severity

suPAR levels were significantly higher among HAP patients with shock. It was also higher among non-survivors and among those with hypoxia, acidosis, mechanical ventilation, prolonged hospital stay, but without statistical significance (Table III). A negative correlation was found between suPAR and minimum SPO2 (Table IV).

When HAP patients were subgrouped according to the median pSOFA score (9 points) on the day of pneumonia diagnosis, suPAR was higher among the subgroup with elevated pSOFA [408 pg/ml (28.5–1939.7) vs 138.9 (22.4–1491.5); *p=0.034*].

Discussion

suPAR is a novel biomarker that has attracted attention in recent years through demonstrating prognostic value in diverse clinical disorders. In the present study, suPAR proved to have a diagnostic value for pediatric pneumonia since its level was significantly elevated among the whole patient cohort compared with controls.

Pneumonia can be diagnosed by clinical and radiological criteria and physicians usually do not need biomarkers for diagnosing it. However, this is not always the case; for instance, suPAR, can be useful for uncovering the nature of a lung's opacity e.g., differentiating

Table IV. Correlations of suPAR with other variables among patients.

Variable	HAP¶¶ patients		CAP‡‡‡ patients	
	suPAR†††		suPAR	
	Spearman correlation coefficient (r_s)	P value	Spearman correlation coefficient (r_s)	P value
Age	-0.13	0.39	-0.31	0.007*
Weight	-0.19	0.21	-0.32	0.004*
Temperature	-0.23	0.12	0.037	0.75
Respiratory rate	0.16	0.29	0.24	0.041*
Heart rate	0.13	0.41	0.26	0.027*
SPO2†	-0.31	0.048*	-0.24	0.041*
RISC‡	NA	NA	0.11	0.51
PRESS§	NA	NA	0.24	0.043*
PIROm¶	NA	NA	0.29	0.012*
Length of hospital stay	-0.08	0.57	0.19	0.1
MV†† duration	-0.23	0.19	0.23	0.53
Sodium	0.07	0.63	0.08	0.47
CRP‡‡	0.14	0.36	-0.01	0.93
WBC§§	0.08	0.62	-0.09	0.41
Platelets	0.10	0.52	0.11	0.37

*statistically significant
†Saturation of peripheral Oxygen; ‡Respiratory Index of Severity Score; §Pediatric Respiratory Severity Score; ¶Predisposition, Insult, Response, Organ dysfunction modified score; ††Mechanical ventilation; ‡‡C-reactive protein; §§White blood cell count; ¶¶Hospital-acquired pneumonia; †††Soluble urokinase plasminogen activator receptor; ‡‡‡Community-acquired pneumonia

Table V. Prediction of severe community-acquired pneumonia* by Soluble urokinase Plasminogen Activator receptor and other biomarkers.

Variable	AUC (95% CI)¶	Cutoff	P-value	Sensitivity	Specificity
suPAR†, pg/mL	0.68 (0.54 – 0.82)	≥4784	0.021*	84.2%	46.4%
CRP‡, mg/dL	0.42 (0.29 – 0.59)	≥ 30.55	0.31	36.8%	66.1%
WBC§, 1000/μL	0.79 (0.64 – 0.94)	≥12.9	<0.001*	78.9%	91.1%
Platelets, 1000/μL	0.39 (0.22 – 0.55)	≤267	0.15	10.5%	78.6%

*Pneumonia severity is diagnosed by PRESS score
†Soluble urokinase plasminogen activator receptor; ‡C-reactive protein; §White blood cells; ¶Area under the Receiver Operating Characteristic curve and 95% confidence interval.

pneumonia from atelectasis or differentiating current pneumonia from opacity persisting from previous pneumonia episodes. Our current study did not specifically address these prospects, but they can be the subject of further research. Furthermore, suPAR can be utilized for monitoring the response of pneumonia to treatment as suggested by a previous adult study.[17]

For better clarification of the role of suPAR, we included both patient with CAP and those with HAP. suPAR was promising in CAP diagnosis, demonstrating high sensitivity and specificity in discriminating patients from controls. Moreover, suPAR was associated with indicators of CAP severity, including respiratory rate, heart rate, SPO2, shock, PICU admission, mechanical ventilation, and mortality.

As far as we know, only two previous pediatric studies, conducted by the same authors, evaluated the role of suPAR in CAP.[18,19] Consistent with our findings, one of these studies[18] reported a significant elevation of suPAR among CAP patients compared with controls, and showed correlation of suPAR with capillary blood saturation, fever, length of hospital stay, and time for defeverscence.

In addition to the relation of suPAR to individual indicators of CAP severity, we found positive correlation between suPAR and both PIROm and PRESS scores, a finding not reported by previous studies.

Of note, we found no correlation between suPAR and CRP which might be due to a difference in the onset or peak of serum level elevation of these markers. Nevertheless, the latter pediatric studies reported correlations of suPAR with CRP and Procalcitonin.[18,19]

Adult studies on the role of suPAR in CAP are also few, but have similarly shown diagnostic and prognostic values, including association of suPAR with mortality and illness severity. Importantly, the AUC for predicting CAP severity by suPAR in our study was 0.68, compared with a value of 0.71 to 0.84 in adult studies.[20,21] A difference in the type of suPAR assay might underlie that variation, as suggested by a previous study which measured suPAR for the same cohort by two different assays, detecting a difference in the AUC for prediction of mortality (0.80 vs 0.68).[22]

The ability of suPAR to diagnose CAP and predict its outcome raises the question of whether a similar association exists between suPAR and other inflammatory respiratory disorders. We did not explore this issue, but previous studies demonstrated a value for suPAR in predicting bronchopulmonary dysplasia[23] and assessment of asthma control.[24]

The inclusion of patients with recurrent CAP in the present study is unlikely to have affected our results since we stipulated that such patients be symptom-free for >14 days after the last episode. In addition, we excluded patients with chronic respiratory disorders who might have had prior subclinical inflammation affecting suPAR levels. Of note, almost all patients with recurrent CAP had only one previous episode that was diagnosed mostly by doctors in other healthcare institutions.

Unlike the diagnostic role demonstrated by suPAR in CAP, we failed to find a significant differences in suPAR levels between HAP patients and controls. Nevertheless, suPAR was inversely correlated with SPO2 and associated with shock and higher pSOFA score, suggesting a prognostic value in HAP.

Studies on the role of suPAR in HAP are scarce. Moreover, they are generally small and conducted on non-pediatric patients with VAP. In contrast to our findings, a small study of adult mechanically ventilated patients showed that suPAR was significantly higher among patients who developed VAP, both on the day of VAP diagnosis and 3 days before.[25] Another study of 180 adults with VAP and sepsis revealed a significant elevation of suPAR levels among patients compared with controls and among non-survivors compared with survivors.[26] Likewise, another study reported a significant elevation of suPAR among adult ICU patients who developed VAP, compared with those who didn't develop it, but suPAR was not associated with mortality.[27]

Undoubtedly, the small sample size precludes us from drawing firm conclusions regarding the role of suPAR in pediatric HAP. However, if our current findings are confirmed by future studies, this will imply that suPAR has a prognostic, rather than diagnostic, value in pediatric HAP.

The next question will be: why did suPAR levels not rise in HAP to the same extent as in the case of CAP? One possible answer comes from the pathogen type. Pathogens causing HAP are generally distinct from those causing CAP and it was noted that different pathogens

trigger different immunological responses due to interaction with different pattern recognition receptors.[28] It is thus possible that suPAR levels vary according to the type of pathogen and, consequently, according to the type of pneumonia (CAP vs HAP).

Another interesting explanation lies in the phenomenon termed "immunoparalysis" which occurs among critically ill patients who develop severe and persistent compensatory anti-inflammatory response syndrome (CARS) that affects mainly the innate immune system.[29] Most HAP patients had been critically ill before they developed pneumonia. It is, therefore, possible that uPAR expression decreased in these patients due to depression of monocytic function in the course of immunoparalysis, with consequent failure of suPAR to rise during HAP to the high levels found in CAP; therefore, lower suPAR levels could represent a risk factor for HAP development among critically ill children.

Indeed, it has been shown that neutrophil migration from the pulmonary circulation utilizes two pathways, one of them is dependent on CD11b/ CD18 (with which uPAR forms a functional complex). Accordingly, uPAR possesses essential role in combating pulmonary infections, particularly those caused by *Pseudomonas aeruginosa*. Moreover, uPAR was shown to be important for neutrophil recruitment into the alveoli during *S. pneumoniae* infection in a CD11b/ CD18-independent way.[30]

On the other hand, it is possible that HAP patients who did not develop immunoparalysis, showed higher suPAR levels, and consequently, more severe inflammatory response, which could explain the occurrence of some aspects of illness severity in these patients.

It should be emphasized that proving the latter hypothesis requires specific laboratory tests for immunoparalysis which can be the subject of future studies.

Generally speaking, suPAR appears to possess some advantages that favor its use in routine practice, including stability in vitro in serum and plasma over time and during repeated freeze-thawing cycles. Additionally, circadian suPAR levels are stable, so the sampling schedule does not affect its measurement.[31]

Limitations of the present study include the small sample size and the limited scope. Further studies are required to determine the onset, peak, and duration of suPAR level elevation in relation to pneumonia. We also need to know whether suPAR can differentiate pneumonia from other respiratory disorders. Moreover, it is unclear whether suPAR can guide antibiotic therapy or prove superior to other markers like Procalcitonin.

Likewise, thorough specific microbiological diagnosis of pneumonia was not made, so we were not able to assess the relation of suPAR levels to the type of bacteria due to the low yield of microbiological cultures, which were taken from blood and, in few cases, from pleural fluid, but not from lower respiratory secretions. The low yield of blood culture in pneumonia is consistent with previous studies.[32]

In addition, we did not evaluate the ability of suPAR to discriminate viral from bacterial infections. Viral studies were not performed due to lack of resources. It was also not possible to diagnose a viral etiology based on chest radiograph (e.g., interstitial infiltrate) due to poor accuracy of this tool. What is more, a significant proportion of pneumonia cases result from mixed viral and bacterial pathogens.[9]

Finally, some of our patients developed HAP in the context of prior infections, including sepsis, which could have affected serum suPAR level. However, the influence of this factor is unlikely to be important since we included in the study only patients in whom the original infections had significantly improved.

suPAR is a promising marker for pediatric pneumonia. It demonstrated clear diagnostic value in CAP but not in HAP. The prognostic value of suPAR was evident in both groups

but more in CAP; among CAP patients, suPAR was clearly associated with morbidity and with mortality while among HAP patients, suPAR was not associated with mortality but was associated with some aspects of disease severity. In other words, suPAR had both diagnostic and prognostic values in CAP but was only prognostic in HAP. Further studies are needed for stringently assessing the value of suPAR.

Ethical approval

This study was approved by the ethics committee of The faculty of Medicine, Menoufia University (Number:1912/9PEDI15) and written informed consent was obtained from parents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MSE, NYS; data collection: MSE, NYS, SES; analysis and interpretation of results: MSE, NYS, SES; draft manuscript preparation: MSE. 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

Promoting adolescent health: health literacy, self-efficacy and internet use

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ABSTRACT

Background. Adolescents are mostly considered as a healthy population; however, failure to acquire positive health behaviors during this period makes them vulnerable to poor health outcomes and long-term chronic disorders. Health literacy is one of the most influential parameters in promoting adolescent health. This study aimed to determine the level and promoters of health literacy in adolescents, emphasize the importance of internet use, and evaluate the relationship between self-efficacy and health literacy.

Methods. A total of 756 adolescents aged 15 and 18 years attending two high schools in socioeconomically different districts in Ankara, Turkey were included in this cross-sectional study. A survey consisting of descriptive questions, a health literacy survey, and a general self-efficacy scale were used to collect data. $p<0.05$ was considered statistically significant.

Results. Among the adolescents who participated in the study, the level of health literacy was inadequate-limited in 56.1%, sufficient in 30.1%, and excellent in 13.8%. A statistically significant correlation was found between health literacy and general self-efficacy levels (r: .412, $p<0.001$). There was also a statistically significant difference between the health literacy groups in terms of the education level of the adolescents' mothers, internet use frequency, and self-efficacy level. The multivariate logistic regression analysis revealed that the participants whose mothers had only received primary school education or no formal education, those that were not using the internet regularly, those that did not search health information on the internet, and those with poor self-efficacy levels were more likely to have an inadequate level of health literacy [odds ratio (OR)=2.6, 95% confidence interval (CI)=1.4-4.9; OR=5.5, 95% CI=1.2-25.1; OR=1.7, 95% CI=1.1-2.9; and OR=3.7, 95% CI=2.6-5.2, respectively].

Conclusions. In this study, it was concluded that the adolescents' health literacy and general self-efficacy levels were related. Furthermore, the health literacy level of the adolescents was associated with internet use and maternal education status.

Key words: health literacy, adolescent, self-efficacy, internet use.

Adolescence is a complex transition period that includes physical growth and the development of secondary sex characteristics, new cognitive skills, and sexual identity.[1] Adult health behavior patterns are set in this critical period.[2] Adolescents are considered as the healthy population who do not require special health services or mostly do not have any chronic disease. However, failure to acquire positive health behaviors during this period makes them vulnerable to poor health outcomes and long-term chronic disorders during both adolescence and adulthood.[3] Furthermore, risky behaviors which frequently begin in adolescence, such as tobacco, alcohol and substance use, risky sexual behaviors, overeating, and lack of physical activity often lead to chronic diseases in adulthood.[4,5] Promoting adolescent health is

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essential to ensure a healthy life course as an individual and support public health.[6]

Today, health literacy (HL) is one of the most essential and effective parameters for promoting health. HL has a multidimensional construction, and it is defined as a combination of knowledge, motivation, and proficiency that allow people to access, understand, evaluate and use health information in order to improve their health, prevent diseases, and make decisions in everyday life to maintain or improve their health and quality of life.[7] Adult studies have shown that differences in HL levels are related to increased emergency service visits and hospitalization, less use of preventive healthcare services, poor overall health status in the population over 65, and higher mortality.[8] Although several adult studies have been conducted on HL, their results cannot be directly generalized to the pediatric population considering the different characteristics of childhood and especially adolescence. Limited adolescent research suggests that obesity, smoking, alcohol use, and drug abuse are related to low HL levels in adolescents.[9,10]

Although HL plays an essential role in health behavior changes, such behaviors are also influenced by psychosocial factors, such as self-efficacy (SE).[11] SE refers to an individuals' belief and confidence in their capacity to perform a specific behavior in diverse situations.[12] High SE indicates more effort being made to attain desirable outcomes despite barriers; therefore, it is one of the crucial components of health promotion behaviors and a determinant for initiating and maintaining positive health behaviors.[13] Additionally, SE has been exceptional for proper self-management for several chronic health conditions, such as diabetes and asthma.[11,14] Furthermore, people with higher SE have been found to be exhibit more ambitious behavior to maintain a healthy diet for weight loss.[15] Lastly, individuals with lower SE and HL are less likely to apply preventative health behaviors.[16] However, despite these adult studies frequently reporting the relationship between disease-based HL and SE status, there are still a limited number of adolescent studies in this area.

The internet is an essential tool for connecting with friends and one of the primary sources for almost every subject in the life of an adolescent.[17] Therefore, internet-based health-related information seeking behavior of adolescents is increasingly being studied worldwide.[18-20] The internet is easy to use, provides the fastest way to reach all types of information, and offers anonymity; therefore, it has been shown that adolescents prefer the internet as a primary source of health-related information rather than traditional health services.[21] In addition, a previous study showed that more than two-thirds of adolescents accessed health-related information on the internet in the USA.[22] This tendency can guide the design of developmentally relevant interventions that may support healthy lifestyle choices and promote HL among adolescents.[23]

This study aimed to determine the level of HL in adolescents, evaluate the relationship between SE and HL, and define significant promoters of HL, such as the internet among adolescents.

Material and Methods

Participants

The participants consisted of high school students aged 15 years and older because the scales used had been previously tested for reliability and validity for this age group in two socioeconomically different districts in the Urban Poverty Mapping Study[24] conducted in Ankara, Turkey. The two high schools participating in the study had 1,582 students during the 2017-2018 academic year. The study was designed as cross-sectional. The number of individuals required for the sample of the study was calculated using the following formula: sample size (n) = [DEFF x Np(1-p)] / [(d2 / Z21 – α / 2 x (N1) + p x (1-p)][25,26] where n refers to the population size (1,582), p refers to the

prevalence of limited HL (0.5), d refers to the absolute precision value (0.3), DEFF refers to the design effect (1), Z1-α/2 was 1.96, and p was accepted as 0.5 considering that the proportion of 50% indicates maximum variability.[27] An estimated minimum sample size of 638 was obtained using the assumptions given above. The convenience sampling method, one of the non-probability sampling methods, was used since the principals of the schools allocated limited time to administer the data collection tools. Using this method, we initially reached 850 participants, but 94 were excluded from the sample because they did not respond to all the questions in the survey. The final data analysis was performed on the data of 756 participants.

Procedure

The study started after receiving approval by local ethics committee of Keçiören Research and Training Hospital (approval reference number: 2012-KAEK-15/1606) and obtaining permission from the principals of the participating schools.

The schools sent a consent letter to the parents to inform them about the study objectives and procedures. The parents that provided consent for their children's participation in the study signed and returned this form. The students completed the surveys in the school over approximately 20 minutes in September 2018.

Measures

The survey consisted of 56 questions to determine sociodemographic characteristics, internet use patterns, health information, HL, and SE.

Sociodemographic information, internet use, and health information

In this section, information on age, gender, parental education status, and school region was collected. In addition, methods to access health-related information (doctors, family, friends, TV, internet, school, and others), internet use frequency, whether health information was accessed using the internet, and most searched health topics on the internet were questioned. As health topics, primary subjects related to adolescent medicine were used, and an open-ended option was provided. Due to the increasing trend toward exercise and sports supplements, these topics were also included as options.

Health literacy

The HL level was measured using the 'Turkish Health Literacy Survey' (THLS, α = 0.92), which was adapted from the original English version of the 'European Health Literacy Survey' (HLS-EUS). THLS comprised 32 items and was rated on a five-point Likert scale to measure the HL level. Total scores were measured and standardized with an index formula between 0 to 50, with 0 representing the 'worst' and 50 representing the 'best' score. The HL levels were classified as 'inadequate' (0–25), 'problematic' (>25–33), 'sufficient' (>33–42), and 'excellent' (>42–50) as in the HLS-EUS study, and the 'inadequate' and 'problematic' levels were combined to a single level called 'limited HL' (0–33) to identify the vulnerable groups.[28] In this study, Cronbach's alpha was 0.90.

Self-efficacy

SE was measured using the Turkish version of the General Self-Efficacy Scale (GSES) developed by Schwarzer and Jerusalem, which included ten items rated on a four-point Likert scale (1 = not at all true; 2 = rather untrue; 3 = rather true, and 4 = exactly true). This scale was designed to measure an individual's subjective belief in her/himself in coping with stressful situations. Higher scores in this scale reflect a higher level of SE.[29] Since there is no cut-off score specified in the literature, we classified the scale scores according to the median score. Thus, the SE levels were categorized as good if the participants scored above the median value and poor if their score was below the median value.

The validity and reliability studies of the Turkish version of the scale were conducted in 2010 with

693 students at various class levels. According to the results of the exploratory factor analysis, the factor loads of the scale varied from 0.45 to 0.75. The explained total variance was 47%. The internal consistency of GSES (Cronbach alpha value) was found to be 0.83. The test-retest reliability of the scale was r = 0.80, p < .001.[29] In our target sample, the construct validity analysis of the Turkish GSES was also evaluated with the exploratory factor analysis. The total variance explained by the scale was 40.1%, and the factor loads of each item varied between 0.485 and 0.680. The internal consistency analysis showed that the scale was reliable, with the Cronbach alpha being calculated as 0.83. The item-total correlation values ranged from 0.377 to 0.571, with no item having a value below 0.30. As a result of the validity and reliability analyses, it was determined that the 10-item scale was valid and reliable for our study group.

Data analysis

Data analysis was conducted with IBM SPSS, v. 23.0 (SPSS Inc., Chicago, IL). The Pearson's chi-squared test was used to compare categorical variables between independent groups in order to examine the differences between the adolescents' HL levels across sociodemographic and health information/ internet use characteristics. The correlation between the SE and HL levels was analyzed using Spearman's correlation coefficient. In order to define the predictors of inadequate HL, the multivariate logistic regression analysis was used. The variable selection method was the 'Enter method', and all the variables were entered in one step. A univariate estimate was performed with the logistic regression analysis, and variables with a significance level of $p < 0.05$ in the univariate analysis were included in the multivariate logistic regression analysis to determine the association of each independent variable with outcome variables. The crude and adjusted odds ratio (OR) and 95% confidence interval (CI) values were also obtained. $p < 0.05$ was considered as statistically significant.

Results

Sample Characteristics

Sample characteristics are shown in Table I. The sample consisted of 756 students aged 15-18 years, and 65.9% were female. Half of the students' parents had secondary or high school education. As the source of health information, the participants most preferred doctors (72.2%), followed by the internet (69.1%). Almost all the participants regularly used the internet, and most (86.5%) sought health-related topics via the internet. Table II presents the percentages of most searched topics. Sports, healthy nutrition, acne, losing weight, and short stature were the most popular subjects.

Health Literacy and Self-Efficacy

Descriptive statistics {mean ± standard deviation [median (minimum-maximum)} for THLS was 32.25 ± 9.13 [32.29 (1-50)], and Table III shows the HL levels according to the THLS scores. Over half of the participants had limited HL (56.1%), while 30.1% had sufficient and only 13.8% had excellent literacy skills. Table IV presents the HL levels by sample characteristics. Age, gender, and school region were not related to the HL level. However, the univariate analysis showed that parental education status, internet use frequency, and accessing health information through the internet resulted in statistically significant differences in the HL level. Accordingly, the adolescents who were using the internet regularly and sought health information on the internet tended to have higher HL levels. Descriptive statistics {mean ± standard deviation [median (minimum-maximum)} for SE was 31.05 ± 5.44 [31 (10-40)]. The level of SE was good (≥median value) in 57.4% (n = 434) of the participants. Figure 1 shows the results of the Spearman correlation analysis, which indicated a moderate positive correlation (r: .412, $p < 0.001$) between SE and HL.

Table I. Sample characteristics.

Variables	n	(%)
Age (n = 756)		
15	293	38.8
16	232	30.7
17	126	16.7
18	105	13.9
Gender (n = 756)		
Male	498	34.1
Female	258	65.9
Maternal education status (n = 756)		
No education or primary school	283	37.4
Secondary or high school	396	52.4
College or university	77	10.2
Paternal education status (n = 756)		
No education or primary school	176	23.3
Secondary or high School	443	58.6
College/university	137	18.1
Socioeconomic level of school district (n = 756)		
Low	252	33.3
High	504	66.7
Methods to access health-related information (n = 756)[±]		
Family	381	48
Friends	69	8.7
Doctors	573	72.2
Television	219	27.6
Internet	548	69.1
School	130	16.3
Others	12	1.5
Internet use frequency (n = 756)		
Everyday	569	75.3
A few days per week	141	18.7
A few days per month	23	3
Never	23	3
Accessing health information through the internet (n = 756)		
Yes	653	86.5
No	102	13.5

[±]:multiple answers allowed

Table II. Most searched health topics accessed through the internet.

Topics (n = 735)[±]	n	(%)
Exercise	443	57.5
Diet, nutrition	429	55.7
Acne	352	45.7
Lose weight	302	39.2
Short stature	193	25
Weight gain	183	23.7
Menstrual irregularities	180	23.3
Sports supplements	156	20.2
Depression	134	17.4
Obesity	81	10.5
Vaccination	72	9.3
Hirsutism	67	8.7
Smoking	61	7.9
Sex	54	7
Alcohol	45	5.8
Sexually transmitted diseases	43	5.5
Drug abuse	27	3.5
Pregnancy	12	1.5
Contraception	6	0.7
Others	39	5

[±]:multiple answers allowed

Table III. Health literacy level according to the Turkish Health Literacy Scale.

