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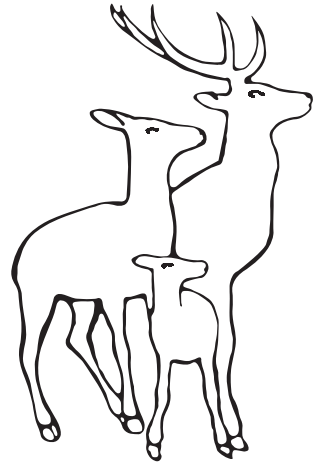

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YAYIN SAHİBİ
Yayınlayan Kurumlar Adına
Elif Nursel ÖZMERT

SORUMLU YAZI İŞLERİ MÜDÜRÜ
Enver HASANOĞLU

DÖKÜMANTASYON
Ali DÜZOVA

YAYINLAYAN
Türkiye Milli Pediatri Derneği,
Hacettepe Üniversitesi Çocuk Sağlığı Enstitüsü
Uluslararası Çocuk Merkezi

EDİTÖR ADRESİ
Çocuk Sağlığı ve Hastalıkları Dergisi
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Faks: (312) 305 22 64

YAYIN İDARE MERKEZİ
The Turkish Journal of Pediatrics Editör Ofisi
Hacettepe Üniversitesi
İhsan Doğramacı Çocuk Hastanesi
06100 Ankara
Tel : (312) 305 26 76
Faks: (312) 305 22 64

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YAYIN ŞEKLİ
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BASIM YERİ
Meteksan Matbaacılık ve Teknik Sanayi A.Ş.
Beytepe No: 3, 06530 Bilkent, Ankara, Türkiye
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Review

Allergic reactions during childhood vaccination and management

Elif Soyak Aytekin, Bülent E. Şekerel, Ümit M. Şahiner

Department of Pediatric Allergy, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Vaccination is one of the most effective public health tools to prevent a variety of infectious diseases. However, concerns about vaccine related adverse effects cause difficulties in clinical practice.

Methods. This review was prepared based on the latest literature available in the PUBMED database in English language (as of March 2021), and all articles with the keywords pediatric vaccine, allergy, hypersensitivity, adverse reaction were evaluated to prepare the article.

Results. Vaccine related confirmed allergic reactions are rare in children, ranging between 0,65-1.45 cases per million vaccine doses. Most of the allergic reactions are self-limited local reactions although in some cases severe anaphylaxis with multisystem involvement can be observed. Allergic reactions may occur because of either the active component (the antigen) of the vaccine, or additional components, such as preservatives, adjuvants, antimicrobials, stabilizers and other substances. Finding the culprit allergen is necessary to prevent future exposure to the allergen and to use alternative vaccines if possible. Diagnosis is largely based on a detailed history and clinical manifestation; also in vivo and in vitro tests may be helpful.

Conclusions. In this review we provide information about hypersensitivity reactions to allergen components of childhood vaccines along with the diagnosis and management of vaccine allergy. Besides the tremendous benefits of vaccination for the health of children, we emphasized that the risk of adverse effects is rare and poses a negligible threat.

Kew words: allergy, anaphylaxis, child, hypersensitivity, vaccine.

Vaccination is effective for preventing a variety of infectious diseases that cause morbidity and mortality. Routine childhood immunization has made the greatest contribution to global health since the 20th century, and throughout that time, the routine childhood immunization schedule has been updated, with new additions of vaccines as well as revisions to the timing and dosing of well-established vaccines. However, vaccine related adverse events are reported in the general population, and cause hesitations and difficulties in clinical practice.

Adverse events after vaccination can be allergic or non-allergic, and both of these reactions can be local or systemic (Table I). Serious adverse events after vaccination occur less frequently than one in 10,000 doses and may become evident after a new vaccine is in widespread use in the general population.[1-3] Vaccine related true allergic reactions are rare, although reported possible allergic reactions to vaccines are frequent.

Local reactions are commonly non-allergic reactions such as pain, redness and swelling, that develops within hours and days at the vaccination site after immunization. Most vaccine related allergic reactions such as contact dermatitis, subcutaneous nodules and maculopapular exanthem, are local type IV hypersensitivity reactions that are triggered by activation of CD4+ and CD8+ specific T cells, in certain situations, monocytes, eosinophils, and

✉ Ümit Murat Şahiner
umsahner@yahoo.com

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Table I. Adverse reactions following immunization.

	Allergic reactions	Non-allergic reactions
Local Reactions	Urticaria	Pain
	Angioedema	Swelling
	Local eczema	Redness
	Allergic contact dermatitis	Abscesses
		Hypertrichosis
Systemic reactions	Systemic urticaria, angioedema	Fever
	Rhino conjunctivitis	Vasovagal syncope
	Bronchospasm	Irritability
	Anaphylaxis	Headache
		Diarrhea
		Muscle pain
		Oculo-respiratory syndrome

neutrophils can also be involved (Fig. 1). These reactions usually occur more than 12 hours after vaccination.

Vaccine related systemic allergic reactions are mostly type 1 hypersensitivity reactions, that may be life-threatening. The symptoms occur rapidly due to releasing of vasoactive mediators by mast cells and basophils (Fig. 2). Bohlke et al.[4] reported 5 cases of anaphylaxis after administration of 7,644,049 vaccine doses, for a risk of 0.65 cases/million doses in a study population consisted of children and adolescents. McNeil et al.[5] identified 18 cases of anaphylaxis after administration of 12,403,201 vaccine doses to 0-17 age group, for an incidence rate of 1.45 cases per million vaccine doses. Non-allergic systemic reactions include fever, vasovagal syncope, and nonspecific symptoms, such as irritability, malaise, diarrhea, muscle pain and headache.

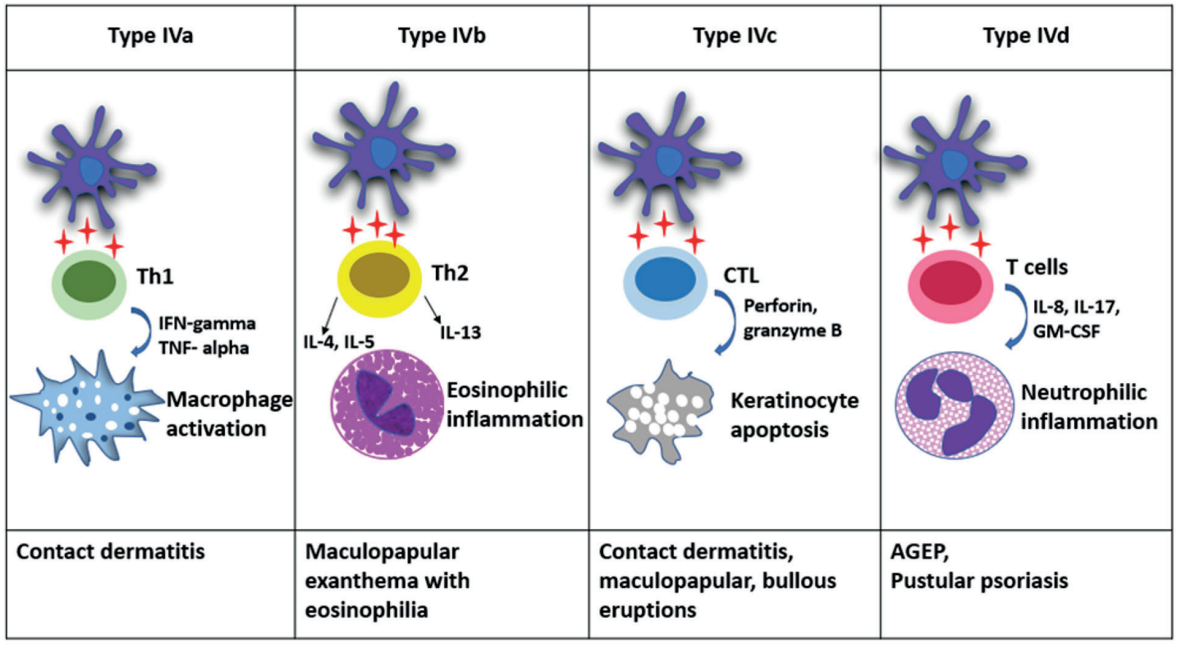

Fig. 1. T-cell mediated (type IV) hypersensitivity reactions.

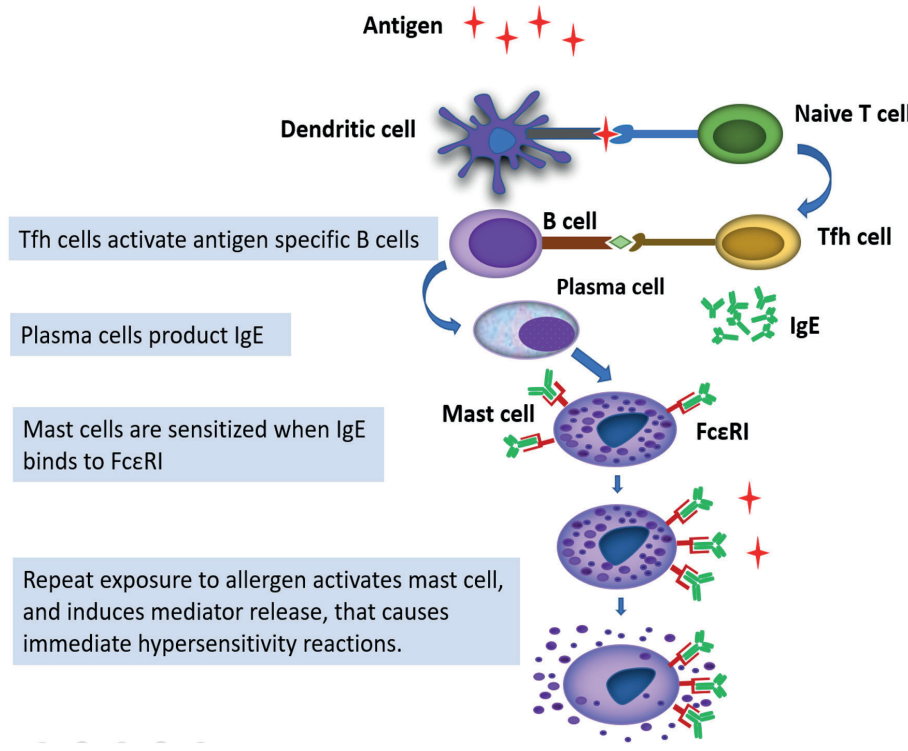

Fig. 2. Mechanism of type I hypersensitivity reactions.

I. Allergic Reactions to Vaccine Components

Vaccines contain an active component (the antigen), and additional components, such as preservatives, adjuvants, antimicrobials, stabilizers and other substances. (Table II)

a. Microbial Antigen

Microbial antigens, which induce the immune response, rarely can cause hypersensitivity reactions. Delayed urticaria, angioedema and skin rash were reported in patients receiving vaccines containing toxoids and pneumococcal antigens.[6] However, most of these hypersensitivity reactions were not confirmed by allergy testing.

b. Gelatine

Gelatine is an animal protein derived from bovine or porcine, and found in drugs, cosmetic products and foods, such as desserts and sweets. Gelatine is also used as a stabilizer in live and inactivated vaccines, including MMR, chicken box, varicella, DTP and Japanese encephalitis. MMR, rabies, varicella-zoster, oral typhoid contain the highest concentrations of gelatine whereas DTP and influenza vaccines contain less, and vaccines with higher gelatine concentration carry higher allergy risks.[7,8]

In children, both immediate and non-immediate systemic allergic reactions were reported with gelatine containing vaccines such as anaphylaxis, urticaria, systemic and local eruptions.[9-11] Prevalence of gelatine allergy appears to be higher in Japanese children, perhaps due to HLA DR 9, common in that population.[12] Administration of the vaccines containing gelatine may also cause secondary food allergy to gelatine in 20 to 25% of children.[9,13] Alpha-gal syndrome is known with delayed anaphylaxis to mammalian meat and gelatine-based products. Children allergic to red meat have a higher risk of gelatine allergy.[14] Recently, gelatine related anaphylaxis after vaccination was reported in a 5 year old patient with alpha-gal syndrome.[15]

Table II. Childhood vaccines and allergic components.

Hepatitis B	Aluminium, yeast, latex, formaldehyde, polysorbate 80
DTaP	Casein, aluminium, 2-phenoxyethanol, yeast, latex, neomycin, polymyxin B, streptomycin, formaldehyde, polysorbate 80
Rotavirus	fetal bovine serum, polysorbate 80, dextran, sorbitol, latex
OPV	2-phenoxyethanol, neomycin, polymyxin B, streptomycin, formaldehyde, lactalbumin
Pneumococcal	Yeast, peptone, polysorbate 80, aluminium
Meningococcal	Casein, aluminium, yeast, latex, kanamycin, formaldehyde, polysorbate 80
MMR	Gelatine, albumin (hen's egg), neomycin, dextran
Influenza	Gelatine, albumin (hen's egg), casein, 2-phenoxyethanol, thimerosal, latex, neomycin, polymyxin B, kanamycin, gentamycin, formaldehyde, polysorbate 80
Varicella	Neomycin, gelatine
Hepatitis A	Aluminium, yeast, latex, neomycin, polymyxin, polysorbate 80
HPV	Aluminium, yeast, polysorbate 80

Since gelatine was replaced with hydrolysed gelatine, allergic reactions to vaccines have decreased significantly.[16] In patients with a suspicion of gelatine allergy, skin test and/or specific IgE to gelatine should be performed. If skin prick test (SPT) is negative, the vaccine can be given with a 60 minute observation at hospital. If SPT is positive, gelatine free vaccine can be given or the vaccine should be administered in graded doses to obtain desensitization.[17]

c. Hen's egg

Egg allergy is the most frequent food allergy in children and the prevalence range is about 50%[18,19], and 50% of children with egg allergies do not tolerate eggs at 6 years of age.[20-22] Many vaccines contain various amounts of egg protein, ovalbumin, such as MMR, influenza, rabies and yellow fever. Egg protein concentrations are higher at vaccines cultured in embryonic eggs (influenza, yellow fever, rabies), and lower at vaccines cultured in chicken embryo fibroblast (MMR). European Union legislation has established 2 μg/ml as the maximum allowed egg protein concentration, which is considered safe in patients with a previous history of egg anaphylaxis.[17] Although vaccines containing egg protein are considered a problem in egg allergic children, several studies have shown that these vaccines are well tolerated and the allergic reaction is similar to the general population.[23,24] Anaphylaxis prevalence due to MMR vaccine was reported in 5,1-12,5 cases per one million doses[5,25], and most of allergic reactions to MMR is observed in children without egg allergies.[26] In 1,061 egg-allergic children, including 335 with previous anaphylaxis to eggs, no systemic reactions were obtained but in about 1% mild reactions were reported with the influenza vaccine.[27,28] All egg allergic children can receive egg protein containing vaccines. Due to low sensitivity and specificity, SPT is not recommended. Children with egg allergies can be MMR-immunized under standard conditions regardless of the severity of the allergic reaction. When influenza vaccine is planned, children with mild allergic reactions should be observed for 30 minutes in primary care after receiving the vaccine[29], whereas children who report previous severe allergic reactions to eggs should receive the influenza vaccine under the supervision of a health care provider who is able to recognize and manage severe allergic reactions.[30]

d. Cow's milk

Cow's milk protein (casein), is used as stabilizer in DTaP, TDaP vaccines. Kattan et al.[31] reported 8 children with a severe cow's milk allergy who reacted with anaphylaxis to booster doses of the DTaP and Tdap vaccines. In addition, oral polio vaccine contains a-lactalbumin, and

in Argentina, four children with cow's milk allergies showed hypersensitivity reactions to oral polio vaccine in three million administered doses.[32] However, most of children with severe milk allergies tolerate DTaP, TDaP and OPV, therefore, in children with cow's milk allergies, these vaccines are not contraindicated.

e. Adjuvants

Adjuvants are molecules, which enhance immunological response when combined with antigens. Aluminium salts (aluminium hydroxide or aluminium phosphate) are the most common adjuvants used in inactivated vaccines, such as DTP, hepatitis A and B, Haemophilus influenza B and conjugated pneumococci vaccines. To date, no immediate hypersensitivity reactions have been documented with adjuvants. However, several contact dermatitis (type IV hypersensitivity) due to aluminium was reported. Itching subcutaneous nodules (vaccination granulomas) and contact allergy to aluminium have been described after vaccination with DTP vaccines in Europe.[33] In a prospective study of 4,758 children, 1,2% developed an itching granuloma following vaccine administration containing adjuvants, and positive patch tests to aluminium was observed in most of the children.[34] However, these reactions are not a contraindication for immunization, if possible alternative vaccines can be administered to prevent local reactions, otherwise the vaccine can be given according to general recommendation.

f. Antimicrobials

Small amounts of antibiotics are used in vaccines (MMR, oral polio, influenza) such as neomycin, gentamycin, polymyxin B, streptomycin and amphotericin B, to prevent bacterial and fungal contamination during the vaccine manufacturing process. No vaccines contain beta-lactams or sulphonamides. Systemic and local allergic reactions with neomycin following MMR vaccination[35,36] and anaphylaxis after rabies vaccine were reported.[37]

g. Latex

Nowadays most vaccines don't contain latex, however, vaccine's vial or syringe may have been contaminated with latex, and allergic reactions may rarely occur. Russel et al.[38] reported that among 160,000 vaccine-associated adverse events, 28 were latex related immediate type hypersensitivity reactions. The most culprit vaccines were inactivated influenza and hepatitis B. If children have a history of severe allergic reaction to latex, alternative vaccines without risk of latex exposure should be administered.

h. Yeast

Vaccines, that are recombinant proteins (Hepatitis B, HPV vaccines), expressed in a culture of *Saccharomyces cerevisiae* (baker's yeast) may contain a trace amount of yeast protein. However, an immediate reaction to these vaccines can rarely happen in yeast-allergic children. In the US, in more than 180,000 vaccine adverse reactions, only 15 were probable or possible anaphylaxis after vaccination of patients with a history of yeast allergies. Of these, 11 cases received hepatitis B vaccine and 4 cases other vaccines.[39] PCV-13, some meningococcal and oral typhoid vaccines also can contain yeast.[17] Children with a history of severe yeast allergy, should undergo allergic evaluation before hepatitis B and HPV vaccine administration.[39]

i. Preservatives

Preservatives such as thiomersal, formaldehyde, 2-phenoxyethanol and phenol are usually added to vaccines to prevent bacterial or fungal growth or contamination.

Thimerosal has been used in influenza, DTaP, pneumococcus, meningococcus, hepatitis B vaccines and reported with contact allergy and rarely with systemic allergic reactions. Nowadays, all pediatric vaccines for children younger than 6 years in the U.S. do not contain thimerosal.[40] After withdrawing thimerosal

from pediatric vaccines, a decrease in contact dermatitis to thimerosal was observed.[41]

Formaldehyde is used in vaccines, such as poliovirus, influenza, hepatitis A-B, and diphtheria and tetanus vaccines, to inactivate toxins from bacteria and viruses. Formaldehyde-containing vaccines rarely can contribute to local or systemic contact dermatitis[42,43], but no IgE-mediated reactions have been reported.

2-phenoxyethanol is used in influenza, DTaP, Tdap and polio vaccines[44], and is well tolerated. However, allergic contact dermatitis rarely have been reported due to 2-phenoxyethanol hypersensitivity after vaccination with DTP.[45]

j. Polysorbate 80

Polysorbate 80 is used as a surfactant, stabiliser and emulsifier in the composition of cosmetics and medications, including vaccines such as HPV, hepatitis A-B, influenza, some pneumococcal and meningococcal vaccines.[44] Despite the frequent use of polysorbate 80 in vaccines, to our knowledge, only one case of anaphylaxis was reported in a 17 year old girl, due to polysorbate 80 hypersensitivity after administration of quadrivalent human papilloma virus vaccine[46], perhaps due to lack of knowledge about the underlying cause of the reaction in similar cases. Polysorbate 80 is also one of the potential causes of the allergic reactions to COVID-19 vaccines(Pfizer-BioNTech) as well as polyethylene glycol.[47]

II. Childhood Vaccines

a) Hepatitis B vaccine

Hepatitis B vaccine is in routine childhood vaccination schedules in many countries and a total of three injections are recommended in the first 6 months of life. Hepatitis B vaccine consist of inactivated HBsAg particles that are obtained from yeast through recombinant DNA technology, and aluminium phosphate or aluminium hydroxide as adjuvant. Anaphylaxis in patients with Hepatitis B vaccine has been rarely reported, probably due to yeast and latex.[39,48,49]

b) BCG vaccine

Bacillus Calmette-Guérin (BCG) is a live attenuated vaccine containing *Mycobacterium bovis* that protects against miliary tuberculosis and tuberculosis meningitis up to a ratio of 80%. According to the World Health Organization (WHO), the BCG vaccine is recommended to all healthy infants in tuberculosis endemic countries. Localized skin reactions following BCG vaccination are common and mostly associated with infection. Self-limiting, local hypersensitivity reactions and dextran associated anaphylactoid reactions have been rarely reported with BCG vaccine.[50-52] In children with weakened immune systems, BCG vaccine may cause local infection at the vaccination site that can be misdiagnosed as allergy, this local infection may also spread to lymph nodes, and cause lymphadenopathy or miliary tuberculosis.[53]

c) Diphtheria, tetanus and pertussis vaccine

WHO recommends diphtheria, tetanus, and pertussis immunization(DTaP) given at 2, 4, and 6 months of age, and booster doses also should be administered at 12 to 15 months of age and again at 4-6 years of age.[54] Tdap vaccine contains reduced diphtheria toxoid, and is used in adolescents and adults. Thereafter, booster doses are recommended every 10 years. The rate of immediate hypersensitivity reactions following DTaP vaccination is 1 cases per 50,000 doses.[49] Anaphylaxis is generally associated with tetanus and diphtheria toxoids.[55,56] Also, Kattan et al.[31] identified 8 children with severe milk allergy who reacted with anaphylaxis to Tdap or DTaP vaccines, which are processed in a broth derived from casein. However, most patients even those with severe milk allergy tolerate these vaccines, so in children with cow's milk allergies, these vaccines are not contraindicated. Aluminium related delayed hypersensitivity reactions (vaccination granulomas and contact allergy) have also been reported after DTaP

vaccination.[33] In the 1990's children who received DTaP, had hypersensitivity reactions with MMR vaccine because of DTP vaccine containing gelatine induced sensitization to gelatine. Therefore gelatine was removed from DTaP products as of 1999.[57]

d) Polio vaccine

Both inactivated poliovirus vaccine (IPV) and live attenuated oral poliovirus vaccine (OPV) have been used worldwide for routine childhood immunization since 1950. A total of three doses IPV are recommended at 2, 4, and 6 to 18 months of age. WHO recommends 4 doses of OPV at birth, 6, 10 and 14 weeks. However, most European countries and the US don't use OPV vaccines after polio eradication. Risk of anaphylaxis after polio vaccine was reported 1,6 per one million doses.[5] OPV may contain trace amounts of antibiotics and cow's milk protein. Children with cow's milk allergies may rarely experience immediate hypersensitivity reactions with the OPV vaccine.[32]

e) Pneumococcal vaccines

Thirteen valent pneumococcal conjugated vaccine (PCV13) is an inactivated vaccine and is routinely recommended for children at 2, 4, 6 months of age and followed by a booster dose at 12 to 15 months of age. The 23 valent pneumococcal polysaccharide vaccine (PPSV23) contains purified capsular polysaccharide antigens of 23 serotypes and licensed for use in people ≥2 years of age.

Allergic reactions to pneumococcal vaccines are rare. Anaphylaxis was reported in a 12 month old infant after PCV-13 vaccine administration due to its carrier protein- a mutant diphtheria toxin CRM(197).[58] In addition, pneumococcal antigen, itself, may be responsible for anaphylaxis.[59,60] Contact allergy to aluminium have also been described with the Prevenar vaccine.[34]

f) Meningococcal vaccines

Current meningococcal vaccine formulations consist of quadrivalent meningococcal conjugate vaccines for serogroups A, C, W, and Y (MenACWY) and monovalent vaccines (serogroup B). Quadrivalent meningococcal vaccines are recommended for adolescents, young adults, and for persons ≥2 months of age who are at an increased risk for meningococcal disease. Meningococcal vaccines contain allergic components such as yeast, casein, aluminium and formaldehyde.[44] However, to our knowledge, no allergic reaction has been reported with these vaccines.

g) MMR

Measles, mumps, and rubella combination vaccine (MMR) is a live attenuated and routine immunization which is recommended at 12 to 15 months of age and 4-6 years of age. The rate of anaphylaxis following MMR vaccination have been reported 5,1-12,5 cases per one million doses.[5,25] MMR vaccine is cultured in chicken embryo fibroblast, and contains negligible or no egg protein. However, there has always been controversy surrounding vaccination of children with egg allergies, although most of the allergic reactions to MMR is observed with gelatine and neomycin and not with egg.[35,61] Although several studies show that MMR vaccination is well tolerated and safe in children with egg allergies[24,62], rare but severe allergic reactions have also been reported.[63] European Academy of Allergy and Clinical Immunology recommends that egg-allergic patients can be MMR immunized under standard conditions (standard vaccine, full dose, no mandatory observation time).[64] The British Society for Allergy and Clinical Immunology (BSACI) guidelines recommend that all children with egg allergies should receive routine MMR vaccination performed by their family doctor/nurse, but in cases of documented anaphylaxis with the vaccine itself, children should be evaluated by an allergist.[65]

h) Influenza

Vaccines for influenza prevention include inactivated influenza vaccines (IIV) (trivalent and quadrivalent) and live attenuated influenza

vaccines (LAIV). Both of these vaccines may be trivalent (two strains of influenza A and one strain of influenza B) or quadrivalent (two strains of influenza A and two strains of influenza B). IIV is approved for use in children ≥ 6 months of age, whereas LAIV is approved for use only in children ≥2 years. Influenza vaccine related allergic reactions are rare, according to VAERS, rate of hypersensitivity reaction was 10.1 , and the rate of anaphylaxis was 0.8 per million doses after influenza immunization.[66] Components in influenza vaccine may cause an allergic reaction such as, 2 phenoxyethanol, gelatine, ovalbumin (hen's egg), microbial antigen and formaldehyde.[67] Nagao et al.[68] identified, IgE antibodies to influenza vaccine antigen and 2-phenoxyethanol were significantly increased in children after influenza vaccination, and this might have enhanced the allergic reaction. Formaldehyde related contact dermatitis and gelatine related anaphylaxis have also been reported in patients after influenza vaccination.[8,42] Influenza vaccines generally contain egg protein (ovalbumin), because the vaccine virus is cultured in embryonated chicken eggs. However, several studies have shown that, both inactivated and live attenuated influenza vaccines are safe in children with egg allergies, even if the patient had severe anaphylactic reactions to eggs.[23,27,28,69] American Academy of Allergy Asthma and Immunology and the American College of Allergy Asthma and Immunology and the American Academy of Pediatrics do not recommend any precautions for influenza vaccine administration to egg-allergic patients regardless of the severity of the egg allergy[70], whereas EAACI recommend that patients with egg allergies should only be immunized with low egg concentrated (<0.12 μg/mL) influenza vaccine, and patients with a previous anaphylaxis history with eggs should be vaccinated under the supervision of a healthcare professional who can recognize and manage anaphylactic reactions.[64]

i) Varicella

Varicella vaccine is a live attenuated vaccine and recommended at 12 to 15 months of age and 4 to 6 years of age. Anaphylaxis incidence due to varicella immunization was reported 3 cases per million doses.[71] Varicella vaccine contains high gelatine content as a stabilizer, and gelatine specific hypersensitivity reactions were observed in children and adults.[10,72,73] Anaphylaxis was also reported in a child with alpha-gal allergy, after varicella immunization.[15] Neomycin is also involved in varicella vaccine, however to our knowledge, no allergic reactions have been documented.

j) Hepatitis A

Hepatitis A vaccine is an inactivated vaccine that is recommended to all children ≥ 12 months of age before potential hepatitis A exposure. A live attenuated hepatitis A vaccine is also available in some countries (India, Nepal, Philippines and Chile) for children ≥ 18 months of age. Hepatitis A vaccine contains many allergic components, however no allergic reaction has been seen after Hepatitis A immunization.

k) Rotavirus

Two live attenuated oral rotavirus vaccines are available. Pentavalent human-bovine rotavirus reassortant vaccinen(RV5) is administered in three doses at 2, 4 and 6 months of age whereas attenuated human rotavirus vaccine (RV1) in two doses at 2 and 4 months of age. Rotavirus vaccines have several allergic components, RV5 contains fetal bovine serum and polysorbate 80, and RV1 contains dextran and sorbitol. But, to our knowledge, no allergic reactions have been reported with rotavirus vaccines. RV1 oral applicator contains latex rubber, therefore RV1 should not be given to infants with a severe latex allergy and RV5 can be preferred for these patients as its dosing tube is latex-free.[74] However, vaccination is recommended if RV1 is the only accessible vaccine, because the benefit

of immunization is considered to be more important than the risk of an allergic reaction.

l) HPV

HPV vaccination is recommended for all children ≥ 9 years of age. HPV vaccines contain synthetically manufactured viruses like particles and additional allergic components such as aluminium, yeast and polysorbate 80. IgE mediated anaphylactic reaction incidence is 1.4 cases per million dose with bivalent HPV vaccine and 26 cases per one million dose with quadrivalent HPV vaccine.[5,75]

III. Diagnosis

Diagnosis of vaccine allergy is largely based on a detailed history and clinical manifestation. The time of onset and duration of the reaction (IgE mediated reactions occur in a few hours after immunization whereas, delayed reactions occur in days) the symptoms (for example anaphylaxis and urticaria/angioedema suggest an IgE mediated reaction, whereas contact dermatitis or maculopapular exanthem suggest a type 4 reaction), history of the previous allergic reaction and possible other allergen (drugs, foods, latex) exposure are important to identify the type of hypersensitivity reaction and the culprit allergen.

When there is a history of vaccine allergy, finding the culprit allergen is necessary, to prevent exposure with products containing this allergen, and to use alternative vaccines without this component, if available. Testing should be performed with allergic components of the suspected vaccines. In vivo and in vitro tests may be helpful. Skin testing can be performed if the patient's history is consistent with IgE mediated reactions. Firstly, SPT (undiluted) should be performed, a positive SPT is suggestive for an allergic reaction, but if negative, intradermal test (1:100 1:10 diluted) should be administered due to its high sensitivity for IgE associated reactions. However, false positivity of intradermal tests may occur due to irritant reactions rather than allergic reactions.[76]

For nonimmediate reactions, in particular contact dermatitis, maculopapular exanthem, subcutaneous nodules, patch testing with culprit allergen, such as aluminium, thiomersal and antibiotics, may be used to identify type IV hypersensitivity reactions.

Specific IgE tests are available for only limited components such as hen's eggs (egg white and ovalbumin), cow's milk (alpha-lactalbumin, beta-lactoglobulin, casein and bovine serum albumin), gelatine, latex and thiomersal. It should be considered that, sensitivity and specificity of both skin tests and specific IgE tests have not been established to confirm or exclude vaccine allergies.

IV. Management

The usual dose of vaccines can be administered keeping the patient under observation at last 30 minutes following vaccination in a facility, where anaphylaxis can be recognized and managed. All patients should be asked whether there have been an allergic reaction following prior vaccinations or if the patient has a known allergy such as medication, food, latex.

Patients with egg allergy (including anaphylaxis) can be immunized with MMR under standard conditions. If the patient has a non-anaphylactic allergic reaction to eggs, influenza vaccine may be also administered under standard conditions. For patients with an egg anaphylaxis history, influenza vaccine should be performed under the supervision of a healthcare professional who can recognize and manage anaphylactic reactions. When milk allergic patients are immunized, there is no need for special precautions.

If a patient has a suspected IgE mediated allergy with other vaccine components such as gelatine/thiomersal/yeast/antibiotics/latex and requires

a vaccine containing these culprit allergens, skin tests and specific IgE tests should be performed. If the results are negative, immunization can be performed in the usual manner, while a positive result requires the vaccine to be given in the form of fractionated doses.

If patients have a history of non-immediate local reactions (contact dermatitis or subcutaneous nodule) with vaccine, the culprit allergen can be established with patch testing. If patch testing is negative, alternative vaccines can be used which do not contain the allergen component, if available. If patch testing is positive/not possible, vaccination is not contraindicated, deep intramuscular administration of the vaccine is recommended to reduce local reactions.[49]

Patients with a history of anaphylaxis to a vaccine, should be administered alternative vaccines which do not contain allergen components (Fig. 3). Where this is not possible, a skin test should be performed with the vaccine. When SPT is negative, the vaccine can be administered in split doses, first 10% of the complete dose and then 30 minutes later, 90% of the dose. When SPT is positive, vaccine can be safely administered under observation using a desensitization protocol, graded dose of vaccine is administered at 15 minute intervals (at the start, 0.05ml of 1:10 dilution, then 0.05ml, 0.1ml, 0.15ml, 0.2ml, of a 0.5ml full-strength vaccine).[77] However, desensitization protocol still carries a risk of anaphylaxis. After the last dose, patients should be kept under observation for 2 hours.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: UMS; literature search, interpretation of the knowledge and manuscript preparation ESA; contribution to the discussion and manuscript structuring BES. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

All authors declare that there is no financial relationship or conflict of interest areas that need to be known regarding the study.

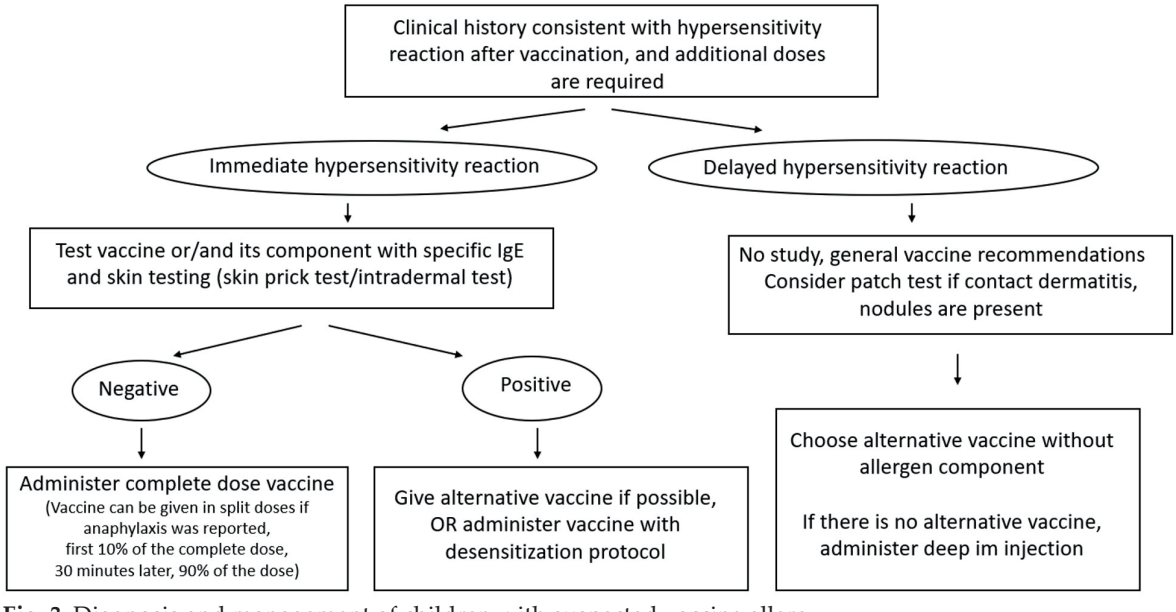

Fig. 3. Diagnosis and management of children with suspected vaccine allergy.

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Original Article

Clinical characteristics, late effects and outcomes in pineoblastomas in children: a single center experience

Ömer Görgün[1,*], Başak Koç[1,*], Rejin Kebudi[1], Johannes E Wolff[2], Abut Kebudi[3], Emin Darendeliler[4]

[1]*Division of Pediatric Hematology-Oncology and* [4]*Department of Radiation Oncology, İstanbul University Oncology Institute, İstanbul, Turkey;* [2]*Division of Pediatric Hematology- Oncology, MD Anderson Cancer Center, Houston, TX, USA;* [3]*Division of Surgical Oncology, Okan University Medical Faculty, İstanbul, Turkey.*

ABSTRACT

Background. Pineoblastomas (PB) are rare tumors of the central nervous system and are more common in children. There is no consensus about standard of care. The objective of this study is to analyze the outcome of children with PB.

Methods. Six patients with PB who were diagnosed between 1990-2012 were evaluated retrospectively. Demographics, age of diagnosis, first complaint, tumor region, diagnosis type, seeding metastasis to the spinal axis or cerebrospinal fluid (CSF), treatment and survival of these patients were recorded.

Results. Three patients had subtotal resection and all patients received chemotherapy and craniospinal irradiation (CSI) after diagnosis. Median follow-up after treatment was 5.5 (range:1-19) years. Two patients are alive with no evidence of disease for 7.5 and 10 years, one of whom was diagnosed with papillary thyroid carcinoma 9.5 years after treatment. One of the patients who died had lived for 19 years after diagnosis.

Conclusions. Pineoblastomas are rare but very aggressive tumors; more effective treatment strategies are needed. Survivors should be followed up for late effects such as second malignancies and endocrine deficiencies.

Key words: pineoblastoma, children, late effects.

Pineoblastoma (PB) is a rare embryonal tumor of the pineal gland.[1] Histologically, PB are classified as WHO grade IV tumors.[2,3] While they typically appear radiographically as focal enhancing mass, PB can also be locally invasive and spread outside the pineal region through the subarachnoid space.[4] In this study, we assessed the demographics, treatment, late effects and outcome of PB patients who were diagnosed and treated in the Istanbul University Oncology Institute (IUOI) during 1990-2012.

✉ Rejin Kebudi
rejinkebudi@yahoo.com

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*Both authors have contributed equally

Material and Methods

During 1990-2012, 516 children with brain tumors were diagnosed and treated in the IUOI. Among these patients six (1.16 %) were diagnosed with PB. Demographics, age of diagnosis, first complaint, tumor region, diagnosis type, seeding metastasis to the spinal axis or cerebrospinal fluid (CSF), treatment and survival of these patients were evaluated retrospectively from patient records. All patients had cranial and spinal axis MRI with contrast at diagnosis and during follow-up. Ethics Committee Approval: Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethical Committee (12.02.2013/ 83045809-3507), Istanbul University Oncology Institute Academic Coordination Council (2013-206).

Results

There were six patients diagnosed with PB between 1990 and 2012. Three of them were female, the median age of diagnosis was 5.75 years (2-14). The demographics of the patients are shown in Table I and modality of treatment, and outcomes are shown in Table II.

All patients had cranial magnetic resonance imaging (MRI) with contrast at diagnosis. Staging included spinal axis MRI with contrast for all patients, and CSF cytology, for all but patient #2 (Table I) in whom lumbar puncture was contraindicated due to increased intracranial pressure. For pathologic diagnosis, three underwent only stereotactic biopsy because the tumor was considered unresectable by the neurosurgeons and three of them underwent subtotal resection of the tumor. In all patients, surgery was followed by craniospinal radiotherapy (CSRT) (except the 2-year-old patient) and chemotherapy.

All patients received CSRT after surgical biopsy or resection. One patient (Table I, pt #1) received eight courses of chemotherapy before radiotherapy (RT) due to her young age (< 3 years old). The disease progressed and RT was initiated, however she died in the intensive care unit without completing RT. Two of our patients (#3 and #4) were re-irradiated after the first RT due to relapse.

As chemotherapy, the patients received the institutional protocols used for embryonal brain tumors in the respective years of diagnosis. The first four patients received 8 courses of vincristine (1.5 mg/m^2, 1st day), etoposide (100 mg/m^2/day x3 days) and cyclophosphamide (1gr/m^2/day) (Table I, pt #1-3) or carboplatin (560 mg/m^2/day) (Table I, pt #4).

One patient (Table I, pt #5) received 8 courses of chemotherapy consisting of vincristine (1.5 mg/m^2), cyclophosphamide (1gr/m^2/day), procarbazine (50mg/m^2) and CCNU (100mg/m^2), all given in one day. She was given growth hormone 3 years after the end of chemotherapy for one year. While the PB remained controlled ever since, her medical history was not unremarkable: She developed papillary thyroid

Table I. Characteristics of patients.

Patient No (#)	Gender	Age (years)	Complaint	Primary Tumor Region	Spinal axis	CSF
1	Female	2	Ataxia	Pineal region	Negative	Negative
2	Female	3.5	Ataxia, tremor	Pineal region	Negative	Not analyzed
3	Male	3.5	Parinaud syndrome vomiting	Pineal region	Positive	Positive
4	Male	8	Parinaud syndrome headache	Pineal region	Negative	Negative
5	Female	10	Headache, vomiting	Pineal region	Negative	Negative
6	Male	14	Headache	Pineal region	Negative	Negative

Table II. Modality of treatment and outcome of patients.

Patient No (#)	Biopsy	Resection	Chemotherapy	Radiotherapy	Survival from diagnosis (months)	Results
1	Yes	No	Yes	Yes*	12	Exitus
2	Yes	No	Yes	Yes	42	Exitus
3	Yes	No	Yes	Yes	24	Exitus
4	No	Subtotal resection	Yes	Yes	228	Exitus
5	No	Subtotal resection	Yes	Yes	126	Alive
6	No	Subtotal resection	Yes	Yes	90	Alive

*: received radiotherapy due to progressive disease, died without completing radiotherapy.

cancer 9.5 years after treatment for PB. She underwent total thyroidectomy and received radioactive iodine ablation therapy. The patient developed seizures 11 years after treatment. Repeated MRI showed there were hyperintense lesions on the subcortical white matter of the left temporal lobe without contrast enhancement. Later, she developed premature ovarian failure. At the time of this report, the patient is 24 years old and continues receiving anticonvulsants, and hormone replacement for thyroid hormone and ovarian hormones. She has good cognitive function and graduated from university.

A fourteen year old male (Table I, pt #6), received postoperative CSRT followed by intensive chemotherapy consisting of ifosfamide (1.8 g/m2/d x 5days, etoposide 100 mg/m2/d x 5 days, and carboplatin 560 mg/m2/day) for eight courses. The tumor responded, but a contrast enhancing residual remained visible by MRI. It could not be resected and did not progress later. The patient is alive, with good cognitive function. Remaining health concerns for him are low testosterone and obesity. He graduated from university and lives independently.

In the first two patients (Table I, pt #1-#2) the tumor progressed under treatment, both of their family declined further treatment and the patients passed away.

The tumor of the third patient (Table I, pt #3) progressed one month after the end of the first line treatment. He received further treatment with a second series of radiotherapy (RT) to the temporal lobe and 12 courses of temozolomide (150mg/m^2/day X 5 days, every 28 days) chemotherapy. The tumor progressed again and treatment was switched to CCNU (100mg/m^2/day, every six weeks), nimotuzumab (150 mg/m^2 /dose once a week for 12 weeks and then every other week thereafter), vinorelbine (25mg/m^2/day on the same day) and intrathecal methotrexate (12 mg once every week for 3 times). The MRI revealed no response to these protocols, and the treatment was switched again to irinotecan (50mg/m^2/day X 5 days) and temozolamid (100mg/m^2/day X 5 days) every 3 weeks. The patient died two years after diagnosis due to progressive disease.

A later recurrence was observed in a patient who had subtotal resection at the age of 8 years (Table I, pt #4). In this patient the residual tumor appeared stable at first, but then progressed after five years. At this time, he was treated with RT to pineal region and 8 courses of vincristine (1.5 mg/m^2), cyclophosphamide (1gr/m^2/day), procarbazine (50mg/m^2) and CCNU (100mg/m^2). After 1 year, new tumor appeared as an intradural mass in the sacral region (S2). The family refused a biopsy. He was treated again with only local RT to sacral region. The patient lived for 19 years after the first diagnosis of the PB. He had cognitive and endocrinological problems. He died due to a traumatic fall in his house.

None of the patients had a family history of malignancy, none had ophtalmologic evidence of retinoblastoma.

In our study, the median survival time was 5.5 (range:1-19) years. Only 2 patients are alive and are long term survivors.

Discussion

The pineal region is compromised of the pineal gland, posterior third ventricle, tela choroidea and velum interpositum. The primary pineal tumors are pineoblastoma (PB) and pineocytoma.[5] Pineoblastomas are rare supratentorial tumors, comprising less than 1% of childhood brain tumors. Pineoblastomas commonly affect children and young adults, and in our study the median age at presentation was 5.75 years (range: 2-14).[6] They mainly occur with a short history of clinical symptoms.[7,8] Signs and symptoms may be nonspecific. Pineoblastomas usually cause third ventricle compression and hydrocephalus leading to vomiting, headaches and somnolence.[7-10] In our center 6 patients with PB were diagnosed among 516 pediatric brain tumors (1.16 %) between 1990-2012 and the most common symptoms were headache, Parinaud's syndrome and ataxia.

Pineoblastomas are embryonal brain tumors similar to medulloblastomas at the histological level, however, at the molecular level there is little similarity. Histologically, these tumors exhibit a classic small round blue cell tumor appearance and Homer Wright and Flexner-Wintersteiner rosettes may also be seen occasionally like medulloblastomas. Positivity for the master regulator of retinal photoreceptor differentiation, CRX, is positive in 100% of pineal parenchymal tumors but it is noticed rarely in medulloblastomas.[1] Similar treatment strategies with high risk medulloblastomas have been suggested for PB.[11] Additionally, PBs -like medulloblastomas- have a tendency to metastasize to the subarachnoid space. All patients should have an additional metastatic evaluation with MRI of spinal axis and CSF cytology. In our study, 1 of 6 patients had spinal axis seeding metastasis with imaging and CSF positivity.

Germ-line RB-1 mutations predispose to pineoblastoma, in addition de Kock et al.[12] have reported that DICER1 is an important susceptibility gene for PB and demonstrated PB to be a manifestation of a germ-line DICER1 mutation. Additionally, mutually exclusive alterations in other microRNA-processing pathway genes as DROSHA, and DGCR8 were common in PB.[13] Liu et al.[14] designed a study of moleculer characteristics of PB and made five groups as PB-miRNA1, PB-miRNA2, PB-MYC/FOXR2, PB-RB1, PPTID. Specification of pineal tumor molecular grouping in CNS tumor classification is suggested to be correlated with clinical characteristics and outcome. None of our patients had an ophtalmic manifestation of retinoblastoma and any of the mutations were not investigated in our cases.