Health Literacy Level (n = 756)	n	(%)
Inadequate (≤25)	155	20.5
Problematic (>25-33)	269	35.6
Sufficient (>33-42)	228	30.1
Excellent (>42-50)	104	13.8

Multivariate Test Results

Table V shows the results of the binary logistic model used to test the association between inadequate HL levels and other variables. In contrast to the univariate analysis, paternal education status was not found to be related to HL in the binary logistic analysis (OR = 0.7; 95% CI (0.4-1.2); p = 0.222). However, maternal education status was statically significant related to HL. Adolescents whose mothers had received primary school education or

no formal education were more than twice as likely to have inadequate HL levels than those whose mothers had graduated from college or university (OR = 2.6; 95% CI (1.4-4.9); p = 0.004). In addition, the adolescents who were not using the internet regularly were more likely to have inadequate HL levels than those using the internet regularly (OR = 5.5; 95% CI (1.2-25.1); p = 0.029). Similarly, the participants who did not search health information on the internet and had poor SE levels were more likely to have inadequate HL levels (OR = 1.7; 95% CI (1.1-2.9); p = 0.027 and OR = 3.7;95% CI (2.6-5.2); p = 0.001 respectively).

Table IV. Health literacy levels by sample characteristics.

Variables (n = 756)	Health Literacy		
	Limited health literacy n (%)	Adequate health literacy n (%)	p* value
Age groups			0.847
15 years	160 (54.6)	133 (45.4)	
16 years	129 (55.6)	103 (44.4)	
17 years	74 (58.7)	52 (41.3)	
18 years	61 (58.1)	44 (41.9)	
Gender			0.378
Male	139 (53.9)	119 (46.1)	
Female	285 (57.2)	213 (42.8)	
Maternal education status			0.001
No education or primary school	176 (62.2)	107 (37.8)	
Secondary or high school	218 (55.1)	178 (44.9)	
College/university	30 (39)	47 (61)	
Paternal education status			0.003
No education or primary school		92 (52.3)	84 (47.7)
Secondary or high School	270 (60.9)	173 (39.1)	
College/university	62 (45.3)	75 (54.7)	
Socioeconomic level of school district			0.023
Low	156 (61.9)	96 (38.1)	
High	268 (53.2)	236 (46.8)	
Internet use frequency			0.005
Everyday	318 (55.9)	251 (44.1)	
A few days per week	74 (52.5)	67 (47.5)	
A few days per month	11 (47.8)	12 (52.2)	
Never	21 (91.3)	2 (8.7)	
Accessing health information through the internet			0.003
Yes	353 (54.1)	300 (45.9)	
No	71 (69.6)	31 (30.4)	
General self-efficacy level			0.001
Poor, <30	237 (73.6)	85 (26.4)	
Good, ≥31	187 (43.1)	247 (56.9)	

*: Pearson's chi-square test

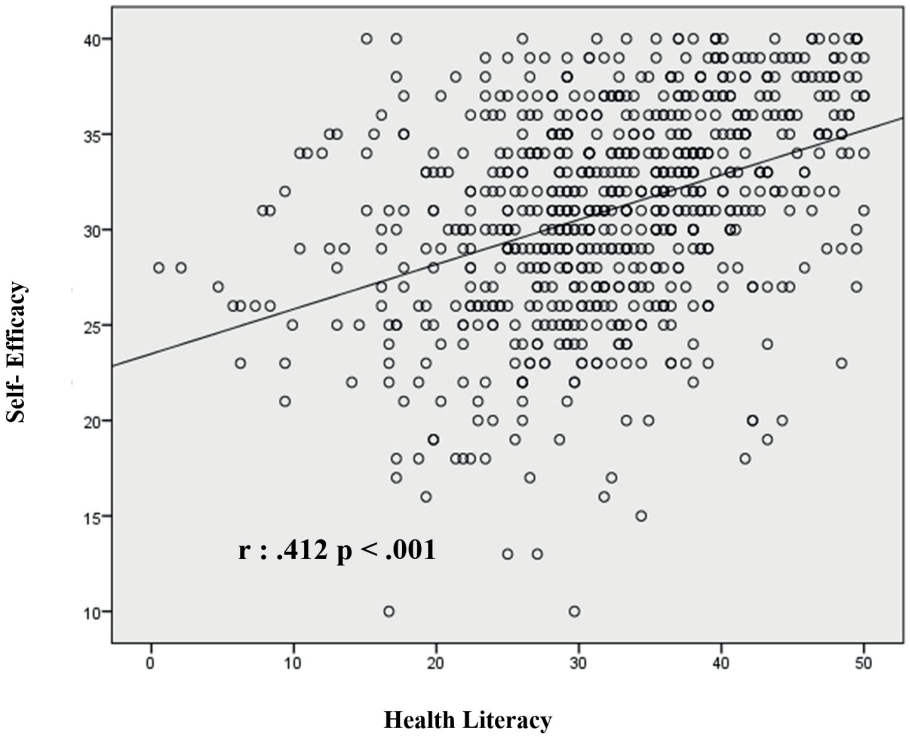

Fig. 1. Correlation between Self-Efficacy and Health Literacy.

Table V. Univariate and multivariate logistic regression models testing the association between inadequate levels of health literacy and other variables.

	Univariate Logistic Regression Analysis			Multivariate Logistic Regression Model		
	Crude Odds Ratio	95 % Confidence Interval	p-value	Adjusted Odds Ratio	95 % Confidence Interval	p-value
Maternal education status (ref: college/university)						
No education or primary school	2.6	1.5-4.3	0.001	2.6	1.4-4.9	0.004
Secondary or high school	1.9	1.2-3.2	0.010	1.7	0.9-2.9	0.077
Paternal education status (ref: college/university)						
No education or primary school	1.3	0.8-2.1	0.218	0.7	0.4-1.2	0.222
Secondary or high school	1.9	1.3-2.8	0.003	1.5	0.9-2.4	0.066
Socioeconomic level of school district: low level (ref: high level)	1.4	1.1-1.9	0.023	1.3	0.9-1.8	0.179
Internet use frequency (ref: Everyday)						
A few days per week	0.9	0.6-1.3	0.467	0.9	0.6-1.4	0.808
A few days per month	0.7	0.3-1.7	0.447	0.8	0.3-1.9	0.636
Never	8.3	1.9-35.71	0.005	5.5	1.2-25.1	0.029
Accessing health information through the internet: no (ref: yes)	1.9	1.2-3.1	0.004	1.7	1.1-2.9	0.027
Poor general self-efficacy level (ref: good level)	3.7	2.7-5.1	0.001	3.7	2.6-5.2	0.001

Discussion

In this study, we assessed adolescents' health promoters. For this purpose, we first measured the HL levels of the adolescents using THLS, which was adapted from HLS-EUS and can be used for both adolescents and adults.[28] HLS-EUS was designed to determine the HL level of individuals living in European Union countries and included 8,000 people from eight different countries. The results were challenging and showed an insufficient literacy level at 12%, with the overall rate of limited HL being calculated as 47%. These results suggested that every other person in Europe required an intervention to improve HL.[30] In addition, THLS was administered in two adult studies in Turkey, and a limited HL level was found in 69.2% and 60% of the participants, respectively.[28,31] Data obtained from different European countries showed that the rate of limited adolescent HL levels varied between 9.3% and 47.3%.[32-35] Furthermore, if we compare the adequate HL levels in our study with those evaluated in an adult THLS study in Turkey, it is clear that the adequate HL level is seen at a higher rate in adolescents than in adults. Previous generations, especially individuals of advanced age have clear disadvantages in accessing internet-based health information. There may also be some loss of HL with senility.[36] When we checked the data of the previous THLS study conducted in Turkey, a limited HL level was found in 63.3% of the participants aged 25-32 years and 66% of those aged 35-45 years.[28] In our study, the rate of limited HL level was 56.1% among the adolescents. The loss ratio of HL was between 7.2 and 9.9%, even in young adults in Turkey. This can be explained by inadequate health policies and HL education. Decreasing HL levels during the transition from adolescence to adulthood indicate the need for new strategies to prevent this loss. Supporting and improving adolescent HL levels may inevitably have consequences not only for adolescence but also adulthood.

It is known that HL is influenced by different factors. Wharf et al.[37] suggested a social-ecological model to understand the influencers of adolescent HL. This model included intrapersonal (characteristics, values, and experiences) and interpersonal (social support, groups, and family) factors. Concerning demographics, Levin-Zamir et al.[38] and Pakkari et al.[34] showed a positive correlation between higher HL and female sex in adolescents. Other adolescent HL studies also showed a significant relationship of HL with age.[39,40] Although some studies support the link between HL and sociodemographic factors, a systematic review of adolescent HL data according to age and sex reported inconsistent results.[6] Our study also showed that sex and age were not related to the HL levels of the adolescents. Also SE is considered to be one of the vital parts of intrapersonal factors, there are only a few studies in the literature reporting the relationship between HL and SE in adolescents.[41,42] Nevertheless, disease-based (adult studies (such as asthma, hypertension, and type 2 diabetes mellitus) are more common and show that patients with chronic diseases require higher HL and SE levels for better disease control.[43-46] We found a significant correlation between HL and general SE in healthy adolescents. Regarding the results of both adult and adolescent studies, we suggest that if higher SE levels can be achieved and maintained in adolescents, this may help increase their HL levels and ability to cope with chronic diseases in adulthood. However, further research is warranted to provide a better understanding of these aspects of adolescent HL.

It is well known that HL is highly related to the education level in adults.[47-50] Moreover, research has shown a significant relationship between high parental education and adequate adolescent HL levels.[37-39,51,52] This may be because parents who have higher education levels better transfer their HL skills to their children or provide them with more resources to effectively access information when needed. In the current study, we found that both parents' education status affected adolescent HL, but our logistic model showed that only

maternal education status was truly effective in adolescents' HL. To our knowledge, this is the first study that emphasizes the relationship between maternal education status and their children's HL level. Although Levin-Zamir et al.[37] showed a significant relationship between maternal education level and adolescent HL, paternal education level was not included as a parameter in their analysis. It is also known that children whose mothers' have higher education status have increased breastfeeding time and healthy nutrition habits, decreased obesity, and easy access to health services.[53-55] Maternal education status affects many parameters in children's lives, and our study revealed that it also affected their HL levels.

Tylee et al.[56] found that adolescents did not tend to seek any healthcare services unless their families asked them to because they feared that their confidentiality might not be protected by healthcare providers. A study designed by the OPINION research group investigated the primary sources of access to health information among the citizens of the European Union and reported that 55.7% of the individuals aged 15-24 years preferred the internet as a primary source, while only 26% of those aged 55 and over resorted to the internet for this purpose.[57] Many United States studies have shown that the most searched health subjects via the internet are sexual activity, contraception, alcohol, sexually transmitted diseases, and drug use by adolescents.[58,59] When we asked our participants about the most searched health-related subjects via the internet, we observed that this was the most popular question and received 2,879 responses. Contrary to the studies conducted in the United States, their most preferred subjects were less selected ones among the Turkish adolescents. Moreover, Ghaddar et al. found that the HL level was significantly higher in adolescents using https://medlineplus.gov/ (United States National Library of Medicine).[41] Today, increased internet use in the presence of health problems suggests that the internet may be a suitable tool for promoting HL in adolescents who use this technology very actively.[60] In addition, in our study, using the internet more frequently and accessing health information through the internet were determined to be related to high HL levels in adolescents. Thus, although cultural differences affect adolescents' interest in health-related subjects, the current study confirmed that the internet was one of the most preferred and reliable sources of health information for adolescents.

Our study had certain limitations. Although the validity and reliability analyses of the HL and SE scales were previously conducted, there is only limited research on the adolescent population and these scales were mainly designed for adults. Further studies should be conducted to support the use of these scales in the adolescent population. In addition, although most adolescents are students, some are employed and have their own families and children. Further studies should be planned to reach wider adolescent populations from different environments.

In line with our results, our recommendations are given below.

Further studies should be planned to measure and promote adolescent HL. HL should not be considered an independent issue, and studies should consider the relationship between HL and SE. Considering that adolescents are active internet users and use the internet as frequently as consulting doctors to seek health-related issues, the internet can be used as a powerful tool for improving HL in adolescents. Reliable sources suitable to the culture of the living environment should be provided under the supervision of healthcare specialists. Furthermore, one of the most critical findings of this study was the significant relationship between maternal education status and the adolescents' HL levels; thus, it is essential to support girls' education, especially in Turkey. This will not only support their future but also contribute to the protection and promotion of health in future generations.

Ethical approval

The study was approved by the local ethics committee of Keçiören Research and Training Hospital (approval reference number: 2012-KAEK-15/1606).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DC, FNAC, ZA, SÖ; data collection: DC, FNAC; analysis and interpretation of results: FNAC, SÖ; draft manuscript preparation: DC, ZA. 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

Does bile reflux reduce *Helicobacter pylori* gastritis?

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ABSTRACT

Background. Chronic abdominal pain is a frequent childhood complaint. This study aims to determine the relationship between bile reflux, which is increasing with the growth in packaged food consumption resulting from the changing food industry, and *Helicobacter pylori* gastritis.

Methods. In this retrospective study, 804 cases where there was an endoscopic examination for abdominal pain were included. We recorded the patients' age, sex, and macroscopic and microscopic endoscopic findings. Patients with chronic diseases were excluded.

Results. Our study included 804 cases. Of patients, 61.8% were female and 38.2% were male. The mean age was 11.56±4.14 years. The *Helicobacter pylori* gastritis rate was found to be 22.3% among all patients. Bile reflux was seen in 192 (23.9%) patients. Only 27 (14.1%) of the 192 patients had *Helicobacter pylori* positivity (p=0.002).

Conclusions. *Helicobacter pylori* gastritis is less common among patients with bile reflux. In another study conducted in our outpatient clinic before the 2000s, the frequency of *Helicobacter pylori* gastritis was found to be 40%, but after 2000 this rate decreased to 22.3% due to bile reflux caused by the changing food industry. This result may be explained by the bactericidal effects of bile acids.

Key words: bile reflux, gastritis, *Helicobacter pylori*.

Chronic abdominal pain is one of the most frequent complaints of childhood and adulthood. It is associated with poor quality of life and psychosocial deprivation.[1] Frequency ranges from 0.3-19% among school children and most of these cases have no organic pathology.[2,3]

Helicobacter pylori (Hp) is a gram-negative bacteria colonizing the human stomach, and which can cause many gastrointestinal diseases.[4] The prevalence of Hp varies worldwide and is affected by many factors such as age, ethnic group, and geographic and socioeconomic conditions.[5] Most people are colonized with Hp in early childhood.[4] Only 10-20% of these have the occurrence of gastroduodenal diseases.[5] Although endoscopy is primarily recommended for the diagnosis of Hp infection, urea breath test, and fecal Hp antigen evaluation are non-invasive tests that can be used.[6]

Bile reflux occurs by retrograde bile movement to the stomach.[7] Bile in the stomach causes clinical symptoms, endoscopically observed macroscopic changes, and histopathological chemical gastritis. There is no gold standard for the diagnosis of bile reflux.[8,9] Bile reflux is divided into primary and secondary reflux. Primary bile reflux occurs without gastric surgery, while secondary bile reflux is usually seen after surgery. Bile bladder dysfunction, gastric, and duodenal dysmotility are risk factors for primary bile reflux.[10] Bile is a strong alkali and causes chemical irritation to the gastric mucosa by changing the gastric pH balance. Bile acids have antibacterial action against Hp.[11] This study aims to determine the relationship between bile reflux and Hp gastritis

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and to show that bile reflux has increased due to the changing food industry and that this may have caused a decrease in Hp infection.

Material and Methods

Our study was retrospective, and data were taken from patients' files. Patients who applied to our pediatric gastroenterology clinic with abdominal pain and who were among the 804 cases that had undergone endoscopic examination between 2000-2019 were included. Patients with accompanying chronic diseases, alarm symptoms like diarrhea, bloody stool, growth retardation, persisting fever, elevated acute-phase reactant levels, and during follow-up, those who were diagnosed with Celiac disease and Familial Mediterranean Fever were excluded.

Diagnosis of bile reflux was made when we endoscopically observed bile in the stomach or detection retrograde bile flowed from the duodenum to the stomach. Patients with secondary bile reflux were excluded from the study.

We recorded the patients' age and sex and their macroscopic and microscopic endoscopic findings. The diagnosis of Hp infection was made by a positive rapid urease test or a positive culture result in addition to positive histopathology according to ESPGHAN guidelines.[12] At least 6 gastric biopsies (antrum and corpus) were taken for the diagnosis of Hp infection.

Statistical analysis

The statistical package for social sciences (SPSS) (Inc., Chicago, IL, USA, 22nd version) was used for data input and to analyze the demographic characteristics of the cases. Demographic characteristics were analyzed using basic definitive statistics. A Chi-square test was used to compare every 2 parameters (bile reflux and Hp infection). A p-value <0.05 was deemed statistically significant.

Clinical research ethics committee approval was received from the Ege University Ethics Committee (19-4T/44).

Results

In our study, 804 cases where all the data were included were enrolled. Of the patients 61.8% were female and 38.2% were male. The mean age was 11.56±4.14 years. Microscopic and macroscopic analyses of the stomach are shown in Table I.

The number of patients where Hp was detected in the gastrointestinal system was 179 (22.3%). Among these 179 patients, 89 of them had chronic active mild gastritis, 59 of them had chronic active moderate gastritis, 13 of them had chronic active severe gastritis, 18 of them had chronic mild gastritis.

The prevalence of bile reflux was 23.9% (n=192). When evaluated according to age; bile reflux occurred in 14.2% in the 0-2 age group, 23.4% in the 3-6 age group, 19.4% in the 7-11 age group, and 27.0% in the ≥12 age group. Of these 192 cases with bile reflux, 108 of them had chronic mild gastritis, 69 of them had chronic active mild gastritis, 13 of them had chronic active moderate gastritis and 2 of them had chronic active severe gastritis microscopically. Only 27 (14.1%) patients had Hp positivity among the 192 patients where bile reflux was detected endoscopically (p=0.002) (Table II).

Discussion

Gastric histopathologic findings were normal in 41.7% of the patients in our study. According to another study that was carried out by Mark et al.[13], 49.0% of 150 patients had normal histopathologic findings similar to our study. The reason for this may be the small tissue specimen taken during the endoscopic procedure, the involvement being patchy, or perhaps problems such as food allergies or psychosomatic disorders in these patients.

Table I. Microscopic and macroscopic findings of the stomach.

	Frequency (n)	Percent (%)
Macroscopic findings		
Normal	205	25.5
Bile reflux	192	23.8
Antral hyperemia	152	18.9
Antral nodularity	152	18.9
Pangastritis	94	11.7
Antral ulceration	9	1.2
Microscopic findings		
Normal	339	41.7
Chronic gastritis mild	201	24.8
Chronic active gastritis mild	181	22.4
Chronic active gastritis moderate	70	8.6
Chronic active gastritis severe	13	1.6

Table II. The relationship between bile reflux and Hp gastritis.

		Helicobacter pylori		Total	p-value
		Positive	Negative		
Bile reflux	Positive	27	165	192	0.002
	Negative	152	460	612	
Total		179	625	804	

Infections due to Hp, which has a commensal relationship with humans, are usually asymptomatic but may cause symptoms related to peptic ulcer or duodenal inflammation in some individuals.[4,5] In developing countries, school children are at risk due to low socioeconomic conditions, poor hygienic drinking water, living in crowded conditions, low personal and environmental hygiene, and food contamination.[14,15]

Hp infection frequency is higher in developing countries than in developed countries.[5] In our study, Hp infection frequency was 22.3%. In Africa, this ratio is between 40-90%.[16-19] According to Hunt et al.[20], while among Ethiopian children between the ages of 10-15 the Hp infection prevalence was 82%, this ratio was between 70-90% in adults.[21] It was noted that in Canada, Hp infection had a ratio of 7.1% in children in the age range of 5-18.[5] This variability maybe because of the different levels of development of these countries. In a local study carried out by Gürakan et al.[21], Hp infection frequency was found to be 52.5%. The decrease in Hp infection after the 2000s was probably a result of the adverse effects of the preservatives and unhealthy oils used in the food industry on bowel movements, and the increase in the use of macrolide group antibiotics may also explain this situation.

Bile reflux was first described in a patient with gastrocutaneus fistula in 1833 and it was perceived as an important problem.[22] Bile reflux without gastric surgery is called primary bile reflux. The risk factors are gallbladder dysfunction, gastric, and duodenal dysmotility. Due to the decrement of migrating complex motor gastric exposure activity to bile, this causes chemical gastritis and clinical symptoms. The prevalence of bile reflux is unknown.[10] Radev et al.[23] found bile reflux prevalence as 10% in adults. According to Slavesku et al.[24],

among children with atrophic gastritis, bile reflux prevalence was found to be 34%. In our study, it was found to be 23.9%. These different ratios may be because of different patient populations. Bile as a strong detergent causes gastric mucosal injury. Histopathologic antral foveolar hyperplasia, vascular congestion, edema in lamina propria, and smooth muscle leave and inflammatory cell shortage can be seen.[25] In our study, 108 of them had chronic mild gastritis, 69 of them had chronic active mild gastritis, 13 of them had chronic active moderate gastritis and 2 of them had chronic active severe gastritis microscopically. Gastritis was detected microscopically in all patients with bile reflux. Only 14.1% of patients had Hp positivity among patients with bile reflux (p=0.002). This may be since bile acids have antibacterial activity against Hp.

The changing food industry may have caused a decrease in Hp infections after the 2000s, due to an increase in bile reflux. The increase in the consumption of cheaper and easily accessible take-home foods over time may have caused a decrease in the consumption of healthy foods. Among these unhealthy products, the poor quality high-fat ratio may cause bile reflux by slowing gastric emptying and causing the release of cholecystokinin, with a negative effect on gastrointestinal system motility.[26,27] Therefore, while Hp gastritis is investigated in the differential diagnosis of patients with abdominal pain, bile reflux should be included in the differential diagnosis and treatment should be planned accordingly.

Ethical approval

Ethics committee approval statement was received from Ege University Ethics Committee in 2019 (19-4T/44).

Author contribution

The authors contribute to the paper as follows: study conception and design: SA, EKT; data collection: FÇ, MS; analysis and interpretation of results: BD, MK; draft manuscript preparation: EKT, MK, FÇ. 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

Could mean platelet volume be used as a marker for activity and severity index of alopecia areata?

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ABSTRACT

Background. We aimed to determine whether MPV can be used as a marker for the activity and the severity index of alopesi areata (AA).

Method. The charts of 71 children who received a diagnosis of AA and 70 age and gender-matched healthy children were retrospectively evaluated. The severity of hair loss was classified as S1 (<25%), S2 (25-49%), S3 (50-74%), S4 (75-95%), S4b (96-99%) (according to the percent of the area involved), alopecia totalis (AT), and alopecia universalis (AU). In the laboratory tests, the results of the complete blood count, anti-nuclear antibody (ANA), thyroid function tests (TSH, free/total T4, free/total T3), and autoimmune thyroid antibodies [anti-thyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibody (AT)] were recorded.

Results. A total of 141 cases including 61 (43.3%) males and 80 (56.7%) females were included. There was no statistically significant difference between the groups according to the mean age ($p>0.05$).

The MPV measurements were statistically significantly higher in the AA group ($p<0.01$). There was no statistically significant difference between the types of AA according to the mean age, gender distribution, the presence of nail involvement, the presence of family history, and the presence of autoimmune disease ($p>0.05$). There was no statistically significant difference between the severity of AA according to the mean age, gender distribution, the presence of nail involvement, the presence of family history, and the presence of autoimmune disease ($p>0.05$).

Conclusion. MPV is helpful in assessing clinical activity in patients with AA. However, prospective studies involving more patients are needed to support our findings.

Key words: alopecia areata, mean platelet volume, inflammation.

Alopecia areata (AA) is a chronic autoinflammatory disease that is characterized by episodic and non-scarring loss of the scalp, eyebrows, eyelashes and other body hairs and by sharply demarcated patches.[1] Although its etiopathogenesis remains unclear, the autoimmune reactions, which target the hair matrix, are thought to be responsible for genetically susceptible individuals.[2] The lifetime risk of AA is 1.7-2%, regardless of age and sex.[3,4] AA is seen especially in young patients and approximately 60% of patients have their first attack before the age of 20.[5] AA can also be accompanied by other autoimmune diseases such as autoimmune thyroid diseases, vitiligo, and lupus erythematosus.[6] Mean platelet volume (MPV) is used as part of the complete blood count in full blood count analyzers and is a common marker used to demonstrate platelet function and activation.[7] It has been shown in recent years that MPV is high in chronic inflammatory diseases and may be used as an inflammatory marker.[8] In this study, we examined the relationship between MPV level and disease severity, nail involvement, family history, disease duration and other accompanying autoimmune diseases in pediatric patients with AA.

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Material and Methods

After receiving the ethical board approval (no:10, in 19.10.2015) of Istanbul Anatolia–North Region Public Hospitals Trust, 71 children under the age of 18 who received a diagnosis of AA at the Dermatology outpatient clinic of Beykoz State Hospital between 2015 and 2017 were retrospectively evaluated in terms of age, sex, disease severity, lesion distribution, nail findings, family history, and accompanying autoimmune diseases. The control group included 70 age and gender-matched healthy children who had no dermatologic or systemic disease. If the duration of AA was 3 months or less, it was considered acute. If the duration of AA was over 3 months, it was considered chronic. The severity of hair loss was classified as S1 (<25%), S2 (25-49%), S3 (50-74%), S4 (75-95%), S4b (96-99%) (according to the percent of the area involved), alopecia totalis (AT), and alopecia universalis (AU). In the laboratory tests, the results of the complete blood count, anti-nuclear antibody (ANA), thyroid function tests (TSH, free/total T4, free/total T3), and autoimmune thyroid antibodies [anti-thyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibody (AT)] were recorded.

Statistical Analysis

NCSS-2007 software program (Number Cruncher Statistical System) (Kaysville, Utah, USA) was used for statistical analysis. Mean, standard deviation, median, frequency, ratio, minimum, and maximum were used for comparing the descriptive data. Moreover, the Mann Whitney U test was used for comparing the parameters without normal distribution between two groups. The Kruskal Wallis test was used for comparing three or more groups without normal distribution. The Fisher-Freeman-Halton test, Fisher's Exact test, and Perason chi- square test were used for comparing the qualitative data. The significance level was considered as $p < 0.05$.

Results

A total of 141 cases including 43.3% (n=61) males and 56.7% (n=80) females who were admitted to the Dermatology outpatient clinic of Beykoz State Hospital between 2015 and 2017 were enrolled in the study. Their ages ranged from 1 to 18 years and the mean age was 12.57±4.38 years.

In the AA group, their ages ranged from 1 to 18 years and the mean age was 12.24±4.69 years. In the control group, their ages ranged from 2 to 18 years and the mean age was 12.91±4.05 years. There was no statistically significant difference between the groups according to the mean age ($p>0.05$) (Table I).

In the AA group, the MPV measurements ranged from 6.4 to 11.4 and the mean MPV level was 8.33±1.15. In the control group, the MPV measurements ranged from 5.4 to 7.7 and the mean MPV level was 6.33±0.41. The MPV measurements were statistically significantly higher in the AA group ($p=0.001$; $p<0.01$) (Table I), (Fig. 1).

The MPV measurements showed no statistically significant differences according to the type of AA ($p=0.344$; $p>0.05$) (Table II). While the MPV measurements showed no statistically

Table I. Age and MPV evaluations according to groups.

		Group		[a]p
		Alopeci areata (n=71)	Control (n=70)	
Age	Min-Max (Median)	1-18 (13)	2-18 (14)	0.551
	Mean±SD	12.24±4.69	12.91±4.05	
MPV	Min-Max (Median)	6.4-11.4 (7.9)	5.4-7.7 (6.4)	0.001**
	Mean±SD	8.33±1.15	6.33±0.41	

[a]Mann Whitney U Test, **p<0,01
MPV: Mean platelet volume

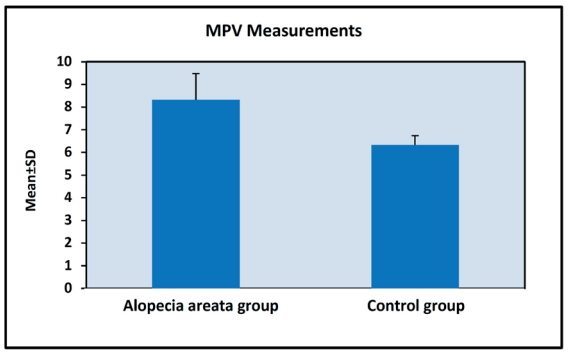

Fig. 1. Distribution of MPV measurements according to groups.

significant difference according to the severity of AA (p>0.05) (Table II), it was remarkable that the MPV measurements were high in the patients with AA severity S2.

The MPV measurements showed no statistically significant difference according to the presence of nail involvement, the presence of family history, the presence of autoimmune disease, and disease duration (p>0.05) (Table II).

There was no statistically significant difference between the types of AA according to the mean age and gender distribution (p>0.05) (Table III).

There was no statistically significant difference between the types of AA according to the presence of nail involvement, the presence of family history, and the presence of autoimmune disease (p>0.05) (Table III). It was remarkable that the patients who had a reticular type of AA had a higher presence of nail involvement than patients who had a siasphio or a ophiasic type of AA (Table III).

There was no statistically significant difference between the severity of AA according to the mean age and gender distribution (p>0.05) (Table IV).

There was no statistically significant difference between the severity of AA according to the presence of nail involvement, the presence of family history, and the presence of autoimmune disease (p>0.05) (Table IV).

Discussion

AA is a chronic inflammatory disease that is characterized by episodic and non-scarring loss of the scalp and/or other hairy areas of the body.[9] Although genetic predisposition, non-specific immune reactions, organ-specific autoimmune reactions, environmental factors, and atopy

Table II. Evaluations of descriptive characteristics according to MPV measurements.

		MPV		p
		Min-Max (Median)	Mean±SD	
Type of alopecia areata	Reticular	6.4-11.4 (8.4)	8.57±1.25	[b]0.344
	Ophiasis	6.8-10.2 (7.7)	8.26±1.16	
	Siasphio	6.8-10.5 (7.7)	8.07±0.99	
Severity of alopecia areata	S1	6.8-10.5 (7.7)	8.05±1.10	[a]0.085
	S2	6.4-11.4 (8.5)	8.52±1.16	
Nail involvement	No	6.4-11.4 (7.9)	8.39±1.19	[a]0.522
	Yes	6.8-9.9 (8.2)	8.12±1.03	
Family history	No	6.4-11.4 (7.9)	8.36±1.19	[a]0.642
	Yes	7.2-9.4 (8)	8.06±0.76	
Autoimmune disease	No	6.4-11.4 (8)	8.30±1.13	[a]0.655
	Yes	6.8-10.9 (7.9)	8.49±1.29	
Disease duration	Acute hair loss	6.8-10.5 (8.5)	8.47±1.07	[a]0.166
	Chronic hair loss	6.4-11.4 (7.7)	8.16±1.25	

[a]Mann Whitney U Test, [b]Kruskall Wallis Test
MPV: Mean platelet volume

Table III. Evaluation of descriptive characteristics according to type of alopecia areata.

		Type of Alopecia areata			p
		Reticular (n=31)	Ophiasis (n=17)	Siasphio (n=23)	
Age	Min-Max (Median)	4-18 (13)	1-18 (14)	4-17 (13)	[b]0.716
	Mean±SD	12.48±4.90	11.41±5.46	12.52±3.87	
Gender; n (%)	Male	17 (54.8)	5 (29.4)	10 (45.3)	[d]0.253
	Female	14 (45.2)	12 (70.6)	13 (56.5)	
Nail involvement; n (%)	No	28 (90.3)	11 (64.7)	16 (69.6)	[c]0.060
	Yes	3 (9.7)	6 (35.3)	7 (30.4)	
Family history; n (%)	No	27 (87.1)	17 (100)	21 (91.3)	[c]0.479
	Yes	4 (12.9)	0 (0)	2 (8.7)	
Autoimmune disease; n (%)	No	23 (74.2)	16 (94.1)	19 (82.6)	[c]0.260
	Yes	8 (25.8)	1 (5.9)	4 (17.4)	

[b]Kruskall Wallis Test, [c]Fisher Freeman Halton Test, [d]Pearson Chi-Square Test

Table IV. Evaluation of descriptive characteristics according to severity of alopecia areta.

		Severity of Alopecia areata		p
		S1 (n=29)	S2 (n=42)	
Age	Min-Max (Median)	1-18 (13)	4-18 (15)	[a]0.167
	Mean±SD	11.28±5.01	12.90±4.40	
Gender; n (%)	Male	12 (41.4)	20 (47.6)	[d]0.603
	Female	17 (58.6)	22 (52.4)	
Nail involvement; n (%)	No	22 (75.9)	33 (78.6)	[d]0.788
	Yes	7 (24.1)	9 (21.4)	
Family history; n (%)	No	28 (96.6)	37 (88.1)	[e]0.390
	Yes	1 (3.4)	5 (11.9)	
Autoimmune disease; n (%)	No	25 (86.2)	33 (78.6)	[d]0.414
	Yes	4 (13.8)	9 (21.4)	

[a]Mann Whitney U Test, [d]Pearson Chi-Square Test, [e]Fisher's Exact Test

have been emphasized, the etiopathogenesis of AA has not been fully elucidated.[9] AA is seen especially in young patients and also approximately 60% of patients have had their first attack before the age of 20.[2] Although AA can be diagnosed easily by clinical examination, diffuse AA may be diagnostic challenging. In this situation, the dermoscopic findings (broken hair, black dot, yellow dot, exclamation hair) facilitate the differential diagnosis; however, histopathological diagnosis may be needed in a small number of cases. The characteristic histopathologic finding of AA is an intense peribulbar and intrabulbar lymphocytic infiltrate of lymphocytes around the anagen follicles in the appearance of a honey bee colony.[9]

In addition to homeostatic functions, thrombocytes interact with endothelial cells, leukocytes (monocytes, neutrophils, dendritic cells, T-cells), and progenitor cells and allow inflammatory cells to migrate to lesion sites and release abundant quantities of inflammatory cytokine and thus provide an inflammatory environment in the lesion area. It has been reported in recent years that MPV also reflects platelet function and activation and may be a marker of inflammation in different chronic diseases.[8]

In our study, the mean MPV levels of the patients with AA were significantly higher than those of the control group. The current hypotheses on the development of AA focuses on the deterioration of autoantigen presentation in the hair follicle as a result of immune privilege in the hair follicles and activation of autoreactive T lymphocytes.[10] The autoreactive T-cell response, which is thought to be involved in the pathogenesis of AA, can be genetically determined.[10] It can also be increased by IL-6 and other cytokines.[11] Although IL-6 is a proinflammatory cytokine produced by various cells such as fibroblasts, macrophages, B and T cells, it plays an important role in the hematopoiesis, immune cell activation, regulation of inflammation, and pathogenesis of autoimmune diseases.[12,13] Inflammation is known to be an important stimulus for platelets. However, it has been suggested that increased levels of IL-6 may stimulate platelet production and release large platelets from bone marrow.[13] Therefore, in our study, the mean MPV levels, which were significantly higher in the patients with AA than in the control group, may be thought to be associated with an increase in young platelets in circulation. When the literature is examined, it is seen that MPV levels vary depending on the severity of systemic inflammation in high- and low-grade inflammatory diseases, but the results are contradictory.[14]

When the relationship between the mean MPV level and the age of disease onset, disease duration, disease severity, family history, nail involvement, and accompanying autoimmune disease was evaluated, there was no difference between the AA and control groups. While the MPV measurements showed no statistically significant difference according to the severity of AA, it was remarkable that the MPV measurements were high in the patients with AA severity S2. When the mean MPV level was examined according to the type of AA, it was seen that the mean MPV level was a little bit higher in the reticular pattern, which is more common in the population.

In this study, although there was no significant relationship between the mean MPV level and the clinical activity markers of the patient group (early onset, family history, ophiasis pattern, nail involvement), we think that MPV can be used as a useful marker in the evaluation of AA.

Consequently, AA is a severe and chronic disease in the pediatric age group. Families often refer to a doctor for medical support and treatment because the disease has social and psychological effects. MPV is a simple marker that does not require advanced or expensive technology and also MPV level is determined in each complete blood count. MPV is helpful in assessing clinical activity at a glance in patients with AA. However, prospective studies involving more patients are needed to support our findings.

Ethical approval

Ethical board approval was received from the Istanbul Anatolia–North Region Public Hospitals Trust (no:10, in 19.10.2015).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Acute demyelinating encephalomyelitis and transverse myelitis in a child with COVID-19

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ABSTRACT

Background. Corona virus disease 2019 (COVID-19) includes a wide range of diseases with varying pathophysiology in children and adults. Although the disease mainly affects the respiratory tract, neurological involvement is also reported in the literature. The most common neurological complaints due to COVID-19 are headache, dizziness and anosmia. Acute necrotizing myelitis, acute demyelinating encephalomyelitis (ADEM), acute axonal neuropathy, acute transverse myelitis, and Guillian-Barre syndrome have been reported as neurological dysfunctions associated with COVID-19.

Case. A ten-year-old male patient presented with complaints of fever, headache and generalized muscle pain. The patient developed inability to walk and significant muscle weakness during the disease course, and he was diagnosed with ADEM and transverse myelitis on magnetic resonance imaging (MRI). As the etiological agent, COVID-19 was detected in both the respiratory panel sample and the cerebrospinal fluid (CSF) sample by the polymerase chain reaction (PCR) technique. Pulse steroid, IVIG, and plasmapheresis treatment were administered. He started to stand with support during follow-up.

Conclusion. We presented a case of COVID-19 related ADEM and transverse myelitis who responded to pulse steroid, IVIG, and plasmapheresis

Key words: acute demyelinating encephalomyelitis, acute transverse myelitis, COVID-19.

Infection associated with COVID-19, which emerged in Wuhan, China in December 2019, spread rapidly all over the world.[1] SARS-Cov 2 infection can classically lead to fever, cough, diarrhea, generalized muscle aches, severe respiratory disease and severe respiratory failure that may progress to acute respiratory distress syndrome (ARDS).[2] The diagnosis of COVID-19 disease is made by viral nucleic acid detection by molecular methods and abnormal liver-metabolic tests supported by hematological panels.[3,4] Although the disease mostly affects the respiratory tract, neurological involvement has also been reported in the literature. The invasion of the central nervous system with COVID-19 occurs completely through immune escape and shortly after infection. The virus enters the central nervous system and causes a series of disease-related consequences.[5] Neurological involvement characteristics of this infection include conditions such as headache, dizziness, epileptic seizures, taste and smell disorders, cerebrovascular events, acute encephalitis, Guillain-Barre syndrome, and acute transverse myelitis.[6,7] Researchers have confirmed that the SARS-CoV-2 virus enters cells through the angiotensin-converting enzyme (ACE) 2 receptors on the surface of human cells and causes the disease.[8] ACE2 receptors found in type 2 alveolar epithelial cells of human lung have become the main target of SARS-CoV-2 virus in the pathogenesis of COVID-19.[9] In addition to highly marked lung, liver and kidney

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injuries possibly associated with the expression of ACE 2 in proximal renal tubules and hepatic bile duct cells, many critical patients with multi-organ dysfunction have been reported during the clinical diagnosis and treatment process.[10,11] ACE receptors are also found on the surface of spinal cord cells. However, it is still not entirely clear whether neurons of the spinal cord are adversely affected by COVID-19.[12,13] In this report, we present a 10-year-old male patient with acute demyelinating encephalomyelitis (ADEM) and transverse myelitis, started with fever and headache and continued with weakness and inability to walk later in the course, which was found to be associated with COVID-19.

Case Report

A 10-year-old male patient, who had been healthy until a month prior, had presented to a physician with weakness, vomiting, severe headache, and fever; he had been treated for sinusitis. A brain magnetic resonance imaging (MRI) study had been performed and found normal. One week after the treatment, the patient had presented to the physician again due to the persistence of headache, severe colic-like abdominal pain attacks, and widespread body pain. He was hospitalized and received ceftriaxone. On the 8th day of treatment, he started to be agitated and have tremors in his left arm and leg. Later in the course, he was unable to walk and use his extremities. The patient whose general condition deteriorated was referred to our pediatric emergency department. At emergency department admission, he had a body temperature of 36.7 °C, a blood pressure reading of 100/70 mmHg, and a pulse rate of 95/min.; his general condition was moderate-to-poor; he was somnolent, got occasionally agitated and was crying. His pupils were isochoric with positive diagnostic likelihood ratio (DLR)\ likelihood ratio (LR), and he had marked neck stiffness. He had positive meningeal irritation signs. He had marked clonus in both lower extremities. His abdominal skin reflex was absent in all abdominal quadrants. His muscle strength was 0/5 in the left upper extremity, 2/5 in the right upper extremity, and 3/5 in both lower extremities. His laboratory tests revealed a WBC of 21,800/mm^3 with a PMNL predominance of %92.5 and a platelet count of 358,000/mm^3; blood glucose was 124 mg/dl, ALT 18 U/L, urea 54 mg/dl, creatinine 1.66 mg/dl; the coagulation tests were normal; he had a sedimentation rate of 38 mm/hour and a negative C-reactive protein (CRP); the urinalysis showed a urinary pH of 6.5, urine density of 1007, negative urinary protein and glucose, trace erythrocytes, and a leucocyte count of 19.5 /hpf. Urine culture was sterile. The patient was admitted to the intensive care unit and meropenem, acyclovir, and clarithromycin were started. The patient was hydrated and his fluid intake and urine output were monitored. His renal function improved during follow-up. Brain and cervical MRI was performed and revealed marked signal increase in basal ganglia and cervical myelitis (Fig.1 a, b, e). Lumbar puncture (LP) was delayed due to his poor general condition. Intravenous methylprednisolone was administered at a dose of 30 mg/kg/day for 5 days. The patient's general condition slightly improved on the third day of methylprednisolone treatment, and thus LP was performed. Cerebrospinal fluid (CSF) pressure was within normal limits, CSF was clear, and no cells were seen. CSF protein level was 38.3 mg/dl, and CSF glucose level was 89 mg/dl. There was no growth in CSF culture or blood cultures. Polymerase chain reaction (PCR) tests for *Neisseria meningitidis, Hemophilus influenzae, Streptococcus pneumoniae*, varicella zoster, herpes simplex virus type 1 and type 2 in the CSF were all negative. No findings other than 2019 ncov PCR positivity was detected in the respiratory panel studied from the nasal swab. Therefore, the 2019 ncov PCR test was reexamined in the CSF sample. As the 2019 ncov PCR test was positive in the CSF, it was thought that the patient possibly had ADEM + transverse myelitis secondary to COVID-19. Methylprednisolone was administered at a dose of 30 mg/kg/day for 5 days, followed by a dose of 30 mg twice a day, after that plasmapheresis was

performed for five days, and then intravenous immunoglobulin (IVIG) was administered at a dose of 400 mg/kg. It was planned that steroid treatment was administered at a dose of 2x30 mg for 4 weeks and tapered in 4-6 weeks. The chest radiography of the patient was normal. The patient was not administered direct treatment for COVID-19. Oligoclonal band, serum anti-mog and aquaporin 4 antibodies in CSF were negative. On the 5th day of plasmapheresis and IVIG treatment, the muscle strength of the patient's left upper extremity was 3-4/5; although his neck stiffness was significantly reduced, it persisted. The patient's clinical status significantly improved. At the end of the IVIG + plasmapheresis treatment, the brain axial T2 and DIR sequences of the control brain and spinal MRI detected regression in the findings (Fig.1 c, d, f).

Informed consent was received from the family.

Discussion

Pediatric acute transverse myelitis is an immune-mediated central nervous system disease.[14-17] Transverse myelitis is a heterogeneous, non-compressive myelopathy characterized by acute- or subacute-onset spinal cord dysfunction due to inflammation.[15]

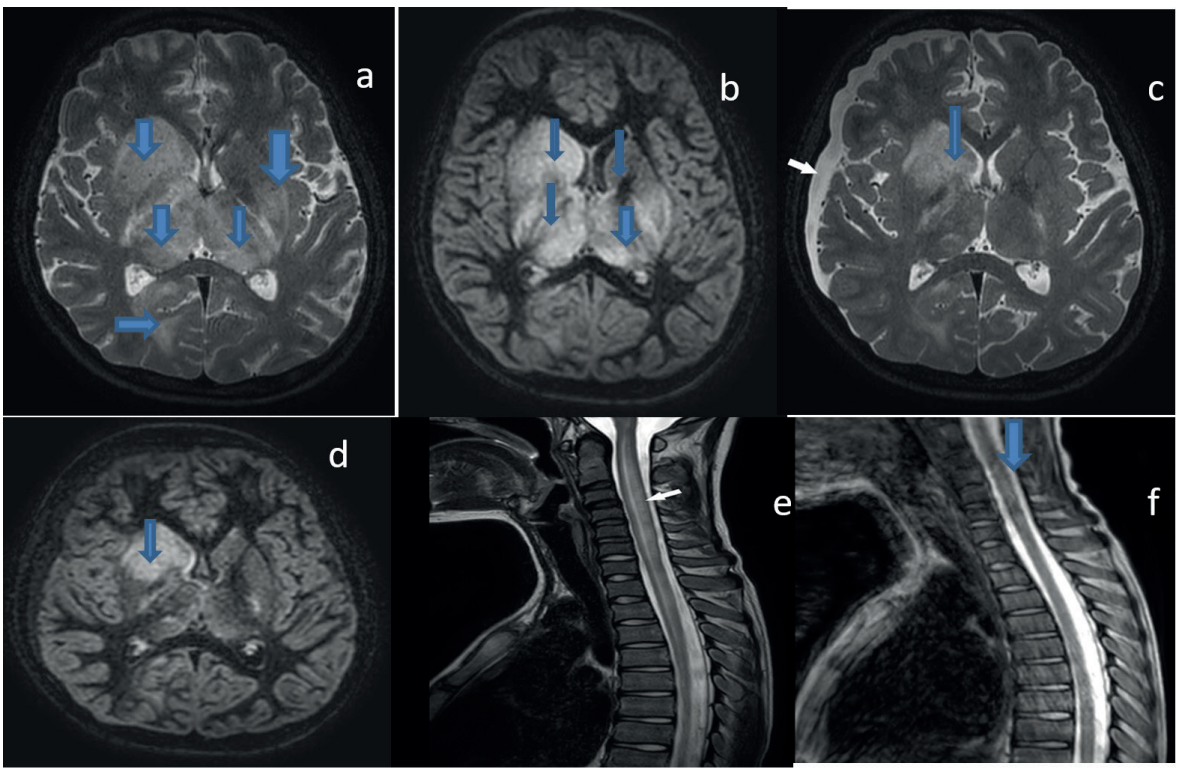

Fig. 1. a-b) T2 axial and axial double inversion recovery (DIR) sequences of brain magnetic resonance imaging (MRI) study revealed no contrast enhancement or diffusion limitation, but marked signal increase, in the right caudate nucleus, bilateral lentiform nuclei, bilateral thalami, partially internal and external capsules, both occipital lobes (predominantly right side) and left hippocampus, and bilateral amygdala (blue arrow). **c-d)** Axial T2 and DIR sequences of the control brain MRI detected regression in the findings, being more pronounced in the bilateral thalamus (blue arrow). In addition, a newly developed subdural effusion (white arrow) was observed on the right side. **e)** On cervical MRI, there was a central signal increase (myelitis?) of a slightly expansive character in the cervicothoracal spinal cord (white arrow), which was more prominent in the cervical region; there was only millimetric punctate enhancement at the C4 level, but no enhancement was observed in other parts. **f)** Sagittal T2 sequences spinal cord expansion signal increase regressed in post-treatment control spinal MRI (blue arrow).