Surgery, radiotherapy and chemotherapy are essential for treatment. Surgery as a cornerstone of the treatment offers rapid symptom relief and is reported to be important for long-term survival.[15] Although gross total resection (GTR) is generally recommended, this is not possible in most cases.[16-20] In a recent retrospective analysis of pediatric and adult PB study, totally 64 patients were included and 42 were children. Twenty-six (61.9%) of the pediatric patients had GTR, and 16 (38.1%) had subtotal resection, however, there was no significant association between the extent of resection and overall survival.[21] In our series, the patients who are still alive had subtotal resection, and all of those that had no resection at all died. Radiation therapy and systemic chemotherapy are also as essential as surgery in this rare aggressive tumor.[19] All of our patients received CSRT, followed by systemic chemotherapy. Craniospinal irradiation followed by local boost to the tumor bed should be standard.[22] On the other hand, maximal surgical resection followed by five cycles of intensive chemotherapy and consolidation with myeloablative chemotherapy and autologous hematopoietic cell rescue (HDCx/AuHCR) and/or CSRT was evaluated in the Head Start I, II, and III study.[23] They found that CSRT and HDCx/AuHCR were statistically associated with improved survival.

Biswas et al.[24] reported a case series where comprehensive CSRT was used in 15 of 17 patients (88%), one patient who received whole brain and ventricular radiation had local and leptomeningeal relapse and died of progressive disease. In a series of 25 patients, progression free survival rates were reported as 47.1 %. 12,5 % and 0 % for those who received CSRT, whole brain RT and focal RT, respectively.[23] In our series, all patients had CSRT except the one, who was less than 3 years old. A recent study from Germany evaluated the treatment of infants with tumors of the central nervous system (1 patient with PB) with Proton Beam Therapy (PBT) and concluded that PBT is feasible for very young children with central nervous system tumors in the short term.[25] None of our patients received PBT, it is not available in our country yet.

According to the data from the Surveillance, Epidemiology and End Results (SEER) between 1973-2007[26], the most important factors were age and degree of spread of tumor in 95 adult patients with PB; younger patients with local disease had the best prognosis. Parikh et al.[20], in 41 pediatric pineoblastoma cases, have reported

that, similar to other primitive neuroectodermal tumors, the most significant predictors of survival are age and metastatic disease status on presentation. In the SIOP/UKCCSG PNET 3 study, relatively good survival for non-metastatic pineal PNETs was reported, and there was no evidence that pre-radiation chemotherapy improved outlook.[27] Although pineoblastomas were classified as primitive neuroectodermal tumors in previous studies, it is classified under embryonal malignancy group in the recent WHO CNS classification.[28]

The analysis of 135 children with pinealoblastoma in the European Society for Paediatric Oncology (SIOP-E) and US Head Start pooled data showed that in children younger than 4 years of age at diagnosis, conventional chemotherapy without RT was not sufficient to induce sustained remissions in PB.[29] Friedrich et al.[30] reported a prospective study about CNS-PNET/PB. In this study, from January 2001 to January 2005, 17 eligible children aged <4 years diagnosed as CNS-PNET/PB were prospectively treated in the trial HIT-2000. In nonmetastatic disease (n= 11) HIT-SKK systemic multiagent chemotherapy followed by CSRT were given. Patients with metastatic disease (M1-M3, n=6) received shorter induction chemotherapy with carboplatin and etoposide, followed by tandem high-dose chemotherapy (HDCT) for those, who had good response to induction therapy. During induction and HDCT, patients received intraventricular methotrexate. Craniospinal RT was given to all patients with poor response to induction or with residual disease. The authors found that short and more intensive induction chemotherapy followed by HDCT was more effective than prolonged moderate induction chemotherapy.[30] In another prospective metacentric trial, including 11 children and adolescents with PB, all patients had surgery followed by hyperfractionated RT accompanied by weekly intravenous vincristine and then 8 cycles of maintenance chemotherapy (lomustine, cisplatin, and vincristine).[31] The authors concluded that this treatment strategy was feasible without major acute toxicity and survival rates were comparable to those of a few other recent studies but superior to those of most other series, including the previous trial, HIT 1991.[31] Gorsi et al.[32] used nivolumab in pediatric patients with recurrent brain tumors with some transient partial responses. Although in this series, there was only one patient with PB who had received only one dose of nivolumab, it was suggested to be a promising option in recurrent brain tumors. Additionally, in a retrospective study with limited small sample size with embryonal tumors, HDMTX combined with dose-intensified multiagent chemotherapy was used; it was suggested that in children with high-risk brain tumors who have a poor outcome, High-dose chemotherapy (HDC) and autologous stem-cell transplantation (auto-SCT) could be an option in treatment and promising to improve survival rates.[33]

A retrospective study from China indicated that aggressive surgery as first-line therapy in PB and younger age was associated with poorer prognosis.[34]

In our series, the patients who lived longer were older than the others. However, they also had resection even it was subtotal, while the others had no resection.

In conclusion, PB are aggressive tumors necessitating intensive treatment including surgery, CSRT and chemotherapy. High dose chemotherapy is promising in some studies, especially in young patients. Metastasis is a poor prognostic factor. Patients should be treated in institutions with dedicated neurooncology experience and they should be followed up for long term side-effects such as second malignancies, and endocrine deficiencies.

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Author contribution

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

Ethical approval

Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethical Committee (12.02.2013/ 83045809-3507), İstanbul University Oncology Institute Academic Coordination Council (2013-206).

For this type of study formal consent is not required.

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Conflict of interests

Johannes Wolff is employee of AbbVie Pharmaceuticals. The other authors declare that they have no conflicts of interest with regard to this research.

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Original Article

Thiol / disulfide balance and oxidative stress parameters in pediatric patients diagnosed with acute and chronic idiopathic thrombocytopenic purpura

Yeter Düzenli Kar[1], Zeynep Canan Özdemir[2], Özcan Bör[2]

[1]Division of Pediatric Hematology, Department of Pediatrics, Afyonkarahisar Health Sciences University, Afyonkarahisar; [2]Division of Pediatric Hematology-Oncology, Department of Pediatrics, Eskişehir Osmangazi University Faculty of Medicine, Eskişehir, Turkey.

ABSTRACT

Background. Changes in oxidative stress and thiol / disulfide balance are thought to play a role in the pathogenesis of idiopathic thrombocytopenic purpura (ITP). Our study investigates total oxidant level (TOS), total antioxidant level (TAS), oxidative stress index (OSI) levels and thiol / disulfide balance in pediatric patients with acute and chronic ITP.

Methods. Thirty four patients with acute ITP, eighteen patients with chronic ITP and thirty three healthy children (control) were included. TOS, TAS, OSI, thiol / disulfide balance were analyzed.

Results. In acute ITP, TAS levels were lower than chronic ITP and control, TOS and OSI levels were higher than control, and native thiol level was lower than chronic ITP ($p < 0.05$). In acute ITP; disulfide level, disulfide / native thiol and disulfide / total thiol ratios were higher than chronic ITP and control, and native thiol / total thiol ratio was lower than chronic ITP and control group ($p = 0.038$, $p = 0.018$, respectively). TOS and OSI levels of the chronic ITP were higher than the control group ($p < 0.05$).

Conclusions. The results of this study have shown that oxidative stress increases in children with acute ITP and chronic ITP, that thiol / disulfide balance is disrupted in favor of disulfide in acute ITP, and that thiol / disulfide balance isn't disrupted in chronic ITP patients whose platelet count is close to normal and who don't require treatment.

Key words: acute, chronic, ITP, oxidative stress, TAS, TOS, thiol / disulfide balance.

Immune thrombocytopenia is an autoimmune disease characterized by isolated thrombocytopenia and defined as a platelet count below 100000 / mm^3 when the number of hemoglobin and white blood cells is normal. In approximately 80% of patients, the platelet count is below 30000 / mm^3.[1] It commonly occurs between the ages of 2-5 years, and its annual incidence is reported to be 1.9-6.4 per 100,000 children.[2] Etiopathogenesis is caused by a complex and multifactorial immune dysregulation targeting platelets and megakaryocytes.[3,4] The best known mechanism is that immunoglobulin G (IgG) type autoantibody coated platelets formed against platelet surface antigens is phagocytized by the macrophages in the spleen via the Fc receptor, leading to shortened platelet life. In recent years, it has been shown that megakaryocyte inhibition develops in the bone marrow due to T-cell-mediated cytotoxicity resulting from immune dysregulation.[5]

In recent years, there are notable studies suggesting that oxidative stress is the mechanism that induces the disease. In idiopathic thrombocytopenic purpura (ITP) patients, it has been shown that gene expression pathways associated with oxidative stress are

✉ Yeter Düzenli Kar
yeterduzenli@yahoo.com

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activated, and that higher level of oxidative stress and lower reduced glutathione / oxidized glutathione (GSH / GSSG) ratios are present compared to healthy controls. In addition, as a result of oxidative damage, it has been shown that patients with ITP develop lipid peroxidation and protein modification.[6] Studies have reported that total oxidant level (TOS) and oxidative stress index (OSI) values are higher and total antioxidant level (TAS) is lower in both acute ITP and chronic ITP patients compared to healthy control groups.[7,8]

Thiols are organic compounds consisting of a sulfur atom and a hydrogen atom attached to a carbon atom and containing a sulfhydryl group (-SH). While thiols in plasma are primarily composed of albumin and proteins, a few are composed of thiols with low molecular weight such as cysteine, cysteinylglycine, glutathione, homocysteine and γ-glutamyl cysteine.[9] Oxidative products such as reactive oxygen species - which occur when oxidative stress increases - oxidize thiol groups to form disulfide bonds. As a result, dynamic thiol / disulfide homeostasis is achieved, and the organism is protected from the damage of reactive oxygen products.[10]

This study investigated TAS, TOS, OSI, total thiol and native thiol levels and the quantity of dynamic disulfide bonds in acute ITP, chronic ITP and control groups, and the relationship between these components was investigated.

Material and Methods

Patient population

Thirty-four children diagnosed with acute ITP (acute ITP group) and eighteen children diagnosed with chronic ITP (chronic ITP group) between May 2018 and August 2019 at the Pediatric Hematology and Oncology Clinic of Eskişehir Osmangazi University, as well as thirty-three healthy children (control group) were included in the study (date: 18.04.2018, no: 80558721/50).

The diagnosis of acute ITP was established based on clinical, laboratory and bone marrow findings and platelet count (platelet count <100,000 / mm^3). Patients whose thrombocytopenia continued for three months from the time of diagnosis were classified as acute ITP, while those with thrombocytopenia continuing for 3-12 months after diagnosis were classified as persistent ITP. Chronic ITP patients consisted of patients with at least 12 months since the time of diagnosis and who had ongoing isolated thrombocytopenia.[3] Approval was obtained from the ethics committee (date: 18.04.2018, no: 80558721/50) and informed consent from legal guardians of the children were obtained.

Exclusion criteria

Patients with cytopenia, hepatomegaly, splenomegaly in other series in addition to thrombocytopenia; patients who had infection and vaccination one to four weeks before diagnosis; patients who had positive viral cereology results (hepatitis, EBV, CMV); and patients diagnosed with collagen tissue disease were excluded from the study. ITP patients using thrombopoietin mimetics were excluded from the study.

Collection of blood samples

Blood samples were collected from chronic ITP patients who did not receive any treatment (intravenous immunoglobulin, corticosteroid) in the past three months. Patients' age, gender, complete blood count parameters (hemoglobin, hemotocrit, red cell distribution width (RDW), platelet count) and C-reactive protein (CRP) values were recorded based on the information in patient files.

Biochemical analysis

Peripheral blood samples were transferred into biochemistry tubes. Serum was separated by centrifuging the blood samples at 2-8 °C for 10 minutes at 1500 × g. Samples were stored at -80 °C until use. The levels of TOS, TAS, OSI, thiol / disulfide and [diannik] disulfide bonds were analyzed in the serum samples.

TAS and TOS were measured spectrophotometrically using Rel Assay Diagnostics Kit (Rel Assay®, Diagnostics kits, Mega Tıp, Gaziantep, Turkey), at 660 nm for TAS and at 530nm for TOS. OSI was calculated by proportioning the TOS values of the samples to the TAS values in terms of percentage.[11-15] Reducible disulfide bonds were reduced to form free functional thiol groups. Formaldehyde was used to remove residual sodium borohydride and DTNB (5,5'-dithiobis- (2-nitrobenzoic acid)) products. Following this, both reduced and native thiol and natural thiol groups were determined. The quantity of dynamic disulfide bonds were calculated by dividing the difference between the total thiol and the native thiol groups by two. After calculating the quantity of native, total thiol and disulfide; the disulfide / total thiol percentage rates, the native thiol / total thiol percentage rates and the disulfide / native thiol percentage rates were also calculated.[9]

Statistical analysis

The data were statistically analyzed using the SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA) program package. All data were expressed as means ± SD, medians (min-max) or numbers. Mann-Whitney U test following Kruskall Wallis were used for comparison of the non parametric variables when appropriate. One-way analysis of variance (ANOVA) was used to compare normally distributed variables. Post hoc Bonferroni and Tamhane's tests were used according to homogeneity of variances. The Chi-square was used to test the association between categorical outcome variables. Spearman rank correlation test was used for analysis of correlations among results. The *p* value was set at 0.05.

Results

Thirty-four children were included in the acute ITP group, 18 children in the chronic ITP group, and 33 children in the control group. There was no statistical difference in gender distribution between the three groups (p> 0.05). Age of the acute ITP group was statistically younger than the chronic ITP group (p = 0.018). In acute ITP group, hemoglobin, hematocrit and platelet values were lower compared to the chronic ITP and control groups (p <0.05), and CRP values were higher (p = 0.048, p <0.001). Platelet values of the chronic ITP group were lower compared to the control group (p <0.001). The characteristic features and laboratory parameters of the patient and control groups are shown in Table I.

In the acute ITP group, TAS level was lower compared to the values of the chronic ITP and control groups (p = 0.012, p = 0.001, respectively), TOS and OSI levels were higher compared to the values of the control group (p < 0.001, for both values), and native thiol level was lower compared to the values of the chronic ITP group (p = 0.025). In the acute ITP group, disulfide level was higher compared to the control group (p = 0.007), while disulfide / native thiol and disulfide / total thiol ratios were higher compared to the values of the chronic ITP and control groups (p = 0.038, p = 0.018, for both ratios), and native thiol / total thiol ratio was lower compared to that of the chronic ITP and control groups (p = 0.038, p = 0.018).

TOS and OSI levels of the chronic ITP group were higher compared to the values of the control group (p <0.001, p = 0.005). TAS, native thiol, total thiol, disulfide levels, disulfide / native thiol, disulfide / total thiol and native thiol / total thiol ratio were similar to those of the control group (P> 0.05 for all). There was no difference in total thiol level between the three groups. TOS, TAS, OSI level and thiol / disulfide balance parameters of the patient and control groups are shown in Table II.

There was a positive correlation between platelet count and TAS level in the patient group (acute and chronic ITP) (r = 0.399, p = 0.014) and a negative correlation between platelet count and disulfide / native thiol, disulfide / total thiol ratio (r = -0.912, p = 0.001, for both parameters).

While 27 of the patients diagnosed with acute ITP went into remission, seven of them became

Table I. Demographic and laboratory data of the children with acute/chronic immune thrombocytopenia and of healthy controls.

	Acute ITP	Chronic ITP	Healthy Controls	P Value
Age (year) (mean± SD)	7.8±3.7	10.9±3.7	9.5±3.9	0.018[α] 0.255[β] 0.547[ϒ]
Sex (Female/male) (n)	15/19	11/7	15/18	0.466[α,β,ϒ]
Hemoglobin(g/dl) (mean± SD)	12.6±0.9	13.2±0.9	12.9±0.95	0.004[α] 0.020[β] 0.996[ϒ]
Hematocrit (%) (mean± SD)	37.4±2.53	38.6±2.57	38.7±3	<0.001[α] 0.004[β] 0.546[ϒ]
RDW (%) (mean± SD)	13.4±0.9	13.2±0.9	13.3±0.8	0.247[α,β,ϒ]
Platelet count (/mm^3) Median (range)	9000 (1000-21000)	91000 (34000-96000)	345000 (178000-490000)	<0.001[α,β,ϒ]
CRP (mg/L) Median (range)	0.5 (0.12-7)	0.3 (0.2-1.5)	0.3 (0.2-0.6)	0.048[α] <0.001[β] 0.124[ϒ]

[α]Acute ITP-Chronic ITP, [β]Acute ITP – Healthy Controls, [ϒ]Chronic ITP- Healthy Controls
RDW; red cell distribution width, CRP; C-reaktive protein.

chronic. No statistically significant difference was found between the Thiol-disulfide balance and TAS, TOS, and OSI levels of patients who became chronic and in remission after one year of follow-up after the diagnosis of acute ITP in Table III.

Discussion

Although the pathogenesis of ITP has not yet been elucidated, increased platelet destruction and decreased platelet production due to loss of immune tolerance to platelet antigens are thought to play a role in the etiopathogenesis. In addition, the immune effects on both platelets and megakaryocytes due to the increase in T-cell-mediated cytotoxicity and the increase in T helper 17 proinflammatory cytokine are thought to play a role in the etiopathogenesis of ITP.[16] A strong relationship between autoimmunity and increased oxidative stress has also been demonstrated in ITP.[17] Free radicals, which normally occur as a product of physiological events in our body, are in a certain balance with antioxidants.6 There are studies suggesting that oxidative stress triggers autoimmunity in both acute and chronic ITP, leading to impairment in platelet structure and platelet dysfunction.[4,7,8,16,18,19] It has also been reported that oxidative stress plays a role in acute ITP becoming chronic.[19]

The results of our study show that TOS and OSI levels are higher in acute and chronic ITP patients compared to the control group, and that TAS level in acute ITP patients is lower compared to the chronic ITP and control groups. Cura et al.[7] found that TAS level was lower and TOS and OSI levels were significantly higher in acute ITP patients compared to the control group. Akbayram et al.[8] reported that they could not detect a significant difference in TAS levels between acute and chronic ITP patients, and that TAS level decreased in both acute ITP and chronic ITP groups compared to healthy controls. In the same study, they found that in cases where oxidative stress and TOS and OSI

Table II. Thiol-disulfide balance and TAS, TOS, OSI levels of children diagnosed with acute and chronic idiopathic thrombocytopenic purpura and healthy control group.

	Acute ITP	Chronic ITP	Healthy control	P value
TAS (μmol Trolox Eqv./L)	1.23	1.34	1,38	0.012$^{\alpha}$
Median (Lower-upper limit)	(0.21-2)	(0.95-2.06)	(1.09-2.020)	0.001$^{\beta}$
				0.805$^{\tau}$
TOS (μmol H2O2 Eqv./L)	30	32,6	12,9	0.489$^{\alpha}$
Median (Lower-upper limit)	(9.55-77.8)	(11.1-70.6)	(1.1-58.8)	<0.001$^{\beta,\tau}$
OSI (U/L)	1.86(0.57-5.93)	1.51(0.78-4.22)	1.06(0.001-5.07)	0.248$^{\alpha}$
Median (Lower-upper limit)				<0.001$^{\beta}$
				0.005$^{\tau}$
Native thiol (μmol/L)	398(114-635)	444(299-600)	428(311-591)	0.025$^{\alpha}$
Median (Lower-upper limit)				0.079$^{\beta}$
				0.344$^{\tau}$
Total thiol (μmol/L)	566(440-792)	565(473-678)	533(480-659)	0.254$^{\alpha,\beta,\tau}$
Median (Lower-upper limit)				
Disulfide (μmol/L)	79.7(10-237)	59(6-113)	58.5(3-138.5)	0.074$^{\alpha}$
Median (Lower-upper limit)				0.007$^{\beta}$
				0.730$^{\tau}$
Disulfide/ nativ thiol (%)	20.9(1.91-206.5)	13.4(1-34)	12,9(0.6-43.4)	0.038$^{\alpha}$
Median (Lower-upper limit)				0.018$^{\beta}$
				0.953$^{\tau}$
Disulfide/ total thiol (%)	14.7(1.8-40.2)	10,5(0,9-20,4)	10.3(0.6-23.2)	0.038$^{\alpha}$
Median (Lower-upper limit)				0.018$^{\beta}$
				0.953$^{\tau}$
Nativ thiol/ total thiol (%)	70.4(19.4-96.3)	78.8(59-98)	79.4(53.5-98.8)	0.038$^{\alpha}$
Median (Lower-upper limit)				0.018$^{\beta}$
				0.953$^{\tau}$

$^{\alpha}$Acute ITP-Chronic ITP, $^{\beta}$Acute ITP – Healthy Controls, $^{\tau}$Chronic ITP- Healthy Controls
TOS: total oxidant level, TAS: total antioxidant level, OSI: oxidative stress index

values increased, the level of Malondialdehyde - which is a good indicator of lipid peroxidation - increased in both acute ITP and chronic ITP patients compared to the control group. Elalfy et al.[4] showed that the reduced glutathione, TAS and catalase (known to be antioxidant systems within the organism) decreased in the acute ITP and chronic ITP groups compared to healthy controls, while the level of malondialdehyde, a lipid peroxidation product, increased. The reason for the difference in TAS level between our chronic ITP group and the control group may be that the number of patients in the chronic ITP group was low and that these patients are composed of patients with close to normal platelet counts who did not require treatment.

Thiols are molecules containing sulfhydryl group (–SH) that can be converted into reversible disulfide bond structures through oxidation by oxidant molecules such as free radicals or reactive oxygen species. This conversion is the earliest indicator of protein oxidation in case of oxidative stress. Disulfide bond structures formed can be reduced to thiol groups again, thus ensuring the thiol-disulfide balance.[9] Disulfide formation can be used as a reliable indicator of oxidative stress, since it reflects the antioxidant status, prooxidant reactions and protein-thiol redox

Table III. Thiol-disulfide balance and TAS, TOS, OSI levels of children diagnosed with acute ITP in remission and acute ITP that becomes chronic group.

	In remission acute ITP	Acute ITP that becomes chronic group	p value
Age (year) (mean± SD)	7.04±3.5	11.07±2.1	0.008
Sex (Female/male) (n)	10/17	5/2	0.199
TAS (μmol Trolox Eqv./L) Median (Lower-upper limit)	1.22(0.21-1.48)	1.27(1.13-2)	0.259
TOS (μmol H2O2 Eqv./L) Median (Lower-upper limit)	29.71(6.44-77.8)	35.22(19.04-41.04)	0.594
OSI (U/L) Median (Lower-upper limit)	1.87(0.57-5.93)	1.68(1.25-3.46)	0.686
Native thiol (μmol/L) (mean± SD)	381±117	405±96	0.626
Total thiol (μmol/L) (mean± SD)	562±76	589±74	0,418
Disulfide (μmol/L) Median (range)	78.5(10-237)	86(46-173)	0.624
Disulfide/ nativ thiol (%) Median (range)	0.22(0.02-2.07)	0.16(0.1-0.64)	0.983
Disulfide/ total thiol (%) (mean± SD)	0.15±0.09	0.15±0.06	0.948
Nativ thiol/ total thiol (%) (mean± SD)	0.68±0.19	0.68±0.12	0.948

TAS: total antioxidant level, TOS: total oxidant level, OSI: oxidative stress index

status in the organism.[20] It has been shown that plasma disulfide level is high in smokers and in degenerative diseases such as obesity, diabetes and pneumonia, and low in proliferative diseases such as multiple myeloma, colon and renal cancer, and aggressive growing tumors.[9] In our study, there was no difference in total thiol between the three groups, while plasma native thiol levels were significantly lower in acute ITP patients compared to chronic ITP patients. Serum disulfide levels of acute ITP patients were statistically higher compared to the control group and chronic ITP group. Disulfide / native thiol (%), disulfide / total thiol (%) and native thiol / total thiol (%) ratios were statistically significantly higher in the acute ITP group compared to both the chronic ITP and the control groups. This indicates that during oxidative stress in the acute ITP group, thiols tend to be reduced and increase the formation of disulfide bonds, and the thiol-disulfide balance shifts towards disulfide direction. In our study, there was no statistically significant difference in disulfide / native thiol, disulfide / total thiol and native thiol / total thiol ratios between the chronic ITP group and the control group. In a recent study by Beyazit et al.[18], in which they investigated the plasma thiol-disulfide balance before and after treatment in patients with acute ITP, they found that the pre-treatment plasma native and total thiol levels were lower in patients with acute ITP compared with the post-treatment values and the levels of the control group. The same study showed that the pre-treatment disulfide level was similar to the control group, while post-treatment disulfide level increased significantly compared to the pre-treatment level.

In our study, there was no significant difference in native thiol and total thiol levels between the acute ITP and the control group. However, it was observed that thiol-disulfide balance shifted towards disulfide increase in acute ITP patients compared to the control group. There was a positive correlation between platelet counts and TAS, and a negative correlation between thiol / disulfide balance in the patient group.

Hemoglobin and hemotocrit values in the acute ITP group were lower compared to both the chronic ITP group and the control group. This result can be explained by the common prevalence of skin and mucosa bleeding such as petechiae, ecchymosis, epistaxis in acute ITP patients at diagnosis.

In our study, in accordance with the literature, the ages of the patients who were diagnosed with acute ITP and became chronic after a one-year follow-up were found to be statistically greater than the patients in remission.[3,8] However, there was no statistically significant difference in thiol-disulfide balance and oxidative stress parameters between patients who became chronic after the diagnosis of acute ITP and those who were in remission. We think that the reason for this is the low number of patients. There is a need for comprehensive studies on this subject.

The limitations of our study are that the number of patients was low and that the platelet counts of chronic ITP patients were close to normal.

In conclusion, the results of this study have shown that oxidative stress increases in children with both acute ITP and chronic ITP; that TAS level decreases significantly in acute ITP; that thiol / disulfide balance is disrupted in favor of disulfide; and that, compared to controls, the thiol / disulfide balance is not disrupted in chronic ITP patients with close to normal platelet counts who do not require treatment. It has been shown that the conversion of native thiol groups to reversible disulfide bonds is increased due to increased oxidative stress in the organism in order to maintain oxidative balance. We believe that showing the increased disulfide / thiol ratios in patients with acute ITP will contribute to elucidation of ITP etiopathogenesis and development of new treatment methods targeting the disulfide / thiol balance in addition to the currently used treatment methods.

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Author contribution

We require that all authors take responsibility for the content of the work submitted. An author contribution section should be given. The contributions of all authors must be described in the following manner: The authors confirm contribution to the paper as follows: study conception and design: YDK, ZCÖ, ÖB; data collection: YDK, ZCÖ; analysis and interpretation of results: YDK, ZCÖ; draft manuscript preparation: YDK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Approval was obtained from Eskişehir Osmangazi University Clinical Researches Ethics Committee (date: 18.04.2018, no: 80558721/50).

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Conflict of interest

No conflict of interest.

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Original Article

Evaluation of medication errors in pediatric patients using antibiotics

Nesligül Özdemir[1], Emre Kara[1], Ayşe Büyükçam[2], Kübra Aykaç[2], Ayçe Çeliker[1], Kutay Demirkan[1], Ateş Kara[2]

[1]Department of Clinical Pharmacy, Hacettepe University Faculty of Pharmacy, Ankara; [2]Department of Pediatric Infectious Diseases, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Medication errors are frequently seen in pediatric patients. Medication error studies on pediatric cases were found to not only be limited but also the collaboration of clinical pharmacists and physicians on this topic was not published in Turkey. This study aimed to identify drug-related problems, especially in antibiotics.

Methods. This study was a point prevalence study with pediatric inpatients that used at least one antibiotic at a pediatric tertiary care reference hospital on November 16, 2016. Medications of patients were evaluated by clinical pharmacists in terms of drug-related problems and by physicians in terms of correct indications.

Results. Eighty-nine hospitalized patients were using antibiotics at the time of the study. The median age was 42 months (range: 1-226 months), and 49 (55.1%) of the patients were male. Clinical pharmacists detected a total of 210 potential drug-drug interactions in 46 (51.7%) patients. Approximately 48.5% of the patients in pediatric wards and 52.4% of the patients in surgical wards had at least one potential drug-drug interaction. A total of 39 medication errors were identified in 36 patients' drug orders. Most of the errors (51.3%) were due to dosing and administration time errors (35.9%). The number of errors per patient in surgical services was higher (0.47) than the pediatric services (0.42). Forty-three percent of errors were antimicrobial-related, and 70.5% of them were classified as dosing errors.

Conclusions. Evaluation of patients' drug usage by a clinical pharmacist in terms of drug-related problems such as drug interactions, side effects and prescribing errors leads to better pharmaceutical care.

Key words: antibacterials, clinical pharmacist, medication errors, pediatrics.

Medication error is defined as 'a failure in the treatment process that leads to or has the potential to lead to harm for the patient'.[1] These errors occur during prescribing, preparing, and administering medications and are frequently seen and increase the likelihood of undesirable effects in patients.[2] In the United States and Europe, medication errors are still a common problem in primary and secondary care centers and they cause high rates of mortality and morbidity.[3]

Many studies evaluating medication errors for adult patients are available in the literature however, the number of studies regarding pediatric cases is limited.[3] Studies have shown that pediatric patients are exposed to 3 times more medication errors than adults.[4] In pediatric patients, the most frequent medication errors are dosing errors which are commonly seen in antibiotic usage.[2,3,5,6] This type of medication error is due to many factors such as a child's age, weight, body surface area, individual differences, and underlying diseases.[3]

✉ Nesligül Özdemir
nesligul.ozdemir@hacettepe.edu.tr

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The development of organs in pediatric patients continues for many years and the organs responsible for drug metabolism and elimination have not yet reached full capacity during neonatal and early infancy. These reasons increase the risk of injury resulting from medication errors compared to adult patients.[7] Therefore, prevention of medication errors in pediatric patients is an important issue. There are too few studies in Turkey regarding the detection of medication errors in pediatric patients.[8-10] However, within our knowledge, there are a few studies conducted by clinical pharmacists and physicians together.[11,12]

The study aims to identify medication errors using drug-related problems classification through a collaboration of clinical pharmacists and physicians in pediatric patients that received at least one antibacterial drug.

Material and Methods

This point prevalence study was conducted at a pediatric tertiary care reference hospital on November 16, 2016. The hospital is a tertiary care reference hospital with 250 pediatric neonatal acute care and intensive care beds and has 215,000 admissions per year. Within the scope of the clinical pharmacy graduate program, clinical pharmacy students have been participating in physician visits in certain services and carrying out clinical pharmacy activities in the hospital since 2014. Ethical approval for this study was obtained for retrospective evaluation of routine clinical pharmacy services performed during rotation in the pediatric infection service with the point prevalence method. The patients taking at least one antibacterial drug on the study day were included in the study. Patients in the pediatric oncology service and bone marrow transplant unit were excluded because of their treatment protocols. Patients' orders were examined in terms of medication errors and analysis and evaluation of errors were done retrospectively. Antimicrobials incorporate antibacterial, antifungal, and antiviral drugs were studied.[13]

Drug indications were evaluated by 3 pediatric infectious diseases physicians whereas drug doses, administration times, and drug-drug interactions were evaluated by 2 clinical pharmacists through the electronic database system, drug orders, and nurse forms. Physicians were informed about drug-related problems identified by clinical pharmacists.

Antibiotic treatments that are not among the first or alternative treatment options in national and international guidelines for the treatment of the patient, or that are not specific to the microorganism, although the causative microorganism has been demonstrated by culture, or that are inappropriate due to patient age or that should not be preferred due to the patient's special conditions (drug reaction or allergy) were considered as not appropriate in terms of indication. The choice of antibiotics, which are not among the first or alternative treatment preferences in international and national treatment recommendations, but are among the antibiotics that can be used for the spectrum of the microorganism and possible diagnosis or the use of age-restricted antibiotic therapies in children with borderline age were considered acceptable in terms of indication.

Appropriateness of doses was evaluated according to the indications in The Harriet Lane Handbook (20th Edition, 2015, Elsevier-Saunders) and as a reference.[14] Updated literature data was also used for the evaluation of doses if necessary from Up-to-date and Nelson Textbook of Medicine 20th Edition.[15,16] For potential drug interactions, the Micromedex Solutions® database 'Drug interactions' section was used. Interactions are rated as contraindicated, major, moderate, and minor. In this database, contraindicated interaction means *the drugs are contraindicated for concurrent use*, major interaction means *the interaction may be life-threatening and/or require medical intervention to minimize or prevent serious adverse effects*, moderate interaction means *the interaction may result in exacerbation of the patient's condition and/or require an alteration in therapy*, minor interaction means *the interaction*

would have limited clinical effects, manifestations may include an increase in the frequency or severity of the side effects but generally would not require a major alteration in therapy.[17] Drug package inserts were also used as a reference for drug interactions.

Medication error is one of the drug related problems and they were evaluated within the scope of the drug-related problems (DRPs). A DRP is defined as an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes by Pharmaceutical Care Network Europe (PCNE).

Drug-related problems were classified according to PCNE classification v7.0.[18] This classification categorizes problems, causes of problems, interventions (the suggestion made by the pharmacist for the solution of the problems), acceptance of interventions (acceptance of the suggestion by the physician), and outcome of interventions (status of implementation of the suggestions). Dosing error is defined as a too low dose (a dose lower than the dose required for the indication) or too high dose (a dose higher than the dose required for the indication or higher than the maximum daily dose for the patient) and drug administration time error is defined as inappropriate timing of administration and/or dosing intervals according to PCNE classification v7.0.

The study was approved by Hacettepe University Ethics Board for Non-Interventional Studies Ethics Committee (date: January 17, 2017, decision number: GO 17/73-25).

Informed consent was not obtained because it was a retrospective observational study, personal data of patients were not used and clinical pharmacists did not make any intervention to patients directly. Following detection of problems, the primary physician of the patient was verbally informed by the clinical pharmacist of the problems and potential solutions determined together with the infectious disease specialist for the detected problems. The physician implemented the suggestions she/he found appropriate (such as dose change, administration time change, drug discontinuation, drug addition).

Mean and standard deviation (SD) were used for data of a normal distribution and median and minimum-maximum were used if the data have a non-normal distribution. A chi-square test was performed to compare categorical variables. Mann-Whitney U test was used for the comparison of continuous variables considering the number of data. SPSS v23.0 was used for statistical analysis.

Results

At the time of the study day, there were 138 patients in the hospital. Eighty-nine (64.4%) of them were on antibiotic treatment during the study and all of them were included in this study. Included patients' median age was 42 months (range 1-226 months) and 49 (55.1%) of them were males.

Twenty-one (23.6%) patients in surgical services (pediatric surgery, neurosurgery, neurosurgery intensive care unit, and cardiovascular surgery); and 68 (76.4%) patients in non-surgical services (pediatric infectious diseases, newborn infectious diseases, hematology, pediatric intensive care unit, adolescent and urology) were evaluated.

Two of these patients (2.2%) were using antibiotics for prophylaxis and all others were on the treatment protocol (Table I). Thirty-two patients (36%) were hospitalized for suspected bacterial infection and 17 patients (19.2%) for bacterial pneumonia according to their primary hospitalization indication.

The total different types of medications in non-surgical services [n=9 (range=1-24)] was significantly higher than surgical services [n = 4 (range=1-13)] (p= 0.032). The median number of antimicrobial drugs used in surgical services was 2 (range=1-3) and in non-surgical services was also 2 (range=1-8). There was no statistically

significant difference between surgical and non-surgical services in terms of the number of antimicrobials (p=0.956) and antibacterial (p=0.909) drugs. Thirty-two (36%) patients were on one, 25 (28.1%) patients were on 2 and 15 (16.9%) patients were on 3 antimicrobial drugs, and the number of antimicrobial drugs ranged from 1 to 8. The most commonly used three antibiotic groups were broad-spectrum penicillins (14.9%), glycopeptides (13.4%), and carbapenems (13.4%) (Table I).

On the day of the study, clinical pharmacists identified 39 medication errors in 36 (40%) patients and the number of DRPs per patient was 1.08 (Table II). Twenty (51.3%) of these errors were due to dosing errors and 14 (35.9%) of them were administration time errors (3 of them were inappropriate dosing intervals; and 11 were inappropriate timing of administration). Seven (35%) of the dosing errors were due to a higher amount per dose administration and the rest of them were lower doses per patient's weight regarding the indication. The number of errors per patient in surgical services was found to be higher (0.47) than the number of errors per patient in non-surgical services (0.42) but this difference was not statistically significant (p>0.05). Forty-three percent of the errors were antimicrobial related, and 70.6% of them were dosing errors, 17.6% of them were timing errors and 11.8% of them were drug-drug interactions.

A total of 210 drug-drug interactions were detected in 46 patients (51.7%). Ninety-one (43.3%) of them were related to antimicrobial drugs in 35 (39.3%) patients. The most common potential interactions were due to fluconazole (23 interactions, 25%), ciprofloxacin (18 interactions, 19%), and amikacin (10 interactions, 11%). Approximately 48.5% of the patients in non-surgical and 52.4% of the patients in surgical services had at least one drug-drug interaction and overall there was no statistical difference between the services (p=0.807). The significance of drug interactions detected in our study is summarized in Table III.

In surgical services, there were 21 patients, indications of antimicrobials in 10 patients (47.6%) were not appropriate, and even though 1 of them (4.8%) was regarded as acceptable based on personal experience of pediatric infectious disease specialist.

In non-surgical pediatric services, 9 (13.2%) patients' antimicrobial indications were not appropriate and 9 (13.2%) patients' indications were considered as acceptable out of 68 patients. The percentage of inappropriate indications in surgical services was significantly higher (p=0.006).

When the drug-related problems were classified according to PCNE classification, 87.2% of the errors were determined as 'the effect of drug

Table I. Indications of antimicrobials.

Diagnoses	Surgical Services, n (%)	Non-Surgical Services, n (%)	Total, n (%)
Blood stream infections	9 (42.8)	23 (33.8)	32 (36.0)
Bacterial pneumonia	4 (19.0)	13 (19.2)	17 (19.2)
Urinary tract infections	3 (14.3)	9 (13.2)	12 (13.5)
Soft tissue infections	3 (14.3)	7 (10.3)	10 (11.2)
Meningitis	1 (4.8)	7 (10.3)	8 (9.0)
Osteomyelitis	1 (4.8)	2 (2.9)	3 (3.4)
Fungal infections	0 (0.0)	2 (2.9)	2 (2.2)
Prophylaxis	0 (0.0)	2 (2.9)	2 (2.2)
Myocarditis	0 (0.0)	1 (1.5)	1 (1.1)
Tuberculosis	0 (0.0)	1 (1.5)	1 (1.1)
Viremia	0 (0.0)	1 (1.5)	1 (1.1)
Total	21 (100)	68 (100)	89 (100)

Table II. Distribution of medication errors.

Medication error type	Surgical Services, n (%)	Non-Surgical Services, n (%)	Total, n (%)
Dosing	8 (80)	12 (41.4)	20 (51.3)
Administration time	0 (0.0)	14 (48.3)	14 (35.8)
Drug-drug interactions	1 (10.0)	2 (6.9)	3 (7.7)
Drug- laboratory interactions	1 (10.0)	0 (0.0)	1 (2.6)
Duplication	0 (0.0)	1 (3.4)	1 (2.6)
Total	10 (25.6)	29 (74.4)	39 (100)

Table III. Number of drug-drug interactions per rates of interaction and per patient.

		Surgical Services, n (%)	Non- Surgical Services, n (%)	Total, n (%)
Contraindicated	Interactions	0 (0.0)	9 (5.0)	9 (4.3)
	Patients	0 (0.0)	7 (7.9)	7 (7.9)
Major	Interactions	12 (41.4)	76 (42.0)	88 (41.9)
	Patients	5 (5.6)	26 (29.2)	31 (34.8)
Moderate	Interactions	11 (37.9)	81 (44.7)	92 (43.8)
	Patients	5 (5.6)	29 (32.6)	34 (38.2)
Minor	Interactions	6 (20.7)	15 (8.3)	21 (10.0)
	Patients	5 (5.6)	9 (10.1)	14 (15.7)

treatment is not optimum' under the problem section, 35% of the problem causes were classified as 'inappropriate drug administration time or interval'. Fifty-one percent of the interventions for preventing errors were 'changing the drug dose', 82% of the interventions were accepted by physicians and fully implemented and 69% of the identified problems were completely resolved (Table IV).

Discussion

In this study, drug orders of pediatric inpatients who were on antibacterial drugs were evaluated by the point prevalence method in terms of medication errors. In a point prevalence study by Grohskopf et al.[19], 54.4% of the patients in pediatric wards and intensive care units were using at least one antimicrobial. In a study conducted by Gerber et al.[20], it was found that 60% of pediatric inpatients were using antimicrobial drugs. Compared with the literature, the antimicrobial drug usage rate was found slightly higher (64.4%) in our study.

In our study, 51.7% of the patients were found to have at least one potential drug interaction. In two other studies conducted on pediatric patients, drug interaction was seen in 45,8% and 49% of the patients, and of those; 0% and 5% of the patients had contraindicated, 10% and 41% of them had major, 28% and 51% of them had moderate, 11% and 39% of them had minor interactions. Also, most of the drug interactions were related to antibiotics (14.8% and 17%).[21,22] In our study antimicrobial drugs accounted for 43.3% of drug interactions and this ratio was quite high compared to the literature.

Forty-seven percent of the patients in the surgical service, 13% of the patients in the pediatric services (21.3% of the patients in total) prescribed antimicrobial drugs were inappropriate for stated indication. In the study of Thiruthopu et al.[23] 53% of the prescribed drugs were found to be 'most appropriate' and the rest were 'eligible'. Compared to the study of Thiruthopu et al.[23] appropriateness of antibiotic indication was higher (78.7% vs 53%) in our study. Ceyhan et al.[24], evaluated the appropriateness of indications for antimicrobials in a point prevalence study including 12 pediatric hospitals and they were found that 54.6% of 1,302 patients had at least

Table IV. Distribution of drug related problems according to PCNE classification V7.0.

Classification	n (%)
Problems	
Effect of drug treatment not optimal	34 (87.1)
No effect of drug treatment/ therapy failure	4 (10.3)
Unnecessary drug-treatment	1 (2.6)
Causes	
Inappropriate timing of administration and/or dosing intervals	14 (35.9)
Drug dose too low	13 (33.3)
Drug dose too high	7 (17.9)
No indication for drug	4 (10.3)
Inappropriate duplication of therapeutic group or active ingredient	1 (2.6)
The Planned Interventions	
Dosage changed	20 (51.3)
Instructions for use changed	15 (38.5)
Prescriber informed only	3 (7.7)
Drug stopped	1 (2.6)
Acceptance of the Intervention proposals	
Intervention accepted and fully implemented	32 (82.1)
Intervention accepted, implementation unknown	5 (12.8)
Intervention not accepted: not feasible	1 (2.6)
Intervention proposed, acceptance unknown	1 (2.6)
Outcome of intervention	
Problem totally solved	27 (69.2)
Problem status unknown[a]	11 (28.2)
No need or possibility to solve problem	1 (2.6)

[a]Problem status unknown because the implementation of the interventions can't be followed or the patients are discharged.

one antimicrobial usage. In 46.7% of patients using antibiotics, an inappropriate indication was detected. The percentage of inappropriate indications in surgical services was found to be higher (80.2%) compared to other services. Compared with the study by Ceyhan et al.[24], the incidence of inappropriate indication was lower in our study in surgical services however it was higher in the surgical services compared to other pediatric services in both studies.

Although only antibacterial prescribed patients were evaluated in our study, at least one medication error was detected in 40% of the patients. Forty-three percent of the errors were related to antimicrobials. The number of medication errors in non-surgical services was found to be higher than in surgical services but the number of medication errors per patient was higher in surgical services. In pediatric patients, drug-related problems are frequently encountered due to many different causes.[25-28] Approximately half of the errors were due to dosing error and 35% of the dosing errors were related to administration time, the rest of them were high or low doses in our study.

The number of patients that had experienced errors during their drug treatment was higher in our study compared to the literature (40% vs 3-37%).[6] In our study, the most frequently observed errors were dose errors and incorrect time of drug administration and they were similar to the literature.[6,26,29] Antimicrobial drug error rates were similar to those of previous studies.[5,30] In other studies, medication errors

were detected mostly in pediatric intensive care units, but in our study, it was seen mostly in non-surgical services.[26,29]

According to PCNE version 7.0, half of our interventions were 'changing the drug dose'. Most of the remaining interventions were related to the modification of the drug instructions. This shows that most of the errors are made in relation to the doses of drugs and necessary precautions must be taken in this regard. To our knowledge and our literature review, the PCNE classification system was used in a small number of studies of pediatric patients in Turkey and our study is among them.

This study has some limitations; firstly, since it was a one-day point prevalence study, the study population was small. There were no control groups. Only, medication errors of patients using antibacterial medications were evaluated. The consequences of errors or interventions on the patient could not be evaluated.

In pediatric patients, medication errors occur frequently. The participation of clinical pharmacists in the multidisciplinary team is beneficial in the detection, prevention, and reduction of medication errors. The PCNE classification system is useful in classifying drug-related problems and clinical pharmacy activities. It will be more meaningful to show the impact of the clinical pharmacist when further studies are conducted with more patients over a longer period of time. It is possible to obtain more accurate and homogenous results by taking all the patients, not only those who use antibacterial.

Ethical approval

Hacettepe University Ethics Board for Noninterventional Clinical Studies approved the study (Approvel date: January 17, 2017, decision number: GO 17/73-25).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NÖ, EK, AB, AÇ, KD, AK; data collection: NÖ, EK, AB, KA; analysis and interpretation of results: NÖ, EK, AB, KA, AÇ, KD, AK; draft manuscript preparation: NÖ, EK, AÇ, KD, AK. 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 are no conflicts of interest.

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Original Article

Tuberculosis risk in the biologic era: tuberculin skin test conversion rates in children with rheumatologic diseases

Canan Özlü[1], Serkan Türkuçar[2], Hatice Karaoğlu Asrak[1], Hatice Adıgüzel Dündar[2], Şevket Erbil Ünsal[2], Nurşen Belet[1]

Divisions of [1]Pediatric Infectious Diseases and [2]Pediatric Rheumotology, Department of Pediatrics, Dokuz Eylul University Faculty of Medicine, İzmir, Turkey.

ABSTRACT

Background. The widespread use of biological treatments has increased the frequency of opportunistic infections such as tuberculosis (TB). The primary objective of our study was to determine the rate of tuberculin skin test (TST) conversion during biological therapy. The secondary objective was to monitor the side effects related to isoniazid (INH) prophylaxis, in the selected subgroup.

Methods. Children with rheumatologic diseases receiving treatment with tumor necrosis factor-alpha (TNF-α) inhibitors, and tocilizumab and canakinumab were included in the study. If baseline screening was negative, TST was performed annually after initiation of biologic therapy. TST conversion was accepted as an increase of at least 6 mm and becoming positive or an increase of 10 mm or more, even in the absence of positivity.