The clinical signs include neurological deficits characterized by motor, autonomic, and sensory involvement in varying degrees. Acute demyelinating diseases such as para-infectious, post-infectious, toxic-drug-related causes, paraneoplastic, autoimmune disorders, and multiple sclerosis and neuromyelitis optica spectrum disorders are held responsible for the etiology of ATM.[14] Acute demyelinating encephalomyelitis (ADEM) is a common postviral demyelinating disease that is mostly seen in children rather than adults. Although clinically heterogeneous, it causes multifocal deficits and encephalopathy. Brain MRI FLAIR images typically show hyperintensity in deep white matter and at the gray-white matter interface. Contrast enhancement usually does not occur, but rarely, punctate contrast enhancement can be seen. Diffusion restriction can also be seen in the early period.[18]

A few cases with neurological findings associated with COVID-19 have been reported in the literature. Acute transverse myelitis was reported for the first time in a 66-year-old Chinese patient with SARS-CoV-2 infection, who presented with acute weakness, urinary and bowel incontinence.[19] A 59-year-old female patient with high fever was admitted to the emergency room with acute onset of ascending flaccid paraplegia in lower extremities, urinary retention, and constipation; she was diagnosed with acute transverse myelitis associated with COVID-19.[14] In another case, a 69-year-old female patient presented to a physician with pain in the cervical region, imbalance, motor weakness, and numbness in the left hand 8 days after the onset of fever and dry cough. The patient's spinal MRI showed an appearance compatible with acute transverse myelitis. Although COVID-19 was detected in the sample taken from the respiratory tract of the patient, it was reported that the COVID-19 test was negative in the CSF sample.[20] The pediatric case in the literature is a 3-year-old girl from Navajo. She presented to hospital with progressive weakness in extremities and reduced sensation. In her examination, she had flask quadriparesia, areflexia, and respiratory failure. Her MRI showed an appearance compatible with transverse myelitis in the cervical region. The patient's nasopharyngeal SARS Cov 2 PCR test was positive.[7]

A 51-year-old female patient was admitted to the hospital with vomiting, dizziness, fever, and difficulty breathing. The SARS cov 2 nasal swab PCR test returned positive. Her deep tendon reflexes were reduced. A contrast-enhanced brain MRI showed signs compatible with ADEM.[18]

In conclusion, neurological symptoms due to COVID-19 can be seen in childhood, albeit rarely. We aimed to emphasize that neurological symptoms should be regarded as a post-infectious complication that occurs after COVID-19 or in the absence of specific COVID-19 symptoms and that they can progress rapidly, for which aggressive first-line treatments should be considered. In addition, the co-occurrence of COVID-induced ADEM and acute transverse myelitis was emphasized because it has not been reported in children in the literature.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HGP, SK; data collection: HGP, SK, MYS, İE, ZAT, YE; analysis and interpretation of results: HGP, SK, MYS, İE, ZAT, YE; draft manuscript preparation: HGP, SK,YE. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Saccharomyces cerevisiae fungemia due to an unexpected source in the pediatric intensive care unit

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ABSTRACT

Background. *Saccharomyces cerevisiae* is one of the microorganisms commonly used as a probiotic. Although it is primarily known as non-pathogenic, it may cause fungemia, particularly in immunocompromised patients or children with a history of long-term hospital stay.

Case. A 6-month-old boy with a history of ventriculostomy, ventriculoperitoneal shunt implantation, and external drainage due to an intracranial mass and hydrocephalus was admitted to the pediatric intensive care unit (PICU) on postoperative day 14 due to respiratory distress and intubated on admission. He was started on broad spectrum antibiotics on day 25 of the admission due to fever and clinical deterioration. Culture of the central venous catheter (CVC) yielded *S. cerevisiae, the* CVC was removed, and the patient was started on caspofungin. We noticed that a patient near this patient was on a probiotic preparation containing *S. boulardii* for diarrhea before PICU admission. His fever subsided on day 2 of caspofungin, and laboratory findings normalized on follow-up.

Conclusions. Probiotics should not be used in PICUs because of the high risk for CVC-related sepsis in critically ill children.

Key words: *Saccharomyces cerevisiae*, sepsis, pediatric intensive care, probiotic, fungemia.

Saccharomyces cerevisiae (Brewer's yeast) is a facultative anaerobic budding yeast used since antiquity for baking and producing fermented beverages, bread, wine, and beer. They are ubiquitous and can be a part of the intestinal, vaginal, pharynx, pulmonary tree, and skin floras, particularly in patients with chronic disorders.[1,2] *Saccharomyces boulardii is a* yeast that is genetically very close or nearly identical to *S. cerevisiae and* is one of the microorganisms commonly used as a probiotic.[3] It is used to treat *Clostridium difficile* associated diarrhea and the prevention of antibiotic-associated diarrhea in humans.[3,4] It is generally non-pathogenic; however, it may cause fungemia, particularly in immunocompromised patients or children with a history of long-term hospitalization.[1,3,4] The incidence of *S. cerevisiae* fungemia is unknown.[5] *S. cerevisiae* fungemia is unusual in previously healthy children, and the leading risk factor is the use of a probiotic or their use by other patients admitted to the same unit in nearby beds.[5] Intravascular catheter and prosthetic valve-related infections were also reported.[3,5,6]

Even though probiotic strains are widely considered safe, there are some safety concerns, especially in severely immunocompromised patients, in whom they may cause sepsis.[7] Probiotics are not used to restore the intestinal flora in pediatric intensive care units (PICU) because of the potential to cause fungemia, especially in patients with central venous catheters (CVCs).[5,6] We hereby present the course and successful treatment of a 6-month-old male with *S. cerevisiae* fungemia due to an unexpected source in our PICU.

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Case Report

A 6-month-old boy with a history of ventriculoperitoneal shunt implantation and external drainage for intracranial mass and hydrocephalus was admitted to the PICU on postoperative day 14 due to respiratory distress. He was intubated on admission, after which he had an oxygen saturation of 96% on a FiO2 of 0.6. His heart rate was 125 beats/minute, his blood pressure was 115/80 mmHg, and his body temperature was 37.3°C. His Glasgow Coma Score was 5. 30 mL of cerebrospinal fluid (CSF) was drained four times daily. He was ultimately diagnosed with an inoperable atypical rhabdoid/teratoid tumor, so he was started on doxorubicin, cyclophosphamide, and vincristine. His white blood cell count was $5.24\times10^3/\mu L$, the total neutrophil count was $3.63\times10^3/\mu L$, hemoglobin level was 9.6 g/dl, hematocrit was 28.4%, platelet count was $652\times10^3/\mu L$, blood glucose level was 101 mg/dL, aspartate aminotransferase 17 IU/L, alanine aminotransferase 10 IU/L, urea 7 mg/dL, creatinine level was 0.03 mg/dL, sodium level was 138 mmol/L, potassium level was 5 mmol/L, chlorine level was 104 mmol/L, calcium level was 8.1 mg/dL, CRP level was 11.3 mg/dL. Blood gas analysis was normal (pH: 7.39, HCO3: 21.8 mmol/L, pCO2: 37 mmHg, laktat 1,6 mmol/L).

The patient had a fever of 39°C on day 20 day of admission. Meropenem, fluconazole, amikacin, vancomycin, and colistin were consequently initiated empirically due to persistent fever. Blood, catheter, CSF, urine, and tracheal aspirate cultures were obtained; peripheral blood, CVC, urine, and tracheal aspirate cultures were negative for bacteria and fungi. A computed tomography scan of the chest was obtained for workup of sepsis of unknown origin.

Repeat cultures were obtained on the following days due to persistent high fever. The culture of the catheter tip yielded *S. cerevisiae* on day 25 of PICU admission. Fluconazole was discontinued and caspofungin was started, and other antibiotics were stopped (Table I). We noticed that our patient had a fever spike the day after a

Table I. Timeline of treatments, fever status, cultures, radiological imaging, respiratory support applied to the patient following PICU admission.

	14th day	20th day	25th day	27th day	39th day	52nd day	70th day
Fever	-	+	+	-	-	-	-
Antibiotic and antifungal therapy		Meropenem, fluconazole, amikacin, vancomycin, and colistin were started	Caspofungin was started and other antibiotics were stopped.	Caspofungin	Caspofungin was stopped	-	-
Cultures			S. cerevisiae was yielded in the CVC tip culture				
Radiological imaging		Thorax computer tomography was normal					
Respiratory support	IMV	IMV	IMV	IMV	IMV	Extubated and oxygene mask	Oxygene mask
Outcome							Transferred to the pediatric oncology ward

CVC: central venous catheter, IMV: invasive mechanical ventilation, PICU: Pediatric Intensive Care Unit.

neighbouring patient with inherited metabolic disease and gastroenteritis was started on a probiotic containing *S. boulardii*.

His fever subsided on day 2 of caspofungin, and laboratory findings normalized. Caspofungin was given for 14 days, until day 39 of PICU admission. He was extubated on day 52 of admission, whereafter he remained clinically stable. He was transferred to the pediatric oncology ward on day 70 of PICU admission.

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

Discussion

Probiotics are becoming progressively available as food supplements and are widely used in the medical industry.[3] Henri Boulard isolated *Saccharomyces boulardii* in 1920 during a cholera, outbreak and it was used as a probiotic for the treatment of gastrointestinal diseases.[3]

S. Boulardii is a yeast used as a dietary supplement. It is used to treat various diseases such as enteral-nutrition-related diarrhea, traveler's diarrhea, antibiotic-related diarrhea, Helicobacter pylori disease, HIV-associated diarrhea, *Salmonella typh*i, and *Clostridium difficile*, Crohn's disease, and other inflammatory bowel conditions.[3,8] *Saccharomyces boulardii* is genetically nearly identical to *S. cerevisiae and* is one of the microorganisms commonly used as a probiotic.[3]

Invasive *Saccharomyces* infections remain rare among invasive fungal infections even though the incidence has significantly increased since the 1990s.[9] The incidence of *S. cerevisiae* fungemia is unknown.[5] Saccharomyces fungemia has been reported to cause endocarditis, liver abscess, and disseminated disease.[5] The main risk factor for Saccharomyces cerevisiae fungemia in previously healthy patients is the use of probiotics or probiotic use by other individuals in the same unit in neighboring beds.[5] Infection of CVCs have also been reported.[5] Predisposing factors are the same as those pertaining to invasive candidiasis, which includes the presence of a CVC, total parenteral nutrition, ICU admission, antibiotic use, and immunosuppression.[1] In a review of 60 cases conducted by Munoz et al.[4], %31 of the patients were immunosuppressed, and %46 were critically ill with invasive *S. cerevisiae*. In this study, 13 patients were ≤ 16 years of age, and 7 patients were ≤ 1 year of age.[4] Many case reports also describe invasive *S. cerevisiae* in hospitalized patients in beds near patients using probiotics.[1,4] The cause of the fungemia was thought to be either translocation through the digestive system or contamination of the CVC through the colonized hands of healthcare personnel after administering probiotics.[3] Our patient was receiving chemotherapy, had a CVC, and was situated near a patient who was on probiotics, so he had several risk factors for disseminated infection. We think it is likely that the probiotic strain was transmitted to our patient through colonized hands of PICU staff.

It has been shown that after a package of freeze-dried yeast is opened, viable cells can be retrieved up to two hours later from surfaces as much as one meter away from the opening site and can persist on the hands of personnel even after vigorous hand hygiene.[8] If the package containing the probiotic is opened in a patient's room, meticulous hand hygiene and the use of gloves may still decrease the risk of infection.8 Yeasts isolated from blood cultures are considered pathogenic, but transient fungemia can occur in immunocompetent patients with no catheter, no valvulopathy, and no organic severe underlying disease.[10] Blood cultures should be repeated and yeast colonies should be identified correctly.[10] Since a patient close to our patient was on probiotics, fluconazole treatment was initiated after the first catheter culture, and the probiotic treatment of the neighboring patient

was discontinued. Recommended treatment for disseminated bloodstream infection related to probiotic use is the removal of the CVC, and amphotericin B (1 mg/kg.day) or fluconazole (10 mg/kg.day), even though reports of strains resistant to fluconazole and amphotericin B have been reported.[5] A literature review by Munoz et al.[4] reported that the most common drugs employed were fluconazole (16 patients) and amphotericin B (28 patients). The mortality rate was 28% (17 of 60 patients) and only one child died. The role of echinocandins was not discussed in this study. However, several case reports of successful treatment of *S. boulardii* with caspofungin can be found.[1,3] Our patient had his CVC removed and he received a 14-day course of caspofungin. Repeat cultures remained negative.

In conclusion, probiotic use is a risk factor for invasive infection and sepsis in critically ill patients. Transmission can occur by air or from patients on probiotics situated on a nearby bed. Therefore, probiotics should not be used in intensive care units due to the risk of central line-related sepsis in critically ill patients.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TK, EG, DBÇ; data collection: EG, DBÇ, EB; analysis and interpretation of results: TK, H.Ö, EG; draft manuscript preparation: EG, DBÇ. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Rhinovirus as a rare cause of acute onset dilated cardiomyopathy due to myocarditis in a newborn: case report and review of the literature

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ABSTRACT

Background. Cardiomyopathies account for 1% of cardiac diseases that mainly originate from myocarditis in the form of dilated cardiomyopathy in the neonatal period. Viruses are the main cause of myocarditis resulting in dilated cardiomyopathy. Rhinovirus is the leading cause of viral respiratory infections though it is rarely severe.

Case. We report a 17 day old newborn with acute onset dilated cardiomyopathy due to myocarditis that developed after a viral respiratory infection caused by Rhinovirus who was admitted to the emergency ward with shock due to heart failure and recovered without any complications. This is the first case reporting the causal role of rhinovirus and myocarditis in the neonatal period.

Conclusions. A comprehensive approach is needed for the diagnosis of myocarditis in the case of unknown etiology and an extensive respiratory panel may be taken into consideration if there is a history or clinical symptoms of respiratory infection.

Key words: dilated cardiomyopathy, myocarditis, newborn, rhinovirus.

Cardiomyopathies (CMs) consist of a heterogeneous group of diseases with a wide spectrum of causes mainly affecting the myocardium resulting in cardiac dysfunction, heart failure and even death. Various classifications of CMs were determined based on anatomical structure, etiology, and pathophysiology.[1] Neonatal CMs include primarily affected myocardium with no valvular or vascular abnormalities that may be acquired or congenital and are responsible for 10% of all pediatric cardiac deaths during the childhood period. Incidence is estimated as 10:100 000 live birth.[2] Neonatal CMs are mostly seen as dilated cardiomyopathy (DCM) in which myocarditis is the major cause of acquired conditions.[3,4] Several microbial agents including viruses, bacteria, parasites, and fungi may be infective pathogens for myocarditis and viruses have a majority rather than other agents. The most common virus detected in the pediatric population is *Coxsackievirus B3* and other viruses like Adenovirus, Cytomegalovirus, Human immunodeficiency virus (HIV), Herpesvirus, *Parvovirus B19, Influenza A and B*, Echoviruses, *Epstein-Barr virus*, and Hepatitis viruses can also be counted in the list.[5-8]

Rhinovirus, a member of the picornavirus family and the enterovirus genus, is the leading cause of viral respiratory infections (VRIs) including the common cold and rare but life-threatening infections such as viral meningitis, encephalitis, myocarditis, and neonatal sepsis-like syndrome.[9] It is responsible for more than %50 respiratory tract infections and it causes worldwide outbreaks. Significant consequences like severe lower respiratory tract infection, myocarditis, or death may be seen in the neonatal period.[7]

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Here we report a case of acute onset dilated cardiomyopathy due to myocarditis developed after VRI caused by Rhinovirus. This is the first case in the literature that reported the causal role of Rhinovirus and myocarditis in a newborn. However, myocarditis is a serious condition that may progress to heart failure, our case was diagnosed, treated effectively, and recovered well without any sequelae.

Case Report

A 17 day old male was administered to the pediatric emergency ward due to poor sucking, feeding difficulty and increased respiratory distress. He was born at 37 weeks to a 20-year old mother via vaginal delivery, weighing 2920 gr. Family history was unremarkable rather than a third-degree consanguineous marriage. His mother was a healthy woman with no history of chronic disease and no routinely used medication, who had upper respiratory tract infection symptoms such as a cough for the last five days. During postnatal adaptation period the baby had no problems and was discharged on the 2nd day of life. On physical examination, he was tachycardic, tachypneic, and dyspneic. His heart rate was 186 beats/min, respiratory rate 68 breaths/min, blood pressure 42/34 (mean 27, <3p) mmHg, body temperature 36.6 °C. He had poor perfusion and weakly palpable pulses in all extremities. His liver was 4 cm palpable below the costal angle. A gallop rhythm was heard on cardiac examination. There was sinus tachycardia with a heart rate of 162 beats/min on electrocardiography. The P wave was normal. There was a low voltage ST segment (less than 10 mm total amplitude in limb leads) and T wave suppression. PR interval was 0.12 seconds, slightly prolonged when corrected to postnatal age and heartbeat (upper limit 0.11 seconds for 160-180 beats/min below 1 month of age). Chest X-ray revealed cardiomegaly and interstitial infiltrations (Fig. 1). On echocardiography, moderate left ventricular and atrial dilatation with mild mitral regurgitation was present. There was moderate left systolic dysfunction whereas ejection fraction (EF) was 43% by Simpson method. One myocardial trabeculation was prominent in the left ventricular cavity. Blood gas parameters taken in the emergency unit were as follows: pH: 7.16, pCO_2: 37 mmHg, bicarbonate: 12.7 mmol/L, and base excess: -13.9 mmol/L. He was intubated after his oxygen saturation dropped below 85% and his blood perfusion became worse, he was then transferred to our neonatal intensive care unit. He was supported with mechanical volume guarantee ventilation. An urgent fluid bolus of 20 mL/kg was administered because of poor peripheral perfusion and milrinone was initiated for inotropic support. White blood cells (8580 cells/L) and platelet count of 197000 per microliter were in normal range and C – reactive protein was negative (0.6 mg/L). On biochemical analyses, all electrolytes, renal and liver markers were in normal range except for creatinine kinase (CK): 7990 U/L (normal range <170 U/L), CK-MB: 302 ng/mL (normal range 0.6-6.3 ng/mL), troponin I: 0.12 ng/mL (normal range 0-0.09 ng/mL). All metabolic screening tests including tandem mass spectrometry and urine organic acids profile were studied and no abnormal results were obtained. Blood samples were taken both for bacterial culture and serum viral markers such as the TORCH

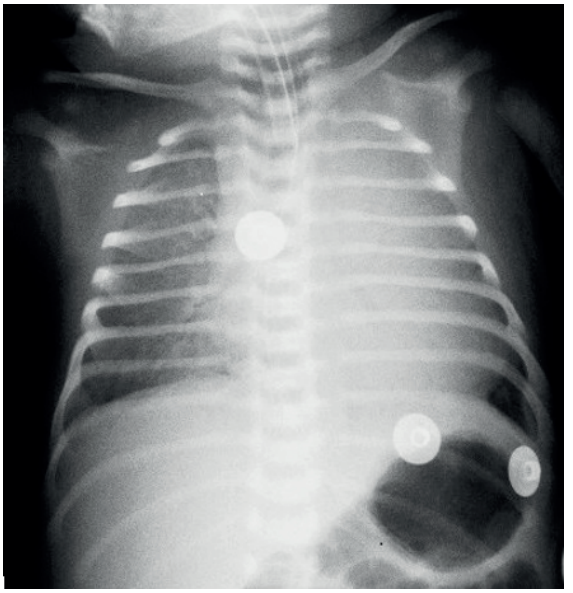

Fig. 1. Cardiomegaly and pulmonary interstitial infiltration on X-ray of the newborn.

group viruses, *Coxsackievirus A and B*, *Parvovirus B19*, HIV, hepatitis viruses (*Hepatitis B* and *C*) were all negative. Thyroid hormones were in the normal range. Extended respiratory panel by PCR (Biofire Diagnostics, Salt Lake City, UT) was analyzed from the nasopharyngeal swab and Rhinovirus was positive. Given the family history of flu for the last five days, this was remarkable and significant for Rhinovirus as the etiology of myocarditis and the patient was diagnosed with acute onset dilated cardiomyopathy due to myocarditis secondary to Rhinoviral respiratory infection. He was reevaluated by a pediatric cardiologist on hospital day (HD) 2. Echocardiography revealed EF of %49 and minimal pericardial effusion so that furosemide was also added for the treatment of congestion. The patient weaned from the ventilator after respiratory stabilization and was extubated on HD 4. Cardiac markers gradually decreased during the follow-up (Fig. 2). All treatments for heart failure were stopped after 3 weeks and EF improved to %53 before discharge from the hospital on HD 27. The patient recovered well without any sequelae and is still being carefully followed in our outpatient clinic, with no signs of heart failure, age-appropriate growth, and neurologic development.

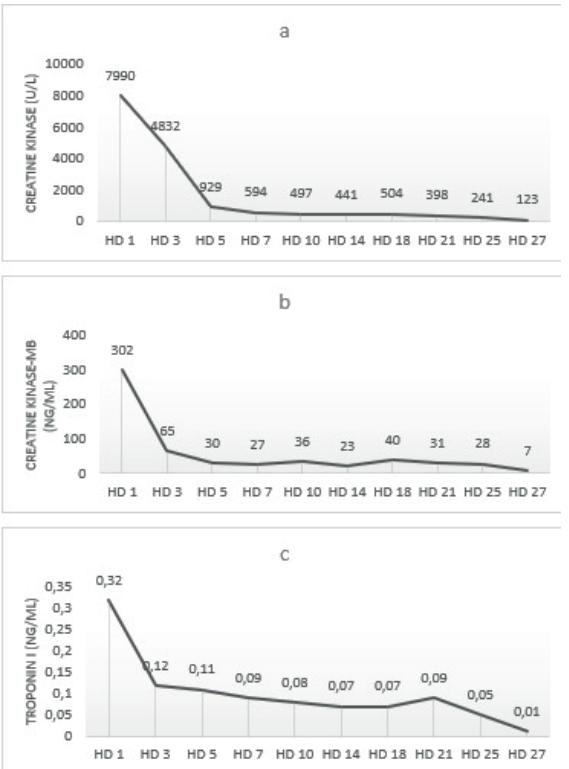

Fig. 2. Changes in cardiac enzymes of the newborn during hospitalization.

Written informed consent was obtained from the parents of the patient.

Discussion

Myocarditis is a common cause of dilated cardiomyopathy among adult patients whereas it is a rarely seen cardiac inflammatory disorder having ominous consequences in the childhood period. Infants, especially newborns are more vulnerable to myocardial damage because of immature structure limiting a quick adaptive response to hemodynamic deterioration There are numerous factors (drugs, metabolic disorders, microbial agents, etc.) underlying the development of myocarditis. With more recent molecular biological techniques, more and more viruses have been identified as the cause of myocarditis. Rhinovirus is one of those mentioned as a causal agent of few cases in the childhood period in the literature. . The reason why myocarditis develops after rhinoviral infections has not been clarified yet but it's thought that both immune mediated and direct cytotoxic mechanisms of myocardium play role in the pathogenesis as it happens in all other virus borne heart diseases.[10,11] A case of a 13 year old girl was presented with heart failure due to myocarditis associated with rhinoviral infection and recovered without any complication.[12] Wiyatno et al reported a 4-year old boy with DCM secondary to respiratory infection and isolated *Rhinovirus C* obtained from nasopharyngeal specimens which was known to be related to more severe illness than other species; *Rhinovirus A and B*.[13] Our main limitation is that we were unable to identify the subtype of rhinovirus with a further detailed molecular PCR method that is mainly more valuable when performed on myocardial tissue.

The clinical presentation of myocarditis varies from mild symptoms to acute heart failure even sudden cardiac death. Unfortunately, myocarditis has a poor prognosis in the neonatal period. Mortality increases up to 75% especially in cases with *Coxsackievirus B* whereas it is about % 25 in the childhood population and gradually decreases. However, there is no precise data about mortality for rhinovirus as the cause of myocarditis in the literature. Our patient was presented with acute hemodynamic deterioration and had to be intubated soon after admission but was able to recover well without any complications.

Both laboratory and imaging techniques are essential diagnostic tools for myocarditis. The main cardiac biomarkers, indicating myocardial damage are creatinine kinase, CK-MB, troponin I, and troponin T. The level of those markers increases due to the inflammatory response involving the myocardium. Especially CK-MB and Troponin I have more diagnostic accuracy to rule out other cardiac diseases. Levels of all cardiac markers were also very high at the time of diagnosis in our cases and declined with time as a response to treatment. The echocardiogram is essential in the diagnosis and follow-up of myocarditis.

The main echocardiographic findings of our case were moderate left systolic dysfunction and only one myocardial trabeculation was prominent in the left ventricular cavity. Bilayered myocardium with prominent trabeculations seen in the left ventricular non-compaction cardiomyopathy (LVNC) is a result of an arrest in compaction during embryonic development.[14] As echocardiography is the main tool to diagnose LVNC many authors focus on the size of trabeculations, the thickness, and the ratio of the compacted wall to non-compacted myocardial area. One trabeculation seen in the left ventricular cavity of our case was accepted as the consequence of DCM which disappeared after the rapid response to heart failure treatment. All medications were stopped at the 3rd week after echocardiographic findings like left systolic function and ventricular dilatation improved and clinical symptoms amended, he was discharged after 27 days of hospitalization without any complication and is still closely followed.

Endomyocardial biopsy is the gold standard for the diagnosis of myocarditis but it should be carried out by an experienced physician due to a high risk of perforation and death. The utility of endomyocardial biopsy is also limited as sampling patchy areas may lead to skipping the inflammatory involvement. We did not confirm the diagnosis of myocarditis with biopsy as our case was a newborn and the high risk of complications weighed against the benefit. To the best of our knowledge, this is the first case report showing that Rhinovirus detected as a viral agent causes myocarditis secondary to respiratory infection in a newborn. A comprehensive approach is needed for the diagnosis of myocarditis in the case of unknown etiology and an extensive respiratory panel may be taken into consideration if there is history or clinical symptoms of respiratory infection. Rapid identification of viral agents may provide prompt diagnosis, appropriate medical treatment, and prevent therapeutic challenges. Physicians may consider using an extended respiratory panel to evaluate the infectious etiology in whom other differential diagnostic diseases were excluded, especially in the neonatal period.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BC, DO; data collection: EA, CY; analysis and interpretation of results: BC, ME, draft manuscript preparation: BC, DO. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Iron deficiency is not always innocent in childhood: a rare diagnosis of collagenous gastritis

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ABSTRACT

Background. Collagenous gastritis (CG) is a very rare disease with still lots of unknowns, characterized by the subepithelial collagenous band in the gastric mucosa associated with a mixed inflammatory infiltrate in the lamina propria.

Case. Iron deficiency anemia is the most common and usually single laboratory finding without any complaint at the time of diagnosis. This entity should be well-known so that we can examine and refer the patient to a pediatric gastroenterologist for differential diagnosis.

Conclusions. The histopathological evaluation, albeit invasive, is essential to exclude this diagnosis. We present a 13-year-old girl with intractable iron deficiency anemia due to CG.

Key words: collagenous gastritis, iron deficiency, child, pathology, endoscopy.

Collagenous gastritis (CG) is a rare disease especially in childhood that is characterized by the subepithelial deposition of collagen bands on histopathology in the gastric mucosa and still has unknown etiology.[1] Iron deficiency anemia, abdominal pain and vomiting are common clinical presentations. The literature indicates that childhood-onset CG has a chronic disease course.[2] This disease has been recognized in recent years and perhaps it is overlooked because it is not considered in more patients. Clinical diagnosis is yet impossible but endoscopic and histopathological evaluation is diagnostic and required.

Case Report

A 13-year-old girl was referred to our pediatric gastroenterology outpatient clinic for the evaluation of a two-year history of intractable iron deficiency anemia. She had a diet rich in meat and meat products and a normal menstrual pattern. At the first visit at the age of 11, the patient did not have any gastrointestinal complaints such as vomiting, dysphagia, abdominal pain, diarrhea, melena.

On physical examination, the patient was obese with body weight in the 97th percentile, height in the 25-50th percentile, body mass index was 27 in the 97th percentile and there was minimal tenderness in her upper abdomen. Laboratory findings showed iron deficiency anemia with hemoglobin (Hb) concentration of 8,6g/dL, mean corpuscular volume (MCV) of 61 fl, serum iron level of 15 mg/dL, serum ferritin of 1 ng/mL and total iron binding capacity (TIBC) of 415 mg/dL with normal infection parameters. Fecal occult blood test was negative. Anemia was treated with oral iron replacement therapy (4 mg/kg/d) for 6 months, but there was no response. Thus, parenteral iron replacement therapy had been administered and Hb level increased up to 12.5 g/dL.

At the age of 13, her Hb level decreased to 9.7 g/dL and the patient was admitted to the pediatric

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gastroenterology department. Parasitic infections were excluded by performing repeated anal band tests and stool examinations. *Helicobacter pylori* infection was excluded by testing fecal *Helicobacter pylori* antigen and her celiac serology was negative.

In the upper gastrointestinal endoscopy for the evaluation of intractable iron deficiency anemia, there was no gastrointestinal bleeding, but endoscopic gastric images were grossly abnormal. The duodenum and esophagus were normal but the gastric corpus-fundus and antral mucosa was nodular. Gastric mucosal edema and erythema, as well as hyperplastic rugae were amongst the other macroscopical findings (Fig. 1). Biopsy specimens were routinely obtained from the duodenum, bulbus, esophagus, fundus, corpus and antrum. In the histopathological evaluation of all of the gastric mucosal specimens epithelial mucosa was normal but the subepithelial layer especially the lamina propria was characterized by the infiltration of chronic inflammatory cells and the deposition of collagen in focal areas. Multiple biopsies from the nodules showed patches of thick collagen bands, hyaline collagen deposition in the subepithelial layer strongly stained with Masson-trichrome and an increased number of plasma cells, eosinophils, and lymphocytes in the lamina propria. All of the gastric specimens revealed the same histopathologic changes (Fig. 2). *Helicobacter pylori* or granulomas were not detected in any of the samples. The patient received the diagnosis of CG by the pathological evaluation.

A high-dose proton pump inhibitor (PPI) with a dose of 2 mg/kg/day and oral iron supplementation was started. After the 6 weeks of treatment, her follow-up physical examination was completely normal but a control endoscopic evaluation was repeated because the Hb level rise was insufficient despite oral iron supplementation and PPI treatment. There was no tissue healing and similar histopathological findings persisted. Despite these findings the patient had no complaints and the physical examination was normal. Colonoscopy was not performed because the mucosal deposition of collagen has been shown usually to be limited to the stomach in childhood. In our case, there were no signs of colitis such as watery diarrhea and abdominal pain. We planned to perform a colonoscopy according to her clinical course to rule out collagenous colitis in case of lower

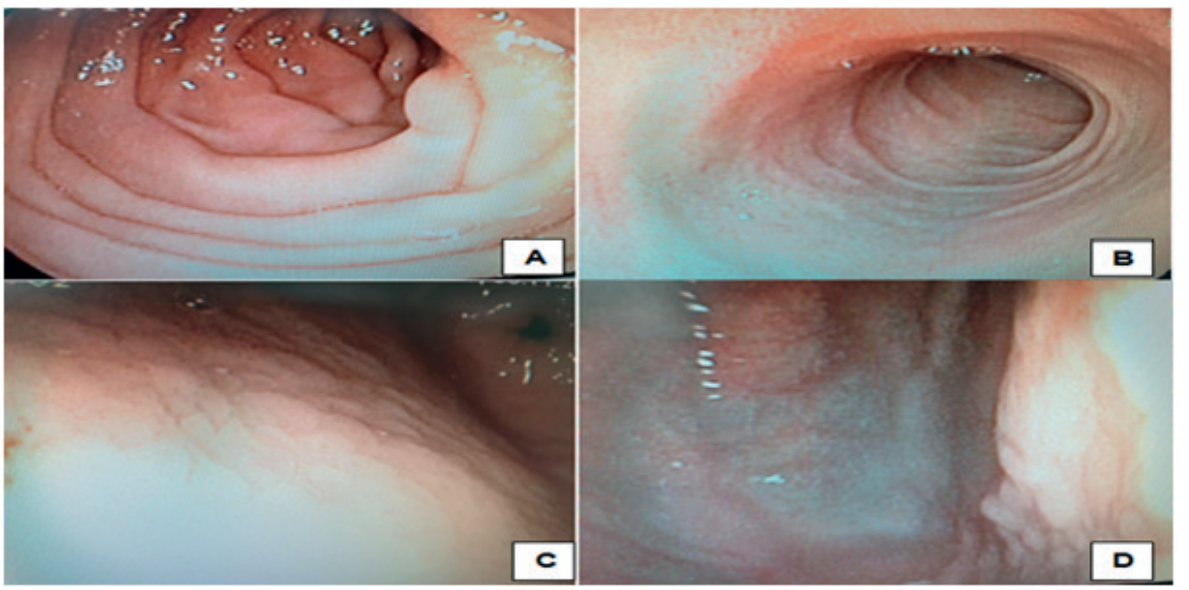

Fig. 1. Endoscopic findings of collagenous gastritis. A,B: duodenum and bulbus; completely normal evaluation. C,D: antrum and corpus; nodular lesions in the greater curvature of the gastric body.

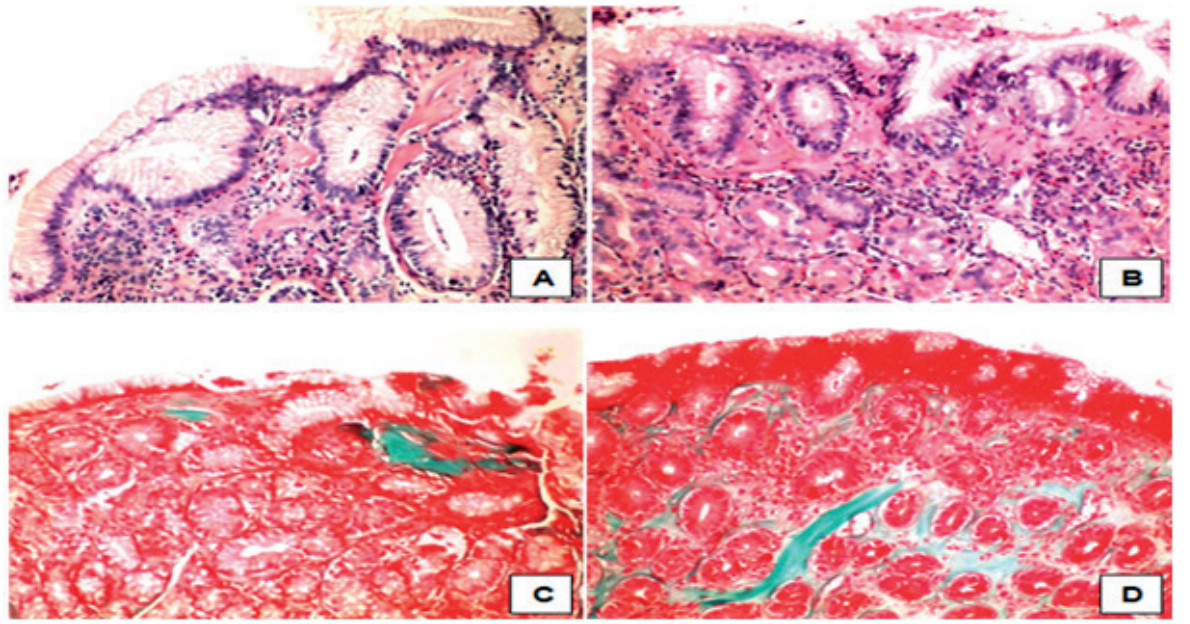

Fig. 2. Histological findings of collagenous gastritis. A,B: HE stain x 200, antrum and corpus ; thick collagenous subepithelial band. Inflammatory infiltrate in the lamina propria. C,D: Trichrome stain, antrum and corpus, thick subepith.

gastrointestinal symptoms. The follow-up was planned with intermittent iron treatment in our outpatient clinic.

The potential concomitant autoimmune diseases were excluded by the negative test results of Antinuclear antibody (ANA), Tissue transglutaminase immunoglobulin (Ig) A, Anti-thyroid peroxidase antibodies. Serum levels of amyloid A and Ig G, A, M were in normal range for her age.

Permission and informed consent was obtained from the patient and the family.

Discussion

Collagenous gastritis is a rare histopathological entity seen especially in children. Clinical diagnosis is impossible but histopathological evaluation is diagnostic and required.[1] In cases in which dietary iron intake is adequate, occult blood loss cannot be demonstrated, infections (parasites, *Helicobacter pylori*…), malabsorption syndromes, allergic diseases are excluded, this disease should be included in the differential diagnosis and endoscopy should be performed when necessary. The first pediatric case was published in 1989 by Colletti and Trainer and female predominance was demonstrated in recent years.[1-3]

GG is characterized by the presence of a subepithelial collagen band (conventionally defined as being >10mm in thickness) in association with an inflammatory cell infiltrate in the lamina propria.[4,5] It has been hypothesized that the deposition of collagen and protein exudate occurs due to an increase in vascular permeability but the pathophysiology is still unclear.[2] Iron deficiency is associated with chronic bleeding from dilated capillaries in the collagen layer.[6] Instead of occult bleeding from the gastric mucosa, decreased iron absorption due to gastric hypochlorhydria can be another mechanism.

In the literature, two phenotypes of the disease have been described: a pediatric-onset and an adult-onset type. Recurrent abdominal pain and iron deficiency anemia, were the most common clinical presentations in pediatric-onset CG but upper gastrointestinal bleeding/hematemesis,

recurrent vomiting and retrosternal pain were also reported.[7-9] In pediatric cases the collagenous mucosal inflammation is generally restricted to the stomach. All pediatric patients who presented with chronic diarrhea and malabsorption were found to have the adult-onset phenotype characterized by concomitant collagenous gastritis and colitis.[4,10-12] Our case presented with only anemia like many other reports in the literature with a prevalence of anemia up to 92% at presentation.[7,8]

The endoscopic image in all cases with CG is grossly abnormal like in ours. Features included nodularity of the gastric mucosa, mucosal edema and erythema, as well as hyperplastic rugae. By contrast, the patients with collagenous colitis had macroscopically normal colonoscopic images. Histopathologic findings of stomach biopsies show characteristic subepithelial hyaline deposits with an inflammatory infiltrate in the lamina propria of the affected areas. In previous histopathologic studies have reported the eosinophil-rich inflammatory infiltrate (>30 eosinophils/ high-power field) rate of 50%–62% in children with CG. The lymphocytic gastritis (>25 surface intraepithelial lymphocytes/ 100 epithelial cells) like pattern was found in <10% of the cases.[8] Follow-up gastroscopies were performed in the largest pediatric cohort (15 patient) study and none of them had evidence of endoscopic or histologic improvement of the gastric mucosal pathology in all treatment regimens like our case.[3] However, in the same study there was no worsening of the mucosal inflammation over time, and no patients developed intestinal metaplasia or advanced mucosal atrophy in the gastric corpus. Moreover, these patients had been evaluated with at least one colonoscopy with multiple mucosal biopsies during the follow-up period. Only one patient with associated collagenous colitis, no additional pathologies were noted in the colonic or ileal biopsies of the remaining patients.

Autoimmune diseases such as celiac disease, type 1 diabetes and psoriasis have been reported in children with CG.[7,8,13] In a study evaluating the frequency of heredity for autoimmune diseases in CG, the rate of positive autoantibodies was 40% but none of these patients had an autoimmune disease during the 11 years follow-up period.[3] In our patient, the autoantibodies were also negative. CG and associated common variable immune deficiency and selective Ig A deficiency have previously been reported.[8,14,15] In a case report of pediatric CG increased serum levels of IgG4 and in another Ig G4-related disease was reported.[3,16]

CG is a chronic disease, but there is still no guideline or effective treatment.[13] There are different treatment strategies with limited efficacy including anti-secretory agents, PPI and H2-receptor antagonists, sucralfate, bismuth subsalycilate, corticosteroids, iron supplementation, hypoallergenic diets, and 5-aminosalicylic acid. Only one study of 15 cases reported that there was no significant difference of clinical, endoscopic and histopathological outcome between PPI and dietary modifications such as cow's milk-free diet, gluten-free diet, and diet free of cow's milk, soy, egg, and wheat.[3] Most of the patients had improvement of anemia with just oral iron supplements and PPI treatment. Iron deficiency recurrence has also been reported after treatment in different studies.[7,12] Improvement of the histological features of both CG and colitis was observed in only one male pediatric patient aged 11 years with adult-type disease who had been treated with oral prednisolone subsequently weaned onto long-term azathioprine treatment.[7] However this patient had additional histological changes in the duodenum on follow-up biopsy.

Childhood-onset CG is rare but should be included in the differential diagnosis in all children with intractable iron deficiency anemia. Endoscopy needs to be considered, even in an otherwise asymptomatic patient. Awareness must be heightened regarding the potentially increased long-term risk for autoimmune/ immune-related diseases. A specific therapy for collagenous colitis has not yet been established but oral iron supplementation and PPI is effective in treating the iron deficiency anemia

in most patients. Careful long-term follow-up, including repeat endoscopies to monitor mucosal involvement is important. Further randomized clinical trials are needed.

Author contribution

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

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Bladder granulocytic sarcoma in a child: case report and literature review

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ABSTRACT

Background. Granulocytic sarcoma (GS) is an extramedullary solid tumor composed of immature myeloid cells. GS has been associated with acute myeloid leukemia (AML), myelodysplastic syndromes or myeloproliferative diseases. Although GS can affect various tissues of the human body, it has rarely been reported in other soft tissues such as the breast, gastrointestinal, respiratory and genitourinary tracts. We report a pediatric case diagnosed with granulocytic sarcoma of the bladder and concomitant AML.

Case. A twelve-year-old previously healthy girl was admitted to the pediatric urology clinic with a ten-day history of hematuria and pollakiuria. Laboratory examinations revealed anemia, thrombocytopenia and neutrophilic leukocytosis. Bone marrow aspiration results were consistent with acute myeloid leukemia -FAB subtype M2-. Abdominal magnetic resonance imaging (MRI) showed an irregularly bounded 12 cm mass on the right side of the bladder. Transurethral resection (TUR) pathology was consistent with granulocytic sarcoma. After a multimodal treatment approach, complete remission was achieved.

Conclusions. Malignant bladder masses are rare causes of macroscopic hematuria in childhood. The diagnostic spectrum is wide, ranging from rhabdomyosarcoma to leukemia involvement. The bladder is a rare site of extramedullary involvement in pediatric patients with AML. Multimodal treatment should be considered on a per-patient basis.

Key words: acute myeloid leukemia, children, granulocytic sarcoma, bladder, treatment.

Granulocytic sarcoma (GS) is an extramedullary solid tumor composed of immature myeloid cells. Initially, it was named chloroma as a word originating from greenish color in the 1850s. GS has been associated with acute myeloid leukemia (AML), myelodysplastic syndromes or myeloproliferative diseases. More frequently than in adults, it is seen approximately 10 % concomitantly at the time of diagnosis of pediatric AML.[1-3] GS can be seen as isolated extramedullary without bone marrow involvement at the time of primary diagnosis or relapse, can proceed to bone marrow involvement or occur later in the course of primary AML disease. Although GS can affect various tissues, the most frequent extramedullary involvement sites in children are; skin, central nervous system, gingiva, bone and, orbit.[1] It has rarely been reported in other soft tissues such as the breast, gastrointestinal, respiratory and genitourinary tracts.[4-8] We report a pediatric case presented with hematuria,diagnosed with granulocytic sarcoma of the bladder and concomitant AML. To our knowledge, this is the 4th pediatric case of granulocytic sarcoma of the bladder in the

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literature. We presented this case due to its rare involvement site, and to discuss treatment modalities.

Case Report

A twelve-year-old previously healthy girl was admitted to the pediatric urology clinic with a ten-day history of hematuria and pollakiuria. Family history was unremarkable. Physical examination revealed no abnormality other than paleness. Vital signs were within normal limits. Laboratory examinations revealed anemia (hemoglobin 7.1 gr/dL), thrombocytopenia (platelet count 45.700/μl) and neutrophilic leukocytosis (white blood cell count was 20300/μl, the neutrophil count was 11000 /μl, lymphocyte count was 6900/μl, respectively). The erythrocyte sedimentation rate was elevated (77mm/hr). Blood chemistry and coagulation parameters were normal with age-appropriate values. Urine analysis revealed three positive erythrocytes, three positive leukocytes and two positive protein with leukocyte esterase negativity. Pediatric hematology-oncology consultation was requested due to bicytopenia before cystoscopy. Peripheral blood smear revealed no abnormal cells. Bone marrow aspiration showed hypercellular marrow with leukemic infiltration; myeloid blasts with large cytoplasm and occasional granulation were 60% of bone marrow cells. Immunohistochemical studies demonstrated positive staining for MPO, lysozyme, CD68, and CD33. Flow cytometry revealed high positivity for CD 13 and 33. Cytogenetic analysis revealed translocation t (8; 21). Consequently, acute myeloid leukemia -FAB subtype M2- diagnosis was established. Abdominal magnetic resonance imaging (MRI) showed a mass originating from the right side of the bladder wall, extending into the lumen, measuring 12 cm in diameter and presenting irregular, lobulated margins (Fig. 1a). Transurethral biopsy pathology showed malignant round cell tumor, immunohistochemical studies were consistent with granulocytic sarcoma (Fig. 2). The patient was started on MRC AML 2012 protocol chemotherapy.[9] After 2 cycles of induction (ADE), bone marrow remission was achieved but pelvic MRI showed residual bladder mass despite marked regression (Fig. 1b). After 4 cycles of chemotherapy (2*ADE+ MACE+MidAC) bladder mass persisted. Transurethral resection was performed to clarify the content of the residual mass. Especially due to the special localization of the tumor, pathological evaluation of the mass was preferred before radiotherapy decision. Pathology results confirmed the diagnosis of granulocytic sarcoma. Radiotherapy was planned to the primary tumor location for local control. Bilateral oophoropexy was performed for fertility preservation. Radiotherapy was applied to the bladder region at the dose of 18 Gy in 10 fractions followed by one

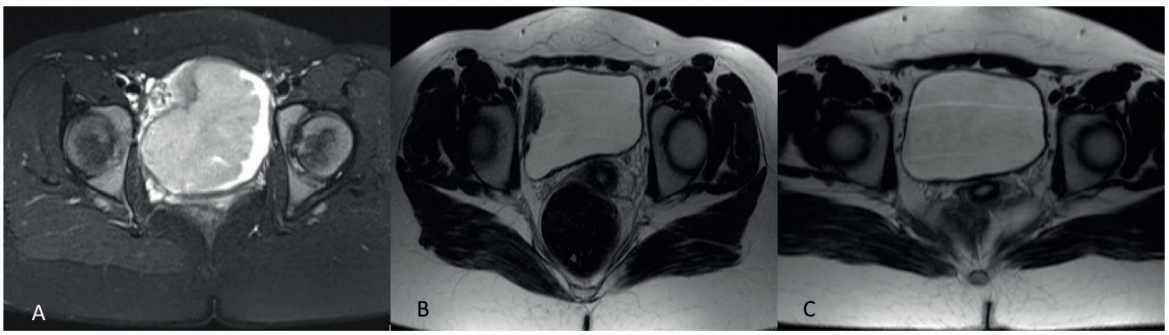

Fig. 1. (A) Initial MR image of bladder mass; T2 weighted fat saturated axial MR image shows hyperintense solid mass with lobulated contour, extending into the bladder lumen. **(B)** T2 weighted axial image reveals marked regression of the lesion after 2 cycles of induction chemotherapy and **(C)** total resolution after the end of the treatment.

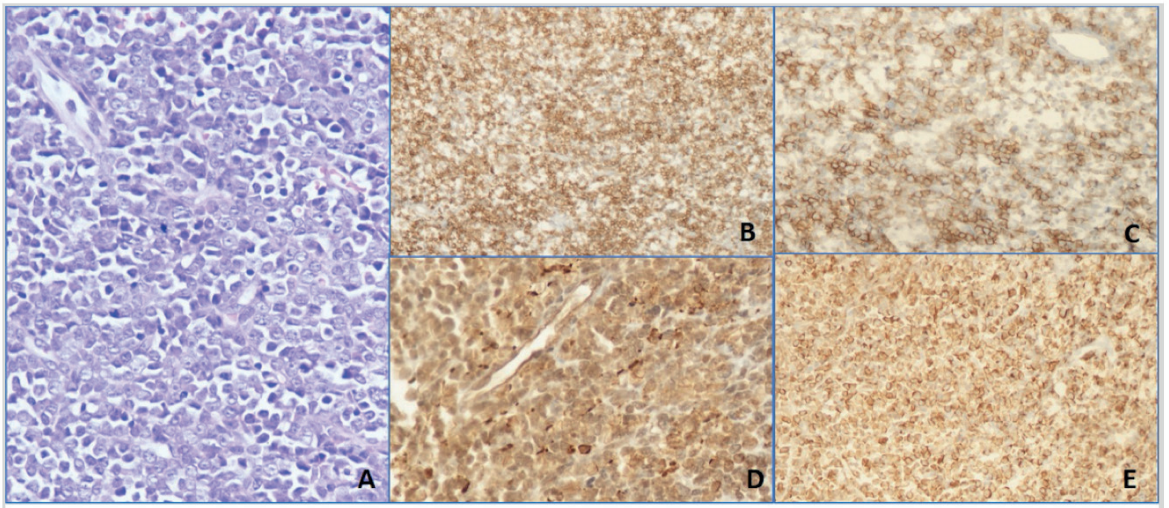

Fig. 2. Transurethral resection pathology: Bladder granulocytic sarcoma (A) Diffuse blastic cell infiltration is seen (H&E, 40X). Tumor cells were positive with CD117 (B), CD56 (C), CD68 (D) and myeloperoxidase (E).

course of chemotherapy (CLASP). At the end of the chemoradiotherapy, MRI showed total resolution of the bladder mass (Fig. 1c). Considering the incomplete response to treatment with persistence of extramedullary involvement at the end of induction and consolidation chemotherapy, she was stratified as high risk despite favorable genetic features of AML. Therefore hematopoietic stem cell transplantation(HSCT) was approved by the HSCT board. She went to allogeneic HSCT from HLA matched unrelated donor. She remains in complete remission for more than 5 years, outpatient follow up is ongoing. Informed consent for publication of this case was obtained from the children and the parents.