Results. 121 patients (female n: 63, 52%) were included in the study. The mean follow-up period was 26.10±14.8 months. 85 of the patients were using TNF-α inhibitors and 18 tocilizumab, and 18 canakinumab. Forty patients had positive TST before biological agents and received chemoprophylaxis with INH. The rate of TST conversion among the 3 biological agents was not statistically significant (20.4% of TNF-α inhibitors, 25% of canakinumab and 33.3% of tocilizumab users). All patients with LTBI received INH prophylaxis, and none of them had active TB.

Conclusions. There was no statistically significant difference among the three biological agents, regarding the seroconversion rates. Patients receiving tocilizumab and canakinumab should also be screened for TB during follow-up. INH related side effects are rare.

Key words: biological agents, tuberculosis, children, TNF-α inhibitors, tocilizumab, canakinumab.

Tuberculosis (TB) is a common infectious disease worldwide. Up to one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*, 5–10% of infected persons will develop active TB disease over their lifetime. In the remaining cases, the bacteria maintain a dormant state without clinical evidence of active TB disease for many years, namely, latent TB infection (LTBI).[1] The main step in TB elimination is the identification of LTBI and inhibition of reactivation with prophylaxis.[2] The risk for active TB disease after infection depends on several factors, such as increased age and children under five, HIV infection, or other immunosuppressive diseases, and/or drugs like biological agents.[3]

Biological agents provide significant treatment advances in several autoimmune and autoinflammatory diseases in children.[4] Treatment with biologic agents, in particular tumor necrosis factor-alpha (TNF-α) inhibitors, is associated with an increased risk of TB. Therefore, screening and treatment for LTBI in patients receiving TNF-α inhibitors are mandatory.[2] Previous studies from Turkey have reported a 10-20 fold increase in the

✉ Canan Özlü
drcananozlu@gmail.com

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risk of reactivation of LTBI in patients treated with TNF-α inhibitors.[5] Biological agents other than TNF-α inhibitors also cause immunosuppression, and can increase the risk of TB. The use of interleukin (IL)-6/ IL-6 receptor-targeted agents (tocilizumab and siltuximab) is associated with an increased risk of TB similar to that observed in TNF-α inhibitors. European Society of Clinical Microbiology and Infectious Diseases Study Group for Infections in Compromised Hosts recommends TB screening in adult tocilizumab users, like TNF-α inhibitors, but does not recommend of those taking IL-1-targeted (anakinra, canakinumab or rilonacept) agents, as the risk of TB with these drugs is reported as "moderate".[6] The guidelines for TB screening in children using biological agents are only available for TNF-α inhibitors, and there is no recommendation for TB screening in children receiving tocilizumab and canakinumab.

Our national guidelines recommend TB screening in children receiving TNF-α inhibitors with a medical history, chest radiogram, tuberculin skin test (TST) and/or interferon-gamma release assay (IGRA) before the initiation of TNF-α inhibitors and according to these guidelines children with negative baseline screening test should be rescreened annually with TST and/or IGRA.[5] Despite the regular use of this screening test, the prevalence of TB in patients receiving biological agents is higher than that of the general population.[3,7-9] In 2017, Cuomo et al.[3] investigated the rate of TB screening test conversion during biological therapy in rheumatic patients with negative baseline screening. There are only a few adult and child studies that investigated the ratio of TB screening test conversion during anti-TNF therapy in rheumatic patients with negative baseline screening[3,7-13], no studies reported the results of rescreening TB in children treated with canakinumab and tocilizumab.

Our study aimed to determine the rate of TST conversion in children receiving biological agents (TNF-α inhibitors, canakinumab, tocilizumab), and the second aim was monitoring the side effects related to isoniazid (INH) prophylaxis.

Material and Methods

Our study was conducted at Dokuz Eylul University Hospital (a tertiary-care pediatric center), between January 2014 and January 2019. We evaluated all children with rheumatic diseases. One hundred and twenty-one patients who had been given biological agents for at least 12 months, were included in this retrospective study. Patients with a history of TB or LTBI, baseline findings suggestive of old or active TB, previous treatment with anti-TB and biological agent were excluded from the study.

The demographic and clinical characteristics (age, gender, type and duration of the underlying disease, type and drug usage duration of immunosuppressive drugs and biological agent treatment) of the 121 patients were recorded. All patients were routinely screened for TB infection before biological agent initiation. They were questioned for possible TB history (close contacts, signs and symptoms consistent with TB) in their families. Patients were physically examined, TST was performed and a chest radiograph was taken. At initial screening, TST with a <5 mm of induration in immunosuppressed, <10 mm in non-immunosuppressed children were accepted as negative, according to the national guideline for diagnosis and treatment of TB.[5] Patients with LTBI at initial TB screening were given prophylaxis with INH for 9-12 months. In this group, TB screening was continued with clinical evaluation in every three months and radiological evaluation with chest radiograph in every 6 months. Children with negative baseline TB screening were additionally screened with an annual TST. TST conversion was accepted as TST increase of at least 6 mm and becoming positive, or TST increase of 10 mm or more even if the absence of positivity.[5,13]

All children with converted TST were administered prophylaxis with INH (10-20 mg/

kg/day, maximum 300 mg/day) after excluding active TB for 9-12 months.[14] After one month of INH prophylaxis, biological agents were implemented if indicated. Transaminase levels were evaluated every 3 months during INH prophylaxis.

Statistical analyses were performed using SPSS 24.0. Categorical variables were shown as percentages, mean values of continuous variables as normal and ± 2SD (standard deviation) values, and non-normally distributed values as median and quartiles (IQR: interquartile range). The relationship between categorical variables and dependent variables were analyzed by chi-square and Fisher's exact test when chi-square assumptions were not met. Statistical significance limit was accepted as $p < 0.05$

Results

One hundred and twenty-one children (63 females) with rheumatic diseases (mean age: 154.35±51.3 months) were treated with a biological agent . The mean follow-up period was 26.10±14,84 months (median 24; range 12-60 months). Adalimumab was the most commonly prescribed drug (43%), followed by etanercept (21.5%), canakinumab (14,9%) and tocilizumab (14.9%). 114 patients used one biological agent, 7 patients switched to another biological agent due to insufficient clinical response. In all 7 (5.8%) patients, switch was performed to adalimumab from etanercept. Juvenile idiopathic arthritis (JIA) was the most frequent indication for a biological agent treatment 95 (78.5%); other indications were autoinflammatory diseases 17 (14%) and uveitis 7 (0.05%) and one patient had Farber disease. Table I presents the data concerning the demographic, clinical and therapeutic features of the 121 patients.

The prevalence of LTBI in our population at the initiation of biological treatment was 33% (40/121). Forty patients had positive TST results (31 TNF-α inhibitors, 6 canakinumab, 3 tocilizumab) and all positive patients were treated with INH for 9-12 months with a diagnosis of LTBI.

Among 81 children using biological therapy and undergoing rescreening for TB, 54(66.6%) were initially treated with anti-TNF, 12(14.8%) with canakinumab, and 15(18.5%) with tocilizumab. Four (4.9%) children were switched to adalimumab from etanercept. At the time of the initial TB screening of 81 children, 63% of patients were receiving methotrexate (minimum three months duration), 5% steroids (2mg/kg,

Table I. Demographic, clinical and therapeutic characterics at initial evaluation in children receiving biological agents.

Variable	Number of patients n (%)
Male sex	58 (48)
Follow up period, months, median	26.10±14
Positive history of TB contact	3 (0,02)
Disease	
JIA	95
Polyarticular JIA	36
Oligoarticular JIA	25
Systemic JIA	8
Enthesitis-related arthritis	21
JPA	5
Autoinflammatory diseases	17
FMF	12
HIDS	4
CAPS	1
Uveitis	7
Farber Disease	1
Biological agents	
TNF- α inhibitors	85
Adalimumab	52
Etanercept	26
Etanercept+ Adalimumab	7
IL-1 Inhibition (Canakinumab)	18
IL-6 Inhibition (Tosilizumab)	18

JIA: juvenile idiopathic arthritis, JPA: juvenile psoriatic arthritis, FMF: Familial mediterranean fever, HIDS: hyper IgD syndrome, CAPS: cryopyrin-associated periodic syndromes, TNF: tumor necrosis factor, IL:interleukin

maximum 60mg/day after pulse steroid in the last month), 5% both steroids and methotrexate. The characteristics of 81 patients in the study are summarized in Table II.

During the annual screening of TB, 19 (23.4%) cases that were initially TST negative became positive, the time interval was 12-60 months (26.85±13.83). The conversion was observed as 20.4% (11/54) for TNF-α inhibitors and 33.3% (3/12) for canakinumab and 30% (5/15) for tocilizumab. No difference was found between biological agent types in terms of TST conversion rates. (p=0.57) (Fig. 1). None of these patients had symptoms of TB. Active TB was excluded in these converted cases by history, physical examination and chest radiography. All converted patients received INH prophylaxis. No patients developed active TB. Detailed information about seroconverted patients are given in Table III.

The factors that may affect TST conversion were also evaluated. There were statistically no difference between the converters and non-converters in terms of gender (p=0.28), age

Table II. Characteristics of 81 patient (converters and nonconverters).

Characteristics	Total (n:81)	Converters (n:19)	Nonconverters (n:62)	p value
Sex (M/F)	34/47	10/9	24/38	0.28
Age (months), mean ± SD	154.35±51.35	157.30±52.25	153.44±51.47	0.51
Disease duration (months), mean± SD	67.52±51.35	61.69±42.6	69.30±42.32	0.44
Follow-up duration (months), mean± SD	26.85±13.83	31.78±11.40	25.33±14.23	0.17
Type of biologic agents				0.57
TNF- α inhibitors	54	11	43	
Adalimumab	31	6	25	
Etanercept	19	3	16	
Etanercept+ Adalimumab	4	2	2	
IL-1 Inhibition (Canakinumab)	12	3	9	
IL-6 Inhibition (Tosilizumab)	15	5	10	
Concomittant treatment	59	12	47	0.52
Steroid	4	1	3	
Methotrexate	51	9	42	0.28
Steroid+ Methotrexate	4	2	2	

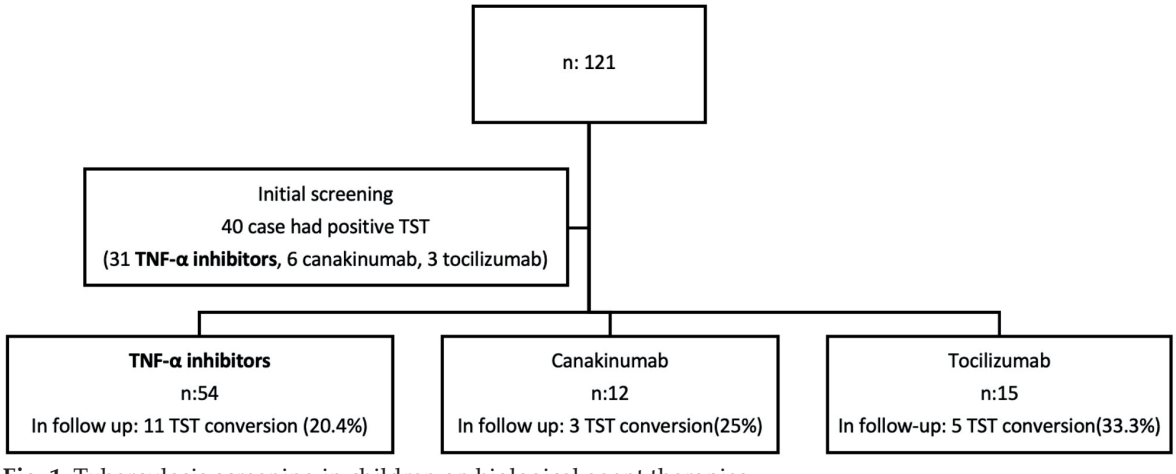

Fig. 1. Tuberculosis screening in children on biological agent therapies.

Table III. Demographics and clinical characteristics of the patients with TST conversion during the use of biological agents.

Case, Sex	Disease	Treatment before biologics	TST value at initial evalution (mm)	Biological agent	Concomittant treatment	Converted TST value (mm)	TST positivity after biologic agent initiation (months)	Chest X-Ray
1, F	ERA	MTX, SSZ	0	Adalimumab	SSZ	13	30	Normal
2, M	ERA	MTX, SSZ	3	Adalimumab	MTX, SSZ	13	36	Normal
3, M	JPA	MTX	0	Adalimumab	MTX	8	42	Normal
4,M	ERA	MTX, SSZ	2	Adalimumab	S	22	30	Normal
5, F	pJIA+ FMF	MTX, SSZ	1	Adalimumab	MTX, SSZ	18	60	
6, M	Uveitis	MTX, S	2	Adalimumab	MTX, S	10	18	Normal
7, F	oJIA	MTX	0	Etanercept	MTX	12	24	Normal
8, M	oJIA+ FMF	MTX	0	Etanercept	C	5	12	Normal
9, F	S+p JIA	MTX, S	0	Etanercept	MTX	11	18	Normal
10, M	JIA+Uveitis	MTX	0	Etanecept/Adalimumab	MTX, S	7	30	Normal
11, F	oJIA	MTX, SSZ	2	Etanecept/Adalimumab	MTX	17	18	Normal
12, F	FMF	C	2	Canakinumab	C	11	24	Normal
13, F	FMF	C	3	Canakinumab	C	10	24	Normal
14, M	FMF	C	3	Canakinumab	C	17	18	Normal
15, M	pJIA	MTX, SSZ	1	Tocilizumab	MTX, SSZ	10	20	Normal
16, M	pJIA	MTX, SSZ	2	Tocilizumab	SSZ	13	12	Normal
17, F	pJIA	MTX	3	Tocilizumab	MTX	10	60	Normal
18, E	Farber	-	1	Tocilizumab	-	16	42	Normal
19, F	pJIA+FMF	MTX, C	0	Tocilizumab	MTX, C	9	15	Normal

ERA: enthesitis-related arthritis, JPA: juvenile psoriatic arthritis, SJIA: systemic juvenile idiopathic arthritis, pJIA: polyarticular juvenile idiopathic arthritis, oJIA: oligoarticular JIA, FMF: Familial mediterranean fever, MTX: methotrexate, S: steroid, C: colchicine, SSZ: sulfasalazine, TST: tuberculin skin test

(p=0.51), type of disease (p=0.46), duration of primary disease (p=0.44), type of biological agent (p=0.57), and concomitant therapy (p=0.52) with steroid, methotrexate, steroid and methotrexate treatment in addition to a biological agent.

A total of 59 patients received prophylaxis with INH. Since 49 of the cases were over 30kg, they received the maximum dose of 300mg INH, while the other ten patients received 10-20mg/kg/day INH. TB disease did not develop in any of the patients who received prophylaxis. One-and-a-half-fold increase in transaminase level was observed in only one patient during INH treatment, it spontaneously regressed, no discontinuation was required.

A 7-year-old girl, who was on canakinumab every 4 weeks for hyperimmune globulin D syndrome (HIDS), developed pneumonia, which did not respond to broad-spectrum antibiotics therapy at the 46th month of canakinumab. Her TST was 0 mm and IGRA was negative. Sputum acid-fast bacilli staining and TB culture were negative. Thorax computed tomography showed lymph nodes at right paratracheal, subcarinal and bilateral hilar, ground-glass opacities and centraciner nodules (budding tree appearance) more prominent in the lower lobes of both lungs. The patient was considered as probable TB and antituberculous treatment was started. The patient improved with antituberculous therapy. Canakinumab was discontinued. In the third month of TB treatment, the patient had to take steroids because of HIDS attack. TB treatment was completed after 12 months. Reactivation was not observed at 24th-month follow-up.

Discussion

This is the first study in literature examining and comparing the conversion rate of TST receiving different types of biological therapies in children. TST conversion rate was 23.5% in this study and no difference was observed in TST conversion rates during the use of different types of biological agents, and TB screening should be performed annually in children using tocilizumab and canakinumab, as in anti-TNF users.

So many guidelines, including our own national guidelines, have recommended repeated TB screening in anti-TNF users. There are as yet few studies investigating the performance of rescreening in patients receiving anti-TNF agents.[13] The rate of conversion ranged from 0-37% for the TST while using anti-TNFs.[8,15-19] In general, the rate of conversion was higher in high-TB-prevalence countries. For example, the conversion rate of TST was 0–12% in low-prevalence countries increasing to 25–37% in high-prevalence areas.[3]

There are only two studies on TST conversion in children using anti-TNF in Turkey. In the study of Acar et al.[11] thirty-two children were given INH treatment for LTBI and 16 (21.9%) of them were started during follow-up. In a study reported by Kılıç et al.[12],14.5% of the patients had been diagnosed as LTBI initially and 4.8% were started on INH during follow-up. The ratio in the first study was similar to ours (20.4%), the second study's ratio was lower attributable to their approach of taking the TST cut-off limit as 10 mm.

No studies have reported TST conversion in children treated with canakinumab and tocilizumab. Cuomo et al.[3] reported TST conversion in tocilizumab users as 15.9%, this rate which was lower than ours (33%), which may be due to BCG vaccination status and differences in prevalence between countries for TB and age groups. Very limited experience has been attained with the use of IL-1-targeted agents in patients with LTBI.[6] In a study from Turkey, it was reported that INH prophylaxis was given in 4 out of 15 children receiving canakinumab for FMF during a 24-month follow-up period because of TST positivity in rescreening.[20] This ratio is similar to ours'(25%).

The reason and clinical significance of this conversion have not been revealed yet. It remains to be clarified whether these results indicate true positive conversion signs of an underlying LTBI, false-positive results or

false-negative initial screening results due to using immunosuppressives such as steroid and methotrexate in children suffering from rheumatic diseases. In the study by Kiray et al.[21] comparing TST response in children with JIA and healthy controls, TST positivity was seen at a lower rate in children with JIA compared to healthy controls due to use of immunosuppressives.

Risk factors that may cause TB test conversion in children using biological agents were evaluated. Longer disease duration, male gender, and older age have been shown to increase TST conversion rates[3,10], but we determined that age, gender, disease type, duration of primary disease, and biological agent type do not affect TST conversion rates.

Our second conclusion from this study is that the rate of transaminase elevation, which can be seen as a side effect due to INH, is low. INH-induced transaminase elevation has been shown in patients using TNF-α inhibitors without severe permanent liver damage.[22] Mutlu et al.[23] reported that hepatotoxicity rate related to INH prophylaxis was 17.3% in 196 adult patients receiving TNF-α inhibitors and INH treatment was discontinued due to progressive hepatotoxicity in 5% of cases. Hepatotoxicity rate was 1.3% due to INH in children receiving TNF-α inhibitors.[11] In this study, one (0.08%) patient had an increase in transaminase that did not require treatment interruption during INH prophylaxis and liver enzymes regressed in follow-up.

Finally, this study has some limitations and results need to be evaluated in this context. Limitations are its retrospective design and relatively small number of patients. All risk factors of TB (vitamin D status, malnutrition, etc) have not been studied. IGRA test was lacking because of its high cost. However, this study is important because it is the first study evaluating the TST conversion rates in pediatric cases using different types of biological agents. Further studies with larger sample sizes are needed to confirm our findings.

In conclusion, TB should be screened not only for those using TNF-α inhibitors but also for other biological agents (canakinumab, tocilizumab) in children with rheumatological disease, especially in an intermediate TB burden area such as Turkey. New TB monitoring guidelines should be established during biological therapy.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: CÖ; data collection: CÖ, HKA, HAD, ST; analysis and interpretation of results: CÖ, HKA; draft manuscript preparation: CÖ, NB, ŞEÜ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Approved by Dokuz Eylül University Medical Faculty Non-invasive Ethical Committee with the decision number 2021/03-46.

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Conflict of interest

There is no conflict of interests.

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Original Article

Could plasma based therapies still be considered in selected cases with atypical hemolytic uremic syndrome?

Sare Gülfem Özlü[1], Bora Gülhan[2], Özlem Aydoğ[3], Emine Atayar[4], Ali Delibaş[5], Gönül Parmaksız[6], Elif Bahat Özdoğan[7], Elif Çomak[8], Mehmet Taşdemir[9], Banu Acar[10], Zeynep Birsin Özçakar[11], Rezan Topaloğlu[2], Oğuz Söylemezoğlu[12], Fatih Özaltın[2,4]

[1]Department of Pediatric Nephrology, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara; [2]Department of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara; [3]Department of Pediatric Nephrology, Ondokuz Mayıs University Faculty Of Medicine, Samsun; [4]Nephrogenetics Laboratory, Department of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara; [5]Department of Pediatric Nephrology, Mersin University Faculty of Medicine, Mersin; [6]Department of Pediatric Nephrology, Adana Başkent University Faculty of Medicine, Adana; [7]Department of Pediatric Nephrology, Karadeniz Technical University Faculty of Medicine, Trabzon; [8]Department of Pediatric Nephrology, Akdeniz University Faculty of Medicine, Antalya; [9]Department of Pediatric Nephrology, Koc University Faculty of Medicine, İstanbul; [10]Department of Pediatric Nephrology and Rheumatology, University of Health Sciences Ankara City Hospital, Ankara; [11]Department of Pediatric Nephrology, Ankara University Faculty of Medicine, Ankara; [12]Department of Pediatric Nephrology, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Atypical hemolytic uremic syndrome (aHUS) occurs due to defective regulation of the alternative complement pathway (ACP) on vascular endothelial cells. Plasma based therapy (PT) was the mainstay of the treatment for aHUS for many years until the introduction of therapies targeting blockage of the complement system. The aim of this study was to evaluate patients with aHUS who had been treated with plasma based therapies alone.

Methods. The outcomes of seven genetically confirmed aHUS patients (2 girls, 5 males) were evaluated by means of clinical presentation, response to plasma therapy, course of the disease during the follow-up period and last status.

Results. The median age of the patients at admission was 6.7 years (IQR 0.7-7.8). Three patients received plasma exchange therapy and the other four patients were treated with plasma infusions. One patient was lost to follow-up after one year; the median duration of follow-up for other patients was 3.7 years (IQR 2.7-6.5). During the follow up, two patients from our historical records when complement blocking therapies had not been in clinical use yet in Turkey, underwent kidney transplantation. One transplant patient experienced an acute rejection episode without graft loss. The remaining five patients had a glomerular filtration rate of more than 90 ml/min./1.73 m^2 at the last visit.

Conclusion. Although we had a relatively small patient population, our findings indicate that PT might still be considered in selected patients particularly in countries where complement blocking therapies are difficult to reach due to their unavailability or costs that are not covered by the health care systems.

Key words: atypical hemolytic uremic syndrome, treatment, plasma infusion, plasma exchange, outcome.

Atypical hemolytic uremic syndrome (aHUS) is an uncommon disease which is characterized with Coombs negative hemolytic anemia, thrombocytopenia and acute kidney injury.[1,2] Dysregulation of the alternative complement pathway (ACP) plays a major role in its pathogenesis. Pathogenic variations in genes encoding complement factor H (CFH), complement factor I (CFI), complement factor B

✉ Sare Gülfem Özlü - sgozlu@ybu.edu.tr
Fatih Ozaltin - fozaltin@hacettepe.edu.tr

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(CFB), complement 3 (C3), membrane cofactor protein (MCP) and complement factor H related (CFHR) proteins1-5 as well as diacyl-glycerol kinase-ε (DGKE), thrombomodulin (THBD), plasminogen, and autoantibodies against CFH are identified in approximately 60-70% of the patients.[2-5]

Plasma based therapy (PT) was the mainstay of the treatment for many years. This therapy provides normal complement proteins through plasma infusion (PI) or removes mutant proteins or autoantibodies through plasma exchange (PE). The efficacy of PT is mainly based on expert consensus, anecdotal reports or retrospective studies.[3,6,7] Although eculizumab, a monoclonal antibody for terminal complement inhibition, has been suggested as a first line therapy for the management of aHUS in recent years, the main limitations of eculizumab are its cost and unavailability in some countries. Therefore, studies are needed to offer alternative approaches. One of them would be PT and therefore place and efficacy of this approach in aHUS patients at the acute stage of the disease should be revisited especially in countries where resources are limited and access to eculizumab is difficult.[3,7]

In the present study, we aimed to evaluate clinical features, response to treatment and outcome of patients with aHUS associated with genetic abnormalities who had been treated with PT alone.

Material and Methods

Patients

In November 2013, the Turkish aHUS registry was established with the participation of 26 pediatric nephrology centers in Turkey to collect information on the demographic, clinical, laboratory and genetic features of pediatric aHUS patients. Management strategies, prognosis and drug safety were also recorded. The registry included a prospective collection of pediatric aHUS patients (i.e. those who are less than 18 years at the time of disease onset) and was updated every 3 months in terms of treatment, complications and outcome.[8]

Diagnosis of aHUS was based on Coombs negative microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Hemolytic anemia was defined as a level of hemoglobin (Hb) less than 10 g/dl and a presence of schistocytes on peripheral smear. Thrombocytopenia was defined as a platelet count of less than 150,000/mm^3. Patients who have Shiga toxin producing E. coli (STEC) infection or other specific infectious diseases and patients with co-existing diseases or drug-related HUS were excluded from the registry. ADAMTS13 activity was screened in all patients and 10% and above activity was considered to be normal. Serum creatinine was measured using the Jaffe method and estimated glomerular filtration rate (eGFR) was calculated using the new Schwartz formula.[9] Proteinuria was defined as ≥ 1+ by urine dipstick test and/or a spot urine protein/creatinine ratio ≥ 0.5 mg/mg in children aged 6-24 months and ≥ 0.2 mg/mg in children older than 24 months of age.[10] Oliguria was defined as urine output <0.5 ml/kg/h or < 500 ml/day/1.73m^2 after immediate neonatal period. Chronic kidney disease (CKD) was defined and staged according to the guidelines of KDOQI (Kidney Disease Outcomes Quality Initiative.).[11]

Renal remission was defined as having an eGFR >90 ml/min./1.73m^2. Hematological remission was defined as having a hemoglobin level of more than 10 g/dl without hemolysis, a platelet count of more than 150,000/mm^3, and a normal lactate dehydrogenase level (<450 U/L). Complete remission was defined as having both hematological and renal remission. Renal failure with complete hematological recovery was considered as partial remission. The presence of proteinuria and/or hypertension and/or eGFR ≤89 ml/min./1.73m^2 for more than a duration of 3 months was defined as renal sequelae.

Genetic analysis

For those patients and/or parents who gave informed consent for genetic screening,

mutational analyses via Sanger sequencing for *CFH, CFI, MCP, CFB, C3, DGKE* and *CHFR5* were carried out at the Nephrogenetics Laboratory of Hacettepe University. *CFHR1-3* deletion was evaluated via multiplex ligation-dependent probe amplification (MLPA) analysis.

Anti-complement factor H autoantibody was searched using the CFH IgG ELISA Kit (Abnova™), according to the manufacturer's recommendations (detection limit 0.6 AU/mL).

The Institutional Ethics Committee of Hacettepe University approved the study on May 2011 (FON10/03-22). Written informed consent was obtained from the parents of each patient.

Statistical analysis

Data were analyzed by using SPSS v.21 (SPSS Inc. Chicago, II, USA). Demographics and clinical data were evaluated with descriptive statistical analysis methods. The mean, median, standard deviation and interquartile range (IQR) were calculated for the numeric variables.

Results

Patient characteristics

Seven patients (5 males, 2 females) with a defined underlying genetic abnormality were included in the study (Table I). Five patients had variations in complement regulatory genes [*CFB* (n=2), *CFH* (n=1), *CD46* (MCP) (n=1), *C3* (n=1)], one patient had a *DGKE* variation and one patient had anti-CFH antibody associated with a homozygous *CFHR1-3* deletion. The median age was 6.7 years (IQR 0.7-7.8). Except for patients #2, #5 and #6, the remaining were diagnosed before the availability of eculizumab in Turkey. None of the patients had a family history of aHUS. Three had parental consanguinity. Diarrhea was not present before the onset of the symptoms. At the time of diagnosis, eGFR was less than 90 ml/min./1.73m^2 in all patients. Two patients with *CFB* variation and one with *MCP* variation had a normal urine output. Patient #7 (with anti-*CFH* antibody/*CFHR1-3* deletion) had neurological involvement characterized by seizures during follow-up.

All patients had hypocomplementemia and all but patient #2 (with *CFB* variation) had varying degrees of proteinuria and hypertension at admission. Demographic variables, clinical and laboratory features, genetic results are summarized in Table I.

Treatment

PE or PI was started on the day of diagnosis in all patients (Table I). Patient #2 was diagnosed after eculizumab approval in Turkey; he was given eculizumab at the time of diagnosis but due to severe anaphylaxis the treatment had to be continued with PE. Overall, patients #2 (with *CFB* variation), patient #3 (with *CFH* variation) and patient #4 (with *MCP* variation) underwent PE with each session of 40-60 ml/kg plasma and four patients received PIs (10-20 ml/kg/day); two received 7 and two received 8 infusions. Peritoneal dialysis (PD) was started in patient #6 (with *DGKE* variation) and patient #7 (with CFH antibody/*CFHR1-3* deletion) and hemodialysis was started in patient #3 (with *CFH* variation). Antihypertensive drugs were administered to all patients (Table I).

Outcome

Patient #5 (with *C3* variation) was lost to follow-up after one year however at the time of the last visit, she had an eGFR of 158 ml/min./1.73m^2. Median follow up duration of the other six patients was 3.7 years (IQR 2.72-6.47). All patients were in hematological remission at the time of discharge. Patient #3 (with *CFH* variation) was discharged with hemodialysis and patient #7 (with anti-CFH antibody/*CFHR1-3* deletion) was followed up on PD. Patient #3 underwent kidney transplantation from a cadaveric donor two years after diagnosis. On the fifth year of kidney transplantation, she suffered from antibody mediated rejection due to incompliance to immunosuppressive medications. She was treated with plasmapheresis and intravenous immune globulin to treat this rejection episode

Table I. Clinical and laboratory features at presentation, genetic results, treatment and outcomes of the patients.

Parameters	Patients						
	1	2	3	4	5	6	7
Genetic abnormality	CFB (Val42Ala; Het.)	CFB (Asp133Asn; Het.)	CFH (Asn1050Tyr; Het.)	MCP (Glu179Gln; Hom.)	C3 c.1976-6C>T (splice site) (Het.)	DGKE (p.Ser41Metfs*2; Hom.)	CFHR1-3 deletion and antiCFH antibody (Hom.)
Age at diagnosis	7 mo.	8 yrs. 5 mo.	4 yrs. 8 mo.	7 yrs. 4 mo.	4 yrs. 4 mo.	5 mo.	4 yrs. 2 mo.
Gender	M	M	F	M	F	M	M
Consanguinity	No	No	No	Yes	Yes	Yes	No
Family history	No	No	No	No	No	No	No
Diarrhea at diagnosis	No	No	No	No	No	No	No
URTI	No	Yes	No	No	Yes	Yes	No
Urine output	Normal	Normal	Oliguria	Normal	Oliguria	Oliguria	Oliguria
eGFR (ml/min./1.73 m^2)	54	59	5.9	69.3	36.5	31.5	21
Hemoglobin (g/dl)	8.7	8.2	8.3	7.3	6.9	6.6	8.8
Platelets (/mm^3)	85,000	43,000	79,000	51,000	46,000	96,000	60,000
LDH (U/L) (N<450 U/L)	1,230	1,575	1,103	2,159	3,334	2,728	5,260
Hypocomplementemia*	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Proteinuria		No	Yes	Yes	Yes	Yes	Yes
Plasma exchange	No	Yes (3 sessions)	Yes (6 sessions)	Yes (3 sessions)	No	No	No
Plasma infusion (10 ml/kg)	Yes (8 times)	No	No	No	Yes (7 times)	Yes (8 times)	Yes (7 times)
Dialysis	No	No	Yes (HD)	No	No	Yes (PD)	Yes (PD)
Duration of follow up	10 yrs.7 mo.	4 yrs. 2 mo.	5 yrs.,2 mo.	3 yrs. 4 mo.	1 yr. 7 mo. (lost to follow-up)	7 mo.	4 yrs. 2 mo.
eGFR at last visit (ml/min./1.73 m^2)	150	124	17.7	140	158	90	124
Hypertension at last visit (current treatment)	Yes (perindopril)	Yes (enalapril)	Yes (propranolol, enalapril)	No	No	Yes (propranolol, amlodipine, enalapril)	Yes (enalapril)
Proteinuria at last visit	No	Trace	Yes (1+)	No	No	Yes (3+)	No
RRT at last visit	No	No	Transplantation	No	No	No	Transplantation

C3: complement C3, CFB: complement factor B, CFH: complement factor H, CFHR1-3: CFH-related 1-3, DGKE: diacyl-glycerol kinase-epsilon, eGFR: estimated glomerular filtration rate, HD: hemodialysis, Het.: heterozygous, Hom.: homozygous, LDH: lactate dehydrogenase, MCP: membrane cofactor protein, PD: peritoneal dialysis, RRT: renal replacement therapy, URTI: upper respiratory tract infection.
(*Hypocomplementemia is defined as a serum C3 level <88 mg/dl)

and then was followed up with conservative therapies for CKD. Patient #7 (with anti-CFH antibody and *CFHR1-3* deletion) underwent kidney transplantation seven years after diagnosis and he is in remission for two years. All but patient #3 had an eGFR > 90 ml/min./1.73 m^2. All but patients # 4 and #5 (with *MCP* and *C3* variation, respectively) received antihypertensive treatment. Proteinuria was detected in patients # 2, 3 and 6 (with *CFB*, *CFH* and *DGKE* variation, respectively). All patients were in hematological remission and never experienced aHUS episode again during the follow-up duration. Outcome of the patients are summarized in Table I.

Discussion

Plasma based therapies had been used for aHUS for approximately 40 years and their efficacies were demonstrated in thrombotic thrombocytopenic purpura.[12] Recently, it has been suggested that response to PT was in part related to the genetic background of the patient and the most favorable response to PT were reported in patients with anti-CFH antibody and *MCP* mutations both in short term and long term.[13,14] Although short term results were acceptable in patients with *CFH* variations, the risk of end stage renal disease (ESRD) or death was reported as 70-80% at the end of one year.[14] Patients with *CFB* and *CFI* variations were reported to be poor responders in these studies.[13,14] After the introduction of complement blocking therapies, there was a sudden change in management that had been in use for 40 years.[15,16] Complement blocking therapies are effective but also do bring significant risks that could be life-threatening including severe infections and allergic reactions. In addition, these drugs remain one of the most expensive drugs world-wide and there is no certain knowledge about how long they should be administered. There are still difficulties to reach these drugs or reimbursement in the healthcare systems in some countries. Therefore, alternative approaches are certainly needed and research on these approaches should be encouraged. This would open a new avenue into individualized treatment in patients with aHUS. In this context, we aimed to evaluate the outcome of seven patients diagnosed with aHUS who had been treated with plasma therapy alone in the present study.

In our study, one patient with *CFHR1-3* deletion/anti CFH antibody and one with *CFH* variation developed ESRD. Both of them were diagnosed with aHUS before eculizumab was approved in Turkey. The patient with *CFHR1-3* deletion/anti-CFH antibody was diagnosed with ESRD soon after the diagnosis and was followed up on PD, underwent kidney transplantation seven years after the first admission. Previous reports have suggested a favorable outcome with a 75% remission rate in patients with CFH antibodies who were treated with PT and additional immunosuppression.[13,14] Recently, it has been reported that outcomes for patients with anti-CFH antibodies were marginally inferior to those without antibodies.[17] Gurjar et al.[18] have reported that some patients with *CFHR1-3* deletion do not have anti-CFH antibodies which led them to suggest that homozygous *CFHR1-3* deletion may also contribute to aHUS antibody-independent mechanisms. Our patient had a worse clinical course than expected which led us to speculate that the co-existence of CFHR1/3 deletion and anti-CFH antibody might have had an additional negative effect on the outcome of the disease. As to the patient with *CFH* variation with poor prognosis she never responded to PE and soon after the disease onset she developed ESRD and underwent kidney transplantation two years after diagnosis. Although PEs have been reported to be effective even in patients with severe renal impairment, the overall prognosis of *CFH* variations is not favorable.[18-20] Davin et al.[21] reported that progression to ESRD was associated with an elevated plasma creatinine level at presentation. In our patient with *CFH* variation, unfavorable prognosis despite duly and intensive PEs could be related to significantly impaired renal functions at admission. Therefore, we think that in both of these patients, complement blocking therapy

(i.e. eculizumab) would also have been ineffective due to very low GFR at presentation even if we had had an opportunity to use it. This observation would confirm once again the fact that sustained low GFR at presentation should be considered a poor long-term prognostic factor in patients with aHUS.

It has been reported that DGKE-HUS patients present with a slowly progressing proteinuric nephropathy and 80% of the patients did not have ESRD at the end of 10 years.[22] In that study, 29 out of 35 aHUS patients were treated with immunosuppression, eculizumab, or PTs at any time of disease course. Sixteen patients were treated with PT and acute improvement was attributed to this management in 10 of them. The authors suggested that the link between DGKE deficiency and the complement cascade was not yet clearly described so therapies targeting complement cascade might not be of benefit.[22] As reported in most of the patients with DGKE-HUS in the literature, our patient with *DGKE* variation was diagnosed in the first year of his life.[4,22,23] At the time of diagnosis, he had oliguria, hypertension and massive proteinuria. He received PIs and underwent PD for 15 days. At his last follow up visit, 7 months after diagnosis he was hypertensive and had 3+ proteinuria and a eGFR of 90 ml/min./1.73 m^2.

CFB variations account for 1-4 % of aHUS cases and have been reported to have a poor prognosis.[14] In contrast to this report, two patients with *CFB* variation in our study had favorable outcomes. One of them (patient #2) was diagnosed after eculizumab approval in Turkey so he was given eculizumab. Because of severe anaphylaxis that developed during the first infusion, we had to switch treatment to PE. The other patient with *CFB* variation (patient #1) received only PIs. Both patients had favorable long-term outcomes. Given the fact that *CFB* variation is a rare cause of aHUS, a clear genotype-phenotype correlation is not possible however it is also plausible that there might be additional modifying factors that would affect the prognosis in CFB-related aHUS.

C3 pathogenic variations that lead to aHUS are also rare and the prognosis in this group is poor with a reported rate of 60 % for ESRD or death at the end of 1 year.[14,24] In a recent report from Japan, the authors reported that *C3* variations were the most frequent genetic abnormality with a rate of 31 % in their cohort. They also reported a remission rate of 92% on the last follow up visit in these patients.[25] In line with these findings, our patient with *C3* variation also exhibited a favorable outcome with complete remission through PT alone.

MCP variations are related to a good prognosis despite frequent relapses.[13,14,26-28] Our patient was treated with three sessions of PEs, he was discharged with renal and hematological remission and he was in complete remission at the last visit. Interestingly, Caprioli et al.[26] have reported a remission rate of 91% in those patients with *MCP* variations who were treated by plasma and 100% who were not and they have concluded that this could be attributed to the fact of MCP, which is a membrane-bound protein. We think that management should be individualized in patients with *MCP* variations given the fact that some patients may manifest extensive microvascular thrombosis and severe hypertension and numerous cases would benefit from PTs.

In 2012, soon after approval by FDA, eculizumab was also available in Turkey. It is a promising life-long therapy for aHUS and the 2016 consensus report suggests that first-line and early treatment is effective and safe in children with aHUS for renal recovery.[2] However, debates still exist for its lifelong administration. It is one of the most expensive drugs in the world; yearly treatment cost per adult patient based on the dosing regimen of administration is reported to be €327.600.[29] There is no published data regarding its cost in children however as of today its yearly cost for a 30kg patient has been estimated as €323.000 in Turkey. Moreover, the risk of development of severe meningococcal infection, possibility of immune mediated drug reactions and severe anaphylaxis as developed in one of our patients

cannot be underestimated.[30,31] Therefore, the efficacy of alternative approaches in aHUS associated with specific complement variations should be explored. In this aspect, our study is of importance in terms of revisiting ancient and ancillary treatment options.

In conclusion, short- and long-term management of patients with aHUS should be tailored individually considering the patient's clinical course, underlying genetic abnormality and current conditions of healthcare systems of countries. We also believe that in countries where eculizumab is difficult to attain or resources are limited; PTs should still be a reasonable alternative in aHUS patients. Although this is a small-sized study, our results indicate that in selected cases plasma based therapies should still be kept in mind for the management of aHUS and more research on the efficacy of these approaches should be promoted.

Author contribution

Study conception and design: OS, FÖ; data collection: SGÖ, AD, GP, BÇA, ÖA, EÇ, ZBÖ, EBÖ, MT; analysis and interpretation of the results: FÖ, BG, SGÖ, ZBÖ, EA; draft manuscript preparation: SGÖ, BG; critically revision of the manuscript: FÖ, OS, RT. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The Institutional Ethics committee of Hacettepe University approved the study on May 2011 (FON10/03-22).

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Conflict of interest

The authors declare no conflict of interest.

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Original Article

Microbiological characteristics and outcomes of children with pleural empyema admitted to a tertiary hospital in southeast China, 2009-2018

Xueya Zhang, Hailin Zhang

Department of Pediatric Respiratory, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

ABSTRACT

Background. Pleural empyema is one of the most serious and life-threatening types of infection in children. The aim of this study was to describe the microbiological characteristics and outcomes of children with pleural empyema.

Methods. A retrospective review was conducted of the medical records of 63 children admitted to a tertiary hospital in China with pleural empyema between January 2009 and December 2018.

Results. The children had a median age of 1 year (range: 2 months to 16 years) and 33 (52.4%) were female. Bacterial isolates included *Staphylococcus aureus* (n=15, 23.8%), *Streptococcus pneumoniae* (n=10, 15.9%), *Pseudomonas aeruginosa* (n=7, 11.1%), *Escherichia coli* (n=2, 3.2%), *Burkholderia cepacia* (n=2, 3.2%), *Enterobacter cloacae* (n=1, 1.6%), *Klebsiella pneumoniae* (n=1, 1.6%), and *Streptococcus constellation* (n=1, 1.6%). All 15 *Staphylococcus aureus* isolates were found to be resistant to penicillin, and the rate of methicillin-resistant *Staphylococcus aureus* was high (66.7%,10/15). Overall, 5 of 10 *Streptococcus pneumoniae* isolates were susceptible to penicillin. Each *Staphylococcus aureus* and *Streptococcus pneumoniae* isolate showed susceptibility to vancomycin. Ceftazidime was effective against all *Pseudomonas aeruginosa* isolates. Of the 63 children, 60 improved, no one died.

Conclusions. *Staphylococcus aureus* and *Streptococcus pneumoniae* were the leading cause of pleural empyema. Antimicrobial susceptibility testing revealed a high percentage of resistance against penicillin while vancomycin provided 100% coverage for these pathogens. *Pseudomonas aeruginosa* is the third most common pathogen mainly detected in those under 3 years old in the summer and have shown to be susceptible to ceftazidime. The prognosis is good after appropriate therapy.

Key words: children, empyema, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia*.

Empyema, defined as the presence of pus in the pleural cavity, is a serious infectious condition with high morbidity and a 15–20% mortality.[1-3] The incidence of empyema in children has varied in recent decades. During 2006, an estimated total of 2,898 hospitalizations of children aged ≤18 years in the USA were due to empyema. The empyema-associated hospitalization rate was estimated at 3.7 per 100,000 children in 2006, compared to 2.2 per 100,000 in 1997.[4] Liese et al.[5] estimated the annual incidence of pediatric parapneumonic pleural effusion and pleural empyema hospitalizations in a nationwide surveillance study and found it to be 18.4 in 2010, which then decreased to 13.7 in 2013, and increased again to 17.3 in 2015 per million children. Generally, early and appropriate antibiotic therapy in children with pneumonia will avoid the development of empyema and its progression. Confirming the predominant pathogen of empyema is important to guide antimicrobial therapy. The bacteriology of pleural infection has changed over time. Recent data demonstrates that the distribution of pathogens causing empyema

✉ Hailin Zhang
zhlwz97@hotmail.com

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differs according to geographical region.[6] Following the introduction of the pneumococcal conjugate vaccines (PCVs), the incidence of empyema in children has changed.[5] This variability according to time period and region has implications for treatment. It is important for clinicians to remain informed of the local bacteriology of empyema in order to inform their choice of antibiotic treatment.

The primary objective of the present study was to describe the microbiological characteristics and outcomes of children with pleural empyema admitted to a tertiary hospital in Wenzhou, China, in order to provide a source of reference for empiric antibiotic therapy.

Material and Methods

The study was approved by the Institutional Review Board of The Second Affiliated Hospital of Wenzhou Medical University (Protocol LCKY2019-199).

Patients

We retrospectively reviewed the clinical data of 63 children aged ≤18 years who had been admitted to the hospital with pleural empyema between 1 January 2009 and 31 December 2018. The inclusion criteria were as follows: (1) Children were aged 1month to 18 years; (2) clinical symptoms of infection, including fever, cough, shortness of breath and other clinical manifestations were confirmed; (3) chest X-ray, computed tomography (CT) or ultrasound scan of the chest provided evidence of pleural effusion; (4) with any of the following additional findings: i) pus aspirated from the pleural space, and/or a positive Gram stain/culture of pleural fluid; ii) pleural fluid with a pH of <7.2, lactate dehydrogenase >1,000 IU/L, glucose<40 mg/dL, and/or a WBC count of ≥50,000 cells/μL; iii) necessary for surgical decortication. Surgical and pathology reports were reviewed to confirm the diagnosis of empyema.[7-9] Children with pleural empyema caused by trauma, surgery, tuberculous pleurisy, or carcinomatous pleuritis were excluded. For the purpose of evaluating the bacteriology of pleural empyema, we assessed the microbiological findings according to the age group. The 63 children were divided into two age groups: <3 years or ≥3 years old. Also, the 63 children were divided into two groups according to the date of the episode: in the first 5 years or in the last 5 years of the study.

Data collection

Data on the children's age, sex, underlying disease, date of the episode, laboratory data, microbiological findings, antimicrobial susceptibility testing, treatments and outcomes were extracted from electronic medical records. Information about vaccination including influenza, conjugated Haemophilus influenza type b (Hib) and conjugated pneumococcal vaccine (PCV) was collected. Haemophilus influenza type b and the 7-pneumococcal conjugate vaccine (PCV7) had been introduced to China in 1999 and 2008,respectively. All of the vaccines were considered as the second-class vaccine. The PCV7 was replaced with the 13-valent pneumococcal conjugate vaccine (PCV13) in 2016.