Discussion

Granulocytic sarcoma is an extramedullary solid tumor composed of myeloid lineage leukemic cells, which can be seen in the course of myeloid leukemia or myeloproliferative disorders. The molecular pathogenesis of the invasion and accumulation of immature cells in various tissues is not fully understood.[10] It has been shown that invasive acute myelogenous leukemia cell lines have higher expression of matrix metalloproteinase 2-9, membrane type 1 metalloproteinase and tissue inhibitor of metalloproteinase 1-2 compared to normal bone marrow cells and less invasive leukemic cell lines.[11-13] Higher expression of metalloproteinases was considered to be associated with the invasion and metastasis capacity of solid tumors.[14,15] It also increases the invasion capacity of leukemia cells and is associated with extramedullary infiltration.[11,16]

In the absence of bone marrow involvement, diagnosing GS can be challenging and requires clinical suspicion. Appropriate immunohistochemical studies are essential for differential diagnosis with non-Hodgkin lymphoma, small round cell tumors and undifferentiated carcinoma, especially for isolated GS.[17,18] GS with skin involvement is mostly seen in M4 (myelomonocytic) and M5 (monocytic) subtypes of AML, while M2 (mature myeloblastic) is more common in patients with GS in other sites.[1,2] The most common cytogenetic abnormalities are t(8;21)(q22;q22) and inv(16)(p13;q22), the latter is associated with extramedullary disease in abdominal sites.[18] The evidence on the effect of extramedullary involvement on favorable genetic features and prognosis is inadequate. In a study of 84 adult AML patients with t(8;21) published in 1997, extramedullary involvement was shown to significantly reduce

survival.[19] A population-based cohort study on 315 children with AML showed that the presence of extramedullary involvement, which corresponds to 23% of total cases, significantly reduced 5-year overall survival.[20]

Despite extramedullary infiltration being relatively common in childhood AML compared to adults, there are a few case reports of bladder involvement in children.[8,21-23] Other rare sites of GS in children are gallbladder, nasal and oral cavity, perineum, small intestine, colon, and testicles.[7,24-28] It shows different signs and symptoms according to localization. Bladder involvement presents with hematuria, pollakiuria, dysuria, fatigue, pallor, urinary incontinence, urinary retention, suprapubic and flank pain. Obstruction and hydronephrosis can lead to acute renal dysfunction and decreased creatinine clearance.

There are a couple of pediatric cases of granulocytic sarcoma of the bladder in the literature (Table I). The first one was a 16-year-old boy who was previously diagnosed with AML, came with isolated bladder GS after bone marrow transplantation. He was treated with radiotherapy but progressed to AML and died.[21] The second case was a 4-year-old previously healthy boy who was diagnosed with AML with bladder GS. The primary mass biopsy was not performed but there were myeloid blasts in the urine with flow cytometric examination.[22] In this case, the presence of myeloid blasts in the urine suggests that leukemic cells may spread within the urinary system, likewise, they do in the cerebrospinal fluid. In this case, remission was achieved with chemotherapy alone. The last case was a 18 month-old previously healthy girl who was diagnosed with bladder GS concomitant with AML. She died with febrile neutropenia after the first cycle of induction chemotherapy.[23] Our patient who had newly diagnosed bladder GS concomitant with AML had achieved complete remission with 5 courses of chemotherapy, local radiotherapy, and allogeneic hematopoietic stem cell transplantation without serious toxicity.

GS should be treated as AML whether there is bone marrow involvement, or not. Even in isolated GS, progression to AML occurs without systemic therapy.[10] Multimodal treatment must include combinations of chemotherapy and radiotherapy, surgery or hematopoietic stem cell transplantation on a patient-specific basis. A study from Children's Cancer Group (CCG) including 1832 newly diagnosed pediatric AML patients shows a higher survival rate in patients diagnosed with AML plus GS in the sites other than skin compared with AML plus skin involvement or isolated AML. In the multivariate analysis, other favorable prognostic factors were low initial leukocyte count, female gender and FAB M2 subtype. According to this study, local radiotherapy to tumor sites did not improve the outcome.[2] However, heterogeneity of the groups with and without radiotherapy constitutes an uncertainty for this result.

In a cohort of 38 adult and child patients with GS, the longest median survival rate with 109 months was observed in patients with genitourinary system involvement compared to other sites.[29] Local treatment with radiotherapy or surgery should be considered for patients with GS and concurrent AML in case of residual tumor despite bone marrow remission after induction chemotherapy likewise for patients with isolated GS as consolidation.[10,30,31] Local treatment modalities can also be used as a part of the initial treatment, in case of a need for palliation and symptom relief or relapse, considering tumor size and localization.[27,31] While lower doses less than 20 Gy are efficient, doses up to 30 Gy can be used for radiotherapy in children for extramedullary involvement of AML.[29] Gonad protective measures for fertility preservation should be considered before radiotherapy to the genitourinary region. Several studies show favorable results with allogeneic HSCT in adult patients with GS, pediatric data did not show a clear benefit for survival despite decreased relapse rates.[9,32,33] For the decision of HSCT in children with AML, reclassification of high-risk patients based on both genetic features and chemotherapy

Table I. Characteristics of reported pediatric cases with granulocytic sarcoma of bladder.

Case	Age	Gender	Past medical history	Symptoms	İmaging	Mass biopsy	Bone marrow examination	Diagnose	Treatment	Bone marrow involvement	Outcome	Reference
1	12 yr	Female	Healthy	Gross hematuria, pollakiuria	Abdomen MRI: Irregularly bounded 12 cm mass extending from right side of bladder wall to lumen	Round cell malignant tumor IH: Positive for LCA, CD 56, MPO, CD 68, CD 117 and negative for desmin, TdT.	60 % myeloblasts AML (FAB M2)	AML Primary diagnose	5 course of chemotherapy (AML MRC12) Local radiotherapy Allogenic BMT	Concomitant with granulocytic sarcoma	CR	Present Case
2	18 mo	Female	Healthy	Bilateral orbital swelling, intermittent gross hematuria, fever, gum swelling	Abdomen CT: Polypoid mass lesions in bladder wall measuring 4 * 2.8 cm	Small-medium sized, round to oval tumor cells with high N: C ratio and collagenous stroma. IH: Positive for vimentin, CD34, CD99, CD44, CD117 and MPO and negative for desmin, tDt, CD3, CD4, CD8, CD10 and CD19.	60 % myeloblasts AML	AML Primary diagnose	Chemotherapy	Concomitant with granulocytic sarcoma	Died	Kumar et al.[23]
3	4 yr	Male	Healthy	A febrile syndrome, abdominal pain, pallor	Ultrasound: Solid, vascularized, heterogeneous, polypoid formation measuring approx. 8*6* 3.2 cm involving posterior bladder wall	Primary mass biopsy: Not performed Urine flow cytometri: Myeloid blasts	AML	AML Primary diagnose	Chemotherapy	Concomitant with granulocytic sarcoma	CR	Kaplan et al.[22]
4	16 yr	Male	AML- FAB M2 BMT	Gross hematuria	Ultrasound: Moderate hydroureteronephrosis on left side Cystoscopy: 30*20mm rounded mass at left ureteral orifice	Large, pirimitive mononuclear cells demonstrating variable cytoplasmic granule formation and nuclear maturation, positive granulocytic differentiation with napthol ASD-chloracetate esterase and lysozyme	Hypocelluler marrow with no leukemic infiltration (blasts 3.5%)	AML relapse	Local radiotherapy	2 months after treatment of granulocytic sarcoma	Died after bone marrow relapse	Cartwright et al.[21]

AML: acute myeloid leukemia, BMT: bone marrow transplantation, CR: complete remission, IH: immunohistochemistry, MRI: magnetic resonans imaging, MPO: myeloperoxidase, CT: computed tomography, yr: year, mo:month.

response improves the outcome according to multicenter studies.[34,35] However the effect of extramedullary tumor response to therapy on the risk groups is not clear. Clinical trials for molecular targeted therapies in selected patients are ongoing.[36]

Malignant bladder masses are rare causes of macroscopic hematuria in childhood. Diagnostic spectrum is wide, ranging from rhabdomyosarcoma to leukemia involvement. The bladder is a rare site of extramedullary involvement in pediatric patients with AML. There is no current clinical consensus guideline for the treatment of this rare disease. Multimodal treatment including chemotherapy, radiotherapy, surgery, hematopoietic stem cell transplantation, and targeted therapies should be considered on a patient-specific basis.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: RT, SK; data collection: RT, ZB, ÖD; analysis and interpretation of results: RT, SK, TO, SA, ÖD, AÜ, DT, ZB, SÇK, AİÇ, ZK; draft manuscript preparation: RT, SK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A case of aplasia cutis congenita following fetal reduction of triplet pregnancy conceived through *in vitro fertilization*

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ABSTRACT

Background. Aplasia cutis congenita (ACC) is a rare congenital localized skin defect that is mostly diagnosed in the newborn or infant period. ACC type 5 often involves the trunk or extremities accompanied by fetus papyraceous (FP) or placental infarcts. The etiology and pathogenesis of this rare type of ACC are not well known. In this case, we report an ACC type 5 with a definite etiology.

Case. We report a preterm infant with ACC type 5, with diffuse bilateral leg lesions found at birth. He was the first baby of dichorionic twin after reduction from a dichorionic triplet pregnancy conceived through *in vitro fertilization*. A fetus papyraceous was found in juxtaposition with the affected baby's placenta. After 37 days of hospitalization, his leg lesions were successfully epithelized with supportive care. He is regularly visiting the Dermatology clinic for scar care and shows normal development without motor limitation.

Conclusions. Herein, we present a preterm infant with ACC type 5 and the placental pathology with fetus papyraceous of the artificially reduced monochorionic co-twin of the affected infant. We suggest a precautious decision in multifetal pregnancy reduction (MFPR) in dichorionic triplets, presenting ACC type 5 as an adverse outcome of MFPR.

Key words: aplasia cutis congenita, fetus papyraceous, multifetal pregnancy reduction, in vitro fertilization.

Aplasia cutis congenita (ACC), defined as the absence of all skin layers at birth, results from disrupted development or degeneration of skin in utero. In 1867, Frieden classified this congenital skin defect into nine groups based on the distribution of the affected area, associated anomalies, and the mode of inheritance.[1] To date, no specific genetic focus is known related to ACC, however, ACC can be related to some genetic or malformation syndromes, such as Adams-Oliver syndrome, Bart syndrome, trisomy 13, trisomy 18, monosomy 4, ectodermal dysplasia, and Johanson-Blizzard syndrome.[1,2] Differential diagnosis of ACC includes focal dermal hypoplasia, dermoid cyst, encephalocele, epidermolysis bullosa, infections (herpes or varicella zoster virus), amniotic band disruption syndrome or birth trauma[3], thus, a thorough examination of the patient and history of familial skin defect, infection, or any birth trauma are important for ACC diagnosis.

Feus papyraceous (FP) formed by mummification of a dead fetus usually occurs during intrauterine death of a co-twin during the late first to early second trimester, whereas earlier or later death of a co-twin typically leads to complete resorption or maceration, respectively.[4,5]

We report a preterm neonate with bilateral symmetric ACC mainly on the lower extremities with FP who was born from a dichorionic triplet pregnancy conceived through *in vitro fertilization* (IVF) that had been artificially reduced to a dichorionic twin pregnancy. We focused on symmetric lower extremity ACC as a consequence of the development of advanced reproductive medicine.

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Case Report

A baby boy weighing 1,990 g was born at 35^{+2} weeks gestation with an unexpected large skin defect in both the legs and trunk. This had been an IVF pregnancy in which three embryos were transferred, two of which survived and one of which split, resulting in two monochorionic fetuses and a third dichorionic fetus. Chorionic villi sampling at 12 weeks gestation demonstrated that they were normal karyotypes. Radiofrequency ablation of the umbilical cord of one fetus was performed at 14 weeks, resulting in a dichorionic twin pregnancy. Afterward, the pregnancy was uneventful until preterm labor pain occurred. The mother was hospitalized due to preterm labor, and cesarean section was performed due to fetal deceleration. The baby boy's Apgar scores were 8 and 9 at 1 and 5 minutes, respectively, and he was cared for in the neonatal intensive care unit of the hospital where he was born.

The parents were examined by special tests including karyotyping before the pregnancy due to recurrent abortion (G2P0), but the result was normal. The mother's age was 39 years, and she did not take any special medication during this pregnancy. There was no family history of ACC, vascular abnormalities, vesiculobullous disease, or thrombophilia.

Physical examination on the first day of life revealed well-demarcated ulcers in a symmetric distribution on both legs. Thin, silky, and transparent membranes were present, and small vessels were visible in both lesions (Fig. 1). Other abnormalities or any dysmorphic features were absent. Without specific diagnostic tests, ACC was diagnosed based on the clinical manifestation. The other live twin was a completely unaffected baby girl with a birth weight of 1,920 g. Formal pathological examination of the placenta after delivery revealed dichorionic and diamniotic twin placentas with microcalcification and a mummified fetus in one chorion (Fig. 2).

No skin biopsy was performed. Based on the clinical manifestation, a diagnosis of ACC type 5 was made. The patient's wounds were treated with a non-adhesive dressing with a pain controller (oral acetaminophen). After the lesions were washed with normal saline, mupirocin ointment (Esrovan Ointment® [JW Shinyak, Seoul, Korea]) and recombinant human epidermal growth factor ointment (Easyef Oint® [Daewoong Pharmaceutical Company, Seoul, Korea]) were applied. Afterward, the dressing area was covered with Physiotulle® [Coloplast A/S, Denmark], medifoam (Medifoam® [Ildong Pharmaceutical Company, Seoul, Korea]) was applied, and the pressure bandage was lightly

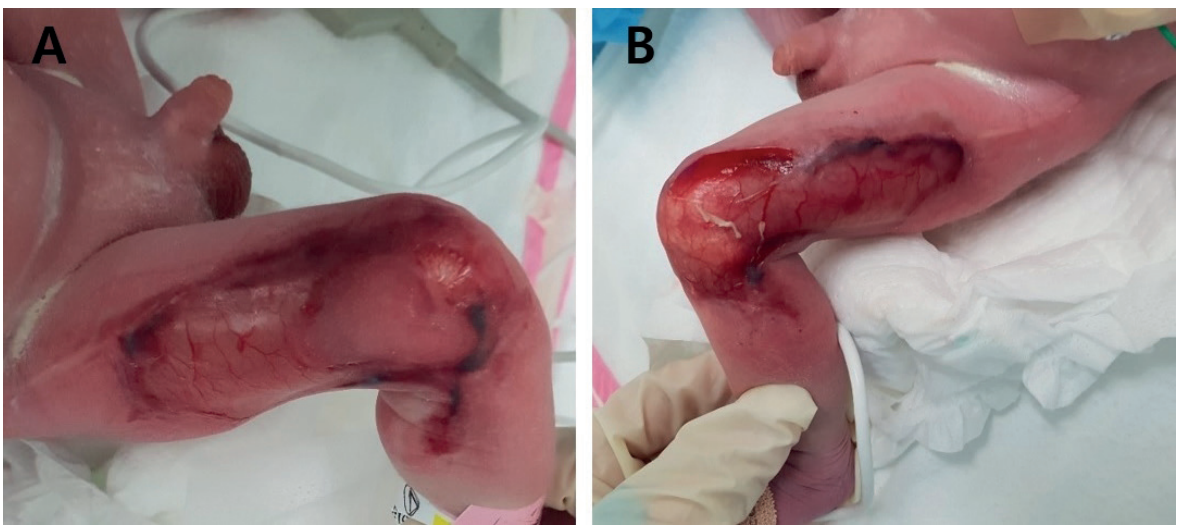

Fig. 1. Symmetric skin defects in both legs of the affected baby on the 1st day of life. (A) Lateral view of right leg. (B) Lateral view of left leg.

fastened to prevent pressure from being applied to it. Because of the large skin defects, active fluid management and prophylactic antibiotic treatment were performed for the first 10 days until minimal epithelization was completed. After one month of dressing in collaboration with the dermatologist and plastic surgeon, the baby's skin lesions were successfully epithelized (Fig. 3 a,b), and he was discharged after 37 days of hospitalization.

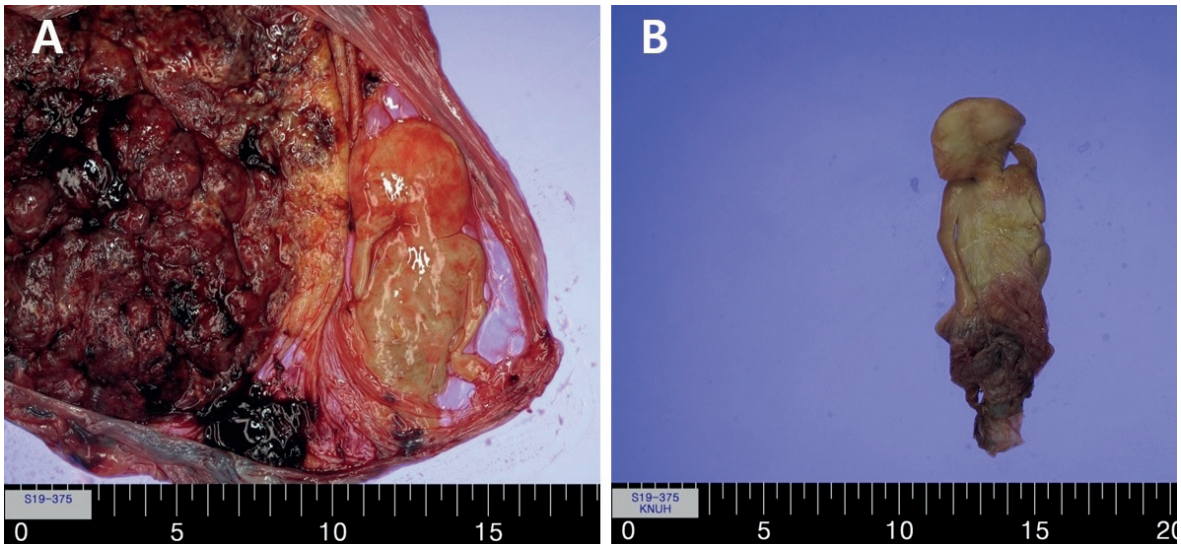

Fig. 2. Placental pathology of the pregnancy. (A) Fetus papyraceous in juxtaposition with the affected baby's placenta. (B) Detached fetus papyraceous with detailed structure.

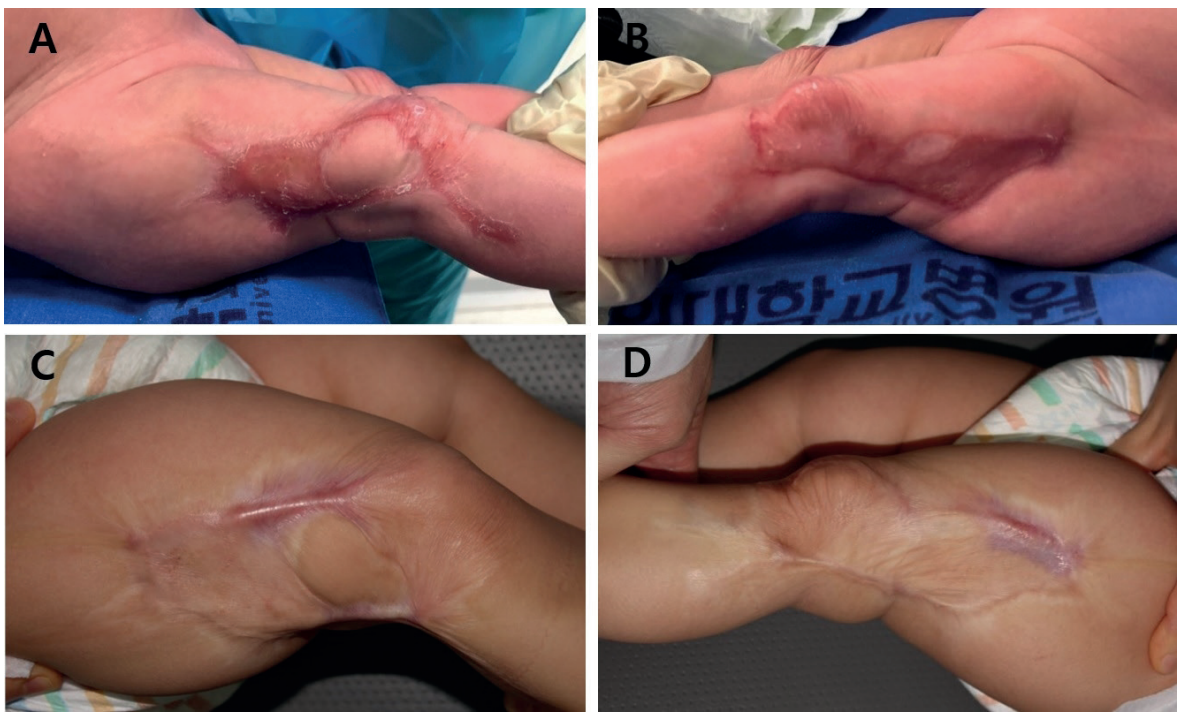

Fig. 3. Progression of aplasia cutis congenital lesions. (A, B) Epithelialized skin defects of both legs at 37 days of life (at hospital discharge). (C, D) Atrophic scar formation of both legs (at 12 months of age).

The infant was followed up at dermatology and pediatrics outpatient clinics. At 12 months of age, the infant showed progressed atrophic scars in both legs (Fig. 3 c,d); thus, treatment with 5-fluorouracil and triamcinolone injection was started, further requiring scar intervention, such as skin grafting. Additionally, he showed normal developmental status per the Korean Developmental Screening Test and level 1 of the Gross Motor Function Classification System without any contracture or motor limitation.

Written informed consent was obtained from the patient's legal guardian for the publication of any potentially identifiable images or data included in this article.

Discussion

The incidence of ACC with FP is likely to be underreported because milder cases often go undetected and because the disappearance of an intrauterine twin at an early stage could be reported as a singleton pregnancy. A recent literature review in Korea revealed only 2 cases of ACC type 5 (ACC with FP or placental infarcts) per the Frieden classification[1] among 59 reported ACC cases in Korea[3], which explains the rarity of the ACC type 5 diagnosis.

The diagnosis of ACC is clinically determined in most cases and biopsies are not routinely performed. Based on the clinical manifestation of a skin defect after birth, ACC type 5 is diagnosed if the following three factors are met: 1) multiparous pregnancy, 2) placental vascular twin anastomoses, and 3) early twin death (FP).[6] A review of 44 cases of ACC with FP found that the mean gestational age of fetal death was 13.3 weeks, with an approximately 1:1 ratio of female to male infants, and the maternal condition was normal prior to delivery in the majority of cases.[7]

Although the exact pathogenesis is unknown, several theories have been proposed regarding skin defects in ACC with FP. Among them, two hypotheses are most plausible. The first is feto-fetal transfusion; the decreased blood pressure of a dying twin can lead to a blood shift from the surviving twin through the shunt to the dying twin. The surviving twin may have hypovolemia and hypotension resulting in ischemia of end-organs such as the skin, sometimes have a watershed area.[8] The second theory is that a dead fetus may pass thrombogenic materials to the viable twin through vascular anastomoses, and the coagulation cascade can be activated in the viable twin, causing ACC and any placental abnormalities.[7] The premature calcifications in our case may reflect placental vascular insufficiency. It is also speculated that any brain injury may occur due to emboli through incomplete anastomosis vessel occlusion after laser ablation. Fortunately, the infant in our case showed normal findings on brain sonography and presented normal development at pediatric and rehabilitation outpatient clinics at 12 months of age.

The incidence of multiple gestation in South Korea has increased sharply over the past two decades (from 1.7% to 4.2% of total births between 2000 and 2018)[9], reflecting a trend toward older maternal age and increased use of infertility treatment. Multifetal pregnancy reduction (MFPR) is a procedure that reduces higher-order pregnancies and was originally intended to improve perinatal outcomes. Typically, in dichorionic triamniotic (DCTA) triplet pregnancies, such as our case, the parents wish to retain a non-monochorionic twin pair after obstetric counselling. Miscarriage or preterm birth are known substantial risks of MFPR; however, an ACC with surviving co-twins is an unexpected event. One case series reported ACC incidence of 8% (2 of 26 newborns after laser ablation at 13 and 14 weeks) after MFPR.[10] One similar case report suggested that the mechanism of ACC was laser burn[11]; however, a baby born after spontaneous co-twin loss during a MCDA pregnancy presented similar truncal and thigh lesions characteristic of ACC type 5,[12] thereby disproving laser burn as the plausible cause.

The assisted reproductive technology (ART) registry of 17 European countries revealed that the multiple delivery rate is approximately 20% after ART.[13] In Korea, under the Korean guidelines, three-embryo transfers are the most frequent transfer number in IVF, and 29.8% of all IVF deliveries are twin or triplet deliveries.[14] With the increase in multiple pregnancies, indications for MFPR may exist, especially in DCTA triplet pregnancies; however, whether MFPR in DCTA triplet pregnancy is superior to expectant management in terms of miscarriage or preterm birth is unknown.[15] Furthermore, this report highlights a distinctive adverse outcome, namely, ACC type 5 with FP, after MFPR. Thus, we suggest that the decision for MFPR in DCTA triplet pregnancy be made with caution, and we present ACC type 5 as an adverse outcome of MFPR in DCTA triplet pregnancy.

Acknowledgment

The parents who consented with the study are gratefully appreciated.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SYK, ESK; data collection: SYK, ESK, JM, KL; analysis and interpretation of results: SYK, ESK, JM, KL; manuscript preparation: SYK, ESK. 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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Case Report

Coats Plus syndrome: a diagnostic and therapeutic challenge in pediatric gastrointestinal hemorrhage

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ABSTRACT

Background. Cerebroretinal microangiopathy with calcifications and cysts formerly known as Coats plus syndrome is a rare multisystemic autosomal recessive disease that affects the eyes, brain, bone, and gastrointestinal system. Intestinal telangiectasia are components of vascular malformations characterized by gastrointestinal system bleedings. Recurrent gastrointestinal system bleedings have been reported as being due to hepatic failure or vascular malformations of the gastrointestinal system tract.

Case. Here we report a patient who presented with recurrent gastrointestinal system bleeding episodes, bilateral exudative retinopathy, intracranial calcification and was diagnosed with Coats plus syndrome. Recurrent gastrointestinal system bleeding was controlled by monthly octreotide treatment.

Conclusions. Coats plus syndrome presenting with vascular malformations should always be kept in mind in a patient with recurrent gastrointestinal bleeding and accompanying systemic physical findings. Octreotide treatment is an important option for patients with life threatening gastrointestinal system bleeding. Long term use of octreotide treatment can be used successfully in selected pediatric cases.

Key words: gastrointestinal bleeding, Coats plus syndrome, octreotide.

Gastrointestinal (GI) hemorrhage is one of the most serious complaints observed in pediatric practice. There are many causes of GI bleeding ranging from those requiring urgent treatment to those that resolve without any therapy. Determining why this is occurring is the art of any medical diagnostic approach and therapeutic options are not always available. Vascular malformations are a rare cause of GI bleeding in children and they are generally difficult to identify.[1,2] We present an adolescent boy with recurrent severe GI bleeding who was diagnosed with Coats plus syndrome (CpS), due to a mutation in the conserved telomere maintenance component 1 *(CTC1)* gene, in order to relate the importance of a multisystem evaluation of pediatric patients with GI bleeding and also to discuss the treatment options.

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Case Report

A 15-year-old male patient was admitted to our clinic with a history of fatigue and recurrent episodes of syncope during a 1-week period. He was born from a consanguineous family (first degree cousins) at full-term gestation after an uncomplicated pregnancy with a birth weight of 1550 grams with severe intrauterine growth retardation. His prenatal and natal history was uneventful except for intrauterine growth retardation. His past medical history included delayed psychomotor development and impaired vision. His developmental milestones were reported to be delayed. An ophthalmologic evaluation at 9 years of age revealed retinal vascular anomalies and capillary telangiectasias requiring laser photocoagulation. His history revealed a syncope 2 months before admission, and his evaluation in another center showed severe anemia. Red blood cell transfusion and oral iron supplementation was the prominent treatment. Physical examination revealed his

growth parameters were within normal limits. He was pale. He had a white forelock in his hair. Lateral gaze palsy was noted in the right eye while medial gaze palsy was prominent in the left eye. Contracture was noted on the left hand. Loss of muscle power was evident in both the upper and lower left extremities. Deep tendon reflexes were increased on the left side. Left-sided dysmetria was noted on cerebellar examination. Choreoathetoid movements were prominent when he lifted his left arm. The remainder of the physical examination was normal.

Laboratory data revealed severe iron deficiency anemia with a hemoglobin level of 5,9 g/dL. In the laboratory evaluation, all the results including liver function tests were within normal ranges except for anemia. Gastroduodenoscopy with duodenal biopsy showed a duodenal ulcer with *Helicobacter pylori*. The patient was transfused with red blood cells and received eradication therapy for *Helicobacter pylori*. He was discharged for outpatient follow-up.

One month later the patient presented melena with a hemoglobin level of 7,1 g/dl. Repeated gastroduodenoscopy showed hyperemic lesions on the bulbus and antrum. No active bleeding source was demonstrated. The colonoscopy and scintigraphy screening for bleeding was normal. The patient was treated conservatively with red blood cell transfusion, proton pump inhibitors and oral propranolol, and then discharged and sent home.

The patient was admitted with a second episode of melena with severe anemia with a hemoglobin level of 7,6 g/dl. Double-balloon enteroscopy was performed, visualizing 200 cm after the pylorus. Most of the mucosa was covered with millimetric ulcers and severe vascularity was noted (Fig. 1). A biopsy revealed edema. Intravenous infusion of octreotide treatment was initiated. The bleeding stopped within days.

The patient was further evaluated for differential diagnosis, due to the accompanying neurological findings. Magnetic resonance imaging (MRI) and computerized tomography of the brain demonstrated amorphous dystrophic calcifications in both temporal lobes and left parietal lobes as well as widespread ischemia in both the thalamus and retrotrigonal periventricular area (Fig. 2). These findings, in conjunction with a physical examination evaluation for neurological, GI, eye and hair outcomes, were suggestive of Coats plus disease. Next generation sequencing identified a homozygous mutation c.2714G> A (p. Arg905Q) (p. Arg905Gln) in the *CTC1* gene confirming CpS. The patient was discharged with monthly intramuscular octreotide treatment. The patient has since been followed for 3 years without any GI bleeding and anemia. Informed consent was obtained from his parents for publication and photographs.

Discussion

We present a case of CpS admitted to our hospital for recurrent severe GI bleeding. The patient's accompanying neurological, ophthalmological, and hair findings along with intestinal lesions were the prominent components for the differential diagnosis. Further genetic evaluation is essential to confirm the diagnosis. The treatment of patients is planned individually according to the bleeding recurrence and accompanying risk factors. The patient benefited from monthly octreotide treatment and showed marked progress.

Coats plus syndrome is an autosomal recessive disease characterized by abnormalities in the eyes, brain, bones, GI system and other parts of the body. In addition, low birth weight, anemia, osteopenia, GI bleeding, portal hypertension, gray hair and nail dystrophies are frequently observed.[3] Recurrent, severe GIS bleeding is increasingly identified in CpS. In addition, cerebral microangiopathy, leukodystrophy, seizures, movement disorders, skeletal disorders and postnatal growth retardation were all reported.[4] Our patient had all of the reported components of CpS with retinal telangiectasias,

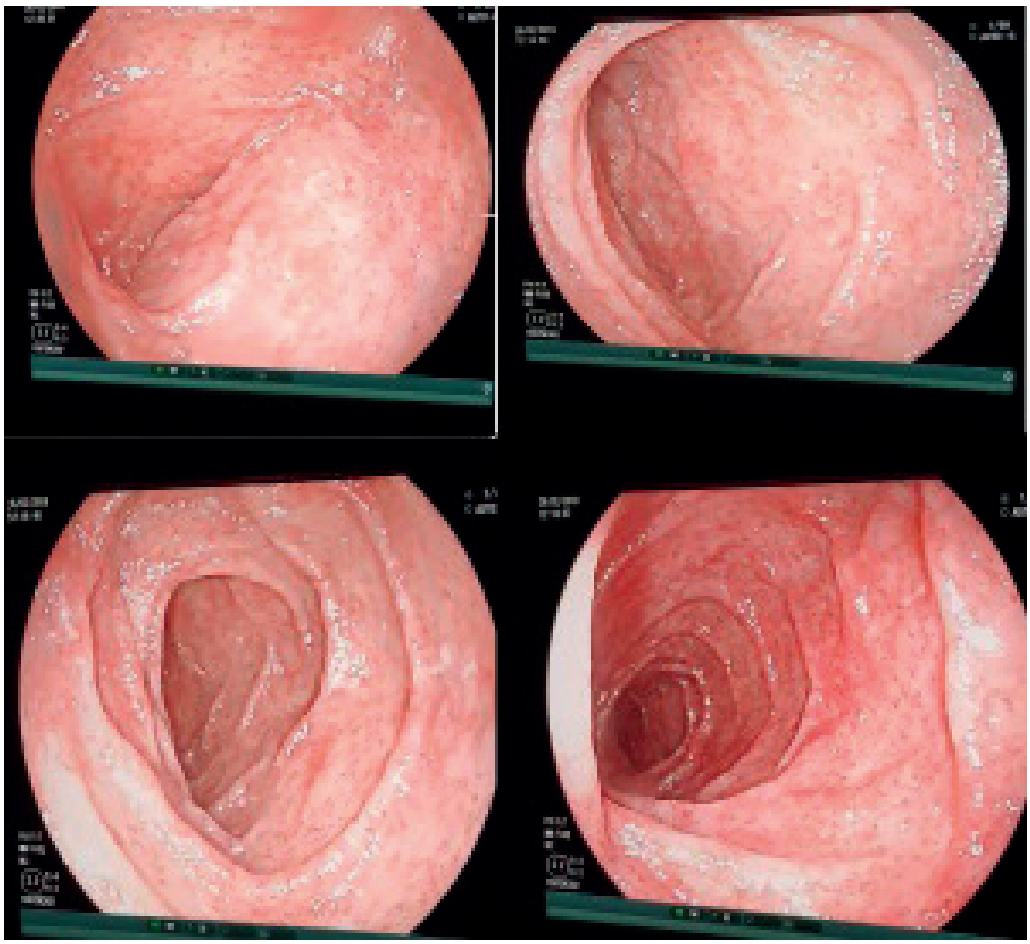

Fig. 1. Double-balloon enteroscopy showing millimetric ulcers and severe vascularity on intestines.

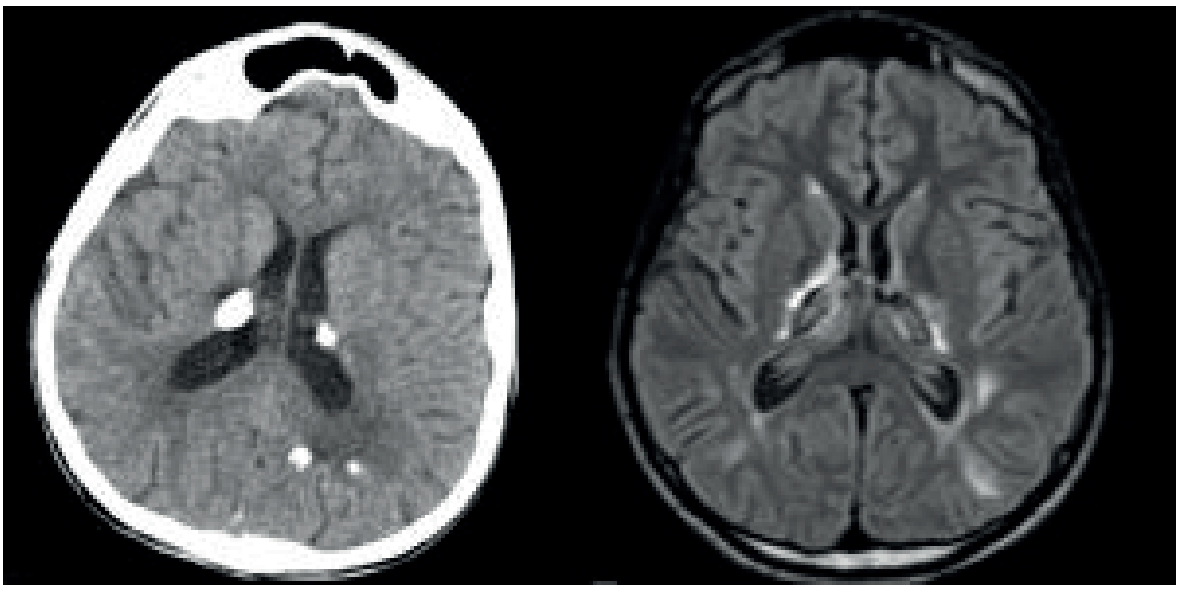

Fig. 2. Cranial computerized tomography scan showing variable and asymmetrically distributed calcifications (Left); Magnetic resonance imaging showed ischemia, gliotic regions and calcifications in FLAIR series (Right).

intracranial calcification, psychomotor retardation, a history of being small for his gestational age, osteopenia, recurrent GI bleeding, liver parenchymal disease and portal hypertension. The diagnosis was a challenge as combining the components was not easy on the first admission. The genetic mutation in the *CTC1* gene was first identified in 2012 (3); thus, further evaluation is necessary to confirm the diagnosis. We identified a previously reported mutation in the *CTC1* gene.

GI bleeding in CpS is an important and life-threatening component of the disease. The cause of bleeding is usually reported to be telangiectasias in the stomach and small intestine. Nevertheless, esophageal variceal bleeding secondary to the development of portal hypertension due to vascular telangiectasia in the liver is not rare.[2] The main pathophysiology for the vascular lesions is obliterative microangiopathy, characterized by thick, sclerotic and calcified vascular walls detected in the eye and brain biopsies of patients with CpS.[5] Our patient had non-cirrhotic portal hypertension. Gastroduodenoscopy and colonoscopy were inadequate for the diagnosis; so double-balloon enteroscopy was needed to show the vascular lesions.

Treatment options in vascular bleeding of the GI system in CpS are generally a challenge since the lesions are often multiplexed. In such cases, endoscopic and surgical treatments may be insufficient.[6] The availability of octreotide in GI system malformations has been demonstrated in many studies. Subcutaneous octreotide therapy has been reported in patients with chronic GI system bleeding due to multiple vascular malformations.[7] Continued monthly subcutaneous octreotide treatment for GI bleeding for intestinal vascular malformations has been reported for up to 15 years.[8] Octreotide treatment has also been an option to control GI bleeding due to angiodysplasia in certain patients.[2] Therefore, we started octreotide treatment for our patient. During the three years of follow-up, our patient's bleeding did not recur and there was no anemia.

It is important to determine the etiology in determining effective treatment approaches in childhood GIS bleeding. However, indicating the exact cause is not always possible or can sometimes be delayed. In this process, the close monitoring and supportive treatment of the patient gain importance. In any GI system when bleeding cannot be controlled in children and adolescents, long term octreotide treatment is also an effective and sustainable treatment option in children. CpS should be considered as a rare cause of GI system bleeding in patients with multisystem involvement. Considering a multisystemic disease that also affects the GI system should be taken into account with any accompanying findings as we have reported here with our patient.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EB, EG; data collection: EB, SB; analysis and interpretation of results: EB, TK, MR; draft manuscript preparation: TK, ET. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A very rare case of a newborn with tetrasomy 9p and literature review

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ABSTRACT

Background. Tetrasomy 9p is a rare genetic condition which usually results from a supernumerary isochromosome derived from the short arm of chromosome 9. Phenotypic findings include multiple congenital anomalies, facial dysmorphism, growth and developmental delays, and also vary according to the presence and degree of mosaicism.

Case. We report on a newborn with tetrasomy 9p who deceased in the newborn period. She had facial features including low-set and anteverted ears, hypertelorism, prominent nasal bridge, and microretrognathia. Bilateral ventriculomegaly, vermian hypoplasia and corpus callosum agenesis were detected on magnetic resonance imaging and double outlet right ventricle (tetralogy of Fallot type), secundum atrial septal defect, and persistent left superior vena cava were displayed by echocardiography. Microarray analysis revealed 38,584 kb tetrasomic region at 9p24.3p13.1. We also present a review of the literature suggesting that there is a recognizable phenotype for this condition and an assessment of cardiac manifestations based on the size and the localization of the breakpoints.

Conclusions. We conclude that cardiac manifestations do not differ according to the localization of the breakpoint. Persistent left superior vena cava seems to be consistent with breakpoints distal to q12, but the present case is different from them by breakpoint p13.1.

Key words: tetrasomy 9p, isochromosome, mosaicism, tetralogy of Fallot, cardiac manifestations.

Tetrasomy 9p is a rare genetic condition which usually results from a supernumerary isochromosome derived from the short arm of chromosome 9 and also demonstrates cytogenetic and phenotypic variability.

Phenotypic features include multiple congenital anomalies, facial dysmorphism, growth and developmental delay. Most common dysmorphic traits are hypertelorism, low-set abnormal ears, bulbous nose and microretrognathia. Intrauterine growth restriction (IUGR), Dandy-Walker malformation, cleft lip and/or palate, congenital heart defects, joint dislocations, hypoplasia of the digits and nails have also been reported.[1]

About 30% of the tetrasomy 9p cases are in mosaic state.[2] Although mosaic cases may be phenotypically normal or mildly affected, most cases in non-mosaic constitution are lethal in the early postnatal period due to severe malformations.[3,4] Herein, we report on a newborn with tetrasomy 9p who died at postnatal 22nd day due to severe cardiovascular collapse.

Case Report

A full-term female infant was born from the third pregnancy of the 44-year-old mother as the first liveborn child. At birth, the child

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weighed 2320 g, and her head circumference was measured at 33 cm (both values below the third percentile). First and fifth minute Apgar scores were 6 and 7, respectively. The parents were not consanguineous. Antenatally the second-trimester triple screen yielded a risk of higher than 1 in 50 for trisomy 18, and detailed fetal ultrasonography showed hydrocephalus and cardiomegaly. The parents refused amniocentesis. The family history was unremarkable except the two spontaneous miscarriages.

The newborn was transferred to our hospital on postnatal day 7 because of hypotonia. She was admitted to the neonatal intensive care unit and intubated because of increased work of breathing and placed on mechanical ventilator. On physical examination, anterior and posterior fontanel measurements were 3.5x4 cm and 2x2 cm, respectively. Metopic suture was not ossified and was palpable. She had low-set and anteverted ears, hypertelorism, prominent nasal bridge, microretrognathia, 3/6 systolic murmur, labial hypoplasia, and bilateral metatarsus adductus (Fig. 1). There were also bilateral single palmar creases, and besides, there were single digital flexor creases on bilateral fifth fingers and a second finger on the right.

Ophthalmological examination revealed no retinal or lenticular pathology. Cranial ultrasonography showed bilateral ventriculomegaly, vermian hypoplasia and corpus callosum agenesis. In addition, hypoplasia of brain stem was detected on cranial magnetic resonance imaging (Fig. 2). Echocardiography showed presence of double outlet right ventricle (tetralogy of Fallot type), secundum atrial septal defect, and persistent left superior vena cava. Abdominal ultrasonography revealed renal bilateral pelvic dilatation and abnormal superior mesenteric arteriovenous drainage. Hypoplasic first rib and costal scalloping was observed on X-rays (Fig. 2).

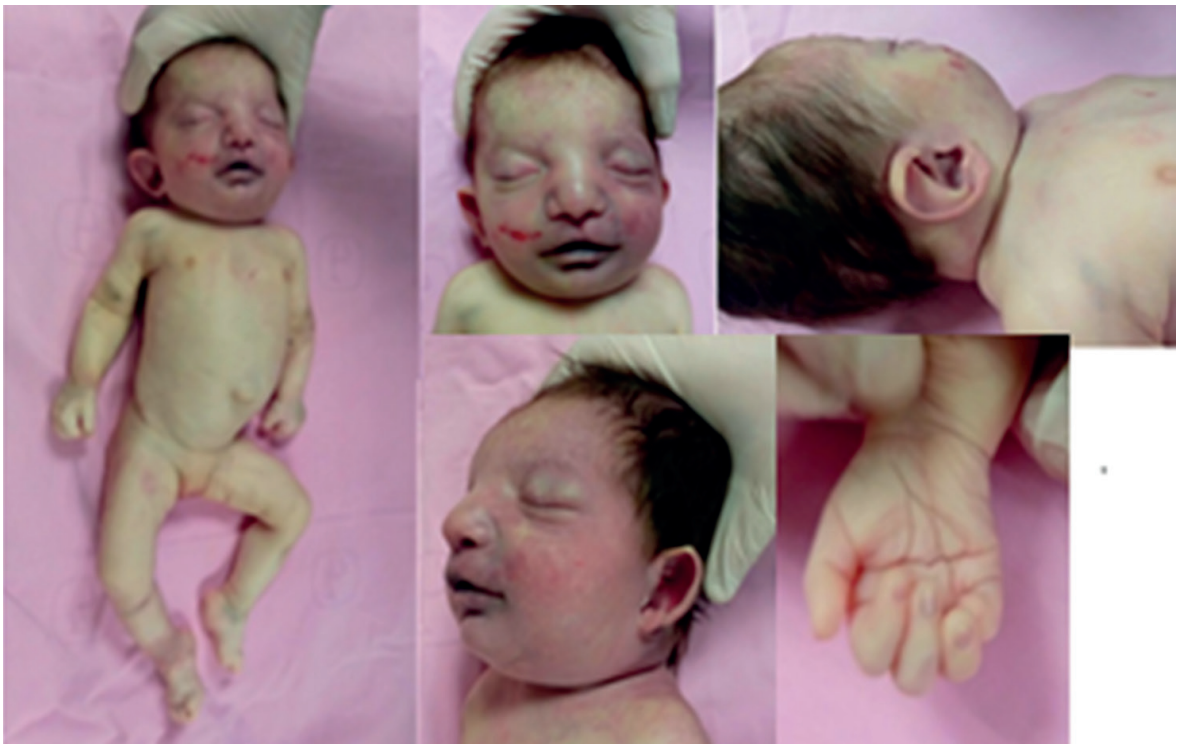

Fig. 1. Overall appearance and facial features of the patient. Hypertelorism, prominent nasal bridge, bulbous nose, low-set and poorly formed ears, microretrognathia, and single palmar crease were remarkable.

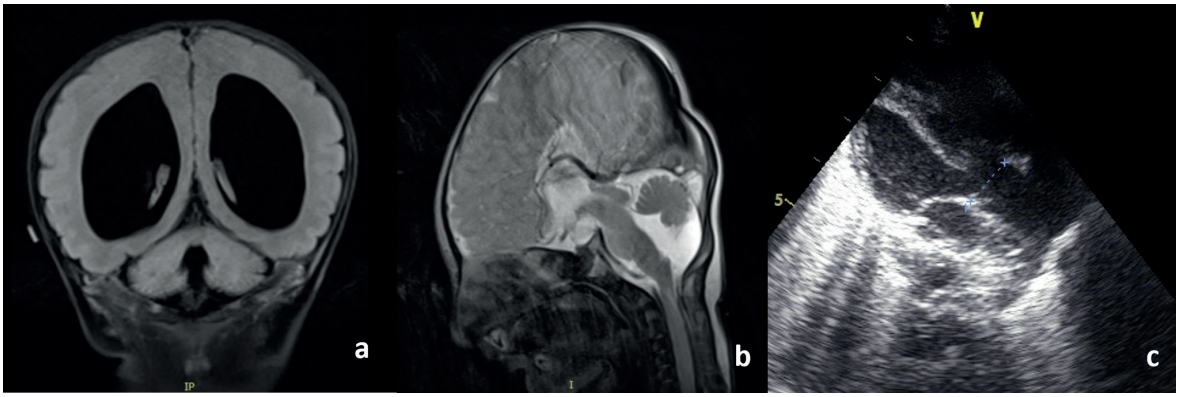

Fig. 2. (a) Magnetic resonance imaging revealed inferior vermian hypoplasia and ventriculomegaly. **(b)** Agenesis of corpus callosum. **(c)** Echocardiography displayed presence of double outlet right ventricle.