Microbiological methods

We also extracted data on the children's microbiology results, blood and pleural fluid cultures were carried out on admission. Bacterial cultures were performed according to standard microbiological methods. Blood and pleural fluid cultures were carried out using BD BACTEC Peds Plus/F vials in the BACTEC system (Becton, Dickinson and Company, Sparks, MD, USA). Confirmation of the species was performed by the VITEK 2 Advanced Expert System (bioMérieux, Marcy-l'Étoile, France). Susceptibility testing of cefoxitin, penicillin, ampicillin, gentamicin, rifampicin, erythromycin, clindamycin, tetracycline, levofloxacin, trimethoprim sulfamethoxazole (TMP-SMZ), cefuroxime, cefotaxime, amoxicillin clavulanic acid, amikacin, ceftazidime, cefepime, ciprofloxacin, aztreonam, imipenem, piperacillin, piperacillin-tazobactam, cefoperazone-sulbactam, tobramycin,

meropenem and ticarcillin clavulanic acid were performed using the disc-diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The minimum inhibitory concentrations (MICs) of vancomycin were determined with a gradient method (Etest, bioMérieux, Marcy-l'Étoile, France). Methicillin-resistant *Staphylococcus aureus* (MRSA) was defined as isolates of *Staphylococcus aureus* that were cefoxitin -resistant by the disc-diffusion method.

Detection of viral pathogens from nasal swabs/ washes or tracheal aspirates was carried out on admission. Samples were examined by direct immunofluorescence assays (DIAs) for respiratory syncytial virus, adenovirus, influenza A, influenza B and parainfluenza I, II and III. *Mycoplasma pneumoniae* antibody was detected by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Counts and percentages were used for categorical variables, and medians were used for continuous variables with a non-symmetrical distribution. Chi squared tests or Fisher's exact test were used to compare categorical variables. P values <0.05 were considered to be significantly significant. Statistical analysis was performed using SPSS for Windows, Version 19.0 (SPSS, Chicago, IL, USA).

Results

Patient characteristics

A total of 63 children were enrolled in the study, of whom 33 (52.4%) were female. Children's median age was one year (range: 2 months to 16 years). Of the 63 children, 40 (63.5%) were aged <3 years (Group 1), and 23 (36.5%) were aged over 3 years (Group 2). Forty-two (66.7%) children were admitted in the winter or the spring (from December to May) and 21 (33.3%) were admitted in the summer or the autumn (from June to November). (Fig. 1). Three children were fully vaccinated with PCV7 and one child received one dose of PCV13. Three children were vaccinated with influenza, while five children were fully vaccinated with Hib. Of the participants 39 were treated with intravenous antibiotics and only 4 children did not receive antibiotic treatment before admission. Patient characteristics are shown in Table I.

Microbiology

Of the 57 children with a pleural fluid culture result available, 29 (50.9%) had a positive culture. Of the 51 children with a blood culture result available, 10 (19.6%) had a positive culture. *Staphylococcus aureus, Streptococcus pneumonia*e and *Pseudomonas aeruginosa* grew in the blood culture in five, three and two patients, respectively. The blood culture results and pleural fluid culture results were both positive in four patients and they were compatible. *Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were the main pathogens. Antimicrobial susceptibility data is presented in Table II. All 15 *Staphylococcus aureus* isolates were found to be resistant to penicillin, and the rate of methicillin-resistant *Staphylococcus aureus* was high (66.7%,10/15). Overall, 5 of 10 *Streptococcus pneumoniae* isolates were susceptible to penicillin. Each *Staphylococcus aureus* and *Streptococcus pneumoniae* isolate showed 100% susceptibility

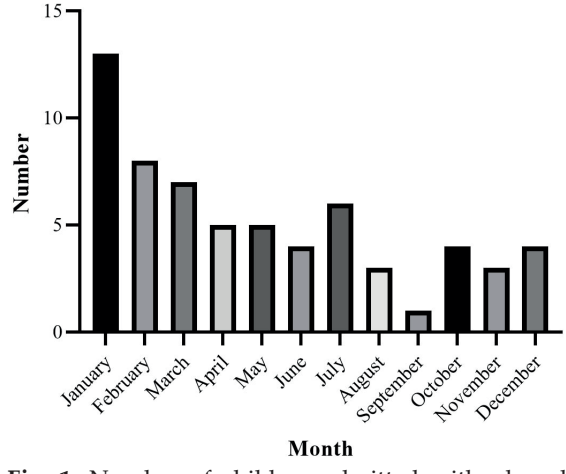

Fig. 1. Number of children admitted with pleural empyema by month, January 2009 to December 2018 (*n* = 63)

Table I. Patient characteristics.

Patient characteristics	<3 years old n=40 n (%)	≥3 years old n=23 n (%)	Total N=63 n (%)	*P*-value
Sex, female	18 (45)	15 (65)	33 (52.4)	0.122
Underlying disease				
Congenital pulmonary airway malformation	5 (12.5)	2 (8.7)	7 (11.1)	0.963
Congenital pulmonary cyst	3 (7.5)	1 (4.3)	4 (6.3)	>0.99
Pulmonary sequestration	0	1 (4.3)	1 (1.6)	—
Tracheomalacia	2 (5)	0	2 (3.2)	—
Intellectual disability	0	3 (13)	3 (4.8)	—
Primary immunodeficiency	2 (5)	0	2 (3.2)	—
Immunosuppression	0	1 (4.3)	1 (1.6)	—
Cause				
Bacterial pneumonia	20 (50)	16 (69.6)	36 (57.1)	0.131
Pulmonary abscess	12 (30)	5 (21.7)	17 (27)	0.477
Septicemia	5 (12.5)	2 (8.7)	7 (11.1)	0.644
Retropharyngeal abscess	2 (5)	0	2 (3.2)	—
Abscess of the chest wall	1(2.5)	0	1(1.6)	—
Laboratory data				
WBC (10^3/mm^3), median (IQR)	23.7 (20.3)	23.6 (14.9)	23.7 (17.7)	0.959
CRP (mg/L), median (IQR)	159.7 (52.4)	149.1 (127.5)	155.8 (84.2)	0.595

CRP: C-reactive protein, WBC: white blood cell, IQR: interquartile range.

to vancomycin. Ceftazidime was effective against all *Pseudomonas aeruginosa* isolates. Children aged <3 years were significantly more likely to have a positive bacterial culture result (65% versus 39.1%, *P*=0.047). (Table III). Three children had more than one bacterial infection detected. A child with a retropharyngeal abscess had *Burkholderia cepacia* and *Enterobacter cloacae* coinfection, and two children had *Streptococcus pneumoniae* and *Staphylococcus aureus* coinfection. One child who had been intubated had *Burkholderia cepacia* which was detected in their pleural fluid. The other types of coinfection are shown in Table III. Coinfection with respiratory viruses or *Mycoplasma pneumoniae* was significantly less common in children aged <3 years than those aged ≥3 years (22.5% vs 56.5%, *p*=0.006).

The number of cases of *Staphylococcus aureus* (eight cases in the first 5 years, and seven cases in the last 5 years) and *Streptococcus pneumoniae* (four cases in the first 5 years, and six cases in the last 5 years) infection remained relatively stable over time. The number of cases of *Pseudomonas aeruginosa* infection declined from five in the first 5 years to two in the last 5 years of the study.

Staphylococcus aureus and *Streptococcus pneumoniae* infections were more common in the winter and the spring. Conversely, *Pseudomonas aeruginosa* was more common in the summer. The distribution of pathogens according to month is shown (Fig. 2).

Treatment and outcome

The study flow chart is presented in Figure 3. Thirty (47.6%) children were treated with empirical antibiotics and a chest tube insertion and did not require surgery. Ten patients received intravenous antibiotics and simple drainage alone, and 20 children required closed thoracic drainage. Thirty-three children (52.4%) required surgery, of whom 21 had an open thoracotomy,

Table II. Antimicrobial susceptibility data for the main pathogens of empyema.

Pathogen	*Staphylococcus aureus* (n=15)			*Streptococcus pneumoniae* (n=10)			*Pseudomonas aeruginosa* (n=7)		
Agent	S	I	R	S	I	R	S	I	R
Penicillin	0	0	15	5	0	5	—	—	—
Cefuroxime	—	—	—	3	2	5	—	—	—
Cefotaxime	—	—	—	6	2	2	—	—	—
Ampicillin	0	0	15	—	—	—	—	—	—
Cefoxitin	5	0	10	—	—	—	—	—	—
Clindamycin	4	1	10	—	—	—	—	—	—
Erythromycin	4	0	11	0	0	10	—	—	—
Rifampicin	12	1	2	—	—	—	—	—	—
Tetracycline	12	0	3	2	0	8	—	—	—
Gentamicin	14	0	1	—	—	—	6	1	0
Levofloxacin	15	0	0	10	0	0	7	0	0
TMP-SMZ	14	0	1	1	1	8	—	—	—
Amoxicillin clavulanic acid	—	—	—	8	2	0	—	—	—
Vancomycin	15	0	0	10	0	0	—	—	—
Amikacin	—	—	—	—	—	—	7	0	0
Ceftazidime	—	—	—	—	—	—	7	0	0
Cefepime	—	—	—	—	—	—	7	0	0
Ciprofloxacin	—	—	—	—	—	—	7	0	0
Aztreonam	—	—	—	—	—	—	7	0	0
Imipenem	—	—	—	—	—	—	7	0	0
Piperacillin	—	—	—	—	—	—	7	0	0
Piperacillin-tazobactam	—	—	—	—	—	—	7	0	0
Cefoperazone-sulbactam	—	—	—	—	—	—	7	0	0
Tobramycin	—	—	—	—	—	—	7	0	0
Meropenem	—	—	—	—	—	—	7	0	0
Ticarcillin clavulanic acid	—	—	—	—	—	—	6	1	0

S: susceptible, I: intermediate, R: resistant, TMP-SMZ: trimethoprim sulfamethoxazole

six had video-assisted thoracoscopic surgery, two had a right inferior pulmonary lobectomy, and one with a congenital pulmonary cyst had a left upper pulmonary lobectomy. Two patients with retropharyngeal abscesses were treated by incision and drainage, and one patient with a thoracic abscess was managed with debridement and drainage. The most common serious complication was pneumothorax. One patient experienced septic shock, and another developed a bronchopleural fistula. One experienced multiple organ failure, and another had hemolytic uremic syndrome. The mean (SD) duration of total hospital stay was 33 (12) days. Of the 63 children, 60 improved, while three did not complete their treatment and left the hospital against medical advice. One was readmitted to the hospital 3 months' post-discharge. Outcomes are summarized in Table IV.

Discussion

According to previous research, pleural empyema is usually secondary to acute bacterial pneumonia. In our study, 57.1% of the cases of empyema were preceded by bacterial pneumonia. Lamas-Pinheiro et al.[10] reported

Table III. Pathogens detected in children with pleural empyema.

Pathogen	<3 years old n=40 n (%)	≥3 years old n=23 n (%)	Total N=63 n (%)	*P*-value
Bacteria	26 (65.0)	9 (39.1)	35 (55.6)	0.047
Staphylococcus aureus[a,b,d]	12 (30.0)	3 (13.0)	15 (23.8)	—
Streptococcus pneumoniae[a,b,d]	8 (20.0)	2 (8.7)	10 (15.9)	—
Pseudomonas aeruginosa[d]	6 (15.0)	1 (4.3)	7 (11.1)	—
Escherichia coli	0	2 (8.7)	2 (3.2)	—
Burkholderia cepacia[b,c]	2 (5)	0	2 (3.2)	—
Enterobacter cloacae[c]	1 (2.5)	0	1 (1.6)	—
Klebsiella pneumoniae	1 (2.5)	0	1 (1.6)	—
Streptococcus constellatus	0	1 (4.3)	1 (1.6)	—
Mycoplasma pneumoniae	3 (7.5)	9 (39.1)	12 (19.0)	0.006
Viruses	6 (15.0)	4 (17.4)	10 (15.9)	0.803
Influenza A virus	1 (2.5)	4 (17.4)	5 (7.9)	0.105
Influenza B virus	1 (2.5)	0	1 (1.6)	—
Human adenovirus	2 (5)	0	2 (3.2)	—
Respiratory syncytial virus	1 (2.5)	0	1(1.6)	—
Human parainfluenza virus type3	1 (2.5)	0	1(1.6)	—

[a]*Staphylococcus aureus* co-infected with *Streptococcus pneumoniae* in one patient aged <3 years.
[b]*Staphylococcus aureus* mixed with *Streptococcus pneumoniae* and *Burkholderia cepacia* in one patient aged <3 years.
[c]*Burkholderia cepacia* co-infected with *Enterobacter cloacae* in one patient aged <3 years.
[d]*Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* grew in the blood culture in five, three and two patients, respectively.

that children with empyema usually had a normal underlying lung. However, in our study, seven of the 63 children (11.1%) had congenital pulmonary airway malformations. Also, several children in our study had underlying conditions, including intellectual disability, immunodeficiency, and immunosuppression. The role of underlying conditions such as these in increasing the risk of empyema requires further research.

Most of the children in our study were aged <3 years. The children in our study were younger than those in a previous study by Eastham et al.[11] conducted in 2004 (median age 1 year versus 5.6 years, respectively). In keeping with the previous research[7], the incidence of empyema was higher in the winter and the spring than in the summer and autumn, probably due to their infective origin.

Table IV. Medical outcomes of the patients.

Outcome	n, %
Serious adverse events	
Pneumothorax	31 (49)
Multiple organ failure	1 (1.6)
Septic shock	1 (1.6)
Bronchopleural fistula	1 (1.6)
Hemolytic uremic syndrome	1 (1.6)
Purulent meningitis	1 (1.6)
Subcutaneous emphysema	6 (9.5)
Pericardial effusion	1 (1.6)
Duration of total hospital stay, mean (SD), day	33 (12)
Outcome	
Clinically improved	60 (95.2)
Ongoing	0
Death	0
Unknown	3 (4.8)
Hospital readmissions	1 (1.6)

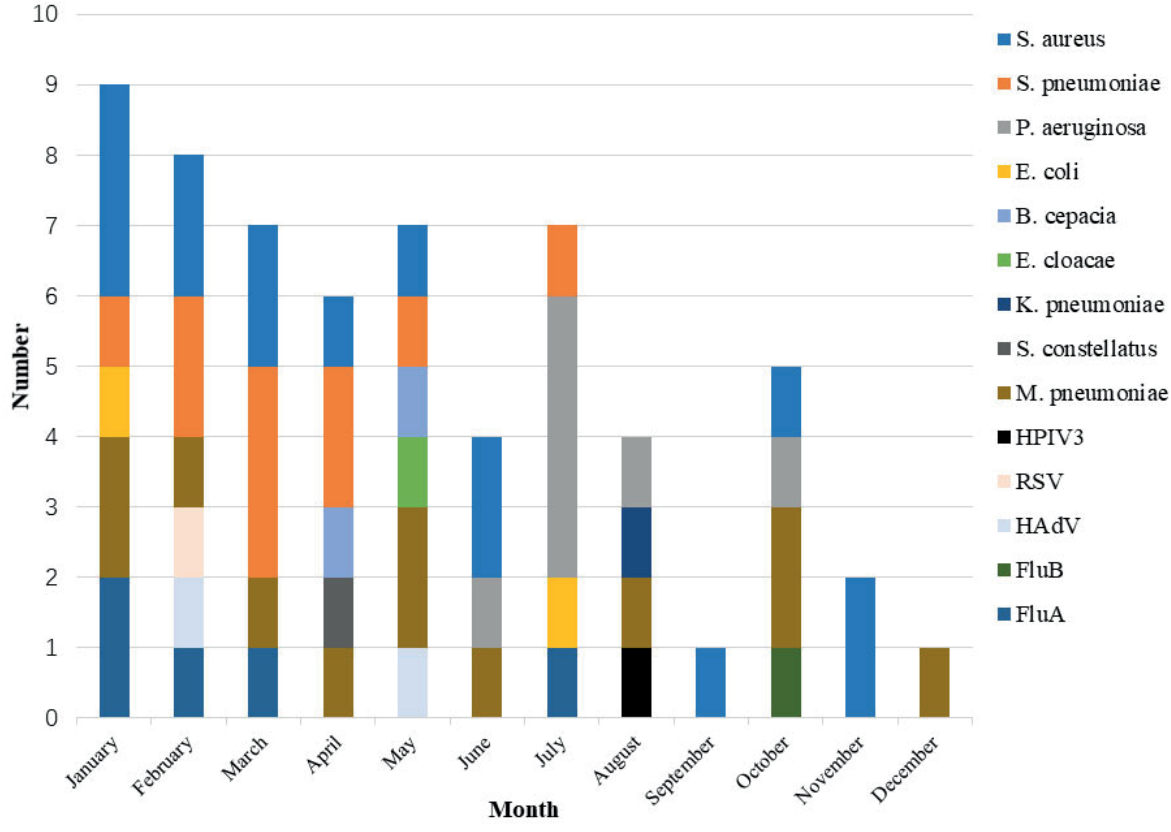

Fig. 2. Causes of pleura empyema according to month. *Staphylococcus aureus: S. aureus; Streptococcus pneumoniae: S. pneumoniae; Pseudomonas aeruginosa: P. aeruginosa; Escherichia coli: E. coli; Burkholderia cepacia: B. cepacia; Enterobacter cloacae: E. cloacae; Klebsiella pneumoniae: K. pneumoniae; Streptococcus constellatus: S. constellatus; Mycoplasma pneumoniae: M. pneumoniae*; Influenza A virus: FluA; Influenza B virus: FluB; Human adenovirus: HAdV; Respiratory syncytial virus: RSV; Human parainfluenza virus type 3: HPIV3.

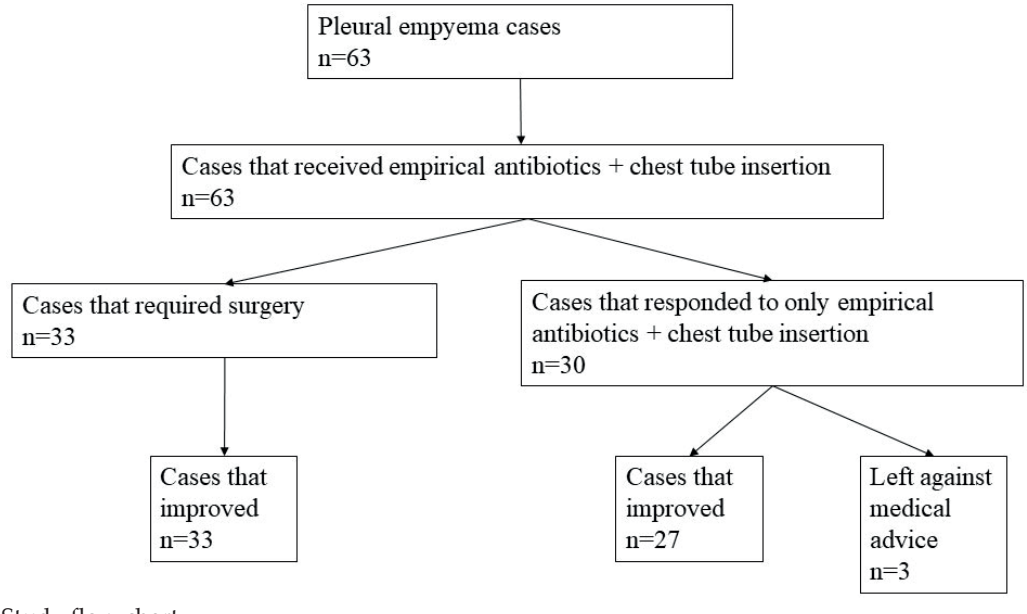

Fig. 3. Study flow chart

In our study, *Staphylococcus aureus* was the most common cause of empyema. This finding is similar to that of previous studies conducted on children in India and New Zealand.[12-14] However, in other studies, the proportion of cases of empyema caused by *Staphylococcus aureus* infection has varied.[15-16] This can be attributed to regional variations in the epidemiology of community acquired *Staphylococcus aureus* infection.[7] The study conducted in New Zealand[11] revealed that the prevalence of MRSA was 26% among 38 children with *Staphylococcus aureus* empyema. In our study, ten of the 15 isolates of *Staphylococcus aureus* (66.7%) were methicillin resistant (MRSA). The high incidence of MRSA could be due to the injudicious use of antibiotics.

Streptococcus pneumoniae was the second most common cause of empyema in our study and 50% were resistant to penicillin. The proportion of cases of empyema caused by *Streptococcus pneumoniae* was relatively low compared to previous studies.[17-19] Lin et al.[20] analyzed the causes of infection among 89 children with empyema thoracic and parapneumonic pleural effusion in Taiwan, confirmed that *Streptococcus pneumoniae* was the most common pathogen. In this study, the number of patients with empyema caused by *Streptococcus pneumoniae* may have been underestimated because we did not use polymerase chain reaction (PCR) to test for *Streptococcus pneumoniae*. Blaschke et al.[21] confirmed that most patients with culture negative empyema were positive for *Streptococcus pneumoniae*.

Pseudomonas aeruginosa was the third most common cause of empyema in this study. This is similar to the findings of a recent study conducted in Iran, which found a prevalence of *Pseudomonas aeruginosa* of 18.1% among 105 children with empyema.[22] According to a previous study, *Pseudomonas aeruginosa* infections occur mainly as a complication of hospital-acquired pneumonia and in patients with chronic lung disease.[23] In contrast to their study, we found that empyema caused by *Pseudomonas aeruginosa* infections occurred as an outcome of community-onset infections; and ceftazidime sustained activity against all *Pseudomonas aeruginosa* isolates. All children with *Pseudomonas aeruginosa* infection did not have underlying lung disease. We hypothesize that the predisposing factors for *Pseudomonas aeruginosa* infections in children differ from those of adults. Of note is that six of the seven children with *Pseudomonas aeruginosa* aged <3 years were diagnosed with empyema in the summer. Therefore, if children under 3 years are diagnosed with empyema in the summer, *Pseudomonas aeruginosa* should be considered as the main cause. The incidence of *Pseudomonas aeruginosa* infection should be continuously monitored but as there were only two cases of *Pseudomonas aeruginosa* infection in the last 5 years of the study, the incidence is too low to determine risk factors for *Pseudomonas aeruginosa* infection in children.

In our study, the most commonly detected coinfection pathogen was *Mycoplasma pneumoniae*, which was significantly more common in children aged ≥3years. In addition, Influenza A virus was the most common virus detected in patients. However, Krenke et al.[19] reported that *Chlamydia pneumoniae* was the most common pathogen (8.6%), and that adenovirus was the most common virus found in patients with empyema (13.8%). In our study, children aged <3 years were significantly less likely to have a coinfection with a virus or *Mycoplasma pneumoniae* than those aged ≥3 years. The observed differences might have been due to age-related differences in immune function and environmental exposures.

In the current study, the duration of total hospital stay was longer than that in previous studies.[11,13,24] One reason for this difference could be the higher rate of *Staphylococcus aureus* infections in our study and the fact that patients required a longer duration of treatment as compared to those with *Streptococcus pneumoniae* infections. Another reason is presumably the occurrence of serious complications. Most of the children with pleural empyema improved, while three of the children who did not complete

their treatment, left the hospital against medical advice. Thus far, the data has shown that prognoses are good after appropriate treatment, a finding that is consistent with previous studies.[25] In addition, children with empyema often have a lower rate of mortality compared to adults, and their long-term prognoses appear to be much better than those for adults.[26]

There are limitations to this study. Firstly, it is a retrospective study with a limited sample size. Thus, there may have been biases in data selection and analysis. Secondly, we did not conduct PCR testing of samples from the children with empyema when the pleural fluid culture was negative. This may have led the prevalence of bacterial infection in children with empyema to be underestimated.

This study described the microbiological characteristics and outcomes of Chinese children with empyema over the past 10 years and confirmed that *Staphylococcus aureus* and *Streptococcus pneumoniae* were the leading cause of empyema in the study setting. Antimicrobial susceptibility testing revealed a high percentage of resistance against penicillin while vancomycin provided 100% coverage for these pathogens. *Pseudomonas aeruginosa* isolates are the third most common pathogens mainly detected in those under 3 years old in the summer and have shown to be susceptible to ceftazidime. The prognosis is good after appropriate therapy.

Author contribution

Author contributions have been stated as follows: study conception and design: HZ; data collection: XZ; analysis and interpretation of results: HZ; draft manuscript preparation: XZ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Institutional Review Board of The Second Affiliated Hospital of Wenzhou Medical University (Protocol LCKY2019-199).

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflict of interest

The authors declare no conflict of interest.

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Original Article

Cost and length of hospital stay for healthcare facility-onset *Clostridioides Difficile* infection in pediatric wards: a prospective cohort analysis

Sevliya Öcal Demir[1], Eda Kepenekli[2], Gülşen Akkoç[3], Nurhayat Yakut[4], Ahmet Soysal[5]

[1]*Division of Pediatric Infectious Diseases, Department of Pediatrics, İstanbul Medeniyet University Göztepe Prof. Dr. Süleyman Yalçın City Hospital, İstanbul;* [2]*Division of Pediatric Infectious Diseases, Department of Pediatrics, Marmara University Faculty of Medicine, İstanbul;* [3]*Division of Pediatric Infectious Diseases, University of Health Sciences, İstanbul Haseki Training and Research Hospital, İstanbul;* [4]*Clinic of Pediatric Infectious Diseases, Başakşehir Çam and Sakura City Hospital, İstanbul;* [5]*Clinic of Pediatric Infectious Diseases, Memorial Ataşehir Hospital, İstanbul, Turkey.*

ABSTRACT

Background. *Clostridioides difficile* (*C. difficile*) is a well-known causative agent of healthcare associated infection, it increases medical cost besides increasing morbidity and mortality. This study was conducted to determine the incidence, and economic burden of healthcare facility-onset *C. difficile* infection (HO-CDI) in children.

Methods. Data was acquired with a prospective cohort study conducted in pediatric wards of a tertiary university hospital between August 2015 to August 2016. The HO-CDI was defined as diarrhea that began after 48 hours of admission with a positive cytotoxic stool assay for the presence of toxin A and/or B of *C. difficile*.

Results. In the 3172 admissions in one year, 212 (7%) healthcare associated diarrhea (HAD) episodes were observed, in 25 (12%) of them *C. difficile* was identified in which 6 (25%) cases <2-year-old. The incidence of HO-CDI was estimated as 8.8/10,000 patient-days. Cases with HO-CDI (n=19) were compared with cases with non-CDI-HAD (n=102); the presence of one of the risk factors for CDI increased the risk for HO-CDI (5,05; 95% Cl: 1.10-23.05; P 0,037), the median length of stay (LOS) attributable HO-CDI was 7 days (IQR,5-10) per admission, whereas for non-CDI-HAD was 2 days (IQR,0-4) (p=0.036). General hospitalization costs in the two groups were similar, specifically estimated costs attributable to HO-CDI and non-CID-HAD were $294.0 and $137.0 per hospitalization respectively (p=<0.0001).

Conclusion. Although in children the incidence of HO-CDI is increasing, its clinical manifestation is still milder and effective infection control measures with antibiotic stewardship can limit related morbidly, mortality, LOS, and cost.

Key words: health-care, HO-CDI, *C. difficile*, cost, pediatric.

Clostridioides (formerly *Clostridium*) *difficile* (*C. difficile*) is an anaerobic, gram-positive, toxin-producing bacillus, *which* exists in spore form in the environment and is a member of the human gastrointestinal system. During or following the usage of broad-spectrum antibiotics, colonic microbiota is disrupted and *C. difficile* starts to multiply and produce toxins leading to diarrhea. Particularly hospitalized elderly patients with comorbidities are more vulnerable to *C. difficile* infection (CDI) than children.[1] But the incidence and severity of CDI is gradually increasing in children.[2] In the United States its incidence in children <18 years was reported as 24.2 cases per 100,000 population in 2011.[3] In Turkey data about pediatric CDI are very limited, Karaaslan et al.[4] reported the incidence of CDI in hospitalized children as 9 per 1000 patients for the years 2013 and 2014.

✉ Sevliya Öcal Demir
sevliyademir@gmail.com

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Healthcare associated CDI is associated with prolonged length of hospital stay (LOS), readmission, extra healthcare cost, increased morbidity and mortality. Recent studies found that the attributable cost of CDI ranges from $3,000 to $15,000 per hospitalization and LOS of 3-7 days in hospitalized adults.[5-7] Only one study has evaluated the impact of CDI in hospitalized children, and they reported the attributable cost of HO-CDI to range from $1,917 to $8,317, and attributable LOS as approximately 4 days.[7]

For there is no prospective study that specifically evaluated additional LOS and attributable cost of HO-CDI in children, we conducted this study to determine the incidence, LOS and healthcare cost attributable of HO-CDI in pediatric patients.

Material and Methods

This prospective cohort study was carried out from August 1 2015 till July 31 2016 at the pediatric wards of Marmara University Medical School. This hospital was founded in January 2011 with a 649 bed-capacity, our patients are of a middle socioeconomic statue. This investigation was conducted at our pediatric department which included patients from the pediatric intensive care unit (PICU) with a 14 bed capacity, the pediatric hematology-oncology unit with a 27 bed capacity, and the general pediatric ward with a 77 bed- apacity.

During 12 months, all admissions of pediatric patients were followed and those aged between 2 to 18 years who were hospitalized for more than 48 hours were recorded daily by one Pediatric Infectious Diseases specialist. Data concerning the patient's age, sex, diagnosis, previous hospitalization, type number and duration of antibiotics used in last 3 months, other medications including chemotherapy, proton pump inhibitors (PPIs), diagnostic tests and treatment for CDI and their costs, duration of hospitalization, outcomes and total hospitalization cost was documented in a data collection sheet.

Health associated diarrhea (HAD) was defined according to the Center for Diseases Control and Prevention (CDC) criteria; diarrhea ≥3 loose or looser-than-normal stools in a 24-hour, of <7 days duration period began after 48 hours of hospitalization. CDI was defined according to the guidance and recommendations from the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA); the presence of symptoms (mainly diarrhea) and either a stool test positive for *C. difficile* toxins or detection of toxigenic *C. difficile*, or colonoscopic or histopathologic findings revealing pseudomembranous colitis. Detection of toxins A and B of *C difficile* was performed by using premier toxins A and B (C. difficile) EIA kit bioMeriux (Marseile, France) according to the manufacturer's instructions. To increase comparability between clinical settings, we used IDSA recommendation for case definition, incidence estimation were used for standardized case definition.[8] To evaluate the impact of CDI on hospitalization cost and LOS, patients with HAD were designated into two groups, the first group had healthcare-facility onset (HO)-CDI (HO-CDI), the other had HAD without CDI (non-CDI-HAD). Because asymptomatic colonization with *C difficile* is common in the neonatal period and infancy,[9-11] children <2-year-old were excluded from the two groups. Firstly, general hospitalization cost was estimated by including all costs associated with hospitalization, and compared the two groups. During the patient stay in pediatric wards, their cost and outcomes were tracked. The specific cost attributable to CDI was calculated with the charge for inpatients, closed beds for isolation, laboratory tests, antimicrobial drugs and other medications used for CDI. Inpatient cost of ICU was not added as an extra cost, because HAD was mild in all and PICU stay was not due to HAD. Also, for other patients when their stay was for their primer disease instead of HAD bed cost was not added as an extra cost. The cost was first recorded in Turkish Lira (TL), then converted to USD ($), using the average exchange rate between TL to USD currency between 1 August 2015 to 31 July 2016 (1TL

= 0.3424$). Attributable LOS associated with HAD was estimated after a daily patient visit to clarify if the patient stayed due to HAD.

The study protocol was approved by the decision of the Clinical Research Ethics Committee of Marmara University Medical School (number: 09.2015.221).

Statistical analysis

Data were analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Frequency and percentage for categorical data, and median (inter quarter range) for continuous data were identified as descriptive statistics. Mann Whitney U-test was used to compare the two groups. Logistic regression analysis was used to analyze risk factors. The results were considered statistically significant in cases that p-value is less than 0.05.

Results

Between August 1 2015 and July 31 2016 1,971 patients had 3,172 admissions for more than 48 hours in pediatric units under surveillance. The repeated admissions mainly belonged to the hematology-oncology patients. During a 12 month follow-up 212 HAD episodes were observed; in 150 (70,75%) the microbiologic agents could not be identified, in 25 *C. difficile*, in 23 rotaviruses, in 7 *Giardia intestinalis*, in 3 adenoviruses, and in 3 *Entamoeba intestinalis* was identified. Because none of our patients had severe diarrhea and some of them were neutropenic colonoscopic examination was not done. CDI was defined according to a positive stool test for *C. difficile* toxins in all of the patients. To estimate HO-CDI incidence, the cases <2-year-old were excluded, the number of cases with HO-CID decreased to 19, total patient-days decreased to 21,520. The incidence of HO-CDI was found as 8,8/10,000 patient-days in children aged between 2-18 years. Other characteristics of our sample group are summarized in Table I.

The distribution of demographic-clinic characteristics and risk factors among the 19 cases with HO-CDI and 102 cases with non-CDI-HAD are shown in Table II. Statistical analysis showed the presence of any of the following risk factors including enteral feeding, PPI, gastrostomy, chemotherapy, immune suppression other than antibiotic usage for CDI increased the risk for HO-CDI 5-fold (5,05; 95% Cl: 1.10-23.05). Antibiotic exposure in the previous 3 months in HO-CDI and non-CDI-HAD groups were %84,2 and %88 respectively, similarly total antibiotic days, type of antibiotics used, were not different in the two groups (Table II), (Fig. 1). Also, repeated hospitalization, hospitalization in the PICU, being a hematology/oncology patient with a malignancy were not statistically different in the two groups.

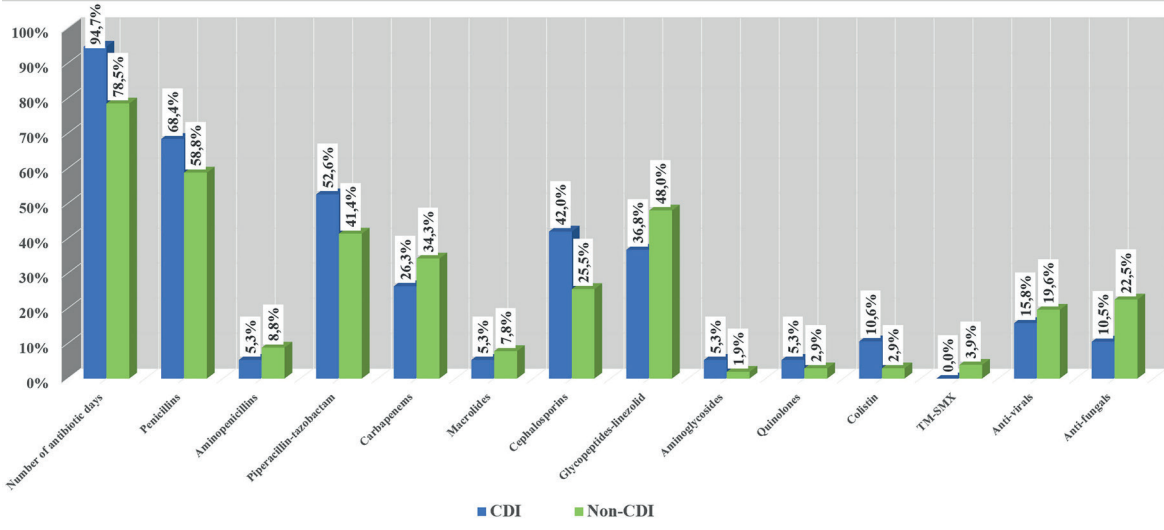
Table I. Demographic characteristics of hospitalized pediatric cases.

		Median (IQR) or N (%)
Total number of patients* (n)		1,971
Total number of hospital admission* (n)		3172
Gender	Boys	1,105 (56.1)
	Girls	866 (43.9)
Age, months		48,0 (12.0-108.0)
Total hospitalization days, n=1971		31114
Mean hospitalization days		6,0 (4.0-11.0)
Hospitalized in (n=1971)		1971
PICU**		113 (5.7)
Hematology-oncology ward + PICU		14 (0.7)
Hematology-oncology ward		346 (17.6)
General wards + PICU		56 (2.8)
General pediatric ward		1442 (73.2)
Healthcare associated diarrhea (HAD) episodes		212
C. difficile		25
Rotaviruses		23
Giardia intestinalis		7
Adenoviruses		3
Entamoeba intestinalis		3
Non-identified		151

*: more than 48 hours, **: pediatric intensive care unit

Table II. Distribution of demographic and clinic characteristics, risk factors in the pediatric cases with HO-CDI or non-CDI-HAD.

Characteristics, N=121		CDI n=19 N (%) or Median (IQR)	Non-CDI n=102 N (%) or Median (IQR)	p
Gender	Male	9 (47.4)	68 (66.7)	0.125
	Female	10 (52.6)	34 (33.3)	
Age, months		48 (36-108)	48 (35-96)	1.000
Hospitalized in	PICU	2 (10.5)	4 (3.9)	0.487
	Hematology-oncology ward + PICU	1 (5.3)	10 (9.8)	
	General wards + PICU	2 (10.5)	8 (7.8)	
	Hematology-oncology ward	9 (47.4)	37 (36.3)	
	General pediatric ward	5 (26.3)	43 (42.2)	
Recurrent hospitalization		13 (68.4)	52 (51.0)	0.212
Length of stay (LOS), (day)		22 (7-44)	16 (10-29)	0.556
Duration of PICU hospitalization, (day)		29 (13-93), n:5	10 (6-28), n:29	0.871
Diarrhea onset day of hospitalization, (day)		9 (2-20)	6 (3-14)	0.634
Duration of diarrhea		4 (3-6)	3 (3-5)	0.109
Presence of any risk factor for HAD		17 (89.5)	64 (62.7)	0.032
Chemotherapy		11 (57.9)	39 (38.2)	0.132
Nasogastric tube		2 (10.5)	13 (12.7)	1.000
PPI		6 (31.6)	17 (16.7)	0.198
Gastrostomy		1 (5.3)	7 (6.9)	1.000
IV Catheter		4 (21.1)	12 (11.8)	0.277
Immunosuppressive treatment		-	1 (1.0)	1.000
Immunodeficiency		1 (5.3)	3 (2.9)	0.500
Surgical operation	None	17 (89.5)	98 (96.1)	0.261
	Cranial	2 (10.5)	2 (2.0)	
	Abdomen	-	1 (1.0)	
	Head/Neck	-	1 (1.0)	
Antibiotic, anti-viral and anti-fungal usage		16 (84.2)	88 (86.3)	0.730
Total antibiotic days		15 (8-31)	13 (5-32)	0.797
Penicillins, (day)		14 (9-17)	11 (5-16)	0.495
Aminopenicillins, (day)		7 (7-7)	4 (2-7)	0.376
Piperacillin-tazobactam, (day)		8 (5-13)	6 (4-10)	0.262
Carbapenems, (day)		15 (10-19)	10 (5-14)	0.535
Macrolides, (day)		9 (9-9)	10 (2-10)	0.450
Cephalosporins, (day)		8 (5-11)	5 (2-10)	0.111
Glycopeptides-linezolid, (day)		10 (8-14)	10 (4-14)	0.393
Aminoglycosides, (day)		8 (2-8)	3 (1-10)	0.215
Quinolones, (day)		8 (8-8)	7 (3-12)	0.788
Colistin, (day)		9 (6-10)	5 (4-6)	0.119
TM-SMX, (day)		-	12 (8-19)	0.382
Anti-virals, (day)		7 (5-8)	11 (6-20)	0.647
Anti-fungals, (day)		10 (10-10)	11 (6-20)	0.197
Mortality	No	18 (94.7)	95 (93,1)	1.000
	Yes	1 (5.3)	7 (6.9)	
Additional treatment usage		18 (94.7)	24 (23.5)	<0.0001



HO-CDI: healthcare facility-onset *Clostridioides difficile* infection, non-CDI-HAD: non- *Clostridioides difficile* infection health care associated diarrhea, CDI: *Clostridioides difficile* infection.

Fig. 1. Type of antibiotics usage in HO-CDI and non-CDI-HAD.

Table III. LOS and extra cost attributable to HO-CDI and non-CDI-HAD.

Cost	HO-CDI (n= 19)	Non-CDI-HAD (n= 102)	p
Total hospitalization cost ($)	7.807 (1.548-11.610)	7.311 (830-9.763)	0.847
Specifically, estimated costs for			
Hospitalization ($)	231.0 (110.0-318.0)	61.0 (0.0-194.0)	<0.0001
Diagnosis ($)	70.0 (24.0-76.0)	51.0 (23.0-49.0)	0.149
Treatment Cost ($)	28.0 (13.0-36.0)	25.0 (10.0-26.0)	0.771
Total ($)	294.0 (163.0-405.0)	137.0 (54.0-180.0)	<0.0001
Length of stay (LOS)	7 (5-10)	2 (0-4)	<0.036

The general total cost of hospitalization in cases with HO-CDI was $7,807 and in cases with non-CDI-HAD was $7,311, which were similar. On the other hand, specifically calculated cost attributable to HAD in cases with HO-CDI (total cost was $294) was higher than in patients with non-CDI-HAD (total cost was $137), this difference was statistically significant (p =< 0.0001). (Table III). The distribution of the cost attributable to HO-CDI was demonstrated in Figure 2, inpatient cost was higher in HO-CDI.

The median LOS attributable HO-CDI was 7 days (IQR, 5-10) per admission, whereas for non-CDI-HAD was 2 days (IQR,0-4) (p=0.036). The mortality rate was not found to be different between the two groups.

Discussion

The diagnosis of CDI in pediatric patients can be challenging due to the high rates of asymptomatic colonization with *C. difficile*. It can be detected in 25-50% of neonates and 40-70% of infants.[12] This is the most important factor for not determining the true incidence of CID in children. To partially overcome this problem, children under 2-year-old were excluded from the analysis. In addition, the presence of predisposing conditions, such as antibiotic exposure, gastric acid suppression, malignancy in most of the patients supported the diagnosis.

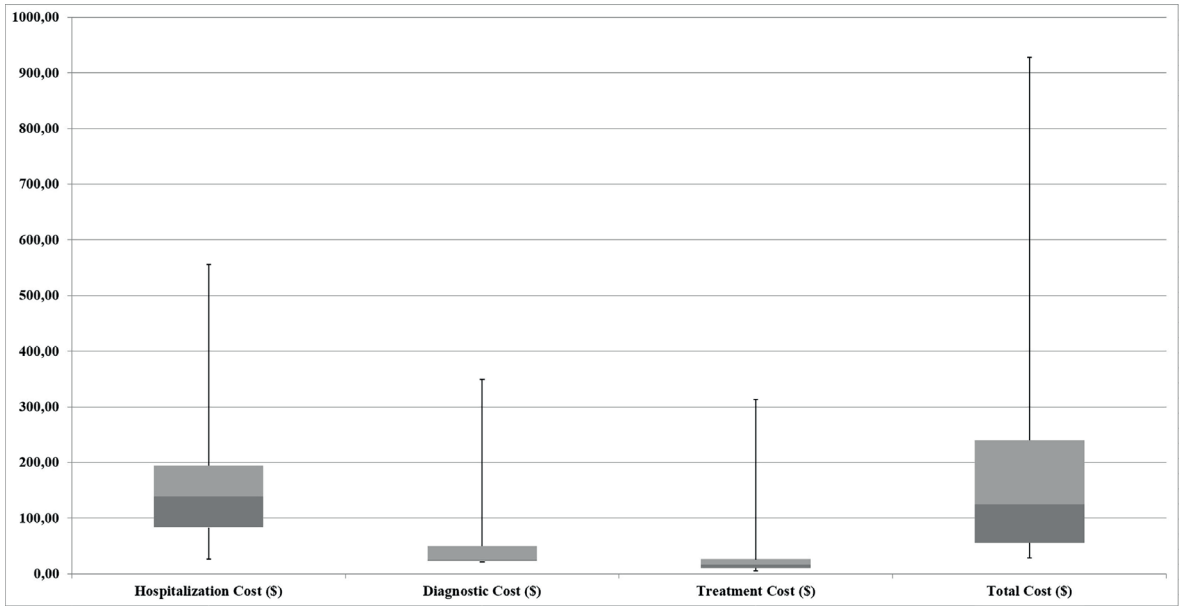


Fig. 2. Distribution of cost in pediatric patients with HO-CDI.

CDI is less frequent in children, the reported incidence was 24.2 cases per 100,000 pediatric population in the United States in 2011, but this was a population-based surveillance.[3] Kukla et al.[13] use case definition according to the IDSA recommendation and found HO-CDI incidence as 2,4 cases/10,000 man-days. Here we found HO-CDI incidence as 8,8 cases per 10,000 patient-day. The incidence of CDI is increasing even in children. This result may be due to the fact that our hospital is a reference hospital, especially for hematology-oncology patients. Indeed, Kukla et al.[13] stated that CID incidence is higher in reference hospitals than in community hospitals. A previous retrospective study from Turkey reported CDI incidence as 9 cases per 1000 patients in 2014 in hospitalized children.[4]

Previous antibiotic exposure is the well-known single most important risk factor for CDI, and in pediatric studies, multiple classes of antibiotics used in the preceding month has been associated with severe and recurrent CDI.[14,15] In a recent study by Khalil et al.[16] antibiotic usage and LOS were reported as predisposing factors for CDI. In this study, there was no difference concerning previous antibiotic exposure, type of antibiotics used and total antibiotic-days between the HO-CDI and non-CID-HAD groups. This may be because we compared HO-CID with non-CID-HO-HAD, rather than cases without HAD, additionally clindamycin which is one of the most blamed antibiotics for CID was never used, fluoroquinolones were used infrequently. Factors such as enteral feeding, PPIs, gastrostomy, chemotherapy and immune suppression were shown to be risk factors for CDI in other studies; PPI usage and malignancy are the most common conditions among hospitalized children with CDI, accounting for 20 to 25% of HO-CDI.[17,18] In the current study, the presence of one of the risk factors defined for CDI, increased HO-CDI risk by 5-fold. But specific risk factors could not define, this result may be due to the small number of patients in the HO-CDI group.

The cost attributable to CDI is expected to be lower in pediatric patients because in children severe disease or complications are not as common as in adults. Indeed, in this study no CDI- related complication was observed. Studies of hospitalized adults with CDI have found related costs ranging from $3,000 to $15,000 per hospitalization and LOS of 3-7 days.[5-7] There was only one study conducted by Mehrotra et al.[7] who evaluated the impact

of CDI on LOS and costs in children and reported that the attributable cost of HO-CDI ranged from $1,917 to $8,317, and attributable LOS as approximately 4 days. In our study we found HO-CDI related costs as $294 per hospitalization. Although this cost is higher than in the non-CDI-HAD group, it is a small difference in contrast to costs determined by previous studies. Milder manifestation of HO-CID in our cases can only partly explained this difference. The prospective design of our study allowed us to follow cases closely and distinguish CID related costs. Retrospective studies have limited clinical and laboratory data, where only the total cost of hospitalization has been obtained, control groups are created from the same population with similar characteristics to estimate attributable cost of CDI. Another limitation is that we only estimated direct medical cost, we could not add the cost of cleaning materials, gloves and gowns, time spent by doctors and nurses, indirect costs such as school absenteeism or parental leave from work.

As expected, HO-CDI resulted in an extension of the duration of hospitalization for ongoing diarrhea, complications associated with the CDI diagnosis or treatment. Studies supported this hypothesis; in hospitalized adults with CDI this time was reported as 3-7 days, likewise in children this is approximately 4 days.[5-7] In our study LOS was similarly 7 days (IQR,5-10) and longer than for non-CID-HAD.

In conclusion, although in children the incidence of HO-CDI is increasing, its clinic is still milder and effective infection control measures and antibiotic stewardship should limit related LOS and cost.

Author contribution

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

Ethical approval

Study protocol was approved by the decision of Clinical Research Ethic Committee of Marmara University Medical School (number: 09.2015.221).

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Conflict of interest

All authors have no potential conflicts of interest to disclose.

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Original Article

Predictive factors of high-flow nasal cannula oxygen therapy failure in children with respiratory distress treated in a Pediatric Emergency Department

Orkun Aydın[1], Elif Arslanoğlu Aydın[2], Ahmet Ziya Birbilen[1], Özlem Tekşam[1]

[1]*Division of Pediatric Emergency,* [2]*Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.*

ABSTRACT

Background. High-flow nasal cannula (HFNC) is widely used as a feasible and tolerable respiratory support method. However, patients should be closely monitored, especially when used with moderate-severe respiratory distress indications. Because these patients can easily develop respiratory failure and escalated care may be required. The aim of this study is to determine the predictive factors in patients treated with HFNC who received escalated respiratory support for HFNC failure.

Methods. A retrospective study of patients admitted with respiratory distress and treated with HFNC therapy between January 2014 and December 2018 was carried out. The variables evaluated were age, gender, vital signs before and two hours post HFNC therapy, underlying disease, use of steroid, salbutamol and antibiotic therapy, blood gase analysis and lactate values, hospitalization in pediatric intensive care unit, respiratory viral panel and need for escalation of respiratory support. HFNC failure was identified requiring noninvasive or invasive respiratory support despite HFNC therapy.

Results. 243 patients receiving HFNC therapy were included in this study. The median age was 11 months [interquartile range(IQR) 5–27]. The diagnosis of 183 patients (75.3%) were acute bronchiolitis and 60 patients (24.7%) were pneumonia. Of 243 patients, 29 (%11.9) received escalated care. 22 invasive and 7 non-invasive respiratory supports were provided. The lower pH on admission was found in the non-responder group. Moreover, heart rate and respiratory rate did not decrease two hours after HFNC therapy.

Conclusions. The careful monitoring of patients receiving HFNC therapy is critical. Because these patients are at risk for needing escalated care. We found that low pH values on admission and high pulse rate and respiratory rate observed at the second hour of follow-up period could be predictive factors for HFNC failure.

Key words: children, high-flow nasal cannula, respiratory distress, respiratory support.

Respiratory distress is an important reason for presentation to pediatric emergency departments (PED). Respiratory distress is usually reversible but when there is failure to treat it, it can cause respiratory arrest and even death. Various respiratory support methods are used in its treatment. It is not completely clear when and which respiratory support modalities including noninvasive or invasive ventilation will be used in these patients. The gold standard for a patient who needs respiratory support is endotracheal intubation, but there are many complications in this method such as volutrauma, barotrauma, ventilator-associated pneumonia.[1,2] Therefore, noninvasive ventilation is used, especially in patients with mild and moderate respiratory failure. High-flow nasal cannula (HFNC) is widely used for noninvasive respiratory support.

It started to be used as a noninvasive respiratory support method in the early 2000s, and its use in critical patient care has increased gradually, especially in recent years.[3] HFNC reduces

✉ Orkun Aydın
orkunaydin.89@gmail.com

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anatomical dead space and resistance in the upper respiratory tract, provides continuous airway pressure, and reduces the work of breathing.[4,5] Also adjustable (FiO_2 21-100%) heated (34- 37°C) oxygen with nearly 100% humidity can decrease mucosal injury and patient discomfort from cold, dry air.[6]

There is still no guideline that determines which patient will be given HFNC therapy. It is generally preferred for similar indications to nasal continuous positive airway pressure (nCPAP). Although HFNC is a feasible method for patients, in some patients HFNC fails and other respiratory support is needed. Also preferring HFNC therapy may negatively affect the prognosis in patients who need invasive respiratory support.[7] For this reason, it is very important to predict in which patient the HFNC treatment will be insufficient. Previous studies have not been able to locate factors predicting failure of HFNC, although the quality of the evidence is very low. However, respiratory acidosis at admission could be related to treatment of failure.[8] In an another study involving patients who were treated with HFNC due to respiratory distress, it was shown that a baseline respiratory rate (RR) >90th percentile, pCO_2>50 mmHg, pH<7.3 could predict HFNC failure.[7]

Considering the increasingly widespread use of HFNC, it remains important to identify the factors that may predict failure in children. Therefore, the aim of this study was to determine the factors that may predict HFNC failure in patients who presented to the PED with respiratory distress.

Material and Methods

Study Design, Setting and Participants

This is a retrospective, observational study. Patients with respiratory distress treated by HFNC therapy within the first 24 hours of admission to the PED were included in this study. The characteristics of the patients admitted between January 2014 and December 2018 were reviewed retrospectively. Medical records of patients were accessed using patient files and computer database. Patients aged 28 days or under were excluded from the study. The study was reviewed and approved by the the Ethics Committee of Hacettepe University (GO 19/185). All patients were anonymous. The parents signed a consent form approving anonymous data use for academic purposes when the patients were admitted to hospital.

High-flow nasal cannula therapy

HFNC therapy was given to patients with moderate and severe respiratory distress. HFNC therapy was provided by Airvo2 (Fisher & Paykel Healthcare).

The initial FiO_2 and flow were determined by the clinicians, it was adjusted as 1-2 L/kg/min flow. The inspired oxygen concentration was adjusted to achieve a SpO_2 >94%.[9,10] All patients who had respiratory distress were monitored in an observation room in the PED. Patients needing an escalation of respiratory support were transferred to the pediatric intensive care unit (PICU).

Definitons

Increase in heart rate (HR) and RR, nasal flaring, grunting, restlessness and use of accessory muscles were accepted as respiratory distress.[11] Initial values of blood gases were dichotomized using pCO_2 greater than 50 mmHg or pH less than 7.3 as markers of severity of respiratory distress.[7]

"HFNC therapy failure" (non-responders) was defined as the need for escalation to an other ventilation support treatment: non-invasive or invasive mechanical ventilation.

The definitive diagnoses of patients were divided into two groups: acute bronchiolitis and bacterial pneumonia. The diagnosis of acute bronchiolitis was made using the guideline of the American Academy of Pediatrics at 2014.[12] Patients who had respiratory distress symptoms (increase in RR, retraction, wheezing)

following fever, cough, two to three days of upper respiratory tract infection findings, and hyperinflation on chest X-ray were considered as acute bronchiolitis. Patients who had sudden fever, cough, toxic appearance, tachypnea, crackles on auscultation, and alveolar infiltration and consolidation on chest radiography were accepted as bacterial pneumonia.[13]

Medical history was coded into 4 binary variables defined by a previous history of atopy (eczema, asthma, reactive airways disease, or allergic rhinitis), genetic abnormalities (chromosomal abnormality, single gene mutation, or ongoing workup), history of prematurity, neurological disease including global developmental delay, muscular dystropy.[7]

Predictive factors

Age (corrected age for premature infants), gender, vital signs before and two hours post HFNC therapy start, underlying disease, use of steroid, salbutamol and antibiotic therapy, blood gases analysis and lactate values, hospitalization to PICU, respiratory viral panel (RVP) and need of escalation of respiratory support were evaluated. All parameters were evaluated during admission and vital signs were evaluated at the admission and second hour of follow-up period. Vital signs at the second hour were examined because of healthier access to medical records and inspired by similar studies. Normal vital signs were evaluated according to pediatric advanced life support (PALS) criteria.[9]

Statistical Analyses

SPSS (Statistical Package for Social Sciences) for Windows 22.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The variables were investigated using visual (histogram, probability plots) and analytical methods (Kolmogorov–Smirnov) to determine whether they were normally distributed. Numerical measurements were presented with mean and standard deviation or medians with interquartile range (IQR) based on distribution; qualitative data with numbers and percentages. According to the distribution of numerical variables, paired samples t-test or Mann–Whitney U were performed to investigate the differences between groups. For categorical variables, a chi-square test or Fisher exact test was performed. The possible factors determined by univariate analysis were then analyzed with a multiple logistic regression model. p value < 0.05 was considered to be statistically significant.

Results

A total of 243 patients who received HFNC therapy were included in this study. 139 (57.2%) of the patients were male and 104 (42.8%) were female (Table I). The median age was 11 months (IQR, 5–27). The age, sex, rate of RSV (Respiratuar Sinsityal Virus) positivity and drug used (steroid, salbutamol, antibiotics) were similar between the two groups. The final diagnosis was acute bronchiolitis in 183 (75.3%) patient, pneumonia in 60 (24.7%) patients. An underlying disease was present in 65.8% of the patients.

There was prematurity in 24 patients, a history of atopy in 31 patients, genetic disease in 61 patients, and neurological disease in 44 patients.

HFNC was well tolerated by all study patients and sedation was not given for any patient. There were no cases of pneumothorax or any other adverse events or complications.

Despite HFNC therapy, 44 (17.6%) patients transferred to PICU from PED and 29 (11.9%) patients required escalation of respiratory support. For 22 patients invasive and 7 patients non-invasive respiratory support were provided. RVP samples were taken from 147 patients, a virus was isolated in 98 patients. The most common agent was RSV (14.4%). It is followed by Humanrhinovirus with 20 patients, Bocavirus with 17 patients, and Influenza with 16 patients. 25 patients were diagnosed with recurrent bronchiolitis.

When the two groups were compared, there was no correlation in terms of age, gender,

Table I. Characteristics of patients with and without HFNC therapy failure.

	Failure (n: 29)	Success (n: 214)	All patients (n: 243)	*p* value
Sex, n (%)				
Male	13 (44.8)	126 (58.9)	139 (57.2)	0.151
Female	16 (55.2)	88 (41.1)	104 (42.8)	
Diagnosis, n (%)				
Bronchiolitis	22 (75.9)	161 (75.2)	183 (75.3)	0.941
Pneumonia	7 (24.1)	53 (24.8)	60 (24.7)	
Comorbidity, n (%)				
Positive	21 (72.4)	139 (65.0)	160 (65.8)	0.427
Negative	8 (27.6)	75 (35.0)	83 (34.2)	
Drugs use, n (%)				
Salbutamol	23 (95.8)	189 (92.6)	212 (93.0)	0.563
Steroid	16 (72.7)	143 (74.1)	159 (74.0)	0.890
Antibiotic therapy	28 (100)	205 (98.1)	233 (98.3)	0.460
Respiratuar Sinsityal Virus, n (%)				
Positive	3 (10.3)	32 (14.9)	35 (14.4)	0.703
Negative	26 (89.6)	182 (85.1)	208 (85.6)	

underlying disease and diagnosis. However, in the non-responders it was found that the HR and RR in the second hour of treatment were higher and also the pH value was lower on admission (Table II).

The results of the logistic regression model are presented in Table III. 102 cases were excluded from the regression model because of missing data. Three continuous variables and one categorical variable were in this model. Just one variable was associated with increased risk for HFNC failure: RR at second hour of initiation.

Discussion

In this retrospective study, possible predictive factors for escalation of respiratory support were determined in patients who received HFNC treatment in a PED. This study is one of the few studies identifying predictors of HFNC failure. Our results show that HFNC is a feasible respiratory support method that can be applied in all age groups of children. However, some patients may need escalation of respiratory support. The failure rate in our study was 11.9% and low pH values on admission and high pulse rate and respiratory rate observed at the second hour of follow-up could be a predictive factor.

Recently, there are no established guidelines for the initiation of oxygen therapy in pediatric patients. HFNC therapy has been extensively used in the last decade and studies continue regarding its use. Many studies have focused on its use in patients with bronchiolitis and HFNC therapy has been confirmed to be beneficial in severe bronchiolitis.[14-16] The physiological benefits generated by the supply of heated and humidified air are proven.[17-19] The reduction in intubation rate is another important benefit confirmed in studies.[20,21] Wing et al.[3] found that the need for intubation and mechanical ventilation decreased after the use of HFNC in their study on patients transferred from PED to PICU with acute respiratory failure. In a similar study, McKiernan et al.[22] examined patients admitted to PICU with bronchiolitis and showed that HFNC treatment reduced the rate of intubation by reducing respiratory rate and work of breathing. But still there is no clear consensus about which patients are the best candidates for this noninvasive respiratory support and which factors can predict HFNC failure.

Table II. Association between patient characteristics and HFNC therapy failure.

	Failure (n: 29)	Success (n: 214)	All patients (n: 243)	*p* value
Age, months (IQR)	8 (3.5-42.5)	11.5 (5-25.5)	11 (5-27)	0.593
Under 2 years (%)	19 (65.5)	160 (74.7)	179 (73.6)	0.289
SpO_2 % (SD)	84.3 (10.7)	86.8 (8.4)	86.5 (8.7)	0.279
HR, bpm (SD)	160.4 (18.4)	158.9 (23.6)	159.1 (23)	0.815
RR, rpm (SD)	63.0 (24.4)	63.0 (16.8)	63 (17.7)	0.924
SpO_2 at 2nd hour, % (SD)	91.4 (12.9)	96.7 (2.5)	96.1 (5.1)	0.337
HR at 2nd hour, bpm (SD)	145.4 (23.0)	133.9 (16.0)	135.2 (17.2)	0.014
RR at 2nd hour, rpm (SD)	56.9 (19.1)	47.6 (11.0)	48.6 (12.4)	0.017
pH (SD)	7.30 (0.07)	7.35 (0.06)	7.34 (0.06)	0.005
pCO_2 (mmHg) (SD)	51.4 (23.6)	44.6 (10.8)	45.5 (13.4)	0.170
SO_2 (mmHg) (SD)	66.5 (25.7)	66.8 (18.6)	66.7 (19.7)	0.694
pO_2 (mmHg) (IQR)	45.7 (30.2-57.2)	37.6 (30.6-48.8)	38.4 (30.6-49.8)	0.236
HCO_3 (mEq/L) (SD)	24.4 (8.8)	23.7 (5.1)	23.8 (5.7)	0.990
Lactate (mmol/L) (IQR)	1.8 (1.1-4.3)	1.6 (1.2-2.1)	1.6 (1.2-2.2)	0.137
Hb (g/dL) (SD)	11.4 (2.04)	11.4 (1.6)	11.4 (1.6)	0.792
WBC (/mm³) (IQR)	11.2 (9.2-19.2)	12.0 (8.7-15.3)	12.0 (8.8-15.5)	0.791
PLT (/mm³) (IQR)	362.0 (291.5-452.5)	349.0 (273.5-436.0)	349.5 (275.7-437)	0.665
ESR (mm/h) (IQR)	12.5 (3.5-24.2)	12.0 (2.7-25.0)	12.0 (3-24.7)	0.918
CRP (mg/dL) (IQR)	1.96 (1.07-4.74)	1.10 (0.45-3.04)	1.26 (0.47-3.42)	0.109
Severe respiratory distress (%)	15 (51.7)	41 (19.2)	56 (23)	<0.001

CRP: *c-reactive protein*, ESR: erythrocyte sedimentation rate, Hb: haemoglobin, HR: heart rate, IQR: interquartile range, PLT: platelet, RR: respiratory rate, SD: standard deviation, WBC: white blood cell.

Table III. Selected predictor variables for multivariable model of high-flow nasal cannula failure.

Variable	OR	95% CI	*p* value
pH	0.002	0.000-30.692	0.209
HR at 2nd hour, bpm	0.896	0.793-1.013	0.080
RR at 2nd hour, rpm	1.058	1.012-1.106	0.012
RSV positive	0.565	0.101-3.168	0.516

HR: heart rate, RR: respiratory rate, RSV: Respiratuar Sinsityal Virus.

High-flow nasal cannula failure rate has been found in different studies. The reason for this may be that the definition of failure is handled in different ways in studies. In some studies, failure was defined as intubation and cardiopulmonary arrest[7], while in others needing escalated care (non-invasive or invasive mechanical ventilation) was defined as a nonresponder.[23] In addition, in some studies, inclusion of patients diagnosed with only bronchiolitis may be another factor.[24,25] Because bronchiolitis diagnosis has been found to be protective for non-responders.[7,26] In this study, non-responder rate was found to be 11.9%. Betters et al.[26] found this rate to be 6% in their study on the use of HFNC outside PICU. This rate was even lower in two randomized controlled trials.[24,25] The rate of these patients was between 6 and 19% in the literature.[20,26-29]

As expected, the predictive factors of HFNC failure also differed. Kelly et al.[7] found that a triage RR greater than 90th centile for age, initial venous blood gas demonstrating pCO_2 greater than 50 mmHg or initial venous pH less than 7.30 were independently associated with

PED in their study of patients under two years of age who underwent HFNC for PED with a higher subsequent need for intubation. In a prospective study investigating bronchiolitis patients who underwent HFNC for less than 12 months in PED, it was found that HR and RR did not decrease in the non-responder group.[28] In another retrospective study, bronchiolitis patients who were taken into intensive care unit in which possible predictive factors of HFNC failure were examined, and on admission RR and pCO_2 were found to be higher in the non-responder group.[29] In a retrospective study examining HFNC failure in patients who were undertaken outside PICU, high FiO_2 requirements, previous history of intubation, and cardiac co-morbidity were associative predictors of HFNC failure.[26] In this study we found that non-responders had lower pH on admission. Also after two hours initiation of HFNC therapy, RR and HR did not decrease. In addition, the pulse, RR and pCO_2 on admission were not related with HFNC failure.

Some patients are at risk for developing respiratory failure and need timely identification for escalated care because in our department HFNC therapy is used in patients with moderate to severe respiratory distress. The objective is not to be late for the necessary escalated care. Some scores used in PED are available for this decision such as the Pediatric Risk of Admission Score[30], the Pediatric Early Warning System Score (PEWS)[31], and the pediatric respiratory assessment measure.[32] Hansen et al.[33] used PEWS in their retrospective study to evaluate clinical response in patients receiving HFNC therapy in the pediatric ward. However, as it is known, clinical respiratory scales are generally used for specific diagnoses (e.g., bronchiolitis, pneumonia, etc.) and there is no validated score for patients receiving HFNC treatment. In our study involving patients treated with HFNC at different ages and diagnoses, no adverse effects such as air leak syndrome, bradycardia, bradypnea, emergency intubation, or cardiopulmonary resuscitation were observed. In a series of cases in the literature, three patients with air leak syndrome were reported.[34]

This study has several limitations. First of all it is not a randomized controlled trial. It is a retrospective study conducted in a single center. For this reason, clinical findings of some patients who were treated with HFNC may not have been reached. Moreover, it does not have a control group so we couldn't control for confounding factors. Another limitation is that the comorbidity is very high because our hospital is a tertiary care university hospital (65.8%). On the other hand, it may indicate that HFNC can be used easily regardless of the underlying disease or the patient's diagnosis. Additionally, the subgroups of patients in our study were not evaluated according to age groups or underlying disease, because there was a large range of ages but a relatively small number of patients, especially in patients with HFNC failure. Not surprisingly, in our study, HFNC failure rate was found to be lower. Possible reasons for that could be a small number of severe patients included in the study and rapid initiation of acute treatment in the PED.

We concluded in this retrospective study that HR and RR didn't decrease in the non-responders group two hours after HFNC initiation and the pH were lower on admission in venous blood gases. However, the need for multicenter randomized controlled studies on this subject is evident to determine predictive factors of HFNC failure.

Ethic approval

The written consents from the patient families were obtained according to the Declaration of Helsinki (1964) and the study was approved by the ethics committee of Hacettepe University (GO 19/185; approval date, March 2019).

Author contribution

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

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Conflict of interest

OA, EAA, AZB, OT declared that they have no conflict of interest.

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Original Article

Investigation of immunity against *Bordetella pertussis* in pregnant women and an overview of the vaccination schedule in Turkey

Serdar Gül[1], Cemile Sönmez[2], Gökçe Ayvaz[1], Selçuk Kılıç[2]

[1]*Department of Infectious Diseases and Clinical Microbiology, Kırıkkale University Faculty of Medicine, Kırıkkale;* [2]*Department of Microbiology Reference Laboratories and Biological Products, Ministry of Health, General Directorate of Public Health, Ankara, Turkey.*

ABSTRACT

Background. Pertussis caused by *Bordetella pertussis*, is a disease leading to significant morbidity and mortality in neonates and infants. Direct protection of the infant may be achieved by maternal and neonatal vaccination. Despite primary vaccination, infants under six months pose the greatest risk of infection with pertussis. Maternal immunization provides a high level of infant protection from birth until immunity is achieved by active vaccination. There is no routine Tdap vaccination recommendation for pregnant women in Turkey. This study was carried out to determine pertussis antibody levels in pregnant women and provide data for improving vaccine planning.

Methods. The study was carried out with 133 pregnant women in Turkey. Antibody titers to pertussis toxin (anti-PT) and filamentous hemagglutinin (anti-FHA) were measured by the commercially available ELISA.

Results. Among 133 participants, 93 (69.9%) were found to be immune according to anti-PT IgG antibody levels. According to anti-FHA IgG antibody levels, 123 (92.5%) participants were considered to be immune. A positive correlation was observed between PT and FHA and the findings were statistically significant (P <0.001, r = 0.343). In the study group, the ages of the participants varied between 17 and 44 years. The mean age of those who were immune was 27.3±5.6, the mean age of non-immune patients was 29.1±6.2 and the difference was not statistically significant (P= 0.14).

Conclusions. Our results reveal that approximately one-third of pregnant women were not immune to pertussis, reflecting many young infants to be vulnerable to pertussis infection until the onset of primary vaccinations, although childhood pertussis vaccination coverage has been high for a long time. We conclude that Tdap vaccine recommendation for pregnant women regardless of previous immunization history may be beneficial for the protection of infants in their first six months.

Key words: pertussis, vaccine, pregnant women, antibodies, seroprevalence.

Pertussis is a highly infectious vaccine-preventable disease caused by *Bordetella pertussis*.[1] Although the incidence of the disease has decreased drastically after the introduction of the whole cell vaccine in the 1940s, pertussis remains a major health problem worldwide. Despite high vaccination coverage, a resurgence of pertussis was observed in some parts of the world in the 1980 and 1990s owing to improved surveillance, waning immunity, bacterial evolution, and the usage of sub potent vaccines.[2] The resurgence of pertussis has led to increased morbidity and mortality in infants too young to be vaccinated.[2,3]

The World health organization (WHO) estimates diphtheria-tetanus-pertussis ($DTaP_3$) immunization coverage at 86% worldwide, reaching more than 90% in 129 (66%) of the 194 WHO member states.[4,5] In Turkey, the pertussis vaccine is administered at the ages of 2, 4, 6, 12-24th months and at the first year of elementary

✉ Selçuk Kılıç
selcuk.kilic@sbu.edu.tr

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school (5-6 years of age) as acellular pertussis vaccine with a total of 5 doses resulting in 98% $DTaP_3$ vaccination coverage in 2018. In Turkey, maternal vaccination with a tetanus toxoid (TT) was started in 1994 and TT was replaced with the tetanus-diphtheria (Td) vaccine in 2004. Currently, Td_2 vaccine coverage among pregnant women reached 52% in 2018.[6,7] Acceptable immunity is likely to be achieved one month after the completion of primary vaccination, which means infants younger than 5-7 months are partly susceptible to pertussis.[8,9] Infants of this age category are at relatively high risk for pertussis-related morbidity and mortality and a majority of pertussis-related deaths occur in infants younger than 3 months old.[10,11] Therefore, preventive measures at this age are crucial and maternal immunization is the most effective measure to protect neonates who are vulnerable to pertussis infection.[12-14] On the other hand, booster doses of pertussis vaccine in adolescents and adults are required to prevent the disease, as the immunity that occurs after the disease is passed naturally or vaccinated against the disease decreases within 4-5 years.[1,15,16]

There is a highly significant correlation between the level of anti-Pertussis Toxin IgG antibody in the serum and protection against pertussis.[13,15-18] However, maternal antibody levels are generally low even in the post-vaccine era, so there is no protective level of antibody transmission, and newborns may remain unprotected.[16-23] In clinical studies, acellular pertussis vaccine during pregnancy has been shown to increase the IgG titers in the mother and the IgG titers transferred to the fetus. Consequently, pertussis risk of newborns tends to decrease.[12,16] Recently, maternal immunization with Tdap has been recommended in several countries such as the USA, the United Kingdom, Argentina, Belgium, Israel and New Zealand.[1,11,23] The Advisory Committee on Immunization Practices (ACIP) has also recommended that pregnant women receive Tdap that should be repeated in every subsequent pregnancy as of October 2012.[3] Tdap is not routinely recommended for pregnant women in Turkey and data on the immune status against pertussis and the level of antibodies in pregnant women are extremely limited.[19,23] The aim of this study was to determine pertussis antibody levels in pregnant women in a city representative of the Turkish population for vaccine planning.

Material and Methods

Patient Population

The study protocol was approved by the Clinical Research Ethics Committee of Kırıkkale University, 01/16.01.2020. The study was conducted in Kırıkkale, which is located in central Anatolia and 70 km east of Ankara, the capital of Turkey. Kırıkkale demographics represents the typical characteristics of a Turkish city with a population of about 300,000. Based on the information from Kırıkkale Provincial Health Directorate, there were 1262 pregnant women in Kırıkkale. The minimum sample size that should be taken with a minimum %10 seronegativity estimation and within the 95% confidence interval was calculated as 125 with the Openepi program. In this study, 133 patients who applied to Kırıkkale University Faculty of Medicine between the dates of 16.01.2020 and 16.06.2020 for routine control in the first trimester of their pregnancies were included. Inclusion criteria were: being pregnant and accepting to participate in the study, exclusion criteria: refusal to participate in the study, a history of disease or medication that suppresses the immune system, having symptoms of acute pertussis disease such as cough, fever and shortness of breath.

After obtaining written consent from the patient, a 5 ml of blood sample was drawn and the samples were centrifuged at 1500 rpm for 15 minutes. Separated serum samples were stored at -20°C until the study date. Laboratory work was carried out using the commercial ELISA test in the General Directorate of Public Health, Microbiology Reference Laboratories and Biological Products Department, National

Vaccine Preventable Bacterial Diseases Serology Laboratory. In addition, data on patients' age, education level, occupation, number of pregnancies, childhood vaccine and Tdap vaccine records, presence of whooping cough, and presence of immunosuppressed individuals or individuals under 1 year old in the same house was collected.

Laboratory Tests

Pertussis Toxin (PT) and Filamentous Hemagglutinin (FHA) IgG antibodies were studied with the commercially available ELISA test. Pertussis Toxin IgG antibodies were studied with the Nova Lisa Bordetella pertussis IgG kit (NovaTec Immundiagnostica GmbH, Germany). The results were evaluated in terms of the Nova Tec Unit (NTU). Antibody> 11 NTU was considered to be a protective level and ≤ 11 was considered as a non-protective antibody level. The sensitivity of the kit was 98.3% with a specificity of 93.0%.

FHA IgG antibodies were studied with Demeditec Bordetella pertussis FHA IgG ELISA kit (Demeditec Diagnostics, Germany). Antibody levels >25 IU / ml were considered protective and ≤25IU / ml were considered as non-protective. The sensitivity of the test was 100% with a specificity of 86%. For both tests, ELISA plates were read in an ELISA reader (Labsystem, Multi Skan Ex, Finland) at 450/620 nm wavelength. Anti PT and anti FHA IgG antibody levels were evaluated using the Alisei Software 2.83 statistical analysis program.

Statistical Analysis

Statistical analyses were conducted by using SPSS 18 software. The suitability of representing the anti-PT and anti-FHA IgG antibody levels with a normal distribution was investigated by using visual (histogram, probability plots) and analytical (Kolmogorov-Simirnov) methods. Since the anti-PT and anti-FHA IgG antibody levels and logarithmic values did not match the normal distribution, the correlation coefficient for these parameters was calculated using the Spearman test. Those with anti-PT antibody level> 11 NTU were considered immune. Chi-square and Fisher's exact test and Mann Whitney U test were used for comparison purposes between groups and it was considered significant if the p value was less than 0.05.

Results

Among the 133 patients included in this study 93 (69.9%) were found to be immune according to anti-PT IgG antibody levels. On the other hand, according to anti-FHA IgG antibody levels, 123 participants (92.5%) were found to be immune. A positive correlation was observed between anti PT and anti FHA, and the findings were statistically significant (P <0.001, r = 0.343) (Fig. 1).

In the study group, the ages of the participants varied between 17 and 44 years. The mean age (±SD) of those who were immune was 27.3 ±5.6, whereas the mean age of non-immune patients was 29.1±6.2. This difference was not statistically significant (P=0.14). Table I shows the two age groups above and below 30 years with their immune and non-immune percentages. It was observed that the immunity rates of age were similar to each other and were not statistically significant (P=0.44). The highest number of patients were high school graduates according to their educational status, but the immune status in all groups was close to each other and

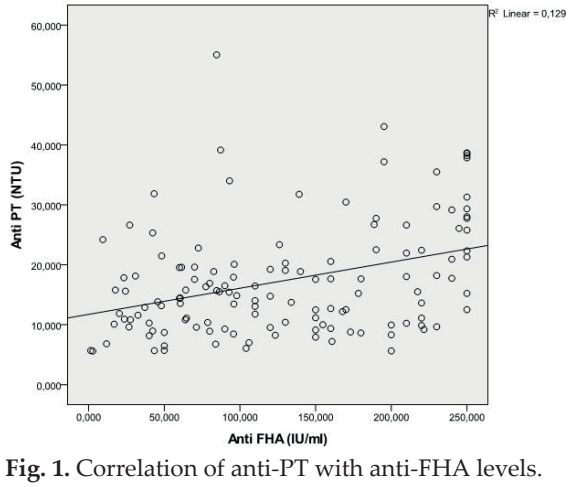

Fig. 1. Correlation of anti-PT with anti-FHA levels.

Table I. Immunity according to patient demographics.

Demographics	Anti PT		anti-FHA		p values
	Immune (%)	Non-immune (%)	Immune (%)	Non-immune (%)	
Age					
17-29	57 (73.1)	21 (26.9)	76 (97.4)	2 (2.6)	0.44
Above 30	36 (65.5)	19 (34.5)	47 (85.5)	8 (14.5)	
Education (n; %)					
Elementary school	19 (70.4)	8 (29.6)	23 (85.2)	4 (14.8)	
Secondary school	14 (70.0)	6 (30.0)	17 (85.0)	3 (15.0)	0.99
High school	41 (70.7)	17 (29.3)	56 (96.6)	2 (3.4)	
University	19 (67.9)	9 (32.1)	27 (96.4)	1 (3.6)	
Occupation (n; %)					
Employee	14 (70.0)	6 (30)	19 (95.0)	1(5.0)	0.99
House-wife	79 (69.9)	34 (30.1)	104 (92.0)	9 (8.0)	
Pregnancy (n; %)					
First pregnancy	36 (69.2)	16 (30.8)	46 (88.5)	6 (11.5)	0.98
Second pregnancy	40 (70.2)	17 (29.8)	53 (93.0)	4 (7.0)	
Three or more	17 (70.8)	7 (29.2)	24 (100)	0	
Vaccination history					
Complete	8 (88.9)	1 (11.1)	9 (100)	0	0.44
No vaccine	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	
Can't remember*	81 (68.6)	37 (31.4)	109 (92.4)	9 (7.6)	

p values were calculated according to anti PT levels.
*This group states that childhood vaccines are complete but do not remember their content.

the difference was not statistically significant (Table I). When the participants were examined according to their employement status (i.e. employed and housewives), the immune status was similar among the groups and the difference was not statistically significant. When the number of pregnancies of the participants were examined, it was observed that there was no correlation between the number of pregnancies and immune status (Table I).

It was found that 118 (88.7%) patients had childhood vaccinations but could not remember whether DTaP vaccine was available. Immune status according to the childhood vaccination is given in Table I. Accordingly, there was no difference observed between the groups. It can be observed that there is a significant number of non-immune participants (40/133; 30.1%), whose infants can be vulnerable to pertussis if not vaccinated immediately.

Discussion

Pertussis continues to cause deaths worldwide. According to the data from WHO, 16 million cases were seen only in 2008 and 195.000 of them were fatal.[1] Infants are in the highest risk group in terms of severe pertussis and they usually get the disease from their adult relatives.[23,24]

Studies show that antibody levels begin to decrease and fall below the protective level 4-5 years after the acellular pertussis vaccine.[1] Therefore, booster doses are needed for long-term immunity in adulthood. The ACIP, therefore, recommends routinely administering the Tdap vaccine to pregnant women since 2012 and repeating this vaccine in every subsequent pregnancy.[1]

Clinical studies are being conducted in the United States and many countries in Europe concerning the results of this vaccination.[1] There

is no routine recommendation of pertussis vaccine for adults and pregnant women in Turkey.[6] The last dose of pertussis vaccine is applied in the first year of primary school (4-6 years old) resulting in a lack of adequate antibody levels in pregnant woman. Although contact with mild or asymptomatic pertussis cases in the society may provide protective antibodies against the disease, a significant part of the society remains susceptible to the disease in adulthood as corroborated with the data presented in the previous section.[25-27]

Studies showing immunity against pertussis in pregnant women are extremely limited in Turkey. In the study conducted by Ercan et al.[19] 72% of pregnant women were found to be immune to pertussis. In our study, the immunity rate was similar 69.9%. In studies conducted in other countries, the rates of immunity in pregnant women vary between 25% and 97.1%.[15,16,27] It is thought that this difference can be attributed to the differences in the vaccination programs, different probability of the prevalence of pertussis disease and differences in disease prevelance rates of different countries.[3,12-14,16,17] Pertussis is usually diagnosed serologically or clinically. According to the Centers for Disease Control (CDC) and WHO, pertussis diagnosis can be established clinically in patients with cough for at least 14 days and the presence of at least one of the following symptoms: paroxysmal cough, post-cough vomiting, or whooping sound in the inspiration.[1] Serologically, antibodies against PT and FHA can be used in diagnosis. Antibodies against FHA antigens are not specific and may occur after *Haemophilus influenza* and *Mycoplasma pneumonia* infections, as well as other *Bordetella* infections. Antibodies developing against PT are specific for *B. Pertussis* and can be used in diagnosis.[1] In our study, the level of immunity in patients was 69.9% according to anti-PT IgG antibodies and 92.5% according to anti-FHA IgG antibodies. In the light of this data, the immune ratio in pregnant women was accepted as 69.9%. There is a rather limited number of studies investigating the level of immunity against pertussis in adults in Turkey. It has been shown that immunity rates vary between 58.1% and 72%.[16,19]

Six of the patients (4.5%) who participated in our study stated that their pertussis vaccine or childhood vaccines were not complete. Other patients reported that childhood vaccines were complete, although they did not remember exactly whether the whooping cough vaccination was complete. The coverage rate of pertussis vaccine between the years 2007-2018 in Turkey varies between 96-98%.[23] This result is supported by the data presented herein with the rate of vaccination in pregnant women being 95.5%. There was no significant difference in terms of pertussis immunity between the patients who stated that their vaccinations were not complete and who had complete their vaccines.

While the average age of patients with pertussis immunity was 27.3 years, the average age of those without immunity was 29.1 years. There was no significant difference in immunity levels when patients were divided into two age groups, over 30 years old and below 30 years old. The reason for this is interpreted as the fact that the current immunity in patients is caused by the cases of whooping cough they encounter in the community rather than the last pertussis vaccine that was applied at the age of 4-6. Similarly, Kurtoğlu et al.[28] stated that protective antibody levels against whooping cough do not decrease in advanced age or even in the population above 70 years of age, which may be due to frequent contact with whooping cough.

The diagnosis of pertussis is difficult in adults and adolescents due to the mild and atypical symptoms, making it extremely challenging to determine the true incidence of the disease. The annual number of pertussis cases reported between 2009-2018 was between 11 and 322[25] in Turkey. In a study by Sönmez et al.[26] in adults with prolonged cough, pertussis was detected in 52 (9.7%) of 538 patients. Similarly, in the study of Gürsel et al.[29] in children with prolonged

cough, pertussis was detected in 12 (23.5%) of 51 patients. The data suggest that pertussis disease continues to be a common persistent public health problem in Turkey and the actual number of cases is significantly higher than reported. Hence, an improved public health policy regarding pertussis vaccination should be a Tdap vaccine administered in the second or third trimester during pregnancy which increases the number of antibodies in the mother and the fetus.[29,30] In some studies, it has been shown that deaths due to pertussis may decrease or even disappear in infants younger than six months of age if the administration of the Tdap vaccine in pregnant women becomes widespread.[30-32] The Tdap vaccine has been found to be safe in pregnant women and it has been reported that it does not increase the risk of hypertensive syndrome, chorioamnionitis, preterm delivery or low birth weight baby.[14,33] In addition, the Tdap vaccine was found to be cost-effective.[34]

Controversy still exists regarding prenatal (After 27 weeks' gestation) or post-partum immunization (up to 14 days after delivery) in the literature. It is emphasized that women should receive the Tdap vaccine during pregnancy regardless of previous immunization history. Given prenatal or post-partum immunization of mothers and development of pertussis in infants, infants whose mothers received prenatal immunization had 50% fewer cases of pertussis compared with those whose mothers received post-partum immunization. Results of cohort studies conducted in the USA and England showed that prenatal maternal Tdap vaccination is 91% effective in preventing pertussis during the first three months of life and 93% effective in preventing pertussis in the first eight weeks.[31,35-37]

In the USA, the National Advisory Committee on Immunization (NACI) and the ACIP of the CDC have recommended routine Tdap vaccination for adults.[1] Additionally, experience in developed countries such as Canada and the USA has demonstrated that an adolescent pertussis vaccine program implemented on a national scale, is safe and can result in a further decrease in the incidence of pertussis. On the other hand, in line with the ACIP recommendation, studies on routine Tdap vaccination in pregnant women are in use in the USA, UK and some European countries.[1-3] In our country, based on the previous data reflecting a significant number of pregnant women who are non-immune to pertussis, we recommend that the Tdap vaccine be implemented for pregnant women regardless of previous immunization history which in turn will be beneficial for the protection of infants in their first six months. Pertussis vaccination rate during pregnancy can be increased by the addition of Tdap vaccine in the routine vaccination schedule or national vaccination campaigns.

In conclusion, the present study revealed that approximately one third of pregnant women are not immune to pertussis, reflecting that newborn infants are vulnerable to pertussis infection. This data supports the need for pertussis vaccination with Tdap in the routine maternal immunization schedule for preventing infant infection in Turkey.

Author contribution

The contributions of all authors must be described in the following manner: The authors confirm contribution to the paper as follows: study conception and design: SG, SK; data collection: GA; analysis and interpretation of results: CS, GA, SG; draft manuscript preparation: SK, SG.

All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the Clinical Research Ethics Committee of Kırıkkale University, 01/16.01.2020.

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Conflict of interest

The authors declare no conflict of interest.

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Original Article

Cerebral sinovenous thrombosis in children: clinical presentation, locations, and acquired and inherited prothrombotic risk factors

Yeter Düzenli Kar[1,2], Zeynep Canan Özdemir[2], Kürşat Bora Çarman[3], Coşkun Yarar[3], Neslihan Tekin[4], Özcan Bör[2]

[1]*Division of Pediatric Hematology and Oncology, Department of Pediatrics, Afyonkarahisar Health Sciences University, Afyonkarahisar; Divisions of* [2]*Pediatric Hematology and Oncology,* [3]*Pediatric Neurology and* [4]*Neonatology, Department of Pediatrics, Eskişehir Osmangazi University Faculty of Medicine, Eskişehir, Turkey.*

ABSTRACT

Background. Cerebral sinovenous thrombosis (CSVT) in children is a rare and life-threatening cerebrovascular disease. Hence, we evaluated its clinical presentations, inherited and acquired prothrombotic risk factors along with the accompanying diseases, the thrombosis locations as well as the outcomes of anticoagulant therapy in children with CSVT.

Methods. The medical records of pediatric CSVT patients treated between January 2011 and September 2018 were analyzed retrospectively.

Results. The study included 29 children, 15 boys (51.7%) and 14 girls (48.3%), with the median age being 11 years (range:3 days-17 years). The most commonly presented complaint in neonates was seizures and in the non-neonatal age groups was a headache. Also, at least one acquired and/or inherited thrombophilic risk factor was identified in 89.7% of the patients. The most commonly acquired prothrombotic risk factors along with the accompanying diseases included infections, central venous catheter, and dehydration, while the most commonly inherited thrombophilic risk factors included heterozygous factor-V Leiden mutation and elevated lipoprotein (a). The most common thrombosis location was found to be the transverse sinus. Also, none of the patients died due to the thrombotic episode. Complications included epilepsy in five patients, hydrocephalus in one patient, and intracranial hypertension in another patient.

Conclusions. Clinicians need to be well aware of the inherited and acquired prothrombotic risk factors in CSVT. It should also be kept in mind that at-risk patients may also present with nonspecific signs and symptoms with no apparent neurological manifestation. The risk of acute complications and long-term sequelae can be substantially reduced if diagnosed early and initiated with appropriate treatment at the early stages.

Key words: cerebral sinovenous thrombosis, children, risk factors.

Cerebral sinovenous thrombosis (CSVT) in children is a rare and life-threatening cerebrovascular condition, and with increased clinical awareness and improved neuroradiological techniques, the disease can now be diagnosed more frequently and at earlier stages. Early diagnosis of CSVT is crucial since the risk of acute complications and long-term sequelae can be substantially reduced if appropriate treatment is initiated within the first few hours.[1] The incidence of childhood CSVT occurs between 0.4 and 0.7 children per 100,000 children per year.[2] The incidence is higher among neonates (30–50%) compared to other pediatric age groups. There is a male predominance with boys accounting for approximately two-thirds of all cases in children.[3]

✉ Yeter Düzenli Kar
yeterduzenli@yahoo.com

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The etiology of childhood CSVT is multifactorial. Also, 33% to 99% of patients have inherited or acquired prothrombotic risk factors. The most well-known acquired prothrombotic risk factors along with the accompanying diseases include catheters, infection, dehydration, chronic inflammatory diseases, nephrotic syndrome, and malignancies.[4] Studies have reported inherited thrombophilic risk factors including antithrombin deficiency, protein C and protein S deficiency, Factor V Leiden and prothrombin G20210A mutations, hyperhomocysteinemia, elevated circulating levels of factors II, VIII, IX, XI, and fibrinogen[5], and high lipoprotein (a) level.[6,7] Recent studies have indicated that mutations in the heterozygous methylenetetrahydrofolate reductase (MTHFR) gene alone cannot increase the risk of thrombosis. Hyperhomocysteinemia occurs in cases with homozygous MTHFR mutation due to the remethylation of impaired homocysteine to methionine. Hyperhomocysteinemia is also considered a strong risk factor for thrombosis in children.[8,9]

The most commonly recommended treatment for CSVT without significant intracranial hemorrhage includes anticoagulation treatment initially with either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) for neonates and children. This initial therapy is followed by LMWH for 6 weeks to 3 months in neonates and LMWH or oral anticoagulant therapy for 3 to 6 months in children.[10] The present study evaluated the clinical presentations, inherited and acquired prothrombotic and accompanying diseases risk factors, thrombosis locations along with the results of anticoagulant therapy in CSVT patients treated in our hospital over the last 8 years.

Material and Methods

1. The medical records of pediatric patients with CSVT who underwent treatment and follow-up in the pediatric and neonatal intensive care units between January 2011 and September 2018 were analyzed retrospectively. The study was approved by the local ethics committee of Eskişehir Osmangazi University Clinical Researches (09.10.2018, no:17). The patients' age, sex, inherited and acquired prothrombotic risk factors and accompanying diseases, neurological symptoms and findings, thrombosis locations, treatment, and outcomes were reviewed from their medical records.

CSVT was diagnosed using brain magnetic resonance imaging and/or brain magnetic resonance venography. The results of etiological investigations were analyzed in all patients which included the complete blood count, cholesterol, triglycerides, lipoprotein (a), protein C, protein S, D-dimer, fibrinogen, antithrombin, homocysteine, C-reactive protein levels, activated protein C resistance, prothrombin time, activated partial thromboplastin time, antinuclear antibody (ANA) and extractable nuclear antigen antibody (ENA) profiles, and antiphospholipid and anticardiolipin IgG antibodies. The genetic mutation analysis in Factor V Leiden G1691A, prothrombin G20210A mutation, *MTHFR* C677T, and *MTHFR* A1298C were evaluated.

Statistical analysis

Data were analyzed with SPSS 17.0 statistical software package (SPSS Inc., Chicago, NY, USA) using descriptive statistics. The Shapiro–Wilk test was used to check whether numerical variables were normally distributed while the Chi-square (χ^2) test (exact method) was used to compare categorical variables.

Results

Patient Population

This study included a total of 29 children. Of which, five were neonates (0–28 days); with two (40%) females and three (60%) male neonates. The non-neonatal age groups (29 days-18 years) included 12 girls (50%) and 12 boys (50%) with a median age of 11 years (minimum 3 days, maximum 17 years) (Fig. 1).

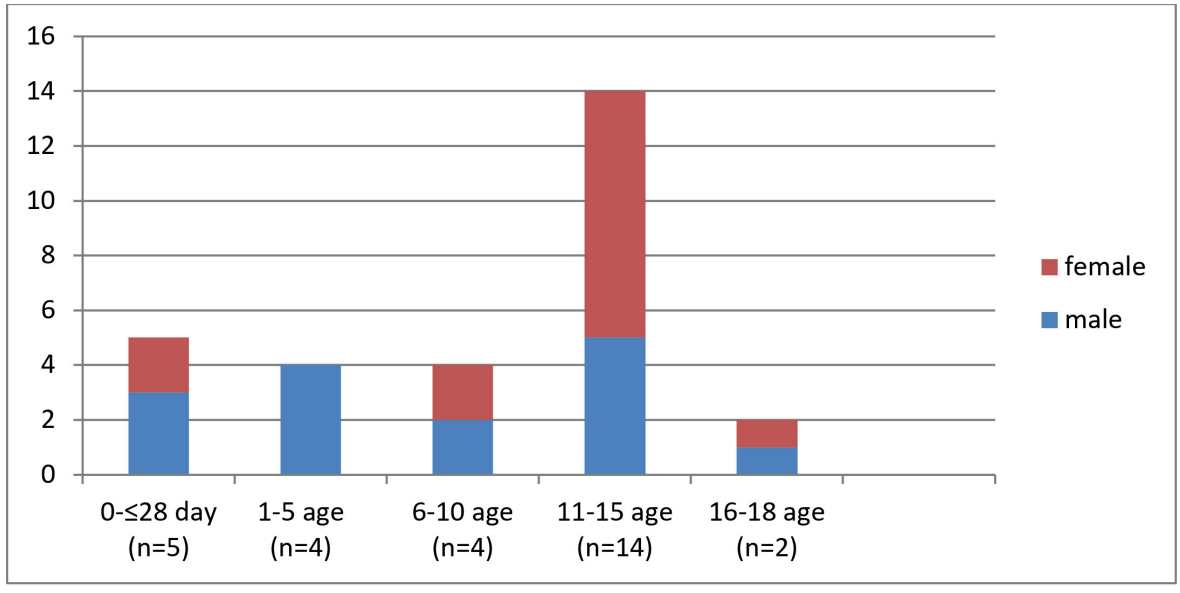

Fig. 1. Sex distribution of pediatric cerebral sinovenous thrombosis patients according to age group.