The baby was fed with an orogastric catheter and was supported parenterally. She received meropenem, vancomycin, amikacin and fluconazole for catheter-related thrombophlebitis and late neonatal sepsis. The newborn's general health condition deteriorated progressively and she died at the 22nd day due to severe cardiovascular collapse.

After informed consent was taken from the parents, peripheral blood was obtained to isolate genomic DNA using standardized protocols for salt precipitation. Microarray procedure was performed using the GeneChip® CytoScan Optima Array (Affymetrix, Santa Clara, USA) following the manufacturer's recommendations. Chromosome Analysis Suite (ChAS®) software (Affymetrix, Santa Clara, USA) was utilized for the analysis, using in-house data and public databases like Database of Genomic Variants (DGV) and Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER). Microarray analysis revealed 38,584 kb tetrasomic region at 9p24.3p13.1, encompassing 492 genes and 165 OMIM genes (arr hg[19] 9p24.3p13.1 (203,861-38,787,480)x4).

Her family provided written informed consent for publication of this report and the accompanying images.

Discussion

Autosomal tetrasomies are rare genetic conditions most commonly caused by isochromosomes and very seldomly by intrachromosomal amplifications.[5] The most commonly encountered isochromosomes are i(8p), i(9p), i(12p) , i(18p) and i(22q).[1] The isochromosomes of the short arms of chromosomes 5, 10 and 20 have also been reported as individual cases.[6-8] U-type exchange is the proposed underlying mechanism for the formation of isochromosomes.[9] Tetrasomy 9p usually results from a supernumerary isochromosome derived from the short arm of chromosome 9. This isochromosome can be grouped into one of the three types: those that originate from 9p alone, those that involve entire 9p with additional heterochromatic band from 9q, or those that involve the entire 9p with additional heterochromatic and euchromatic bands from 9q.[10,11]

Tetrasomy 9p was first reported in 1973 by Ghymers and 72 cases have been reported since then.[1,2,7-9,12-15] Clinical manifestations of the disease include psychomotor retardation (%73), ear deformity (69%), skeletal anomalies (57%), hypertelorism (56%), microretrognathia (46%), urogenital-renal anomalies (43%), eye anomalies (43%), bulbous nose (40%), congenital heart

disease (40%), cleft lip and/or palate (33%), clino-camptodactyly (26%), down slanting lips (24%) and microcephaly (20%) (Table I).[1,2,14,16-20] Although most isochromosomes are maternal in origin, no correlation between maternal age and this chromosomal pathology seems to exist. In the reported study the mean maternal age was found to be 33.9 years. The present patient had lots of these clinical findings and her maternal age was 44 years.

Sometimes, tissue-specific mosaicism for isochromosomes is observed; such as i(12p) found in skin fibroblasts in Pallister-Killian syndrome or i(9p) in lymphocytes with a lower level in fibroblasts. Mosaicism causes diagnostic difficulties, especially prenatally.[13] It is recommended to study fetal blood samples with uncultured prenatal specimens, to prevent missed diagnosis, until noninvasive prenatal screening using cell-free fetal DNA from maternal blood presents data from all set of chromosomes, and becomes widely available.[11-14]

On the other hand, prenatal ultrasonographic findings are valuable for the morphological diagnosis, and most frequent findings in tetrasomy 9p are ventriculomegaly, Dandy-Walker malformation, intrauterine growth retardation, genitourinary anomalies, and cleft lip and/or palate.[15,19,21,22] The present patient had ventriculomegaly detected prenatally, consistent with these reports. Facial dysmorphic features were also consistent with the condition, such as open sutures, wide fontanels, hypertelorism, prominent nasal bridge and microretrognathia.

According to the already existing literature, 78% of patients have central nervous system malformations, with ventriculomegaly and Dandy-Walker malformation being the most frequently detected anomalies.[1,2,16,19,21-33] The present patient had bilateral ventriculomegaly, vermian hypoplasia, corpus callosum agenesis and brain stem hypoplasia.

This condition is very rarely encountered, and affected individuals do not survive, particularly when in non-mosaic condition. Therefore, a clear delineation of the phenotype is difficult to establish. The degree of mosaicism and the size of the isochromosome expectedly contribute to the phenotype and survival rates.[21] We consider that the condition in the present patient was non-mosaic, although no second tissue was sampled for karyotype analysis.

During the last 20 years, researchers have observed a wide variety of cardiac anomalies in tetrasomy 9p patients. In the presented

Table I. Clinical findings of tetrasomy 9p and comparison of our patient's findings with them.[1,2,14,16-20]

	Literature n:73	Overall	The Present Patient
Maternal age	33.9 (n:66)	33.9	44
Psychomotor retardation	24/33	73%	
Low-set / malformed ears	47/68	69%	+
Skeletal anomalies	38/67	57%	+
Hypertelorism	32/57	56%	+
Micro/retrognathia	31/68	46%	+
Urogenital anomalies	29/67	43%	+
Ophthalmological anomalies	23/54	43%	-
Bulbous tip of nose	27/67	40%	+
Cardiac defects	27/68	40%	+
Cleft lip/ palate	22/67	33%	-
Clino- camptodactyly	17/66	26%	+
Down slanting lips	16/66	24%	+
Microcephaly	13/66	20%	+

case, echocardiography revealed pulmonary stenosis, double outlet right ventricle (tetralogy of Fallot type), secundum atrial septal defect and persistent left superior vena cava. Lloveras et al.[32] and Leichtman et al.[34] previously reported atrial septal defect in tetrasomy 9p cases. Dhandha et al.[21] published three patients with severe congenital heart disease. The malformations included ventricular septal defect, right ventricular outflow tract stenosis, hypoplastic left ventricle, aortic stenosis, thick tricuspid valve and also persistent left superior vena cava. All three patients had isochromosome 9p with segmental euchromatic material from 9q. Cardiac findings have been reported mostly associated with isochromosomes involving 9q segments, but the patient presented here had major heart defects despite being tetrasomic only for segments from 9p. Persistent left superior vena cava (PLSVC) is a noteworthy cardiac finding in tetrasomy 9p and previously reported cases have been associated with isochromosomes additionally involving 9q region.[8] Infundibular stenosis of the right ventricular outflow tract and overriding aorta were reported previously, but to the best of our knowledge this is the first patient with double outlet right ventricle (tetralogy of Fallot type) with a tetrasomy 9p.[32]

Cardiac manifestations of tetrasomy 9p according to the breakpoint are summarized in Table II.[1-4,10,11,13-19, 21-45] We conclude that cardiac manifestations do not differ according to the localization of the breakpoint. Persistent left superior vena cava seems to be consistent with breakpoints distal to q12, but the present case is different from them such that the breakpoint was p13.1. Besides, double outlet right ventricle is being reported for the first time in tetrasomy 9p with a breakpoint at p13.1.

It is known that prognosis of tetrasomy 9p is poor; almost half of patients died during the first year of life, and most of deaths occurred within the first 3 months of life.[1] The present patient passed away at the 22nd day.

In conclusion, tetrasomy 9p presents with a recognizable constitution of facial dysmorphic features and congenital anomalies. Cardiac anomalies are frequent and diverse, also including double outlet right ventricle (severe form of tetralogy of Fallot type); however, no genotype-phenotype correlation seems to exist. Microarray analysis is useful in detecting the degree of mosaicism as well as the exact size of the copy number abnormality, thereby allowing a more precise establishment of genotype-phenotype correlations in further patients.

Acknowledgment

The authors thank the patient's family and all the medical teams involved for their cooperation, and Dr. İlker Ertugrul (Department of Pediatric Cardiology Diseases, Hacettepe University Faculty of Medicine, Ankara, Turkey) for cardiologic evaluation.

Ethical approval

Ethics committee approval was received for this study from the Hacettepe University Non-Invasive Clinical Trials Ethics Committee (Approval number: GO 20/1151). Research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Author contribution

Conseption and design: MS, SO; supervision: HTC, POSK, GEU, SY; data collection and preccessing: MS, SO, GK, HTC, SY; analysis and interpretation: MS, SO, GK, GEU; literature review: MS, SO; writer: MS, SO, GK, HTC, POSK, GEU; critical review: POSK, GEU, SY.

Conflict of interest

The authors declare that there is no conflict of interest.

Table II. Cardiac manifestations of tetrasomy 9p according to the breakpoint.

Cardiac manifestations	Number of reported patients -Breakpoint p10 or more proximal	Number of reported patients -Breakpoint q12 or more distal	Present patient -Breakpoint p13.1
Atrial septal defect	2	-	+
Patent foramen ovale	2	-	-
Atrioventricular septal defect	-	1	-
Ventricular septal defect	-	7	-
Patent ductus arteriosus	3	1	-
Truncus arteriosus	-	1	-
Persistent left superior vena cava	-	4	+
Juxtaductal aortic coarctation	1	-	-
Overriding aorta	-	1	-
Pulmonary hypertension	1	-	-
Septal hypertrophy	-	1	-
Biventricular hypertrophy	1	-	-
Asymmetry of the cardiac ventricles	1	-	-
Hypoplastic left ventricle	-	2	-
Hypoplastic left atrium	-	1	-
Cardiac echogenic focus in prenatal ultrasound	1	-	-
Infundibular stenosis of the right ventricular outflow tract	-	1	-
Bicuspit aortic valve	1	-	-
Aortic stenosis	-	1	-
Thick tricuspid valve	-	1	-
Double outlet right ventricle (tetralogy of Fallot type)	-	-	+

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Short Communication

The pediatric asymptomatic SARS-CoV-2 IgG seropositivity in the Turkish Republic of Northern Cyprus

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ABSTRACT

Background. While children were initially thought to have serious contributions to the coronavirus disease 2019 (COVID-19) transmission, recent studies suggest otherwise. However, the possible effect of asymptomatic pediatric spread still has not yet received enough attention. The aim of our study was to estimate asymptomatic infection rates among children in the Turkish Republic of Northern Cyprus, by using pediatric patients admitted to a university hospital without any COVID-19-associated symptoms.

Methods. Blood samples collected from 80 pediatric patients with no symptoms and history of COVID-19 infection, who were admitted to a university hospital between September 2020 and January 2021, were included in the retrospective study. Isolated serum samples were tested by Dia.Pro SARS-CoV-2 IgG ELISA assays.

Results. The patient group included 40 (50%) male and 40 (50%) female patients. The average age of children was 7.6 ± 4.0 years, with min-max ages ranging from 2 to 15 years. Among the 80 patients tested, only one (1.3%) was detected positive by the Dia.Pro IgG ELISA kit.

Conclusions. The asymptomatic seropositivity reported in our study suggests the use of randomly performed serologic tests to monitor SARS-CoV-2 infection among the pediatric population in schools that would contribute to the public health fight against COVID-19.

Key words: SARS-CoV-2, serology, seropositivity, IgG, children.

Since its first detection in March 2020, there have been more than 2,600 cases and at least 14 deaths that were officially attributed to Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in the Turkish Republic of Northern Cyprus (TRNC). While COVID-19 spread was under control, thanks to the non-pharmaceutical interventions implemented[1] during the early stages of the pandemic, lifting some control measures in the second half of the year resulted in a continuously elevated number of confirmed cases, that still continues today.[2]

One control measure implemented globally was school closure to reduce social contact between children. The data motivating this implementation was mainly based on past experiences with influenza outbreaks, for which children were the major transmitters. While the SARS-CoV-2 transmission dynamic appears to be different since current accumulating evidence suggests children are not the major driver of COVID-19 spread and are rarely involved in secondary transmission[3,4], this was also thought to be due to lower testing and exposure in children, which should be addressed for more reliable conclusions.[5] Today, more attention has

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been drawn on pediatric transmission due to an increased number of cases and the emergence of new variants of the virus.[6,7] Initial studies conducted early in the pandemic regarded children as the 'silent spreaders' of SARS-CoV-2 due to the higher rate of asymptomatic COVID-19 infection in the pediatric population than that reported for adults.[4,8] However, despite data on the asymptomatic spread of SARS-CoV-2, this mode of transmission by children, which may spread the virus for extended periods[9] and can act as a potential source of undetected community transmission, has not yet received enough attention in the literature.[4] This could be especially important for emerged variants of the virus with quicker transmission rates.[10]

The aim of our study was to retrospectively evaluate asymptomatic SARS-CoV-2 exposure among a group of pediatric patients, who were admitted to a university hospital, without any complaint associated with or any history of SARS-CoV-2 infection, in the TRNC after the school closure implementation was lifted (i.e. after September 2020). For our study, serologic testing was preferred as it can detect antibodies from both past and present infections. The results would provide data on pediatric asymptomatic infection rates that may contribute to future studies on the planning of health care policies.

Material and Methods

General characteristics of the TRNC population

According to the latest data provided by the TRNC Statistical Institution (TSI) in 2011, the total population of TRNC was reported to be 286,257 with 150,483 (52.6%) male and 135,774 (47.4%) female subjects. The numbers of subjects with ages of <19 years, 20-49 years, 50-64 years, 65-84 years and >85 years were 73,517 (25.7%); 150,105 (52.4%); 39, 377 (13.8%); 21, 435 (7.5%); and 1,823 (0.6%), respectively.[11]

Ethical approval

This retrospective study was approved by Near East University (NEU) Scientific Research and Evaluation Ethics Committee (Project No: YDU/2020/86-1226). All patient databases were obtained using the hospital information system after receiving informed consent from the parents. Absence of any past SARS-CoV-2 infection was confirmed by hospital records and interviews with parents.

Serum samples

Blood samples collected for routine testing from 80 children who were admitted to Near East University Hospital for routine control or with complaints other than those associated with COVID-19 between September 2020 and January 2021 were included in the study. Centrifuged blood specimens were stored at 4 ºC until analysis. Serum samples were then separated and used for enzyme-linked immunosorbent assay (ELISA) for retrospective detection of SARS-CoV-2 IgG antibodies.

ELISA

To evaluate IgG production, isolated serum samples were analysed by CE-*in vitro* diagnostic-certified Dia.Pro COVID-19 IgG kit (Diagnostic Bioprobes, Sesto San Giovanni, Italy), following the manufacturer's guidelines. The kit is involved in detection of antibodies against SARS-CoV-2 nucleocapsid (NC) and spike (S) antigens.

The kit has sensitivity of more than 98% as indicated in the manufacturer's manual. It was also reported to have specificity and sensitivity rates between 84%-98% and 92%-98%, respectively, by independent studies using samples from symptomatic and asymptomatic SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR)-confirmed patients.[12,13]

Results

Patient characteristics

A total of 80 children (40 boys and 40 girls) who were admitted to the Near East University Hospital without any symptoms associated

with SARS-CoV-2 infection were included in our retrospective study. The age of pediatric patients ranged between 2 and 15 years, with an average age of 7.6 ± 4.0 (SD) years.

Among those, 31 (38.5%) were with a pre-diagnosed underlying condition including neurodevelopmental (n=11, 13.8%) and nephro-urological disorders (n=10, 12.5%). While 17 (21.3%) children admitted to the hospital for routine check-up control, the remaining displayed complaints such as gastrointestinal (n=20, %25), dermatologic (n=12, 15%), and genitourinary (n=10, %12.5) symptoms. Among those with complaints, seven (8.8%) were concluded not to be associated with any disease, condition or disorder and the rest were diagnosed with conditions such as infection/ inflammation (n=26, 32.5), nephro-urological disorder (n=12, 15%), hematological disorder (n=12, 15%), and neurodevelopmental disorder (n=11, 13.8%) (Table I, supplementary data).

SARS-CoV-2 IgG seropositivity results

The seropositivity rate of IgG antibodies in the collected serum samples was investigated by using Dia.Pro anti-SARS-CoV-2 ELISA IgG kit. Analysis of 80 serum samples revealed only one specimen (patient number 51) with IgG seropositivity (1.3%).

Discussion

Current literature does not have any data on SARS-CoV-2 seropositivity among asymptomatic children in Northern Cyprus. The aim of our study was to fill this gap in the literature by using blood samples collected from children who were admitted to a university hospital with complaints not associated with COVID-19 and no history of a SARS-CoV-2 infection. The study covered a period of five months after the international flight restrictions and school closure implementations were lifted, during which 1,394 subjects were reported positive out of 182,723_molecular tests performed in the country.

Serologic tests were preferred for our investigation as they can detect antibodies produced during both present and past infections. Such serologic studies would provide data for health-care policies, and on the immune status of children in the region. According to the latest data provided by TSI in 2011, the population younger than 20 years of age constituted 25.7% (n=73,517) of the overall (n=286,257) TRNC population.[11]

Our study reported 1.3% IgG seropositivity in the pediatric population. Furthermore, there has not been any multisystem inflammatory syndrome or Kawasaki syndrome cases[14] observed in the pediatric patient population in the hospital during the time that the study was conducted. The only patient with an IgG+ result was a two-year old girl who had a complaint of anal itching and was diagnosed with parasitosis caused by *Enterobius vermicularis*. However further testing, such as a neutralization assay, is required to confirm the data due to cross-reactivity previously suggested between SARS-CoV-2, and parasites.[15]

The asymptomatic seropositivity rate detected in our study is similar to the global seroprevalence previously reported for populations younger than 20 years of age (2.3%: 1.0 – 3.6%).[16] Since children are more likely to be infected at home during widespread school closures[5], the low seropositivity rate is thought to be due to reduced exposure because of the school closure implementation that was in practice between March-September 2020. On the other hand, considering the asymptomatic infection rate among children that ranged between 15% to 42%[17], our data also indicates that children in the country are generally susceptible to SARS-CoV-2 infection, and highlights the importance of prevention and control measures for the pediatric population.

In our study, IgM seropositivity rates were not investigated which underestimates the possible impact of early infections on the seropositivity rate reported. While IgM-based tests were more sensitive in the detection of symptomatic than

Table I. Clinical characteristics of the pediatric patients.

Clinical characteristic	Study group n (%)
Patient	80 (100)
Gender	
Girl	40 (50)
Boy	40 (50)
Age	
Average ± SD	7.6 ± 4.0 yrs
Range (min – max)	2 – 15 yrs
Pre-diagnosed condition*	
No pre-diagnosed condition	49 (61.3)
Allergy	
Asthma	6 (7.5)
Endocrine disorders	
Celiac disease	1 (1.3)
Type-1 diabetes	1 (1.3)
Gastrointestinal disorders	
PFIC	1 (1.3)
Hematological disorders	
Iron deficiency	1 (1.3)
Neoplasm	1 (1.3)
Nephro-urological diseases	
Chronic kidney disease	2 (2.5)
IgA nephropathy	2 (2.5)
Nephrolithiasis	2 (2.5)
Nutcracker syndrome	2 (2.5)
Proteinuria	1 (1.3)
Vesicoureteral reflux	1 (1.3)
Neurodevelopmental disorders	
Attention-deficit/hyperactivity	2 (2.5)
Cerebral palsy	1 (1.3)
Developmental delay	1 (1.3)
Epilepsy	4 (5)
Joubert syndrome	1 (1.3)
Precocious puberty	2 (2.5)
Rheumatological disorders	
Juvenile idiopathic arthritis	2 (2.5)
Reasons for admission#	
Routine check-up	17 (21.3)
Dermatologic symptoms	
Hair loss	1 (1.3)
Paleness	10 (12.5)
Soft tissue inflammation	1 (1.3)
Endocrine symptoms	
Overweight	1 (1.3)
Gastrointestinal symptoms	
Abdominal pain	12 (15)
Diarrhea	4 (5)
Nausea	3 (3.8)
Vomiting	1 (1.3)
Genitourinary symptoms	
Anal itching	2 (2.5)
Faul smelling urine	1 (1.3)
Painful urination	3 (3.8)
Recurrent infection	1 (1.3)
Urinary incontinence	3 (3.8)
Neurological symptoms	
Headache	1 (1.3)
Speech delay	1 (1.3)
Respiratory symptoms	
Nasal blockage	1 (1.3)
Skeletal symptoms	
Swelling on foot	1 (1.3)
Swelling on knee	1 (1.3)
Swelling on neck	1 (1.3)
Leg pain	1 (1.3)
Trauma	1 (1.3)
Others	
Fatigue-malaise	11 (13.8)
Loss of appetite	1 (1.3)
Nasal bleeding	1 (1.3)

PFIC:progressive familial intrahepatic cholestasis
*Three patients had mixed underlying conditions.
#One patient had complaints of both nausea and vomiting.
†Thirteen patients were diagnosed with more than one disorder/infection.

Table I. Continued.

Clinical characteristic	Study group n (%)
Diagnosis[†]	
No diseases	7 (8.8)
Allergy	
Asthma	6 (7.5)
Urticaria	1 (1.3)
Ear Nose Throat disorder	
Nasal polyp	2 (2.5)
Endocrine disorders	
Obesity	2 (2.5)
Rickets	6 (7.5)
Gastrointestinal disorder	
Celiac disease	1 (1.3)
Constipation	2 (2.5)
PFIC	1 (1.3)
Hematological disorder	
Inguinal lymphadenitis	1 (1.3)
Iron deficiency anemia	9 (11.3)
Lymphadenitis	1 (1.3)
Neoplasm	1 (1.3)
Infection/inflammation	
Cellulitis	1 (1.3)
Gastroenteritis	12 (15)
Parasitosis	2 (2.5)
Urinary tract infection	11 (13.8)
Nephro-urological disorder	
Chronic kidney disease	2 (2.5)
Diabetic nephropathy	1 (1.3)
IgA nephropathy	2 (2.5)
Nephrolithiasis	2 (2.5)
Nutcracker syndrome	2 (2.5)
Nonorganic enuresis	1 (1.3)
Proteinuria	1 (1.3)
Vesicoureteral reflux	1 (1.3)
Neurodevelopmental disorder	
Attention-deficit/hyperactivity	2 (2.5)
Cerebral palsy	1 (1.3)
Developmental delay	1 (1.3)
Epilepsy	4 (5)
Joubert syndrome	1 (1.3)
Precocious puberty	2 (2.5)
Rheumatologic – Orthopedic disorder	
Juvenile idiopathic arthritis	2 (2.5)
Trauma	2 (2.5)

PFIC:progressive familial intrahepatic cholestasis
[*]Three patients had mixed underlying conditions.
[#]One patient had complaints of both nausea and vomiting.
[†]Thirteen patients were diagnosed with more than one disorder/infection.

asymptomatic SARS-CoV-2 infections[12], this could be due to delayed sampling time as it is difficult to identify the onset of the asymptomatic infection. Furthermore, in a recent study, detection of IgM and IgG antibodies, especially in combination with nucleic acid-based tests and pulmonary computed tomography, was suggested as a preferred method for detection of asymptomatic SARS-CoV-2 infections.[18] Screening for SARS-CoV-2-specific IgA antibodies can also be considered for future studies since like IgM, IgA production starts and peaks within the first 7 days of infection.[19]

In our study, because of its retrospective nature, it was not possible to collect molecular and/or serological data from other family members and investigate the effects of asymptomatic children on SARS-CoV-2 transmission. Therefore, future community-based studies with the inclusion of subjects of different ages at multiple time points would not only contribute to the relevant literature on the role played by asymptomatic children, but also influence policy decisions during the pandemic in Northern Cyprus.

In addition to the limitations described above, another limitation that prevented us from drawing comprehensive conclusions in our study was the low sample size. Furthermore, since asymptomatic patients are unlikely to be hospitalized, community-based studies are recommended for the estimation of the actual asymptomatic infection rate among the pediatric population in the country.

To conclude, our study reported an asymptomatic SARS-CoV-2 seropositivity rate of 1.3% among children in Northern Cyprus, which can further increase due to the lifting of school closure implementation. Therefore, rapid antigen or RT-PCR tests that may be performed along with serological assays[20,21] are recommended to continuously monitor SARS-CoV-2 infection among the pediatric population for the control of COVID-19 spread in the country.

Ethical approval

Near East University (NEU) Scientific Research and Evaluation Ethics Committee (Project No: YDU/2020/86-1226)

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TS; data collection: UG, AAS, ER, BS, CD, IB, KS, ND; analysis and interpretation of results: UG, AAS, ER, MS, TS; draft manuscript preparation: UG. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Erratum to "Tuberculosis risk in the biologic era: tuberculin skin test conversion rates in children with rheumatologic diseases" [Turk J Pediatr 2021; 63: 978-985]

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After publication of the original article it came to the attention of authors that there was a typographical error in Table III of the manuscript. The converted TST value (mm) of the 8th case was erroneously written as "5" this; value should be corrected as "6". The authors would like to apologize for this and any inconvenience it may have caused.

Table III. Demographics and clinical characteristics of the patients with TST conversion during the use of biological agents.

Correction: Converted TST value (mm) of the 8th case should be "6".

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