Clinical presentation (Neurological symptoms and findings)

All five neonates with CSVT presented with seizures (100%), accompanied by hemiparesis in two patients (40%), change in consciousness in two (40%) patients, and respiratory failure in one (20%) of the patients. Patients in the non-neonatal age groups presented with a headache (87.5%), vertigo (50%), diplopia (29.2%), papilledema (29.2%), hemiparesis (29.2%), change in consciousness (25%), seizures (20.8%), facial paralysis (12.5%), ptosis (8.3%), and numbness (8.3%) (Table I).

Table I. Distribution of the patients age, sex, and neurological symptoms and findings.

Neonates (0–28 days)	n	%
Seizures	5	100
Change in consciousness	2	40
Hemiparesis	2	40
Respiratory failure	1	20
Infants, children, and adolescents (29 days–18 years)		
Headache	21	87.5
Vertigo	12	50
Hemiparesis	7	29.2
Double vision	7	29.2
Papilledema	7	29.2
Change in consciousness	6	25.0
Seizures	5	20.8
Facial paralysis	3	12.5
Ptosis	2	8.3
Numbness	2	8.3

Acquired and inherited thrombophilic risk factors

At least one acquired and/or inherited thrombophilic risk factor was identified in 26 patients (89.7%), while the other three patients (10.3%) showed no known risk factors. Seven of the 21 patients with acquired risk factors also had inherited thrombophilic risk factors. The most commonly acquired risk factors included infections (24.1%), a central venous catheter (6.9%), and dehydration (6.9%), while the most commonly inherited thrombophilic risk factors included heterozygous factor V Leiden mutation (13.8%) and elevated lipoprotein (a) level (13.8%) (Table II). All five of the neonates had an acquired risk factor and accompanying diseases (dehydration in two, severe infection [sepsis] in two, and ventriculoperitoneal shunt

Table II. Acquired and inherited thrombophilic risk factors of the patients.

Risk Factors	n (%)
Acquired Prothrombotic Risk Factors	21 (72.4)
Infection (4)*	7 (24.1)
Jugular venous catheter	2 (6.9)
Dehydration (1) ≠	2 (6.9)
Obesity	2 (6.9)
Chronic kidney disease + oxalosis (1) µ	1 (3.5)
Juvenile idiopathic arthritis (1) ∞	1 (3.5)
Nephrotic syndrome	1 (3.5)
Mental retardation + epilepsy	1 (3.5)
Congenital heart disease	1 (3.5)
Acute leukemia	1 (3.5)
Hereditary spherocytosis	1 (3.5)
Hydrocephalus + ventriculoperitoneal shunt	1 (3.5)
Inherited Thrombophilic Risk Factors	13 (44.8)
Factor V Leiden mutation (heterozygous)	4 (13.8)
Lipoprotein(a) elevation (>30 mg/dL)	4 (13.8)
Prothrombin G20210AI mutation (heterozygous)	2 (6.9)
Hyperhomocysteinemia + homozygous/compound heterozygous *MTHFR*	3 (10.3)
No known risk factors	3 (10.3)

*Accompanied by heterozygous factor V Leiden mutation in 2 patients, lipoprotein(a) elevation in 1 patient, hyperhomocysteinemia + homozygous/compound heterozygous MTHFR in 1 patient.
≠Accompanied by lipoprotein(a) elevation in 1 patient.
µAccompanied by heterozygous factor V Leiden mutation and hyperhomocysteinemia + homozygous/compound heterozygous MTHFR in 1 patient.
∞Accompanied by heterozygous prothrombin G20210AI mutation in 1 patient.

and hydrocephalus in one neonate). One of the neonates with dehydration also had elevated lipoprotein (a) level as an associated inherited risk factor. However, no inherited risk factors were identified in the other neonates.

Of the two neonates with infection as an acquired risk factor, one developed transverse and sigmoid sinus thrombosis following sepsis, while the other developed cerebral and Galen vein thrombosis after sepsis. Cases associated with infection in the other age groups included three patients developing lateral sinus thrombosis after otitis and mastoiditis, one developing cavernous sinus thrombosis after periorbital cellulitis, and the other developing bilateral transverse sinus thrombosis after meningitis.

Thrombotic Locations

Of the CSVT patients in this study, 44.8% had thrombosis in a single anatomic venous system, while 55.2% showed involvement of multiple venous systems. The most common anatomic site of thrombosis was the transverse sinus (Table III). None of our patients had thrombosis with intracranial hemorrhage.

Treatment

Treatment was initiated with unfractionated heparin or LMWH in all patients. LMWH was preferred over UFH due to its ease of administration, particularly in newborns and infants, patients with high hemorrhage risk, and the one for whom continuous vascular access was not possible.[8]

Table III. Thrombosis locations of patients diagnosed with central sinovenous thrombosis.

Thrombosis Location	n (%)
Single anatomic venous system	13 (44.8)
Transverse sinus*	9 (31.0)
Sagittal sinus	1 (3.5)
Jugular vein thrombus	1 (3.5)
Cavernous sinus	1 (3.5)
Sphenoparietal sinus	1 (3.5)
Multiple anatomic venous systems	16 (55.2)
Transverse sinus + Sigmoid sinus*	5 (17.2)
Bilateral transverse sinus	3 (10.3)
Bilateral sagittal sinus	1 (3.5)
Sinus rectus + Inferior sagittal sinus	1 (3.5)
Sinus rectus + sinus confluence	1 (3.5)
Transverse sinus + Galen vein$^{\alpha}$	1 (3.5)
Sagittal vein + Transverse sinus	1 (3.5)
Cerebral vein + Galen vein$^{\beta}$	1 (3.5)
Jugular vein + Transverse sinus	1 (3.5)
Transverse sinus + Sigmoid sinus + Jugular vein	1 (3.5)

*1 neonate, *2 neonates, $^{\alpha}$1 neonate , $^{\beta}$1 neonate.

All neonates received anticoagulant therapy of LMWH for 3 months. In three non-neonatal patients, anticoagulation was initiated with UFH for the first 5 to 7 days, which was then continued with an oral anticoagulant (warfarin) administration. Twenty-one patients started treatment with LMWH, where 15 continued treatment with LMWH while six of them were switched to oral anticoagulant (warfarin) after 5 to 7 days. All patients were treated for at least 3 to 6 months. None of the patients had anticoagulant-related bleeding complications.

Outcomes

The mean follow-up time was 24 months. None of the patients died due to their thrombotic episodes. Five patients developed epilepsy, one patient developed hydrocephalus, and one patient developed intracranial hypertension. Two epilepsy patients and one hydrocephalus patient were neonates while the non-neonatal age group showed other complications. One patient's ptosis resolved after about one year.

Discussion

CSVT is a rare cerebrovascular disease that can occur in children of any age starting from the neonatal period, and carries a high risk of mortality and neurological sequelae. It is observed more commonly in males (56–75%).[6,8,11-16] In the present study, male predominance was observed in the neonatal group consistent with the literature, whereas males and females were equally represented in the other age groups. The incidence of CSVT is reported to be highest in the neonatal period.[6,11,14,17-20] Beside the neonatal period, it was more common in the 6–11 year age group (36.84%) and in the 15–18 age group patients (35.29%).[15,21]

CSVT patients can exhibit various signs and symptoms such as headaches, seizures, papilledema, cranial nerve palsies, motor weakness, and altered mental status. The most common neurological symptoms and findings in the literature include seizures in neonates[4], whereas the headache was reported in 90% of

adults and 60% of children.[22] All the neonates in this study presented with seizures. The most common neurological symptoms and findings in the non-neonatal age groups were headache (87.5%), dizziness (50%), hemiparesis (29.2%), and seizures (20.8%). Less frequent findings were diplopia, papilledema, numbness, ptosis, and facial paralysis. Although seizures were more common in neonates, they may also occur in any child with CSVT. In addition to seizures, two of our neonates showed changes in consciousness, two showed hemiparesis, while one developed respiratory failure. The incidence of seizures in all childhood CSVT was reported as 26.9% by Lolli et al.[20], 34.8% by Ozcan et al.[5], 37.5% by Javed et al.[16], 37.9% by Heller et al.[11] and 40% by Sèbire et al.[14]. Hemiparesis was reported at a rate of 13–37.5% in the literature, which was consistent with our findings. Alterations in mental status may have manifested as irritability, stupor, or coma.[6,23,24] Clinically, seizures and coma were reported as poor prognostic factors[23], while isolated headache was considered a favorable prognostic factor.[24] Isolated headache was the most commonly presented complaint in most of our patients. Also, none of our patients went into a coma or died due to CSVT.

Acquired prothrombotic risk factors along with the accompanying diseases reported in the etiology of CSVT include infections, dehydration, surgery, jugular or subclavian central venous catheters, solid tumors, leukemia and lymphomas, anemia, autoimmune diseases, renal diseases, obesity, metabolic disorders, birth asphyxia, and cardiac malformations.[13] In this study, 10.3% of the patients showed no known acquired prothrombotic risk factors and accompanying diseases or inherited risk factors, while the other 89.7% showed at least one acquired and/or inherited risk factor. Similar to our study, Wasay et al.[19] and Lolli et al.[20] reported that 90% and 84.3% of all childhood CSVT cases, respectively, indicated the presence of one or more acquired prothrombotic risk factors and/or inherited thrombophilic risk factors. Acquired prothrombotic risk factors were identified in 70.5% of patients in the study by Heller et al.[11], 77.4% by Carvalho et al.[18], 81.5% by Kenet et al.[25], 86.8% by Viera et al.[12], 88.8% by Suppiej et al.[26], and 100% by Sèbire et al.[14]. The prevalence of acquired prothrombotic risk factors along with the accompanying diseases in the present study was 72.4%, which is consistent with the literature. Infection was the most commonly acquired prothrombotic risk factor in our study. Most infection-associated cases occurred in patients with head and neck infections.[6,20,22,27] Also, head and neck infections were most common in our patient series, with three out of seven cases associated with infection occurring after otitis and mastoiditis, while one case occurred after periorbital cellulitis, and one after meningitis.

In 44.8% of our patients, at least one inherited thrombophilic risk factor was identified In previous large-scale studies conducted in Turkey, inherited thrombophilic risk factors were reported in 30–54% of patients.[28-30] According to the literature, factor V Leiden mutation is the most commonly detected inherited thrombophilic risk factor in venous thromboembolism.[5,21,28,29] In the present study, the most commonly inherited thrombophilic risk factors were factor V Leiden mutation (13.8%) and elevated lipoprotein (a) level (13.8%). According to a study by Heller et al.[11], these were also the most commonly identified inherited thrombophilic risk factors. Population-based studies have shown that homocysteine metabolism-related gene polymorphisms such as *MTHFR* C677T and *MTHFR* A1298C do not alone cause thrombosis but increase the risk of thrombosis and cardiovascular disease when accompanied by elevated plasma homocysteine levels. Particularly, two known polymorphisms of the *MTFHR* gene (C677T and A1298C) have been associated with high homocysteine levels.[5] Homocysteine is an amino acid that is formed as an intermediate product while converting methionine to cysteine and requires Vitamin B for conversion to either methionine or cysteine in reactions. Both B vitamin deficiencies (B_6, folic acid, B_{12}) and mutations in the MTHFR

gene plays a role in the conversion of folate to its active form and are risk factors for hyperhomocysteinemia.[22,31] In our study, hyperhomocysteinemia with homozygous/compound heterozygous *MTHFR* mutation was the third most commonly inherited thrombophilic risk factor, which was detected in 10.3% of the patients.

The involvement in more than one anatomical region (multiple venous sinuses) was reported as more common in pediatric CSVT cases, with the transverse sinus being the most frequently involved region.[5,19,32] Multiple venous sinus thrombosis (55.2%) and transverse sinus thrombosis were also more common in the present study. Wasay et al.[19] observed multiple venous sinus involvement in 74% of their patients, with transverse sinus involvement being the most common (73%). Ozcan et al.[5] also reported that multiple sinus involvement was more frequent and reported the transverse sinus as the most commonly involved region (69.6%). Viera et al.[12] reported the transverse sinus to be most commonly involved as well (67.9%). In contrast, Lolli et al.[20] reported that 40.7% of patients had multiple sinus involvement while thrombosis was most frequently located in the transverse sinus in neonates (27.3%) and the cortical veins in older children (31.3%). Superior sagittal sinus thrombosis was common in some studies, which was detected at frequencies of 100% by Javed et al.[16], 62.4% by Heller et al.[11], and 47.4% by Bonduel et al.[15]. Of the five neonates in our study, thrombosis was located in the transverse and sigmoid sinuses in two neonates; in one neonate, it was located in the transverse sinus thrombosis; in another neonate, the location was in transverse sinus and Galen vein, and cerebral vein and Galen vein in another neonate.

According to the scientific statement from the American Heart Association/American Stroke Association, anticoagulation is the main treatment except in the case of otogenic lateral sinus thrombosis. The type, dose, and route of the anticoagulant agent were selected based on the individual patient's circumstances. For patients with CSVT and hemorrhagic infarction, otitis media/mastoiditis, head trauma, or neurosurgery, a multidisciplinary approach for anticoagulation should be undertaken.

American College of Chest Physicians Evidence-Based Clinical Practice Guidelines recommends anticoagulation treatment initially with either UFH or LMWH and subsequently with LMWH or vitamin K antagonists (VKA) for a minimum of 3 months for children with CSVT without significant ICH. If CSVT occlusion or ongoing symptoms persist after the initial 3 months of therapy, further administration of anticoagulation was suggested for 3 more months.[7] For CSVT patients with significant hemorrhage, the radiologic monitoring of the thrombosis was suggested for 5 to 7 days, and anticoagulation was suggested if thrombus extension was noted at that time or initial anticoagulation was suggested for children without hemorrhage in close follow-up.[7,10] However, to choose one of these two options, the multidisciplinary decision has to be considered. In children with CSVT and recurrent risk factors (e.g., nephrotic syndrome, L-asparaginase therapy), prophylactic anticoagulation was suggested at times of risk factor recurrence. Thrombolysis, thrombectomy, or surgical decompression may be required only in children with severe CSVT, where no improvement was observed after initial anticoagulant therapy. Also, supportive care and neuroprotective measures such as replacement of intravenous fluids, oxygenation, the elevation of the head of the bed to 30°, and treatment of seizures are important too. Children with CSVT should be followed up for increased intracranial pressure and papilledema. Repeated MRV venography is required during the follow-up therapy to decide the duration of anticoagulation.[7]

None of our patients had thrombosis with intracranial hemorrhage. Therefore, anticoagulation was initiated in all our patients immediately upon diagnosis. Also, studies in children have reported that LMWH and UFH are safe in children, where anticoagulant therapy reduces mortality, preserves cognitive

functions, and lowers the rate of recurrent thrombosis.[7,24]

The limitations of our study were as follows: a small number of patients, a single-center center experience-based study, and the retrospective nature of the study.

In conclusion, CSVT in children is a rare and life-threatening condition. Early diagnosis is critical since the risk of acute complications and long-term sequelae can be significantly reduced with an appropriate treatment approach implemented in the early stage. Therefore, clinicians need to be well aware of the clinical picture of CSVT and it is important to keep in mind that these patients may not have neurological symptoms such as seizures, diplopia, papilledema, numbness, ptosis, or facial paralysis, and may only be presented with nonspecific symptoms and findings, such as headache, dizziness, fatigue, and respiratory failure. Also, thorough knowledge of the acquired prothrombotic risk factors along with the accompanying diseases and inherited prothrombotic risk factors facilitates the early diagnosis of patients at risk.

Ethical approval

The study was approved by the local ethics committee (09.10.2018, no:17).

Author contribution

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

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Conflict of interest

No conflict of interest.

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Original Article

Turkish reference ranges for the left fetal modified myocardial performance index

Kadriye Yakut[1], Doğa Fatma Öcal[2], Filiz Öztürk[2], Merve Öztürk[2], Cem Yaşar Sanhal[3], Şevki Çelen[1], Yaprak Üstün[1]

[1]Department of Perinatology , Etlik Zubeyde Hanim Women's Health Teaching and Researching Hospital, Ankara; [2]Department of Perinatology ,Turkish Ministry of Health, Ankara City Hospital, Ankara; [3]Department of Perinatology, Akdeniz University Faculty of Medicine, Antalya, Turkey.

ABSTRACT

Background. This study aimed to assess fetal cardiac left ventricular function in healthy pregnant women by calculating the modified myocardial performance index (Mod-MPI) and to construct reference ranges for the Turkish population.

Methods. One-hundred-two randomly selected healthy singleton pregnant women ranging between 25 and 39 gestational weeks were included in the study. Left fetal Mod-MPI was measured for each pregnant woman. Women with chronic systemic diseases or fetuses with chromosomal or structural abnormalities were excluded from the study. Mitral valve (MV) and aortic valve (AoV) clicks were used as landmarks to define the following time periods that were used to calculate the Mod-MPI: isovolumetric contraction time (ICT), isovolumetric relaxation time (IRT), and ejection time (ET).

Results. The mean Mod-MPI was 0.42±0.10. The mean IRT, ICT, and ET were 43.5±10.2, 27.27±8.1, and 170.5±16.9, respectively. A significant correlation was found between Mod-MPI and gestational age, umbilical artery systolic/diastolic (UA S/D) ratio and the middle cerebral artery pulsatility index (MCA PI) values (r=0.199, p=0.047, r=-0.328, p=0.001, and r=-0.0349, p=0.001, respectively)

Conclusions. The current study's results will be a reference for future studies, especially studies investigating pathological conditions that impact fetal cardiac function.

Key words: Modified myocardial performance index, healthy pregnancy, normal ranges, obstetrics.

The myocardial performance index (MPI) is a ratio obtained by Doppler ultrasound to assess heart functions.[1] MPI may be applied to the right or left ventricles of the heart. MPI is calculated by dividing the sum of the isovolumetric contraction time (ICT) and the isovolumetric relaxation time (IRT) by the ejection time (ET).[1] Since there may be inter- and intraobserver variations, the MPI has been modified and valve clicks have been used as landmarks to optimize the measurements.[2] The prognostic value of the MPI with other strain and remodeling indices has been studied in athletes with hypertrophic cardiomyopathy.[3,4] In the present study, we hypothesized that conditions that influence fetal cardiac functions will result in changes in the MPI values. Fetal growth restriction, twin-twin transfusion syndrome, maternal diabetes, preeclampsia, fetal hydrops, fetal anemia, and cholestasis are examples of these conditions.[5-15] MPI values may be a guide for fetal interference and timing of the delivery in cases that affect cardiac function. However, it is important to ensure that the measurement of MPI is consistent and accurate. There is no universal reference range for an optimal MPI measurement methodology and no guidelines for normal values. Therefore, the present study aimed to measure MPI in healthy fetuses,

✉ Kadriye Yakut
yakutkadriye@hotmail.com

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and to standardize our own reference values and measurement method before conducting research on MPI measurements in relation to pathological conditions.

Material and Methods

Study Design and Patient Selection

Between November 2018 and February 2019, 102 randomly selected healthy singleton pregnant women ranging between 25 and 39 gestational weeks were included in the study. Ethical approval was obtained from the Zekai Tahir Burak Women's Health and Research Hospital's ethics committee before the study was conducted (Review board number: 21/2019). The study was performed in accordance with the ethical standards described in the original 1964 Declaration of Helsinki, and revised in 2013. Written informed consent was obtained from the patients included in the study. Pregnant women with a chronic systemic disease, such as diabetes, hypertension, heart disease, goiter, and kidney disease, or those with a history of drug use that would affect cardiac functions were excluded from participating in the study. The results of double-triple screening tests, detailed ultrasonography, and oral glucose loading tests were evaluated. Pregnant women with low risk and normal screening results were included in the study.

Mod-MPI Measurement

All ultrasonographic measurements were performed by one obstetrician, who had at least 10 years of experience. The observer evaluated each patient and obtained three measurements. The mean of all measurements was recorded.

Ultrasonographic measurements were performed using a Voluson 730 Expert (GE Medical Systems, Waukesha, WI, USA) ultrasound machine and 2-7 MHz curvilinear probe during the absence of fetal movements. Both mechanical and thermal indices were kept below 1.0. Gestational age (GA) was calculated according to the first day of the last menstrual period, and it was confirmed by the first-trimester crown rump length (CRL) measurement. Initially, fetal biometry was performed. Biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), femur length (FL), and estimated fetal weight (EFW) were evaluated and recorded. Subsequently, amniotic fluid index, umbilical artery (UA) Doppler, and localization and structure of the placenta were examined, and all the measurements were recorded. Fetal echocardiographic measurements were performed after it was determined that the patient had no obstetric pathology.

Doppler and fetal echocardiography were performed based on the International Society of Ultrasound and Obstetrics and Gynecology (ISUOG) Practice Guidelines.[16] The modified MPI (Mod-MPI) method was used to measure fetal left ventricular MPI, as described by Hernandez-Andrade et al.[2] Prior to the Mod-MPI measurement, the apical four-chamber view of the heart was obtained. The women for whom we were unable to view a favorable fetal position to obtain an optimal cross-section were evaluated at different times. The Doppler sample was opened at 3–5 mm and placed at the lateral wall of the ascending aorta to include both the mitral valve (MV) and the aortic valve (AoV). The insonation angle was maintained below 15 degrees. The wall motion filter was calibrated at 300 Hz, and the Doppler sweep velocity was 5 cm/s. Doppler gain was lowered in order to clearly see the valve clicks. The clicks of the opening and closing of the AoV and the MV were obtained. Early ventricular filling (E) and active atrial filling (A) waves were viewed. ICT was described as the time interval beginning with the closing of the MV and ending with the opening of the AoV. IRT was described as the time interval beginning with the closing of the AoV and ending with the opening of the MV. ET was described as the time interval beginning with the opening of the AoV and ending with the closing of the AoV (Fig. 1). Mod-MPI was calculated by using the following formula: (ICT+IRT)/ET.

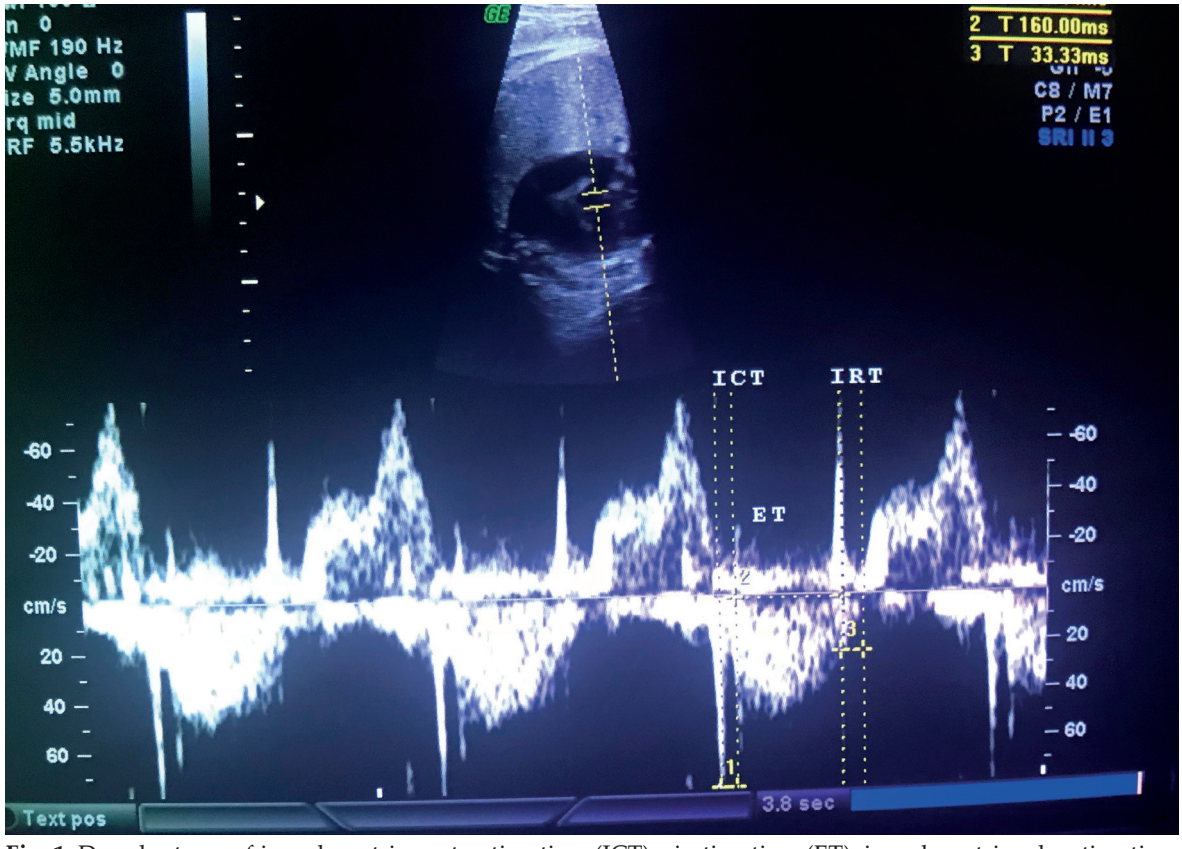

Fig. 1. Doppler trace of isovolumetric contraction time (ICT), ejection time (ET), isovolumetric relaxation time (IRT), and E/A wave peak velocities.

All the results and demographic characteristics, such as age, gravidity, parity, number of abortions, number of living children, and gestational week were recorded.

Statistical Analysis

All the statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, version 21.0, released 2012, Armonk, NY, USA: IBM Corp.). Descriptive statistics were reported as mean±standard deviation (SD), median (minimum-maximum), and mean+95% reference interval. Spearman's correlation test was used to analyze the correlations between the parameters. P <0.05 was considered to be statistically significant.

Results

According to our results, the median age, body mass index (BMI), gravidity, parity, number of living children, number of abortions, EFW, and gestational week were 27 (20–43), 28.7 (19.6–44.4), 2 (1–6), 1 (0–4), 0 (0–4), 0 (0–3), 2300 (716–4030), 34.6 (25.4–39.1), respectively.

Table I shows the ultrasonographic measurements for the pregnant women. Accordingly, the mean Mod-MPI was 0.42±0.10 while the mean IRT, ICT, and ET were 43.5±10.2, 27.27±8.1, and 170.5±16.9, respectively.

The results for the normal gestational ranges in the Turkish population are shown in Figure 2 as scatter plots, with the estimated mean and

Table I. Ultrasonographic measurements of the pregnant women in the study.

	Mean±SD	Median (Min-Max)
UA-Doppler RI	1.09±0.34	0.6 (0.2-4.5)
UA-Doppler S/D	2.66±0.30	2.6 (1.3-4.2]
UA-Doppler PI	0.98±0.38	1.0 (0.3-2.7)
MVP	47.75±31.30	50.0 (0.0-76.0)
ICT	27.27±8.10	26 (12-48)
IRT	43.5±10.20	44 (23-70)
ET	170.4±16.90	173 (123-213)
Mod-MPI	0.42±0.10	0.4 (0.21-0.67)
MCA-PI	1.97±0.69	1.8 (0.97-3.7)
UtA-PI	3.82±0.27	0.82 (0.49-2.41)
DV-PI	0.68±0.41	0.63 (0.12-1.79)
TCD	41.02±6.65	42 (26.7-52)

UA: Umbilical artery, RI: Resistance index, S/D: Systole/diastole, PI: Pulsatility index, ICT: Isovolumetric contraction time, IRT: Isovolumetric relaxation time, ET: Ejection time, Mod-MPI: Modified myocardial performance index, MCA: Middle cerebral artery, UtA: Uterine artery,
DV: Ductus venosus, TCD: Trans cerebellar diameter, MVP: Maximum vertical amniotic pocket

percentile curves for ICT, IRT, ET, and Mod-MPI. ICT and IRT were constant during the pregnancies, while ET decreased and Mod-MPI increased throughout the gestational period.

Spearman's correlation test was used to assess the relationship between the parameters (Table II). The correlation coefficient analysis results revealed that there was a significant correlation between Mod-MPI and GA, and UA systolic/diastolic (UA S/D) ratio and the middle cerebral artery pulsatility index (MCA PI) values (r=0.199, p=0.047, r=-0.328, p=0.001, and r=-0.0349, p=0.001, respectively) (Fig. 3). Mod-MPI increased with advanced GA; moreover, Mod-MPI decreased as UA S/D and MCA increased. However, no statistically significant correlation was found between Mod-MPI and the women's age, EFW, BMI, UA Doppler PI/RI (resistance index), ductus venosus (DV)-PI, and uterine artery (UtA)-PI values (p>0.05). A negative correlation was found between ET and gestational week (r=0.238, p=0.018) (Fig. 4). All of the UA doppler parameters (S/D, PI, and RI)

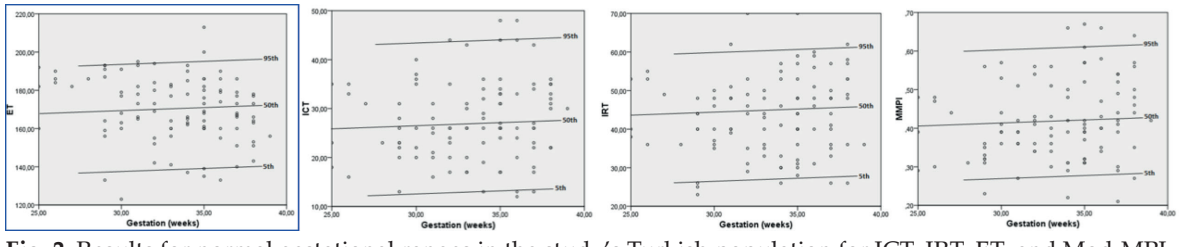

Fig. 2. Results for normal gestational ranges in the study's Turkish population for ICT, IRT, ET, and Mod-MPI.

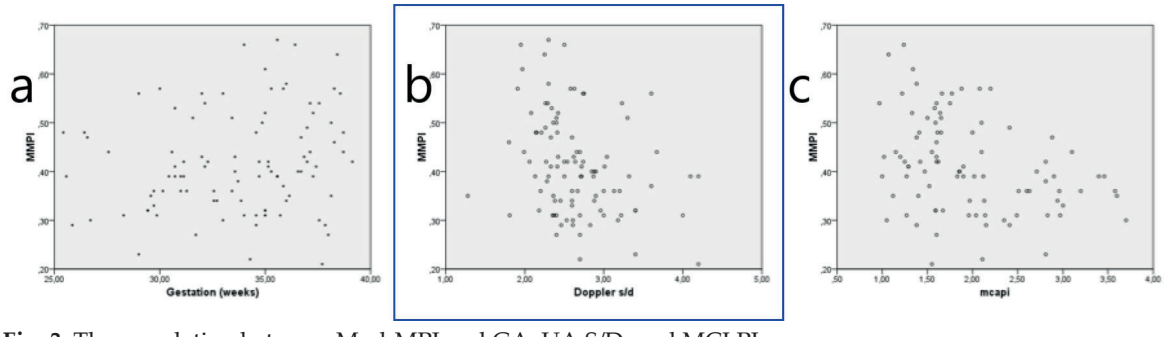

Fig. 3. The correlation between Mod-MPI and GA, UA S/D, and MCI PI.

Table II. The relationship between the study's parameters*.

		Mod-MPI	ICT	IRT	ET
Age (year)	r	-0.021	-0.071	0.123	0.086
	p	0.849	0.511	0.257	0.427
GA (week)	r	0.199	0.117	0.131	-0.236
	p	**0.047**	0.245	0.194	**0.018**
EFW	r	0.178	0.077	0.170	-0.204
	p	0.097	0.473	0.114	0.056
BMI	r	0.078	-0.045	0.027	-0.071
	p	0.462	0.670	0.802	0.500
UA-S/D	r	-0.328	-0.131	-0.421	0.169
	p	**0.001**	0.198	**0.000**	0.096
UA-PI	r	-0.181	0.028	-0.303	0.067
	p	0.080	0.790	**0.003**	0.520
UA-RI	r	-0.200	-0.085	-0.316	0.092
	p	0.053	0.413	**0.002**	0.376
MCA-PI	r	-0.349	-0.339	-0.149	0.192
	p	**0.001**	**0.001**	0.158	0.068
DV-PI	r	0.160	0.210	0.066	0.007
	p	0.261	0.139	0.646	0.961
UtA-PI	r	-0.206	-0.121	-0.162	0.091
	p	0.065	0.282	0.149	0.420
TCD	r	0.109	0.154	-0.060	-0.219
	p	0.390	0.226	0.639	0.081

*Spearman correlation test was used. GA: Gestational Age, EFW: Estimated Fetal Weight, BMI: Body Mass Index, UA-S/D: Umbilical artery systole/diastole, UA-PI: Umbilical artery Pulsatility Index, RI: Resistance Index, MCA: Middle Cerebral Artery, DV: Ductus Venosus, UtA: Uterine artery, TCD: Trans cerebellar diameter, Mod-MPI: Modified myocardial performance index, ICT: Isovolumetric contraction time, IRT: Isovolumetric relaxation time, ET: Ejection time.

were found to have a negative correlation with IRT (r=-0.421, p=0.000, r=-0.303, p=0.003, and r=-0.316, p=0.002, respectively) (Fig. 5). A negative correlation was also found between ICT and MCA-PI (Fig. 6).

Table III shows the means and the 95% reference intervals for the Mod-MPI, ICT, IRT, and ET values based on the gestational week. There was an increase in the Mod-MPI from 25 to 39 weeks of gestation, with means±SD of 0.37±0.087 at 25 weeks and a mean of 0.42±0.11 at 39 weeks.

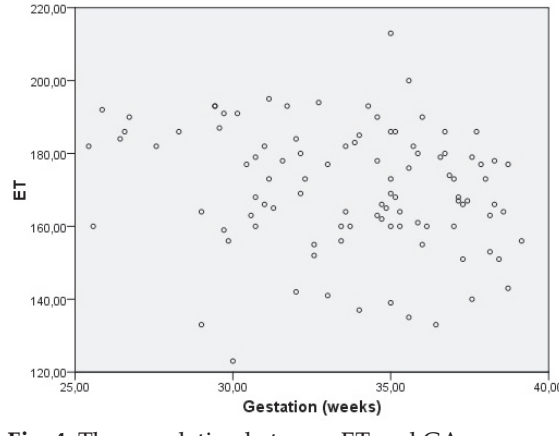

Fig. 4. The correlation between ET and GA.

Table III. Means and 95% reference intervals for Mod-MPI, ICT, IRT and ET throughout pregnancy.

Gestational age weeks	Mod-MPI mean	Mod-MPI 95% reference interval	ICT mean	ICT 95% reference interval	IRT mean	IRT 95% reference interval	ET mean	ET 95% reference interval
25	0.39	0.15-0.62	25.3	3.6-47.0	43.7	23.4-63.9	178.0	137.3-218.7
26	0.42	0.17-0.67	28.0	2.1-53.9	48.0	22.1-73.9	186.7	179.1-194.3
27	0.44	0.18-0.67	31.0	4.2-42.6	49.0	23.4-82.1	182.0	142.0-196
28	0.31	0.13-0.53	23.0	17.2-32.0	36.0	25.4-40.2	186.0	175.2-192.4
29	0.35	0.27-0.43	23.3	18.7-27.8	36.3	27.9-44.6	172.0	153.4-190.6
30	0.44	0.37-0.51	30.9	23.5-38.3	41.7	36.0-47.4	165.9	145.8-185.9
31	0.39	0.32-0.45	23.9	19.1-28.6	46.8	39.3-54.5	178.9	167.8-189.9
32	0.43	0.35-0.50	26.9	19.6-34.1	44.6	33.4-55.8	168.6	153.8-183.4
33	0.41	0.34-0.48	26.3	19.0-33.5	41.1	35.9-46.3	165.4	177.5-135.8
34	0.40	0.29-0.50	26.7	21.0-32.3	41.2	32.0-50.4	171.0	157.5-184.5
35	0.43	0.37-0.49	29.9	25.5-34.4	43.8	37.5-50.0	172.0	161.3-182.7
36	0.45	0.36-0.55	27.5	16.4-38.6	48.9	40.6-57.2	169.6	153.7-185.6
37	0.41	0.34-0.49	25.8	19.9-31.7	41.6	35.7-47.6	166.7	158.0-175.4
38	0.42	0.31-0.54	30.7	28.6-32.7	36.1	40.3-57.9	156.0	143.8-164.2
39	0.47	0.39-0.56	30.0	26.6-34.7	49.1	40.3-57.9	163.1	153.8-172.4

Mod-MPI: Modified myocardial performance index, ICT: Isovolumetric contraction time, IRT: Isovolumetric relaxation time, ET: Ejection time.

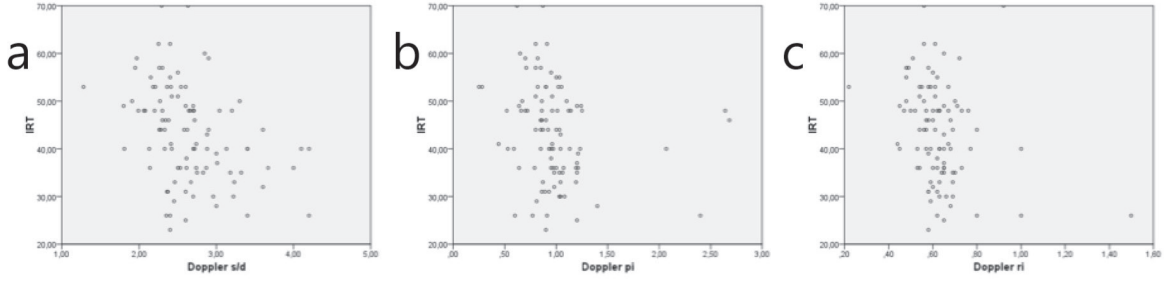

Fig. 5. The correlation between IRT and UA S/D, PI, and RI.

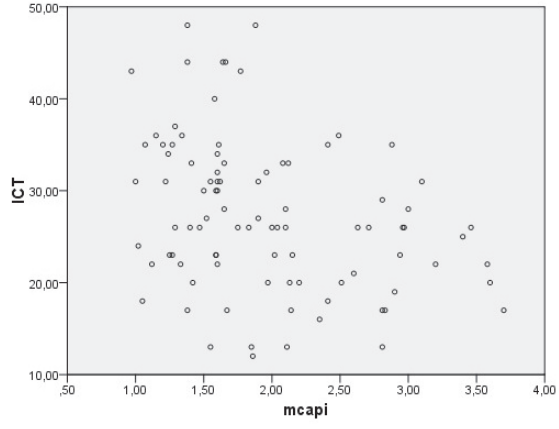

Fig. 6. The correlation between ICT and MCA PI.

Discussion

The main findings of this study were:

1. The normal reference of Mod-MPI was 0.4 (0.21–0.67).
2. There was a positive correlation between the Mod-MPI values and GA. The Mod-MPI values significantly increased with GA.
3. There was a negative correlation between the Mod-MPI values and UA S/D and MCA-PI.

There are several ways to evaluate cardiac functions during the intrauterine period. MPI is one of the most common methods used to evaluate cardiac functions, globally. Conditions that disturb cardiac systolic functions result in the lengthening of the ICT and shortening of the ET. Thus, the systolic dysfunction results in prolonging the MMPI values. Previously we investigated the utility of Mod-MPI in fetal growth restriction (FGR). There was no difference between the groups in terms of Mod-MPI values. MPI values in healthy fetuses were 0,4 (0,3–0,7). However, IRT values, the earliest influenced component of MPI, were significantly lower in the FGR group than in the control group.[17] Another study performed by Kır et al.[18] investigated the importance of MPI in children diagnosed with isolated ventricular septal defect (VSD). The MPI values were found to be significantly higher in children with VSD than in healthy children, and the mean MPI values in healthy children were 0,26 ± 0,005. This average value is lower when compared to fetal MPI values; it may be related to changes in fetal cardiac geometry and the myocardium's functional capacity in the intrauterine environment.

We found a negative correlation between UA S/D and the Mod-MPI and IRT values. IRT is an important component of fetal cardiac function. Disruption of IRT results in reduced calcium uptake and a reduction in fetal cardiac function. Moreover, IRT is the earliest influenced component of MPI in cases of pathological conditions. The pathological increase in UA S/D means a disruption of blood supply to the fetus. Previous studies have demonstrated that pathological conditions that could influence cardiac functions also alter the Mod-MPI values.[5-15] In the present study, all of the UA S/D values were in the normal ranges based on GA. Therefore, we could not interpret this result objectively.

The present study showed that the normal Mod-MPI measurements of our pregnant population (0.4 [0.21–0.67]) were consistent with the reference intervals reported in previous studies.[15-19] Additionally, in our study the Mod-MPI values increased with GA, which is similar to the results reported by Cruz-Martinez et al.[19] who found a positive correlation between Mod-MPI and GA.

The literature reports a wide range for the mean MPI values. Although some previous studies advocate that the mean MPI is a constant value throughout pregnancy[20-22], it has also been reported that the MPI values are GA-dependent.[23] Moreover, some studies have demonstrated that the MPI values decrease with GA.[24,25] This inconsistency could be due to differences in the techniques used to measure the MPI. The ultrasound device settings are also important. Hernandez-Andrade et al.[2] used a Mod-MPI measurement technique with an insonation angle ranging between 0-30 degrees and a sweep speed of 15 cm/s. In the present study, we used an insonation angle ranging between 0-15 degrees and a sweep speed of 5 cm/s. Using a high sweep speed ensures optimal magnification for the correct placement of the caliper when obtaining the measurements. We increased the sweep speed to 5 cm/sn, as reported by Sanhal et al.[13]

The Doppler sample placement point is another challenge of Mod-MPI measurements.[26] Applying valve clicks when measuring MPI may decrease the inter- and intraobserver variations. However, valve clicks also have a thickness, so Mod-MPI values may be altered

if they are measured from the beginning of the valve clicks. Furthermore, original or reflected valve clicks may be used for the measurement. Although the beginning points and the end points are different, the peaks of the original MV and AoV closure clicks match the peaks of the reflected valve clicks. In the present study, we used the peak point of the clicks to prevent variation in the caliper placement due to the click interval. Similarly, Meriki and Welsh suggested using the peaks of the clicks to obtain the MPI measurements.[27] Other authors have also suggested using the beginning points of the clicks to place the calipers.[15,19,27]

To calculate the MPI, we preferred using the left Mod-MPI measurement. However, the use of the right MPI measurement has also been reported.[28] Paytoncu et al.[29] also evaluated fetal cardiac function via measuring MPI from the fetal left and right ventricles and tricuspid and mitral annular plane systolic excursions (TAPSE and MAPSE). They included 152 fetuses in their second and third trimesters in the study. Similar to us, they found the left ventricle mean MPI value as 0,47 ± 0,16. However, in this study, they did not detect a difference between the gestational week's progression and the MPI values.[29] Theoretically, the right MPI measurement seems to be more prudent, since the fetal heart is thought to be right dominant during the intrauterine period.[26,30] Although evaluation of the right ventricle provides early findings on cardiac dysfunction, MPI assessment requires two different planes for right ventricle pulmonary and tricuspid valve measurements. This makes it difficult to evaluate the right ventricle. However, the AoV and MV components of the left ventricle can be easily determined in the same section, and a Doppler examination can be performed. Moreover, more studies have reported on left ventricular MPI data than right ventricle MPI data. Nevertheless, reports on the clinical applications of fetal left MPI are controversial.

Previous studies have reported on the reproducibility of Mod-MPI measurements, and the reproducibility rate was found to be low when the GA was less than 27 weeks. In earlier GA weeks, the interobserver correlation rate also decreases. However, in the late periods of pregnancy, both measurement repeatability and interobserver correlation increase. This may be due to the relative increase in the size of the fetal heart, and, thus, to a more accurate placement of the Doppler caliper.[26]

If MPI is to be used as a useful clinical parameter, an acceptable reference range for normal fetuses should be presented.[27] Consequently, it is necessary to establish a standard for the factors that will cause variations, such as the device, the device settings, and the caliper placement technique.

To minimize the interobserver differences and obtain a standardized method with possible repeatability and reliability, automated MPI measurement techniques have been researched, and their routine usability has been communicated. With the introduction of routine use in automated methods, the relationship of MPI with fetal pathologies will be understood more clearly in the future.[31,32]

Previous studies have reported that a learning process is required in order to perform accurate MPI measurements. For example, Cruz-Martinez et al.[30] reported average numbers related to the completion of the learning process associated with left Mod-MPI measurements, such as 42 for ET, 77 for ICT, and 83 for IRT.

Before conducting this study, the obstetrician who performed the MPI measurements had previously measured, on average, 100 healthy pregnant women; however, these measurements were not included in the study.

In conclusion, this study's results contribute to the field in several ways. We generated normal gestational reference ranges for Mod-MPI for the Turkish population. We used an objective method for caliper placement and ultrasound machine settings. The study's sample size was relatively larger than the sample sizes of some previous studies.[21,23,24,26] These results will be a reference for future studies, especially those

investigating the pathological conditions that impact fetal cardiac functions.

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None.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: KY, DFÖ; data collection: KY, DFÖ; analysis interpetation of results: MÖ, FÖ, CS; draft manuscript preperation: KY, YÜ, ŞÇ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Ethical approval was obtained from the Zekai Tahir Burak Women's Health and Research Hospital's ethics committee before the study was conducted (Review board number: 21/2019).

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Conflict of interest

The authors report no conflicts of interest.

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Original Article

Parental knowledge about familial Mediterranean fever: a cross-sectional study

Deniz Gezgin Yıldırım[1], Sevcan A. Bakkaloğlu[1], A. Şebnem Soysal Acar[2], Necla Buyan[1]

[1]*Division of Pediatric Rheumatology,* [2]*Department of Pediatrics, Gazi University Faculty of Medicine, Ankara, Turkey.*

ABSTRACT

Background. The life-long course, long-term complications, necessity for regular treatment, and potential side effects of the medications must be well understood by parents of pediatric familial Mediterranean fever (FMF) patients. The aim of this study was to assess parental knowledge and to investigate how parents obtained scientific information about FMF.

Methods. One hundred and seventy-one pediatric FMF patients and their parents were enrolled in this cross-sectional study. Three-part questionnaires, including forms on socio-demographics, knowledge and perceptions of FMF, and how to get information about FMF, were administered to parents.

Results. In the analysis of the knowledge questions, 90.1% of parents were aware of colchicine as an effective drug for FMF, but only 39.2% of them were aware that there is no vital risk during FMF attacks. Caregivers preferred to obtain information from physicians (98.8%), websites (47.9%), seminars (3.5%), and books (1.7%). The knowledge scores of parents were significantly higher among those whose children were using anti-interleukin-1 therapy in addition to colchicine relative to those on colchicine alone ($p = 0.04$). There was a positive correlation between knowledge level and parental educational status ($p = 0.0001$).

Conclusions. Knowledge scores among parents of pediatric FMF patients are unsatisfactory. The parents whose children have a severe disease course and a need for anti-interleukin-1 therapy are more knowledgeable. For parents, continuing education programs including books, seminars and web-sites giving information about the course, prognosis, complications and treatments of FMF should be employed immediately after the diagnosis and thereafter.

Key words: childhood, familial Mediterranean fever, pediatric rheumatology, parental knowledge.

Familial Mediterranean fever (FMF) is the most common monogenic, chronic auto-inflammatory disease characterized by recurrent attacks of fever, pleuritis, pericarditis, peritonitis and arthritis. The attacks are self-limiting and typically resolve within 24-72 hours.[1] Mediterranean fever *(MEFV)* gene mutation causes hyperactivity of inflammasomes which leads to an increase of interleukin-1β (IL-1β) and resultant severe inflammation.[2] The prevalence of FMF is changing among communities, and the disease is reported in approximately 1/1000 people in Turkey.[3] Colchicine is a cheap, well tolerated, and life-long treatment that prevents the development of amyloidosis and must be used daily by oral delivery.[4] The inflammatory attacks generally occur before 20 years of age in 90% of FMF patients, so patients are generally diagnosed with FMF in childhood.[5] As a result, parents take responsibility for their children to take regular colchicine every day and go to routine outpatient follow-up visits.[5] Therefore, having a life-long disease with long-term complications, the necessity for life-long regular treatment, and the side effects of the therapy must be well understood by parents of pediatric FMF patients. Treatment compliance

✉ Deniz Gezgin Yıldırım
gezgindeniz@gmail.com

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of pediatric FMF patients might be affected by social and demographic factors, such as education, age, and social network of parents. Parental knowledge about chronic diseases, such as rheumatic diseases, epilepsy, thalassemia, neurofibromatosis, and hypospadias, has been investigated previously.[6-11] However, to date, no data has been reported about the parental knowledge of pediatric patients with FMF.

We, herein, aimed to evaluate parental knowledge and perceptions of FMF and to investigate how parents obtained scientific information about it.

Material and Methods

FMF patients aged 4-16 years who had been suffering from FMF for six months or more and their primary caregivers were asked to participate in this cross-sectional study by a pediatric rheumatologist and a social worker. All patients were being followed by the Pediatric Rheumatology Department at the Gazi University Faculty of Medicine between June 2018 and November 2018. Patients who had an additional chronic disease were excluded from the study. All patients were evaluated clinically according to the Tel Hashomer Criteria.[12] Demographic data of patients and parents were recorded. All patients were receiving colchicine treatment. Resistance to colchicine therapy was quantified as experiencing one or more attacks per month despite receiving the maximally tolerated dose of colchicine for ≥ 6 months. Partial response to colchicine therapy was accepted as a decrease in attack frequency. Complete response to colchicine was accepted as resolved inflammatory attacks and serum acute phase reactant levels.[13] FMF patients using maximum tolerated colchicine dose regularly in everyday without forgetting were accepted as good compliance, while FMF patients with missing colchicine doses were accepted as non-compliance to the therapy. Biologics targeting IL-1 were started with some FMF patients due to partial response, resistant, non-compliance or intolerance to colchicine, or secondary amyloidosis development.

Pras activity scores were used for evaluating disease severity in FMF patients.[14] The FMF severity score comprised of age at FMF onset, frequency of attacks, presence of arthritis, erysipelas-like erythema, amyloidosis, and the required dose of colchicine prophylaxis necessary to control FMF symptoms. Escalating scores indicate mild (score, 1-5), moderate (score, 6-9), and severe (score, >10) FMF activity.[15]

Three self-administered surveys which were developed by authors, were given to caregivers:

a. Caregivers' socio-demographics form: Caregivers (mother or father) were asked to provide personal demographic information, including age, gender, education status, and having FMF or not.

b. FMF parental knowledge and perceptions form: The knowledge section of the questionnaire contained 14 items. To evaluate the parents' knowledge level of FMF, we posed a set of 14 questions to all participants. This non-standardized questionnaire was generated by authors. These questions included whether FMF is a contagious or a genetic disease or a disease that should be followed-up regularly in a pediatric rheumatology department; whether or not children with FMF have a vital risk during attacks or have lower IQs than their peers; whether or not colchicine is an effective treatment agent; whether or not drugs for FMF are addictive or have side effects such as infertility; whether or not FMF resolves spontaneously over time or worsens with age; whether or not FMF patients can work actively and join sport activities; and whether or not FMF symptoms get worse or irreversible damage develops in internal organs, such as kidneys, when medication is not used regularly (Table II). Each item is rated on a 3-point scale (Yes, No, I don't know). Each correct answer was given 1 point. The total score of knowledge was graded between 0 (the lowest grade) and 14 (the highest grade) points.

c. Getting information about FMF form: Parents were asked to a respond to a total of four statements about where they obtain information about FMF, such as from physicians, books, symposia, or web sites.

This study was approved by the Gazi University Medical Faculty Ethics Board (11.06.2018/456) and was applied in accordance of the Declaration of Helsinki. Informed consents were obtained from each participants' caregivers.

Statistical analysis

Statistical analysis of the data was performed by Statistical Package for Social Sciences (SPSS) software version 15 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). The differences between two independent groups were compared by using independent sample t-test for normally distributed variables or Mann-Whitney U test for non-normally distributed ones. Correlations between variables were evaluated by Pearson or Spearman correlation coefficients for variables. A p value of less than 0.05 was considered to be significant.

Results

One hundred and seventy-one pediatric FMF patients aged 4-16 years and their primary caregivers were enrolled in this study. Three parents refused to participate in the study. The caregivers' and patients' characteristics are summarized in Table I. Of the 171 study participants, 142 (83.0%) were mothers. The median paternal and maternal ages were 41 (27-56) and 39 (24-55) years, respectively. While half of the mothers had graduated from primary school (56.1%), a majority of the fathers had graduated from secondary/high school or university (64.3%). All patients were using oral colchicine, but 30 (17.5%) of them were nonadherent to this daily oral treatment and 16 patients (9.4%) were additionally receiving anti-IL-1 treatments, either anakinra or canakinumab. A total of 117 (68.4%) patients

Table I. The demographic data of caregivers and familial Mediterranean fever patients.

Characteristics	Median (min.-max.)	n (%)
Parents		
Total		171 (100)
Mother		142 (83)
Father		29 (17)
Age of mothers (years)	39 (24-55)	
Age of fathers (years)	41 (27-56)	
Education status of mothers		
Primary school		96 (56.1)
Secondary - high school		59 (34.5)
University		16 (9.4)
Education status of fathers		
Primary school		61 (35.7)
Secondary - high school		76 (44.4)
University		34 (19.9)
Children		
Total		171 (100)
Male		75 (43.9)
Female		96 (56.1)
The feature of age groups		
Preschool age		38 (22.2)
School age		133 (77.8)
Age of present time (years)	12 (4-16)	
Family history of FMF		117 (68.4)
Attack frequency in a year		
≤2 attacks per year		138 (80.7)
>2 attacks per year		33 (19.3)
Frequency of emergency visits		
≤2 visits per year		157 (91.8)
>2 visits per year		14 (8.2)
FMF symptoms		
Abdominal pain		152 (88.9)
Fever		170 (99.4)
Arthralgia		113 (66.1)
Myalgia		37 (21.6)
Arthritis		66 (32.3)
Chest pain		41 (24.0)
Erysipelas-like erythema		28 (16.4)
Amyloidosis		3 (1.8)
Compliance of colchicine treatment		
Adherent		141 (82.5)
Nonadherent		30 (17.5)
Response of colchicine treatment		
Complete		152 (88.9)
Partial		12 (7)
Resistant		7 (4.1)
Response to anti-IL-1 treatment		
Complete		13 (81.2)
Partial		3 (18.8)
PRAS activity score*	6 (3-15)	
Mild course		54 (31.6)
Moderate course		86 (50.3)
Severe course		31 (18.1)

CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, FMF: familial Mediterranean fever, IL-1; interleukin-1.
*The Pras activity score evaluates the severity of the disease with scores of 2–5 for those having mild activity, 6–10 for moderate activity, and > 10 for severe activity.[15]

had a positive family history for FMF. The median Pras activity score was 6 (3-15).

All knowledge-related questions are presented in Table II. Of 171 parents, 154 (90.1%) knew that colchicine is an effective drug in FMF treatment. In contrast, only 67 (39.2%) knew that there is not a vital risk during attacks of FMF disease.

In evaluating parents' sources for gathering knowledge about FMF, we determined they preferred obtaining information from physicians (98.8%), web-sites (47.9%), seminars (3.5%), and books (1.7%) (Table III).

Parental knowledge scores and patients' Pras activity scores were compared by demographic findings and treatment responses (Table IV). There were no significant differences in knowledge scores of parents by either patients' or parents' age, gender, education status, or compliance with colchicine use ($p > 0.05$). However, the knowledge scores were significantly higher in parents whose children needed to use anti-IL-1 therapy in addition to colchicine ($p = 0.04$). There were no correlations between parental knowledge scores and Pras activity scores in mothers' ages, fathers' ages, patients' ages and colchicine compliance ($p > 0.05$) (Table V). There was, however, a positive correlation in knowledge level and parental educational status ($p = 0.0001$).

Table II. Percentage of correct answers to questions comprising the knowledge score of parents of children with familial Mediterranean fever.

Items	% of correct answers
FMF is a contagious disease	85.4
FMF is a hereditary genetic disease	71.3
There is a vital risk during attacks of FMF disease	39.2
Colchicine is the effective drug in FMF treatment	90.1
Drugs of FMF are addictive	49.7
FMF is a disease that affects intelligence	63.2
FMF is a disease that passes by itself over time	62.0
FMF gets worse with age	46.8
FMF patients cannot work actively	68.4
FMF patients cannot do any sports	74.3
FMF disease gets worse when colchicine is not used regularly	71.9
Medications of FMF have side effects such as infertility	61.4
Damage to internal organs such as kidneys can develop when colchicine is not taken regularly	83.6
FMF is a chronic disease that must be followed-up regularly by a pediatric rheumatologist and/or a pediatrician.	80.1

FMF: familial Mediterranean fever.

Table III. Parents' sources of information for familial Mediterranean fever.

Item	%
I have information about FMF from physicians	98.8
I have read a book about FMF	1.7
I have participated a seminar about FMF	3.5
I have followed a web-site about FMF	47.9

FMF: familial Mediterranean fever.

Table IV. Demographic findings and treatment responses by parental knowledge and by patients' Pras activity scores.[12]

Factor	Knowledge	p value	Pras activity score*	p value
Parents				
Mother	8.9±2.9	0.36	7.14±2.4	0.20
Father	9.5±2.1		6.69±2.3	
Mother's age				
30-50 years	8.9±2.7	0.18	7.05±2.4	
51-70 years	10.1±3.1		7.19±2.3	
Father's age				
30-50 years	8.8±2.8	0.17	7.08±2.5	0.89
51-70 years	9.6±2.6		7.02±2.3	
Mother's education status				
Primary school	8.9±2.7	0.43	6.54±1.9	0.04
Secondary/high school/university	9.3 ±2.8		7.33±2.6	
Father's education status				
Primary school	8.9±2.9	0.53	6.62±2.1	0.012
Secondary/high school/university	9.1 ±2.7		7.53±2.6	
Patients with FMF				
Age				
4-7 years	8.7±3.1	0.49	6.74±1.7	0.33
8-16 years	9.1 ±2.6		7.17±2.6	
Age at diagnosis				
< 10 years	8.8±2.9	0.22	7.27±2.4	0.03
> 10 years	9.5 ±2.2		6.45±2.3	
Compliance of colchicine use				
Regular	8.9±2.8	0.53	7.18±2.5	0.43
Not regular	9.3±2.4		6.63±2.0	
Response to colchicine				
Complete response	8.8±2.4	0.04	6.73±2.3	0.001
Incomplete response	10.1±2.8		9.94±1.5	
Anti-IL-1 therapy				
Not users	8.9±2.4	0.04	6.73±2.2	0.001
Users	10.1±2.7		10.38±1.9	

FMF: familial Mediterranean fever, anti-IL-1: anti-interleukin-1.
*The Pras activity score evaluates the severity of the disease with scores of 2–5 for those having mild activity, 6–10 for moderate activity, and > 10 for severe activity.[15]

Discussion

This study has evaluated parental knowledge, perceptions, and the ways parents access scientific information about FMF. This study showed that parents of FMF children did not have adequate knowledge about FMF, and they mostly tried to get information from their physicians and web-sites. Furthermore, we found that the parents whose children had a severe disease course and needed to use anti-IL-1 therapy in addition to colchicine were more knowledgeable than the others about FMF disease. Also, the level of educational status of parents was positively correlated with knowledge about FMF disease. Surprisingly, the knowledge level of parents was insufficient

Table V. Correlation between parental knowledge and patients' Pras activity scores

Factor	Knowledge		Pras activity score*	
	r value	p value	r value	p value
Child age	0.12	0.09	0.04	0.55
Mother age	0.13	0.09	0.01	0.87
Father age	0.11	0.10	0.06	0.43
Age of onset symptoms	0.13	0.09	-0.38	0.0001
Attack frequency in a year	0.28	0.0001	0.44	0.0001
Mother education	0.35	0.0001	-0.16	0.03
Father education	0.33	0.0001	-0.20	0.008
Response of colchicine treatment	-0.14	0.04	0.40	0.0001
Use of anti-IL-1 treatment	-0.07	0.36	0.29	0.0001
Compliance to colchicine treatment	0.04	0.58	-0.09	0.24

Anti-IL-1: anti-interleukin-1.
*The Pras activity score evaluates the severity of the disease with scores of 2–5 for those having mild activity, 6–10 for moderate activity, and > 10 for severe activity.[15]

even though Turkey is one of countries where FMF is most commonly seen.

Al-Eid et al.[7] reported that the majority of parents have insufficient knowledge regarding rheumatic diseases, and proposed to increase health education programs to enhance awareness of pediatric rheumatic diseases in parents. Wickwar et al.[6] recommended a questionnaire to evaluate the knowledge level of parents about methotrexate therapy in pediatric rheumatic diseases. Although FMF is a chronic, life-long, and hereditary disease[4], there is still misinformation and a lack of knowledge about FMF. For example, 28.7% of parents were not aware that FMF is a hereditary disease, 60.8% of parents were not aware that FMF attacks do not pose a vital risk to patients, 50.3% of parents were not aware that FMF drugs are not addictive, and 53.2% of parents were not aware that FMF does not get worse as time goes on. Incorrect beliefs might occur due to the lack of education of parents. Higher education may provide easier access to information about healthier lifestyles and illnesses. In this study most of the mothers had graduated from primary school (56.1%), while most of fathers had graduated from secondary or high school (44.4%). We found a positive correlation between education status and the knowledge level of parents. We concluded that mothers adapt well to the idea of having a chronic disease like FMF in their children and can gain knowledge about FMF even if they have lower education status. This follows from the fact that many questions were answered correctly by the majority of the responders; for example 90.1% of parents think that colchicine is an effective drug in treating FMF, 85.4% think that FMF is not a contagious disease, 83.6% think that FMF may progress to chronic kidney disease without regular colchicine usage, and 80.1% think that FMF must be followed by a pediatric rheumatologist and/or a pediatrician.

One of the most important attitude problems in parents is not using the therapy on time for their children with FMF. Parents' ideas about addiction and side effects of treatments and children's fears of the subcutaneous needle treatments may lead to irregular use of treatments. In the present study, half of the parents thought that FMF drugs are addictive, and 38.6% thought that FMF drugs cause infertility over time. Delayed treatment due to late diagnosis may cause the development of complications, such as amyloidosis, and enhance morbidity and mortality in patients as well as bring severe economic and psychologic burden to families and communities.[16] In a society, the training of patients with chronic diseases and their caregivers could improve the

course of these diseases and the quality of life of patients and family members.[17] Patient and caregiver education aims to develop a sense of responsibility and improved health by building positive habits, such as healthy diet, regular prescription drug use, and regular follow-up to keep the chronic disease under control. Parents' correct perceptions and adequate knowledge about chronic diseases have an important effect on the successful management of the disease course. Programs about raising the knowledge and awareness of FMF could ensure the process of parents' acceptance of their children's disease and provide compliance with the treatments. Compliance is often a marker of patients' and parents' understanding and adaptation to a chronic disease, such as FMF. Poor compliance with colchicine use may increase attack rates of FMF.[4] The 82.5% of our patients found to be compliant represent a good compliance rate for colchicine, and we did not find any significant differences in parental knowledge scores between patients with poor and good compliance with colchicine usage. Poor colchicine response and higher disease severity with attacks cause parents to be more motivated to cope with FMF. This situation causes patients' relatives to take a more effective attitude towards obtaining information about FMF. There is a significant relationship between anti-IL-1 use and the knowledge scores about FMF; therefore, using anti-IL-1 therapy as an add-on therapy in FMF might encourage parents to read and know more about FMF to take care of their children properly and to make their lives better. Kinkar et al.[18] demonstrated that the parents of children who had been earlier diagnosed with epilepsy, had more knowledge about epilepsy as a chronic disease, but in our study, interestingly, there were no significant differences in evaluation of knowledge scores between ages of parents and children's age of diagnosis. Furthermore, we did not find any correlations between parental age, patients' age, patients' age at disease onset, and parental knowledge. Therefore, we concluded that the parents' efforts to learn more information about the disease did not show any difference by the age of the patients at diagnosis or by parental age.

The main limitations of this study were the small sample size from a single centre and the lack of a control group. Another limitation of this study was the questionnaire used, which is not validated and based on a previously published/validated pediatric scale.

In conclusion, having a good level of knowledge about FMF is important to increase patient compliance with treatment and, therefore, to prevent not only acute attacks but also long-term complications. However, in our study population, the knowledge about FMF among parents was unsatisfactory. The parents whose children have a severe disease course and, therefore, require anti-IL-1 add-on therapy were more knowledgeable. Educational tools, including books, on-line or live seminars, and websites giving information about FMF disease course, treatments, prognosis, and complications should be provided immediately after diagnosis and should be continuous.

Ethical approval

This study was approved by the Gazi University Medical Faculty Ethics Board (11.06.2018/456).

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Author contribution

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

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Conflicts of interest

The authors declare no conflict of interest.

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Original Article

The relationship between immature platelet fraction and severity of acute bronchiolitis

Murat Doğan[1], Mehmet Köse[2], Mehmet Adnan Öztürk[1], Melih Hangül[1], Hümeyra Aslaner[3]

Departments of [1]Pediatric Emergency, [2]Pediatric Pulmonology and [3]Family Medicine, Erciyes University Faculty of Medicine, Kayseri, Turkey.

ABSTRACT

Background and objectives. Acute bronchiolitis is one of the most common reasons for hospitalization in infants. Although patients with acute bronchiolitis generally have a good prognosis, death can also occur. In this study, we evaluate the immature platelet fraction (IPF) as an indicator of the severity of acute bronchiolitis.

Methods. In our study, 179 patients diagnosed with acute bronchiolitis were divided into three groups as mild (n: 48; 26.8%), moderate (n: 104; 58.10%) and severe (n: 27; 15.1%) bronchiolitis. There were 80 healthy children in the control group. The diagnostic capacity of IPF and hematological parameters (platelet distribution width (PDW), mean platelet volume (MPV), white blood cell count (WBC), and platelet count (PLT)) values to predict severity of acute bronchiolitis was evaluated using receiver operating characteristic (ROC) curves and their respective areas under the curves (AUCs) calculated with 95% confidence intervals.

Results. The IPF value of patients with acute bronchiolitis was significantly higher than the healthy group ($p < 0.001$). In addition, a positive correlation was observed between clinical severity of bronchiolitis and IPF. The ROC curve analysis indicated that the IPF cut-off point for predicting severity of acute bronchiolitis was >3.2% (Sensitivity of 84%, specificity of 97%). We found that the AUCs for IPF, MPV, PDW, WBC and PLT were statistically significant for bronchiolitis relative to the healthy control group. The parameter with the greatest AUC value was IPF.

Conclusion. The IPF may present for diagnosing and evaluating the clinical severity of acute bronchiolitis in children.

Key words: acute bronchiolitis, immature platelet fraction, children.

Acute bronchiolitis is the most common lower respiratory tract infection in children younger than 1 year.[1] Viruses are the most common cause of acute bronchiolitis, especially respiratory syncytial virus. Bronchiolitis is characterized by airway obstruction and edema, increased mucus production, and the loss of airway epithelial cells. The clinical severity of bronchiolitis ranges from mild cases that can be treated on an outpatient basis to severe cases that require mechanical ventilation or extracorporeal membrane oxygenation in intensive care units.[2] Acute bronchiolitis might be associated with morbidity and mortality in children. Currently, there is no biomarker that can predict the severity of acute bronchiolitis. The lack of any single biomarker for acute bronchiolitis that may be applied across all clinical scenarios is a recurring problem and has led many clinicians and researchers to pursue alternative diagnostic strategies.[2]

Platelets play an important role in hemostasis, inflammation, allergic reactions, and angiogenesis, as well as the repair and renewal of tissues. They also secrete mediators such as chemokines, cytokines, and coagulation factors,

✉ Murat Doğan
doktormurat033@hotmail.com

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which provoke a strong inflammatory response and tissue regeneration. Platelet production in bone marrow increases and platelets are redistributed during the inflammatory response.[3] In addition to platelet count (PLT), platelet indexes include platecrite, mean platelet volume (MPV), platelet distribution width (PDW), and a new inflammatory parameter called the immature platelet fraction (IPF). The IPF is the percentage of reticulated platelets that can be measured in the blood, and it may be used to quantify the production of bone marrow platelets. The IPF correlates directly with the thrombopoietic rate, increasing when platelet production rises and decreasing when production falls. Inflammation causes changes in the bone marrow, leading to increases in platelet production and specifically of the circulation of immature platelets.[4-6] There may be a relationship between the number of immature platelet fraction (IPF) in circulation and the severity of inflammation. Diagnostic accuracy studies performed over the last few years suggest that IPF levels can provide clinically relevant information regarding inflammatory activity and disease prognoses.[5-8] Here, we evaluate the relationship between changes in platelet parameters such as IPF and the clinical severity of acute bronchiolitis.

Material and Methods

A single center prospective case control study of children aged 1-24 months who were hospitalized with acute bronchiolitis was performed between December 2018 and May 2019. In accordance with American Academy of Pediatrics guidelines, a diagnosis of acute bronchiolitis was based on at least two of the following signs: chest retractions, tachypnea, and the first episode of wheezing or rales on auscultation following a viral upper respiratory tract infection in children aged younger than 24 month.[9] During the study period, 330 acute bronchiolitis patients were admitted and 179 patients were included in the study. 80 children seen in Erciyes University Medical School Children's Hospital in Social Pediatrics outpatient clinic for routine control were included in the study as the control group. The patients had no health problems at this time were taken as a control group, which were similar to the patient group in terms of age and gender. Before the study, the all groups were informed about the study and informed consent were taken. On admission, the clinical severity score (CSS) for acute bronchiolitis (*i.e.*, a composite clinical score including respiratory rate, retraction, wheezing, and general condition) was used to evaluate patients, as previously described by Wang et al.[10] An adapted score from Wang et al. used for disease severity.[11] Moderate bronchiolitis was defined by a score of >5 and severe by a score of >10. Bronchiolitis severity score (BSS) was recorded for each patient at the time of presentation. Each patient with bronchiolitis was classified into one of three groups, depending on whether they had mild, moderate, or severe bronchiolitis. Inclusion criteria were: aged 1-24 months, first wheezing episode, no previous disease history, and no previous medication. Exclusion criteria were: chronic disease, premature birth, birth weight < 2500 g, malnutrition, passive smoking, proven immune deficiency, proven or suspected acute bacterial infection, previous treatment with bronchodilators or corticosteroids, or having symptoms for more than 7 days.

Data collection

Complete blood count measurements (including white blood cells, MPV, and platelets) were recorded from the blood samples taken on the first day of hospitalization using a BC-6800 analyzer (Mindray, Shenzhen, China). Eosinophil counts (%) determined by automated blood analyzer. The IPF was recorded using flow cytometry and the reticulocyte/platelet channel of an automated hematology analyzer (Sysmex, Kobe, Japan) with a fluorescent dye containing polymethrin and oxazine. In cases of our study, blood samples were taken before starting steroid treatment. The IPF is the fraction (%) of immature platelets in the total

platelet population and reference range for the IPF has been determined 1-5%. The range of IPF reference values in a healthy population is 1.1-6.1% (with Sysmex analyzer).[12,13] Data from each patient recorded in the emergency room included: age, sex, disease history, medication, birth history, whether this was the first attack of bronchiolitis, weight, vital signs (*i.e.*, heart rate, respiratory rate, tympanic temperature, and oxygen saturation when breathing ambient air, which was measured using pulse oximetry and expressed as SpO_2). Complete blood counts and IPF data were obtained from blood samples taken for routine testing of these children at the time of admission. This study was conducted with the approval of the Ethical Committee of Erciyes University Faculty of Medicine on 23/02/2018 (Project number: 2018/91). All study procedures were performed in accordance with the ethical principles of the 1964 Declaration of Helsinki. No financial support has been received from any institution or organization.

Statistical methods

In our study, power analysis was performed using G * Power 3.1 analysis program to determine the sample size. The number of patient to participate in the study with a population size of 330 was determined as a minimum of 178. (α-value: 0.050, ß-value: 0.800). Statistical analyses were performed using SPSS software (ver. 21.0; IBM, Chicago, IL, USA). Continuous variables are expressed as medians (minimum–maximum) and categorical variables as values and percentages. Categorical data were compared using chi-square tests. For non-normally distributed data, the Mann–Whitney U test was used to determine whether or not differences between groups were statistically significant. Spearman's method was used to evaluate correlations between the IPF and other blood parameters if variables were not normally distributed. The most discriminating biomarkers for acute bronchiolitis were identified by drawing receiver operating characteristic (ROC) curves for each biomarker, and calculating sensitivity and specificity, as well as positive and negative predictive values. Areas under the curves (AUCs) for IPF, PLT, MPV, PDW, and white blood cell count (WBC) were evaluated for the acute bronchiolitis versus the control group. Logistic regression was used to identify associated factors and to calculate odds ratios and 95% confidence intervals. A p-value < 0.05 was considered statistically significant.

Results

In total, 179 patients aged 1–24 months were included in this study, with a median age of 8 months (range, 1–24 months): 116 (64.800%) of these patients were male and 63 (35.200%) were female. The control group included 80 healthy children, also with a median age of 8 months (range, 2–24 months): 49 (61.200%) of them were male and 31 (38.800%) were female. The patient and control group did not differ significantly in age or sex (p = 0.619 and p = 0.583, respectively). Patients had significantly higher WBC, PLT, MPV, IPF, and PDW values compared with the control group (p = 0.000). The frequency of eosinophilia did not differ significantly between the patient and control groups (p = 0.756; Table I). At admission, the median body temperature of patients was 37°C (36–39.3°C) and their median oxygen saturation was 91% (75–99%). In total, 99 patients had a chest X-ray finding (*e.g.*, diffuse interstitial consolidation or increased aeration), 68 patients were given antibiotics, and 71 patients received steroid therapy (all patients in the severe group and 42.300% of patients in the moderate group). All patients were provided with supportive therapy such as hydration, oxygen, and salbutamol nebules if indicated. The patient group included 48 (26.800%), 104 (58.100%), and 27 (15.100%) children classified as having mild, moderate, and severe bronchiolitis, respectively. The median CSS was 6 (range, 2–12). No significant correlation was found between acute bronchiolitis CSS and age, sex, body temperature, oxygen saturation (%), PLT, WBC, proportion of lymphocytes, or proportion of eosinophils (p > 0.05). The MPV value of the

Table I. Comparison of demographic and laboratory characteristics of the patients and healty group.

	Patient Group Median (Min-Max)	Healty Group Median (Min-Max)	p
Age (month)	8(1-24)	8(2-24)	0.619
Sex male (n)(%)	116 (64.800)	49 (61.200)	0.583
IPF (%)	5.500(0.700-18.800)	1.950 (0.800-4.100)	<0.001
MPV (fL)	9.400(8-11.800)	8.600(7.400-9.900)	<0.001
PDW (%)	9.800(7.700-15.400)	9.250(7.700-12.100)	0.027
WBC (10^9/L)	10.260(2.040-24.440)	6.720(3.140-11.500)	<0.001
PLT (10^9/L)	266(74-631)	288 (101-449)	<0.001
Eozinofil (%)	0.300 (0.000-9.100)	0.200 (0.000-9.100)	0.756

Mann Whitney U test was used. p <0.05 was considered statistically significant.
IPF: immature platelet fraction, MPV: mean platelet volume, PDW: platelet distribution width, WBC: white blood cell count, PLT: platelet.

Table II. Comparison of the clinical severity of acute bronchiolitis and laboratory findings.

	Mild Median (Min-Max)	Moderate Median (Min-Max)	Severe Median (Min-Max)	p
IPF (%)	3.350(0.700-9.600)	5.600(1.400-18.700)	10.800(3.600-18.800)	<0.001
MPV (fL)	9(8.500-11.300)	9.500(8.000-11.800)	10.200(8.700-11.700)	<0.001
PDW (%)	8.90(7.800-14)	9.950(7.700-13.600)	11.100(8.400-15.400)	<0.001
WBC (10^9/L)	4.090(2.810-31.000)	11.570(4-23.800)	9.790(4.190-23.800)	0.422
PLT(10^9/L)	220.500(88-430)	231(74-631)	218.00(88-631)	0.335
Eozinofil (%)	0.500(0.000-4.700)	0.150(0.000-9.100)	0.400(0.000-9.100)	0.280

Kruskal Wallis Test and Post Hoc Dunn correction were used. p <0.05 was considered statistically significant.
IPF: immature platelet fraction, MPV: mean platelet volume, PDW: platelet distribution width, WBC: white blood cell count, PLT: platelet.

mild clinical severity group was significantly lower than those of the moderate ($p = 0.002$) and severe ($p = 0.000$) group, but MPV values did not differ significantly between the moderate and severe groups ($p = 0.142$). IPF values differed significantly among the mild, moderate, and severe groups ($p < 0.001$). A positive correlation was observed between the CSS and the IPF ($p < 0.001$). The mild clinical severity group had a significantly lower PDW compared with the moderate and severe groups ($p = 0.000$), but the PDW values of the moderate and severe groups did not differ significantly (Table II).

ROC curve analyses were used to evaluate the performance of each biomarker in distinguishing acute bronchiolitis patients from controls, and in assessing acute bronchiolitis severity. The AUC for IPF, MPV, PDW, WBC, and PLT was 0.950, 0.850, 0.580, 0.790, and 0.710, respectively. ROC curve analysis suggested that the cut-off for using IPF to predict bronchiolitis was >3.2, with a sensitivity of 84% and a specificity of 97%. The positive and negative predictive values of the IPF were 98.700% and 73.500%, respectively. The AUC was greatest for IPF (Fig. 1). IPF, MPV, and PDW ROC curves showed significant sensitivity and selectivity in patients ($p < 0.001$; Table III). For all patients, this was the first bronchiolitis attack.

Discussion

We found that the IPF, which is a new inflammatory platelet index, was greater in patients with acute bronchiolitis than in healthy individuals and was positively correlated with the clinical severity of acute bronchiolitis.

Table III. ROC analysis of ınflammation markers in the prediction of acute bronchiolitis.

Parameter	Cut-off	Sensitivity %	Specificity %	PPV	NPV	AUC (95% CI)	p
IPF (%)	>3.200	84	97	98.700	73.500	0.950(0.910-0.970)	<0.001
MPV (fL)	>9	78	72	86.400	59.700	0.850(0.800-0.890)	<0.001
PDW (%)	>10.100	44	75	79.800	37.500	0.58(0.520-0.640)	0.017
WBC (10^9/L)	>9.940	56	95	96.200	49.600	0.790(0.730-0.830)	<0.001
PLT(10^9/L)	>424	37	97	97.100	41.100	0.710(0.650-0.760)	<0.001

IPF: immature platelet fraction, MPV: mean platelet volume, PDW: platelet distribution width, WBC: white blood cell count, PLT: platelet, PPV: positive predictive value, NPV: negative predictive value, AUC (95% CI): area under the receiver operating characteristic curve (95% confidence interval).

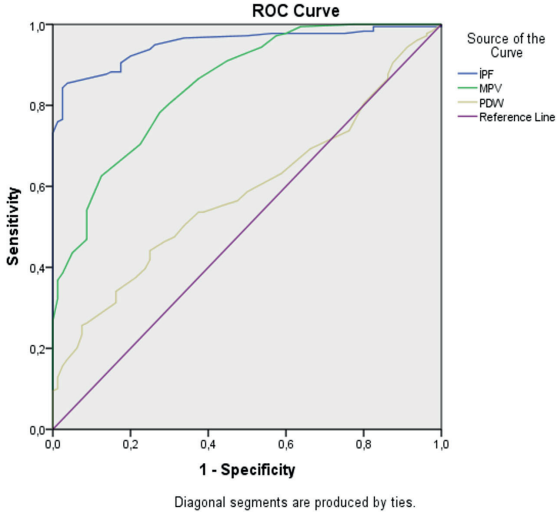

Fig. 1. Receiver operating characteristic curve (ROC) analysis of Immature platelet fraction (IPF), Mean platelet volume (MPV), Platelet distribution width (PDW).

To our knowledge, this is the first study to investigate the relationship between acute bronchiolitis and IPF. Some cytokines and mediators are activated as part of the response to inflammatory conditions. Therefore, we think that there may be a positive correlation between the severity of inflammation and the immature platelet fraction in the bone marrow. One of the biochemical and hematological systemic inflammatory markers is supposed to be IPF, an index of platelet activation and production rate in the bone marrow.[3] Diagnostic accuracy studies performed over the last few years suggest that IPF levels can provide clinically relevant information regarding inflammatory activity and disease prognoses.[7] De Blasi et al.[6] reported that IPF levels increased in patients with sepsis before sepsis was observed clinically, and they also found that IPF was a better biomarker for sepsis than procalcitonin or C-reactive protein. Park et al.[5] found that IPF levels were significantly higher in patients with sepsis than in individuals who did not have sepsis. Rodolfo et al.[7] evaluated the IPF as a biomarker for sepsis diagnosis and severity and found that it was correlated with sepsis severity scores and had the highest diagnostic accuracy for sepsis among all the clinical and laboratory parameters assessed. Another study found that IPF was strongly positively correlated with MPV and PDW, and attributed this to an increase in the number of larger and wider platelets that appeared after the destruction of pro-inflammatory cytokines and endotoxins as part of a severe inflammatory response.[8] Together, these findings suggest that IPF levels increase in inflammatory diseases such as sepsis and may be used as an indicator of the inflammatory response. In our study, there were significant differences in inflammatory markers (IPF, MPV, PLT, and WBC) between acute bronchiolitis patients and healthy individuals. These inflammation markers can be used to predict the clinical severity of acute bronchiolitis, but the marker with the highest sensitivity and specificity was IPF (AUC:0.950). Our observation that the IPF was greater in patients with acute bronchiolitis than in healthy individuals is consistent with previous studies. The clinical severity of acute bronchiolitis was positively correlated with the IPF. Therefore, patients with acute bronchiolitis and high IPF values (>3.2) should be monitored closely after admission to the emergency department.

Platelets play an important role in inflammatory responses, and the PLT increases in many inflammatory diseases. Studies have found that patients with acute bronchiolitis have higher PLTs than healthy individuals.[14,15] Our patient group had significantly greater PLT values than our healthy control group, and this observation is consistent with previous studies. Our severe bronchiolitis group had higher PLT values compared to our mild bronchiolitis group, but the difference was not statistically significant. During an inflammatory response, depolymerization occurs in the microtubular structure of platelets, and changes also occur in the structure of actin that becomes polymerized; consequently, platelets change their shapes and these changes are reflected in the MPV.[15] Changes in MPV can be observed before changes in platelet number, so changes in MPV may be useful for predicting inflammation at an early stage and determining its subsequent severity.[16-18] Among our study population, the MPV was significantly higher in those with moderate and severe bronchiolitis than in those with mild bronchiolitis or in healthy controls, so MPV values may be used to estimate prognoses and the severity of acute bronchiolitis; patients with an MPV value greater than 9 fL should be monitored carefully for clinical deterioration. Several previous studies have evaluated the relationship between acute bronchiolitis and MPV. Gökçe et al.[3] found that patients with acute bronchiolitis had higher MPVs than their healthy counterparts, but found no statistically significant differences in MPVs between different bronchiolitis groups. Higher MPVs were observed in children with influenza A respiratory tract infections than in healthy children.[19] MPVs were also found to be higher in children with pneumonia, asthma, or sepsis than in healthy children.[20-22] Gasparyan et al.[23] found that MPV values were increased in patients with mild inflammation, and they attributed this finding to the transfer of large activated platelets to the site of infection as the severity of inflammation increased. Together, these results suggest that the MPV may also be used to estimate prognoses and the severity of other inflammatory diseases.

PDW, another platelet volume index, shows the variation in platelet diameters and varies by platelet activation. An increase in PDW may occur as a result of swelling, disruption, or platelet immaturity.[15] In our study, PDW values were significantly greater in patients with moderate and severe bronchiolitis cases than in those with mild bronchiolitis or healthy controls. Ergül et al.[15] found greater PDW values in children with acute bronchiolitis than in healthy children. PDW may also be greater in patients with sepsis than in healthy individuals, and may be used as a prognostic indicator for patients with sepsis.[22-24]

There are several limitations to the current study. Firstly, our study was not a multi-center study. In addition, the study population was not evaluated for the type of virus and the patients did not have other inflammatory markers such as CRP and procalcitonin.

In conclusion, the IPF is a novel indicator of inflammation, and it is also a new marker for evaluating the clinical severity of acute bronchiolitis. Larger prospective studies are needed to clarify the clinical significance of using IPF values to assess patients with acute bronchiolitis.

Ethical approval

This study was conducted with the approval of the Ethical Committee of Erciyes University Faculty of Medicine on 23/02/2018 (Project number: 2018/91).

Author contribution

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

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None.

Conflict of interest

The author declares that there are no conflict of interests.

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Original Article

Clinical follow-up of children with high vitamin B12 values: should we worry?

Davut Albayrak[1], Canan Albayrak[2]

[1]*Department of Pediatric Hematology and Oncology, Medicalpark Samsun Hospital, Samsun;* [2]*Department of Pediatric Hematology and Oncology, Ondokuz Mayis University Faculty of Medi-cine, Samsun, Turkey.*

ABSTRACT

Background. Requests of Vitamin B12 test increased with the widespread use of autoanalysers. Although the cause of requests was deficiency suspicions, an important ratio of high levels of Vitamin B12 were reported to physicians by laboratory. Ratios of values of high Vitamin B12 among test request in adults are reported as 14-20% in present three monocentre studies and one multicentre study. There is no report on children with high vitamin B12 for both ratio in lab requests or clinical follow up.

Methods. We evaluated the records of 40 children (23 male /17 female) with high B12 values (>1000 pg/ml) retrospectively. Children were otherwise healthy children and were seen at outpatient pediatric clinics. Additionally, vitamin B12 values of 13 acute lymphoblastic leukemia patients at diagnosis time were retrieved to enlighten possible role of lymphocytes.

Result. Children did not have any malign or chronic diseases causing the high Vitamin B12 values. Holotranscobalamin levels were normal or slightly above. Two patients did develop leukemia later. Our follow up showed that high vitamin B12 values slightly decreased at 3 months and then remained unchanged later. The high numbers of T and B cells are not the source of vitamin B12 elevation.

Conclusions. Our study suggests that high-vitamin B12 values are usually benign in children but some patients may develop leukemia later. We suggest that patients should be followed up for some time after testing for severe hematological diseases.

Key words: vitamin B12, cobalamin, children, pediatric, holotranscobalamin.

Vitamin B12 (cobalamin, Cbl) is one of the most important vitamins that can be dissolved in water. It is absorbed in terminal ileum, transported by haptocorrins and stored in the liver. It is involved in methyl transfer and nucleotide synthesis. In children, deficiency leads to megaloblastic anemia and affects neurologic development. The importance of deficiency in children is well understood. There are many studies about vitamin B12 deficiency.[1-3]

The widespread use of autoanalysers in hospital laboratories has made vitamin B12 testing, like many tests, widespread, inexpensive, fast and accessible everywhere. Thus, all doctors, especially pediatricians, internist and neurologists, have started to request vitamin B12 tests from every patient in which the deficiency might have an impact or relation. Important data have accumulated in laboratory recording systems due to the increased requests. Analysis of these data showed that a significant proportion of the vitamin B12 tests were higher than normal.[1-3] Thus, a new research area related to vitamin B12 levels was opened. The new questions are what is the meaning of this high vitamin B12 (hCbl) level found incidentally high in a patient and how to manage such a patient. These questions are very important, because the high levels of Cbl are seen in malign or proliferative diseases of myeloid and lymphoid

✉ Davut Albayrak
davutalb@omu.edu.tr

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series diseases such as promyelocytic leukemia, chronic myeloid leukemia (CML), autoimmune lymphoproliferative syndrome (ALPS), systemic inflammatory syndrome, polycythemia vera, hypereosinophilic syndrome, solid cancers, liver diseases such as acute hepatitis, cirrhosis, hepatocellular carcinoma, kidney disease, autoimmune disease and some infection diseases such as HIV and malaria.[1-4]

Elevation of Cbl may be one finding of these diseases or is likely to be the first presenting finding of a developing disease. This possibility makes it clinically important.

The ratio of hCbl among vitamin B12 samples that reached the laboratory is large enough to attract attention. Its ratio is 14%, 18.5% and 20% in three monocentre studies, and 18.5% a multicentre study. They also examined the relationship between high levels of Cbl with holotranscobalamin levels in the blood samples of these patients. They reported that 1% of samples are from patients with myeloproliferative disease.[4-7] However, there is no report on clinical follow up of adult or child patients with high vitamin B12 values.

The management of children with a hCbl value is still an important and unanswered question. There is no publication in children in terms of laboratory and clinical short and long-term follow-up. There is no report on clinical follow up of adult or child patients with hCbl values.

In this article, we present clinical and laboratory follow-up of patients who were referred to a pediatric hematology outpatient clinic by pediatricians because of vitamin B12 elevation. We also point to possible causes of hCbl and research subject areas for patients with hCbl levels.

Material and Methods

The records of 40 children with high Cbl value (>1000 pg/ml) were evaluated retrospectively. These patients were seen in our outpatient pediatric hematology clinic in the years of 2017-2020. Most of them were referred from other pediatric outpatient clinics for hematologic evaluation. The vitamin B12 levels had been requested during routine outpatient examinations for suspicion of deficiency. After history and physical examination, vitamin B12 (Normal value 200-800 pg/ml), holotranscobalamin (Normal value 37–171 pmol/L), hemogram, ferritin, iron, ALT, AST, creatinine, folate values and abdominal ultrasonography of the patients were examined. They were also followed-up for any disease related with hCbl level such as myeloproliferative or lymphoproliferative disease. Cbl levels were measured initially and at third and sixth month. The patients were followed up clinically between 1 and 3 years.

In addition, vitamin B12 levels in 13 patients with acute lymphoblastic leukemia (ALL) having high vitamin B12 level at diagnosis were retrieved to discuss the possible lymphocyte origin of this high level.

Categorical data were expressed as number and percentage (%). Scale variables were expressed as mean± standard deviation (SD) and range. Student's t test was used for comparison of means. Categorical variables were compared with Chi- Square. $P<0.05$ was assessed as statistically significant. The statistical relationship between two variables was calculated by Pearson Correlation analysis.

Results

The records of 40 children with hCbl value (>1000 pg/ml) were evaluated retrospectively. Twenty three patients (58%) were male. The average age of patients was 3.12±1.43 year. Initial vitamin B12 are 1267,89±382,01 pg/ml and vitamin B12 in the third month are 1162.09±381.96 pg/ml, in the sixth month are 1169.64±351.74 pg/ml.

Demographic and laboratory findings are represented in Table I. Cbl levels decreased slightly in the third month. Difference of Cbl values between initial and in third month and sixth month was p=0.03 and statistically significant. But difference of values between

Table I. Demographic and laboratory findings of children with high Cobalamin (Cbl) values.

	Mean	SD	Range
Male/Female	23/17		
Age (year)	3.18	±2.11	1-6.5
Hemoglobin (g/dL)	12.11	±0.88	9.6-13.6
White Blood Cell (x10^9/L)	9.52	±3.53	3.75-15.49
Lymphocyte (x10^9/L)	4.63	±1.59	2.65-8.64
C-reactive protein (mg/L)	0.23	±0.4	0.01-1.39
Ferritin (ng/ml)	40.74	±17.6	9-77
Holotranscobalamin (pmol/L) (normal values 25-165 pmol/L)	147.07	±60.06	82.80-630
Initial Cbl (pg/ml)	1267.89	±382.01	1000,9-2001
At third month Cbl (pg/ml)	1162.09	±381.96	606.8-2000
At sixth month Cbl (pg/ml)	1169.64	±351.74	466.2-2000

third and sixth month was not significant (p>0.05). Holotranscobalamin levels are 147,07±60,06 pmol/L (normal values 25-165). Holotranscobalamin levels were normal %67 and slightly above in 33%. Correlations between vitamin B12 and holotranscobalamin levels was r=0.24. There was no correlation between vitamin B12 and white blood cells, neutrophil, lymphocyte, platelet, hemoglobin and ferritin levels.

The follow-up period of the patients lasted from one year to three years. Two patients developed leukemia at follow-up. The first patient was a 2-year-old female patient and she developed ALL after two months of first admission. The second patient was a 6 year old male and he developed chronic myeloid leukemia (CML) after two years of the first hCbl level.

Characteristics of children with ALL are given in Table II. Cbl levels of ALL patients were normal range excluding one B cell ALL patient whose vitamin B12 level was 1154 pg/ml. In two patients with delta-gamma T cell ALL, Cbl levels were normal.

The research was reviewed and approved by institutional review board of Ondokuz Mayis University on clinical studies with date 15/02/2021 and number B.30.2.ODM.0.20.08/26-61, and that participation involved informed consent. There is no financial assistance.

Table II. Characteristics of children with acute lymphoblastic leukemia.

Male/female	6/7
T cell	2
B cell	9
deltagamma T cell	2
Mean cobalamin±SD (pg/ml)	504.59±333.91
Median cobalamin, range (pg/ml)	429.4 (166.2-1154)
Mean White Blood Cell ±SD (x109/L)	30.35±52.57
Median White Blood Cell, range (x109/L)	10.9 (1.03-184)

Discussion

There is no report on clinical follow up of adult or child patients with Cbl values, although clinicians need to frequently decide about these patients. There is not enough publications about the meaning, importance, management and reasons of hCbl in a person who looks healthy in other respects and does not use vitamin B12 drugs. Our study is the first study to show what can be seen with clinical follow-up of patients. It has the potential to trigger other clinical studies.

Clinicians have been encountering more patients with hCbl results in the last decades. Patients and parents read the reasons for the hCbl from the internet and come to the physician with true or false information, fear and questions. Fear is due to the fact that the hCbl is associated

with many hematological and chronic diseases such as juvenile myelomonocytic leukemia, CML, polycythemia vera, hypereosinophilic syndrome, chronic lymphocytic leukemia, acute leukemias, lymphomas, myelodysplastic leukemia, autoimmune lymphoproliferative syndrome, liver and kidney diseases.[1-3] They want satisfying and detailed information. Patients live through a referral chain starting from their own doctor through a pediatrician to pediatric hematologist.

There have been many developments in recent years that have caused us to see more patients with hCbl. This increased our need for a management algorithm. We know better the relationship of Cbl deficiency and diseases.[1-3,8] Ideas are being developed about how vitamin B12 deficiency changes the clinics of other diseases. Autoanalysers offer fast, inexpensive, and results available on the same day and then we can use these results to decide patient treatment. Doctors are very concerned about the consequences of Cbl deficiency and use auto analyzer facilities abundantly. They request measurement of plasma Cbl for their patients and intend to find a deficiency. But, see both high and low Cbl test results.[4-7] Our cases are also determined by test request for deficiency suspicion.

To understand the cause, significance, possible side effects and possible new research areas of Cbl elevation, we must understand the structure, synthesis by bacteria and archea, transporting to human by food chain.[9-12]

Cbl is a haptocorrin compound with cobalt in its center. Only a limited number of prokaryotes (some bacteria such as Pseudomonas denitrificans and archaea such as Thermosipho africanus) can synthesize cobalamins. These prokaryotes are a part of intestinal microbiata. Other living things take cobalamins from bacteria and archaea by a food chain. In the intestine, the synthesis of this molecule is by two pathways.[13] The first path is anaerobic synthesis and studied in Salmonella typhimurium, the second pathway is aerobic synthesis and studied in Pseudomonas denitrificans.[14,15]

If the bacteria in our gut synthesize Cbl, why is Cbl deficiency occurring? Cbl, are synthesized by the bacteria and archaea, but it remains in the living bacteria until it dies.

Most of bacteria are found in colons and it is after terminal ileum where the Cbl receptors are located.[9-13] This is an important detail because the host may not absorbe Cbl in its own bacteria. For this reason, animals usually do not use these sources except that very small part.

In humans, Cbl -containing proteins are hydrolyzed in the stomach and intestine. Free Cbl now binds to intrinsic factor secreted by parietal cells of the stomach. Cbl and intrinsic factor complex bind to cubulin receptors of epithelia of terminal ileum and enters epithelia cell by active transport by contributions of amnionless, megalin, serum paraoxonase/ arylesterase and receptor-associated-protein.[1-4] In addition, 1% of an oral vitamin B12 is also passively absorbed. Then Cbl is secreted to portal vessels. In plasma, cobalamin is bound to either transcobalamin II (TC) as active form (holo-TC) or other haptocorrins transcobalamin I, transcobalamin III (HC) as storage of Cbl. holo-TC constitutes 6 to 20% of plasma Cbl but HC binds to 80–94% of them. Transcobalamin II is produced by mainly hepatocyctes and additionally enteric epithelia, endothelia and monocytes. Haptocorrins are a part of secondary granules. Filtered holotranscobalamin are reabsorbed by megalin receptors in renal tubules. Cells receive Cbl by holotranscobalamin II receptor CD 320. It is converted to methyl Cbl in cytoplasm and adenosyl Cbl in mitochondria. Methyl Cbl is a co-factor of methyl transferases, including methionine synthase (MetH) and corrinoid iron-sulfur protein (CFeSP). It is required for methionin synthesis from homosistein. Methionin is a methyl donor. In the methylation deficiency, thymidine synthesis and methylation of nucteotides are affected. Cbl deficiency also effect uracil synthesis. In bacteria, new molecules and enzymes are affected by methyl cobalamin levels. In mitochondria, Adenosyl cobalamin are required as a cofactor to convert methylmalonyl CoA to succinyl CoA.

Adenosyl Cbl is involved to lipid metabolism by this way.[9-12]

Cbl supply to cell may be deficient despite hCbl levels, because HC has higher affinity than TC. If intestinal supply of Cbl is insufficient, present Cbl remain in the high affinity HC pool, low affinity TC cannot find sufficient vitamin B12 to bind. Low holoTC levels cause Cbl deficiency even in hCbl levels.[9-12] Our cases have normal range holo-TC. There is no clinical deficiency. On the other hand holo-TC levels were normal although Cbl levels were high. That is, TC synthesis did not increase by some cause increasing HC levels

High levels of plasma Cbl in each disease are explained by different mechanisms.[9-12] Secretion of haptocorrin from leukocyte granules is responsible for high hCbl levels in lymphoprolipherative diseases, such as, automimmune lymphoproliperative disease (ALPS), myeloma and myeloprolipherative diseases such as polycytemia vera and other cancers. In autoimmune disease, hCbl levels are due to production from leukocytes, decreased uptake and decreased filtration by TC autoantibodies. In ALPS, Cbl level increases 15-20 fold of normal. In a study, HC is in lymphocyte lysate of ALPS-FAS subjects but not that of controls. TC is not present in lymphocytes of both groups. HC is present in granulocytes of both ALPS-FAS patients and controls.[16] This also explains why there is no increase of vitamin B12 in lymphocytosis patients. Our acute lymphoblastic leukemia patients with T, B and delta gamma T cells also show malign lymphocytes which were not usually the source of hCbl levels. Delta -gamma T cells have the same differences from alfa -beta T cells. Two delta gamma T cell leukemia/ lymphoma patients have normal values. We saw hCbl levels in myeloid leukemias such as JMML, CML and acute myeloid leukemias.

In kidney disease, high levels may be caused by a decreased renal Cbl clearance. In hepatic disease, release from lysed hepatocytes and decreased uptake by hepatocytes cause increased levels of TC.[1-4]

hCbl values in healthy children cannot be explained by suggested mechanisms hCbl associated disease. In our cases, kidney and liver diseases and abdominal tumors were excluded by abdominal ultrasonography and liver and kidney function test. Hematological disease was excluded by normal WBC, peripheral smear and clinical follow up. We need new explanations. For this reason, we look at the deficiency associated diseases and mechanism to see patterns and use it to explain the high levels in otherwise healthy children. The present list of variables are: oral intake by food or drugs, gastric intrinsic factor, consumption by intestinal parasites and bacterial overgrowth, bacterial flora, bacteriophages in flora, terminal ileum inflammation or resection, ph changes in gut, genetic disease of cubam receptor complex, transcobalamin II deficiency, haptocorrin releasing from leukocytes or malign cells, TC uptake by liver, TC reabsorption in kidney, holo-TC receptor deficiency and competition for limited Cbl between high affinity haptocorrins and TC.[1-4] Now thinking about these variables: oral intake was excluded initially. Intrinsic factor does not rate limiting for absorption although low levels cause deficiency. Cubilin expression is rate-limiting step with pH dependency because 5 pg daily absorption is saturated amount of oral diet.[17,18] Increasing expressions of absorption receptor complex may be related with high vitamin B12 levels. Mutation studies of these genes may give a relation.

The relationship between Cbl levels with genetic factors was investigated in deficient and normal persons. Heritability was estimated to be 59% in a study of monozygotic and dizygotic twins. This suggests that genetic effect is significant.[19] Recent updates on whole genome SNP in deficient and control samples give a list of genes related to Cbl deficiency.[20] A counterpart of these studies in patients with hCbl values may give a candidate gene list. Passive absorption rate is 1% of oral high doses and 500mcg give 5 pg of passive absorption.[21] But there was no oral b12 treatment in our patients. Dietary intake is also important. The mean serum Cbl in vegans

was 33% lower than in vegetarians and 57% lower than in omnivores.[22] Diets of our patients were typical children's diets.

In children with hCbl level, all known diseases should be excluded first by hematologic, biochemical and radiologic investigation. As in our cases, hCbl levels did not have any other abnormalities. In these patients, we should think of new causes increasing levels of Cbl. One of them may be microbiota.[23] Horses do not eat soil or meal but their vitamin B12 levels are normal. Their normal values are found to be associated with helicobacter colonization because helicobacter can synthetize Cbl and its upper intestinal colonization is appropriate for the absorption of vitamin B12 by dead bacteria. In humans helicobacter colonization is common and associated with peptic ulcus and antibiotic is used extensively. Does Cbl deficiency deepen after helicobacter therapy in a vegetarian? We did not know the role of helicobacter colonization in our patients. Another possibility is an increase of intestinal colonization. But there is opposite clinical finding present in intestinal duplication by Cbl consumption by increased bacterial colonization. Other possibilities are feeding habits may cause preferential increase of Cbl synthesis in bacteria and archaea by ingredients. Pickles, vinegar, butyrate foods may be examples of ingredient sources.[23]

In our study, leukemia developed in two of 40 children. The ratio 2/40 may be a coincidence. Annual risk of developing new leukemia in children is 4/100.000 in the literature.[24] That is 8/100000 new cases in two years. The difference is significant when we compare 2/40 and 8/100000 with the chi-square test (p=0,000). This important association should be validated with larger series.

We recommend that the following examinations be performed initially in a patient presenting with hCbl: WBC, peripheral smear, vitamin B12, holotranscobalamin, ferritin, folic acid, liver and kidney function tests and abdominal ultrasound. If there is cytopenia, tests should include direct coombs, autoantibodies and double negative ratio in alpha beta T lymphocytes for ALPS and ALPS like diseases. In next visits, a limited test may be sufficient such as WBC, peripheral smear and Cbl. If there is a new abnormality in WBC or additional complaints, the test panel should be expanded appropriately. Patients should be followed up for two years for the development of leukemia and myeloproliferative disease. hCbl is not toxic. Meat restriction was not recommended because it is the main source of vitamin B12, iron and zinc. We are not afraid of Cbl elevation itself, but we do fear that it may be the first sign of a hematological disease such as leukemia.

Future clinical studies may be conducted in groups that can conduct laboratory research on pathways from oral intake to final clearance of Cbl and full genome analysis. This cooperation will provide sufficient material to find the causes leading to hCbl and to establish a cause and effect relationship.

In conclusion, a significant number of children seen in outpatient clinics due to other reasons have hCbl. Our study suggests a few patients may develop leukemia later. We suggest that patients should be followed up for two years after initial investigations for hematological, liver and kidney diseases showing hCbl levela. On this subject, longer and more detailed studies with larger case series are needed.

Author contribution

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

Ethical approval

The research was reviewed and approved by institutional review board of Ondokuz Mayis University on clinical studies

with date 15/02/2021 and number B.30.2.ODM.0.20.08/26-61, and that participation involved informed consent.

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Conflict of interest

The authors have no conflict of interest to inform.

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Case Report

Hematopoietic stem cell transplantation complicated with EBV associated hemophagocytic lymphohistiocytosis in a patient with DOCK2 deficiency

Elif Soyak Aytekin[1], Deniz Çağdaş[1], Çağman Tan[1], Büşranur Çavdarlı[2], Işıl Bilgiç[3], İlhan Tezcan[1]

[1]*Division of Pediatric Immunology,* [3]*Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara;* [2]*Department of Medical Genetics, Ankara Bilkent City Hospital, Ankara, Turkey.*

ABSTRACT

Background. Dedicator of cytokinesis 2 (DOCK2) deficiency is a rare autosomal recessive combined immunodeficiency presenting with very early onset, severe bacterial and viral infections. In DOCK2 deficiency; T, B and NK cell numbers are decreased and functions are impaired resulting in severe atrophy of secondary lymphoid tissues. The aim of this report is to provide information on clinical and laboratory features and hematopoietic stem cell transplantation (HSCT) outcomes of a DOCK2 deficient patient. The patient was diagnosed by using a targeted next generation sequencing primary immunodeficiency (PID) panel. Lymphocyte subsets were measured by flow-cytometry.

Case. Here, we describe a patient with DOCK2 deficiency presented with severe combined immunodeficiency. He underwent HSCT without conditioning regimen before the genetic diagnosis and developed hemophagocytic lymphohistiocytosis(HLH) due to Epstein-Barr virus (EBV) infection.

Conclusions. Genetic testing is necessery for early diagnosis of DOCK2 deficiency. The curative treatment should be HSCT soon after diagnosis.

Key words: DOCK2 deficiency, hemophagocytic lymphohistiocytosis, hematopoietic stem cell transplantation, severe combined immune deficiency, EBV associated hemaphagocytic lymphohistiocytosis.

Dedicator of cytokinesis 2 (DOCK2) deficiency is a rare autosomal recessive combined immunodeficiency presenting with very early onset, severe bacterial and viral infections. It is caused by homozygous or compound heterozygous mutations in the DOCK2 gene. The curative treatment is hematopoietic stem cell transplantation (HSCT) soon after diagnosis.

DOCK2 protein is predominantly expressed in hematopoietic cells and regulates leukocyte activation/migration by stimulating and activating Rac. Rac is a small signaling G protein, which is a subfamily of the Rho family of GTPases. T and B cell migration to peripheral lymph node is mediated by CCL21, CXCL12 and CXCL13, homeostatic chemokines expressed in high endothelial venules and stromal cells. It is shown that in vitro stimulation of DOCK2-deficient (DOCK2(-/-)) T and B cells with CCL21, CXCL12 and CXCL13 do not stimulate chemotactic response leading to severe atrophy of secondary lymphoid tissues.[1,2]

B and NK cell functions are impaired in addition to T cell number/function defects in DOCK2 deficiency. Sanui et al.[3] showed that TCR-mediated Rac activation was almost totally abolished in (DOCK2-/-) cells. DOCK2 regulates T cell activation through Rac protein activation to provide immunological synapse formation.[3] Rac activation is impaired in plasmacytoid

✉ Deniz Çağdaş
deniz.ayvaz@hacettepe.edu.tr

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dendritic cells similar to lymphocytes in DOCK2 deficiency, resulting in the reduction of motility and the loss of polarity during chemotaxis. Recently Moens et al.[4] showed that reactive oxygen species production are also partially impaired in DOCK2-deficient neutrophils.

As far as we know only 11 patients were reported.[4-6] Here, we present a DOCK2 deficient patient who underwent HSCT without conditioning regimen and died because of Candida pneumonia after Epstein-Barr virus (EBV) associated hemophagocytic lymphohistiocytosis (HLH).

Case Report

A one-year-old boy was admitted to Hacettepe University Ihsan Dogramacı Children's Hospital with recurrent diarrhea. He had a normal birth-weight, received vaccination according to the routine immunization schedule. During the follow-up period, he had been hospitalized once due to perianal abscess and three times for pneumonia, including Cytomegalovirus (CMV) pneumonia at 4.5 months of age. His parents were consanguineous (first-degree cousins) and had a four-year-old healthy sister.

On physical examination, his body weight was 12 kg (50-75 percentiles (p)), height, 74 cm(<3p), head circumference, 46,5 cm (25p). He had hepatosplenomegaly (both 4 cm below the costal margin).

Laboratory tests showed normal total lymphocyte count (8400/mm3 (3200-12300)), normal immunglobulin levels (IgA:79,7mg/dl (17-69)), IgG:2880mg/dl (463-1006), IgM:51,9mg/dl (46-159)) with low CD3 (31% (53-75)), CD4 (8% (32-51)), normal CD16/56 (4% (3-15)) and high CD19 (65% (16-35)) ratio and very low naive CD4+ T cells (0.2% (57.1-84.9)), naive CD8+ T cells (0.4% (28.4-80.6)) and recent thymic emigrants (1% (31-81)), suggestive of severe combined immunodeficiency. Monthly intravenous immunoglobulin (IVIG) treatment (400 mg/kg/dose), trimethoprim sulfamethoxasole and fluconazol prophylaxis were started. At 14 months of age, HSCT (6,6X108nucleated cells/kg) was performed from his HLA-identical father without any conditioning regimen. He was discharged three weeks after HSCT as he was well and an increase in CD3 after HSCT compatible with engraftment was recorded.

After receiving the informed consent from the parents, next-generation sequencing (NGS) primary immunodeficiency (PID) panel of 266 genes (Thermofischer-Ion Torrent Platform) was performed on the patient and a homozygous DOCK2 mutation (NM_004946.2 c.1773_1774insG) (p.L592Afs*76) was found. The mutation was novel, not found in the healthy population (Gnomed, 1000G). Mutation taster predicts that the variant is disease-causing (CADD_Score:35). This is a null variant that causes a frameshift defect. The probability of being loss-of-function intolerant (pLI) score of the DOCK2 gene indicates that the gene function is not tolerant for loss-of-function variants. The c.1773_1774insG variant is classified as a pathogenic causative variant for the disease according to the American College of Medical Genetics and Genomics 2015 guidelines.[7] The variant was detected as a heterozygous state in the parents by another NGS platform (Miseq-Illumina San Diego, CA) using PCR primers designed in-house and was visualized with ALAMUT® VISUAL (Interactive Biosoftware: France) software. We gave genetic counseling to the parents. Although there were other consanguineous marriages in the family, our patient was the only affected person.

Three months after HSCT, the patient was admitted to another center with a swelling on the neck. Physical examination revealed cervical lymphadenopathy, massive hepatosplenomegaly (6 cm and 9 cm below the costal margins respectively), and maculopapular rash on arms and trunk. As the skin biopsy was compatible with grade 1 graft versus host disease, cyclosporine was started. We did an extensive infection workup. It included blood

cultures, polymerase chain reaction (PCR) testing for mycobacteria and Herpes family, respiratory viruses, and parvovirus. Epstein–Barr Virus viremia (EBV DNA, 991 copies/mL) was present, and acyclovir was started. Despite the broad-spectrum antibiotic therapy, the fever continued, and the patient was evaluated for hemophagocytic lymphohistiocytosis (HLH). Anemia (hemoglobin:7,8g/dl) thrombocytopenia (59000/mm3), neutropenia (500/mm3), hyperferritinemia (6932μg/L) and hypertriglyceridemia (743mg/dl) were observed. Microscopic evaluation of bone marrow aspiration biopsy showed megaloblastic changes. However, hemophagocytosis was not present. Based on laboratory and clinical findings, the patient met the criteria for HLH. Corticosteroids and cyclosporine treatment were given according to the HLH-2004 protocol. Etoposide was not given as EBV DNA was high (20634 copy/ml). The chimerism analysis was low (6%), and the patient was given lymphocyte infusion from his EBV IgG positive donor for EBV reactivation. A second HSCT with conditioning regimen and interferon alpha-2b treatment was planned, but it could not be performed as the patient developed pneumonia. Candida inconspicua was isolated from bronchoalveolar lavage culture. Despite antifungal treatment, the pneumonia did not improve. Unfortunately, the patient died of respiratory insufficiency and multiorgan failure.

Discussion

DOCK2 deficiency is caused by compound heterozygous or homozygous mutations of the DOCK2 gene (Fig. 1). It is one of the rare CIDs reported in 11 patients.[4-6] Six out of 11 underwent HSCT (Table I). Three patients received myeloablative while one received reduced-intensity conditioning regimens. One patient underwent HSCT from the match related sibling without conditioning, and she was well. In case of severe viral infections in CID, it is difficult to decide to give a conditioning regimen to the patient. As he had a very low T-Cell Receptor Excision Circles (TREC) (1% (31-81)) and T cell activation and had a history of severe cytomegalovirus (CMV) infection, we performed HSCT from his HLA-identical father without giving any conditioning regimen. After the diagnosis of DOCK2 deficiency, and due to the low level of chimerism, we planned a second HSCT. However, the patient died from EBV-associated secondary HLH and fungal pneumonia.

Patients with DOCK2 deficiency are highly prone to bacterial, viral, and fungal infections. Four out of the eleven patients previously reported died from infections (Table I). Two of the patients who died had multiple organ failures after varicella infection. The others died from recurrent CMV and Klebsiella infection and sepsis, respectively. The present patient had

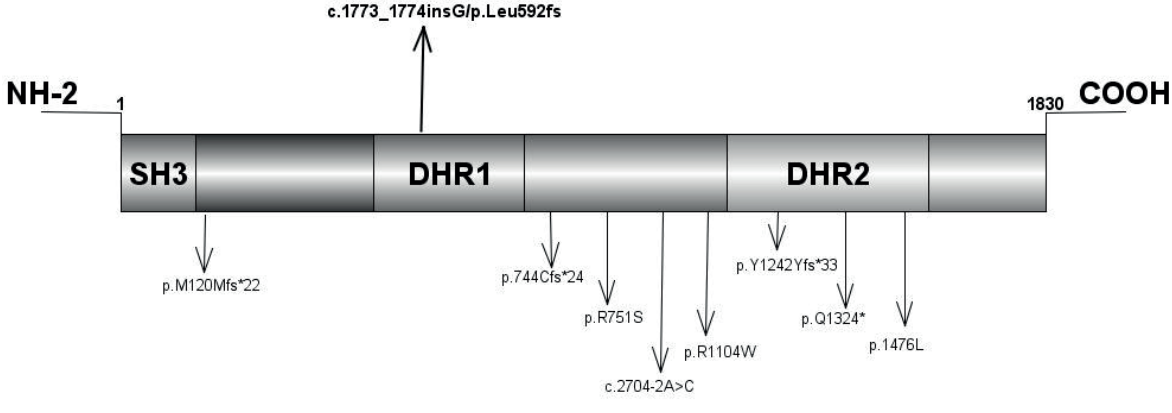

Fig. 1. Structure of DOCK2 protein showing the different domains and defined mutations.

Table I. DOCK2 defective patients, review of the literature.

Patients	Dobbs et all. P1	Dobbs et all. P2	Dobbs et all. P3	Dobbs et all. P4	Dobbs et all. P5	Moens et all. P6
DOCK2 mutation	p.Y1242Yfs*33	p.R1104W p.Q1324*	p.R751S	p.F744Cfs*27	p.M120Mfs*22 p.P1476L	c.2704-2 A>C
Ethnic origin	Lebanese	Finnish	Turkish	Turkish	Hispanic	Moroccan
Age at evaluation	5 months	2.5 years	6.3 years	1 years	4 months	One months
Sex	Male	Female	Male	Male	Male	Male
Symptoms at onset	bronchiolitis caused by RSV	granulomatous inflammation in lungs caused by M. avium intracellulare	meningoencephalitis caused by mumps	neonatal-onset chronic diarrhea, oral moniliasis	İnterstitial pneumonia	Ulcerative perianal dermatitis, *E. coli* pyelonephritis
Other symptoms and findings	recurrent pneumoniae	recurrent otitis media, pneumonia and diarrhea, recurrent thrombocytopenia and thrombosis	recurrent upper and respiratory tract infection	growth failure, hepatomegaly	Rectal fistula	nephrotic syndrome, Omenn Syndrome
Viral infections	RSV	M. avium intracellulare	Mumps, Varicella	parainfluenza virüs, type 3 adenovirus, CMV, K. pneumoniae		-
HSCT	+	+	-	-	+	-
Donor	MMRD	MUD			MRD	
Conditioning regimen	myeloablative conditioning with busulfan and fludarabine	reduced-intensity conditioning with treosulfan, fludarabine, and alemtuzumab			Myeloablative conditioning with busulfan and cyclophosphamide	
Cause of death	Alive	Alive	Varicella pneumonia	Sepsis (K.pneumoniae)	Alive	ARDS and capillary leak syndrome

ATG: anti-thymocyte globulin, GVHD: graft versus host disease, HSCT: hematopoietic stem cell transplantation, MMRD: mismatched related donor, MRD; matched related donor, MUD: matched unrelated donor, NA: not available

early-onset severe CMV infection. After HSCT, he suffered from EBV-associated secondary HLH and died from Candida pneumonia. In this case, IVIG was started even though immunoglobulin levels were normal. Because the patient's clinical and immunological features were compatible with severe combined immunodeficiency.

HLH is a rare and highly fatal multisystem inflammatory syndrome. HLH is common in patients with primary immunodeficiencies, particularly those with abnormal T cell functions. However, secondary HLH after transplantation in PID is uncommon.[8,9] Asano et al.[10] reported that secondary HLH developed in 37 out of 5427 HSCT cases, and four out of 37 were PID. Ali et al.[11] reported EBV-associated HLH in three out of 408 patients who underwent allogeneic HSCT, and all three patients died. Epstein–Barr virus is the most frequently associated infectious agent with HLH.[11] In these cases, it is challenging to control HLH and EBV.[10] Therefore, EBV titer monitoring is needed in the patients before and after the HSCT. In the present case, the HLH-2004 protocol was given without etoposide, because of high EBV DNA copy numbers (20634 copy/ml). Anti-CD20 antibody (rituximab) improves outcomes in patients with EBV-associated lymphoproliferative disorders by depleting B cells.[12] However, we could not give the patient the rituximab therapy. Because the patient's fever persisted, and we could not rule out sepsis. HLH could have possibly occurred in the patient due to graft failure.

In plasmacytoid dendritic cells, induction of type I interferons (IFN) critically depends on

Table I. Continued.

Patients	Moens et all. P7	Moens et all. P8	Moens et all. P9	Alosaimi et all. P10	Alosaimi et all. P11	Our patient P12
DOCK2 mutation	c.2704-2 A>C	c.2704-2 A>C	c.2704-2 A>C	Del 902-1078	Phe848fs	p.L592Afs*76
Ethnic origin	Moroccan	Moroccan	Moroccan	NA	NA	Turkish
Age at evaluation	NA	NA	NA	2 weeks of age	5 months	16 months
Sex	Female	Male	Female			Male
Symptoms at onset		NA	NA	chronic diarrhea, recurrent sinopulmonary infection, CMV viremia	chronic diarrhea, recurrent pneumonia, oral candidiasis	recurrent diarrhea
Other symptoms and findings		NA	NA	-	NA	perianal abscess, recurrent pneumonia, Growth retardation, EBV associated HLH
Viral infections	Varicella	NA	NA	CMV	-	CMV, EBV, Candida inconspicua
HSCT	-	+	+	+	-	+
Donor	NA	NA	MRD	MUD		MRD
Conditioning regimen		NA	Without conditioning	myeloablative conditioning with busulfan, fludarabine, and ATG		Without conditioning
Cause of death	Varicella infection	Alveolar hemorrhage after HSCT	Alive	Alive	Sepsis	GVHD Pneumoniae

ATG: anti-thymocyte globulin, GVHD: graft versus host disease, HSCT: hematopoietic stem cell transplantation, MMRD: mismatched related donor, MRD; matched related donor, MUD: matched unrelated donor, NA: not available

IFN regulatory factor 7 (IRF-7) and IkappaB Kinase IKKα, which activates IRF-7 after binding.[13] In DOCK2-/- plasmacytoid dendritic cells, phosphorylation of IKK-α and nuclear translocation of Interferon Regulatory Factors (IRF-7) were impaired, resulting in a selective loss of IFN-α/IFN-β induction. Recombinant IFN-α2b replacement protects DOCK2 deficient fibroblasts from virus-induced cell death. During the HLH process, we planned to give interferon-alpha 2b treatment to the patient. However, we could not give as we lost the patient within a few days.

In conclusion, DOCK2 deficiency is a rare form of CID. Its diagnosis is challenging because these patients may have normal lymphocyte count on routine laboratory analysis. Accurate diagnosis usually requires genetic testing, and early diagnosis may increase the chance of effective treatment. The curative treatment should be HSCT soon after diagnosis. Further studies are needed to define other treatment options for DOCK2 deficient patients.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ESA, DC, IT; data collection: ESA, IB; analysis and interpretation of results: CT, BC, DC; draft manuscript preparation: ESA, DC, İT. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Case Report

A case of pediatric psoriasis achieving remission after allogenic bone marrow transplantation

Dilara Güler[1], Gülhan Gürel[1], Gülsüm Şeyma Yalçın[2], İbrahim Eker[3], İrem Nur Durusu[1], Çiğdem Özdemir[2], Özge Vural[4]

Departments of [1]Dermatology, [2]Pathology and [3]Pediatric Hematology, Afyonkarahisar Health Sciences University Afyonkarahisar; [4]Department of Pediatric Hematology, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Psoriasis is an inflammatory skin disease in which the cells and molecules of innate and adaptive immunity are involved in the pathogenesis. Aplastic anemia is a bone marrow deficiency syndrome that is characterized by an extreme reduction in the number of blood cells as a result of failure in hematopoiesis. Allogenic hematopoietic stem cell transplantation is a promising treatment for Aplastic Anemia and it is important to note that other comorbid diseases like psoriasis- since both have some common pathogenetic mechanisims- might achieve remission after treatment.

Case. We present a 12-years-old male patient who underwent bone marrow transplantation for aplastic anemia and his psoriasis vulgaris lesions completely regressed. The final follow-up visit on day 150 also revealed no sign of the pre-transplantation skin and scalp lesions.

Conclusions. This is the first case of pediatric psoriasis together with aplastic anemia that achieved complete remission of psoriasis after bone marrow transplantation. Our case report needs to be supported by prospective studies involving larger patient populations.

Key words: aplastic anemia, autoimmune, bone marrow transplantation, pediatric, psoriasis.

Psoriasis is an inflammatory disease that is mainly associated with dermatological manifestations, and affects approximately 2-3% of the general population.[1] The prominent mechanisms in the pathogenesis of psoriasis are immune-mediated processes. Previous studies have demonstrated that the infiltration of the dermis and epidermis by CD4+ and CD8+ T cells and dendritic cells plays a key role in its pathogenesis, hence the cytokines released from these cells are involved in the immune processes.[2,3]

✉ Dilara Güler
dsaritepe78@gmail.com

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Aplastic anemia is a bone marrow deficiency syndrome that is characterized by an extreme reduction in the number of blood cells as a result of failure in hematopoiesis. Immune-mediated mechanisms manifest in the destruction of hematopoietic cells by lymphocytes are involved in the occurrence of aplastic anemia. A vast majority of patients diagnosed with severe aplastic anemia are cured with allogenic stem cell transplantations from a histocompatible sibling.[4,5]

Patients undergoing allogeneic stem cell transplantations may also experience remission in several immune-mediated diseases that are comorbid with aplastic anemia. Previous studies have reported the remission of psoriasis following allogenic stem cell transplantation, most frequently in adult patients in which the transplant indication was malignancy.[6-8] A further study in literature reports on a pediatric

case with psoriasis improved remission after a transplantation for Ewing's sarcoma.[9] Herein, we present a case of pediatric psoriasis that achieved complete remission upon bone marrow transplantation.

Case Report

A 12-years-old male patient who diagnosed with psoriasis four years previously was admitted to the pediatric hematology clinic with fever and fatigue. Physical examination revealed leukoplakia of the hard palate, diffuse scaly, erythematous plaques and petechial rash all over the body. There was nothing unusual in the patient's medical history, aside from psoriasis, nor any drug and/or toxic substance exposure that could cause pancytopenia. A complete blood count revealed severe pancytopenia. A bone marrow aspiration revealed cellularity of <5%, there were no megakaryocytes, while rare myelocytic and erythrocytic series were noted. The patient was thus diagnosed with aplastic anemia. While attempting to determine the etiology of the aplastic anemia, the patient was administered blood component transfusions, when he developed symptomatic anemia and thrombocytopenia, as well as supportive therapies involving antimicrobial treatments when he was febrile. While the patient was undergoing treatment in this regard, the patient was consulted to the department of Dermatology due to an increase in the psoriatic lesions. It was ascertained from the patient's medical history that he had presented to the Dermatology outpatient clinic many times before with nummular, scaly, erythematous plaque lesions on the trunk, left elbow and inguinal region for about 1.5–2 years. The first histopathological examination had been performed 4 years ago and was reported as psoriasis vulgaris. The patient was treated with topical corticosteroids, topical calcipotriol and methotrexate at different times. He had only been receiving intermittent topical corticosteroid therapy for the previous six months.

Dermatological assessment revealed annular, scaly, erythematous plaques on the trunk and upper extremity. Scaly, mildly erythematous-to-yellowish, greasy plaques were also noted around the eyes and scalp (Fig. 1abc). Upon the recent dermatological assessment a new punch biopsy was performed. The histopathological examination revealed parakeratotic foci on the surface, scale crust formations within parakeratoses and loss of granular cell layer immediately underneath the parakeratotic foci. There were granular cell layer of 2-3 lines in the non-parakeratotic areas. As well as irregular acanthosis, mild spongiosis, occasional lymphocyte exocytosis and focal suprapapillary thinning were seen. There were

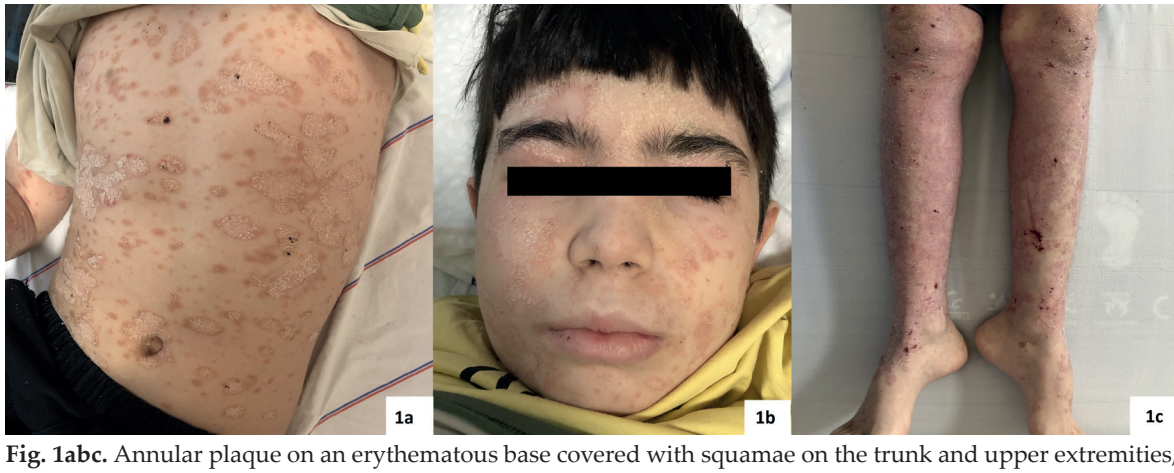

Fig. 1abc. Annular plaque on an erythematous base covered with squamae on the trunk and upper extremities, and squamous plaque lesions with erythema and a yellowish, greasy look around the eyes and on the scalp before treatment.

tortuous, dilated and congested capillaries in some areas of papillary dermis. The superficial dermis featured with scattered melanophages and slight perivascular lymphocytic infiltrate around the superficial vascular plexus. Deeper sections indicated that the parakeratotic foci had a tendency to be located at the follicular ostia (Fig. 2abc).

Considering the clinical and histopathological findings, the patient was diagnosed with sebopsoriasis, and treatment was initiated with systemic itraconazole, topical corticosteroids and ketoconazole. At the same time period examinations were made to investigate the etiology and to classify the aplastic anemia and resulted with the diagnosis of a very severe acquired aplastic anemia. The bone marrow transplant had to be delayed due to the COVID-19 pandemic, and the patient required more transfusions and antimicrobial supportive therapies as time went by. Seven months after the aplastic anemia diagnosis, the patient underwent a bone marrow transplantation from his fully-matched brother without any psoriasis diagnosis. A conditioning regimen was administered using cyclophosphamide (total dose 200 mg/kg intravenously) over 4 days and ATG Fresenius (total dose 35 mg/kg) intravenously over 4 days. Umbilical cord-derived mesenchymal stem cells (MSC) were applied for graph versus host disease (GVHD) prophylaxis, and cyclosporine A and methotrexate were used. Dermatological assessment on day 7 following the allogeneic stem cell transplantation, revealed complete remission of the cutaneous lesions of the patient (Fig. 3abc). He was discharged on day 43 with follow-up by the Pediatric Hematology department on an outpatient basis. The patient experienced no complications during follow-up in the outpatient setting, and the final follow-up visit on day 150 also revealed no sign of the pre-transplantation skin and scalp lesions. We obtained consent from his family to publish this report and to include his photograph.

Discussion

Psoriasis is an inflammatory skin disease in which the cells and molecules of innate and adaptive immunity are involved in the pathogenesis.[10] While it may occur in any period of life, psoriasis has two peak onset periods at the age of 20–30 years and at the age of 50-60 years. Symptoms are reported to start under the age of 20 years in 35–50% of patients.[11] Primary cells involved in the pathogenesis of the disease are dendritic cells, Th1, Th17 and Th22 lymphocytes. IL-12 and IL-23 released from the inflammatory myeloid dendritic cells induce the release of IL-17, IFN-γ, TNF-α and

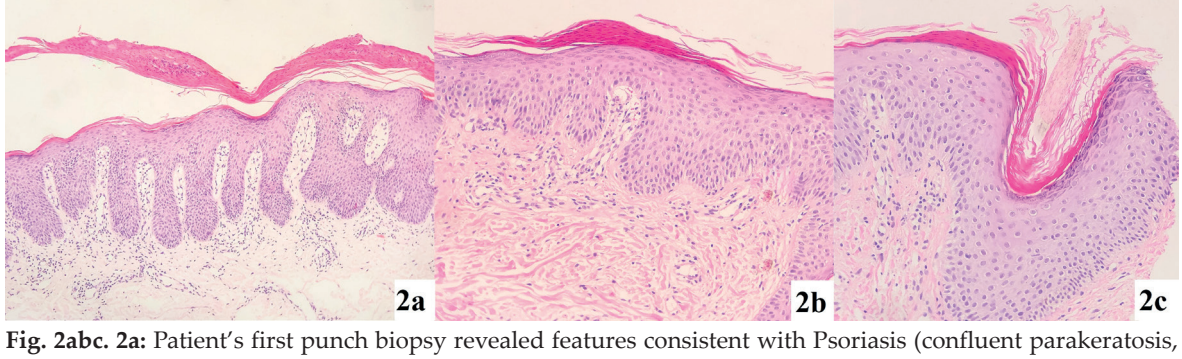

Fig. 2abc. 2a: Patient's first punch biopsy revealed features consistent with Psoriasis (confluent parakeratosis, neutrophilic parakeratosis, loss of granular cell layer, regular acanthosis, elongated rete ridges, suprapapillary thinning, dilated capillaries)(Hematoxylin and eosin, x100) **2b:** Patient's recent punch biopsy showed parakeratotic focus, loss of granular cells underneath the parakeratosis, minimal spongiosis, tortuous and dilated capillaries in the dermal papilla and minimal perivascular lymphocytic infiltrate (Hematoxylin and eosin, x200) **2c:** The parakeratotic focus was located at the follicular ostium in deeper sections of the patient's recent biopsy (Hematoxylin and eosin, x200).

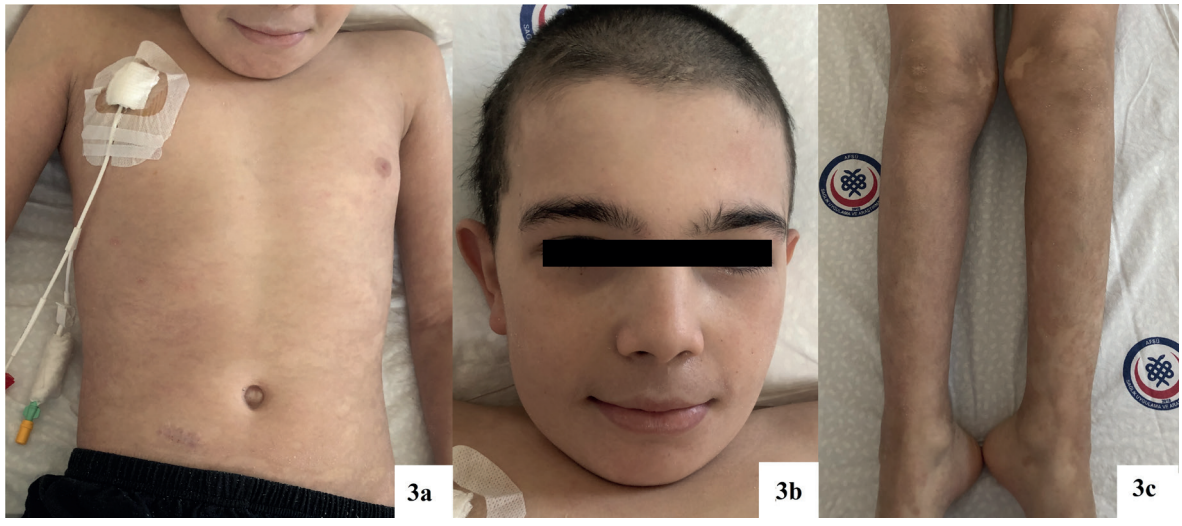

Fig. 3abc. Complete remission in psoriasis vulgaris at visit on the 7th day after bone marrow transplantation.

IL-22 -which are known as psoriatic cytokines- from Th1, Th17 and Th22.[10]

Aplastic anemia is a disease characterized by pancytopenia and hypoplastic bone marrow.[12] Previous studies have found that abnormally activated CD8+ T lymphocytes, dendritic myeloid cells, Th1 cells and some small molecules released from them such as IFN-γ, TNF-α and IL-2 play the main role in pathogenesis of the disease.[13] The etiology of aplastic anemia includes viral infections, environmental toxins, inherited and acquired mutations. The curative treatment of the disease is bone marrow transplantation.[14]

Complete remission of psoriasis after bone marrow transplantation for aplastic anemia has not been reported in a child. In the literature, there are some case reports that have shown remission in psoriasis after bone marrow transplantation.[6-8] A 40-year-old male patient reported by Kishimoto et al.[6] achieved a complete remission in palmoplantar pustular psoriasis following bone marrow transplantation for the treatment of Acute Myelocytic Leukemia (AML). Another case reported by Yokota et al.[7] 36-year-old male patient with psoriasis vulgaris who developed drug-related aplastic anemia and achieved complete remission in psoriatic plaque and nail symptoms, despite not receiving any antipsoriatic treatment after the bone marrow transplantation. We believe that the alteration of the host's immune system cells may have been involved in the remission of the psoriasis.

Occurrence of GVHD has significant correlation with the number of platelet transfusions and the number of red blood cell (RBC) transfusions in allogeneic hematopoietic stem cell transplantation (HSCT) from HLA-matched sibling donors for severe aplastic anemia. Our patient had a high transfusion burden of platelet and RBC before transplant and because of this he had a significant risk of GVHD.[10] On the other hand, there are some studies reporting increased T cell activation in patients with aplastic anemia. Some clinical conditions which have been seen in aplastic anemia may be due to mesenchymal disorders. MSC infusion may be an effective treatment modality in aplastic anemia. MSC infusion inhibits T-cell-mediated hematopoietic stem cell (HSC) destruction and aids in the formation of the environment that contributes to hematopoiesis. There are some studies using MSC infusion alone or in combination with HSCT in the treatment of aplastic anemia.[11] Autoreactive T-lymphocytes are also thought to contribute to psoriasis through the ineffective control of proinflammatory cells.[12] For all these reasons, in our patient with both psoriasis,

aplastic anemia and heavy transfusion load, MSC was given in the aim to reduce the risk of GVHD, increase the likelihood of engraftment and we hoped it would be effective in treating psoriasis, despite the transplantation from HLA full matched sibling.

The main emphasis of our case report is the effect of HSCT on psoriasis. HSCT indication for this case was very severe acquired aplastic anemia. But after the transplantation, the patient's psoriasis also went into remission completely. Basically, the elimination of the autoreactive clone in the bone marrow with HSCT, that causes psoriasis, may explain the remission of the autoimmune disease after transplantation. Additionally, the high dose of immunosuppressive treatment given during the conditioning regimen may be effective in the remission of the disease. This explains the remission of psoriasis as early as 7 days after HSCT when the effects of the transplanted clones are not observed yet. Another important point is the successful engraftment in the patient, although the amount of CD34 positive stem cells that can be given to the patient is very low. MSC therapy applied to the patient may also have been effective in providing this, by modulating the immune responses and maintaining an environment supportive of hematopoiesis.[13]

In a study, the status of psoriasis after allogenic HSC transplantation was evaluated in patients with diagnosis of psoriasis with hematological malignancies. Ciurea et al.[14] demonstrated that allogeneic transplantation is effective in the treatment of disease in patients with psoriatic skin lesions and arthritis. They saw a rapid recovery after transplantation and the lesions did not recurred in the seven-year follow-up.[14] Kaffenberger et al.[15] also suggested that psoriasis is likely to remit after allogeneic HSCT, but it is likely to recur after autologous HSCT, in their report of 19 cases with psoriasis and had undergone allogenic or autologous HSCT. MSCs were not used in these transplants also.

Neither Ciurea nor Kaffenberger give information in their reports about the chimerism of the patients. But Chakrabarti et al.[16] reported a patient with severe psoriasis achieved remission after nonmyeloablative allogeneic HSCT due to non-Hodgkin's lymphoma. MSCs were not used in this transplant also. They concluded that if long-term remission is achieved in autoimmune diseases after allogeneic HSCT with low-intensity conditioning regimens, allogeneic transplantation may become an option for the treatment of some autoimmune diseases. All of these findings suggested that in our patient the allogenic HSCT may be the prominent cause of psoriasis remission rather than MSC.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DG, GG, İE; data collection: DG,GSY, İE, CO, OV; analysis and interpretation of results: DG,GG,GSY, İE; draft manuscript preparation: DG, GG, GSY, İE, İND. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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Case Report

Primary spinal multifocal intradural-extramedullary Ewing sarcoma in children: presentation of a case and review of the literature

Eren Müngen[1], Nilgün Kurucu[1], Tezer Kutluk[1], Kader K. Oğuz[2], Figen Söylemezoğlu[3], Bilgehan Yalçın[1]

[1]*Departments of Pediatric Oncology, Hacettepe University Faculty of Medicine and Cancer Institute Ankara; Departments of* [2]*Radiology and* [3]*Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey.*

ABSTRACT

Background. Primary spinal, intradural, extramedullary Ewing sarcoma (PSIEES) is exceptionally uncommon. Here, we present an interesting pediatric case with a PSIEES diagnosis confirmed by the presence of a specific fusion protein in the tumor tissue and who then developed a cerebellar recurrence. We also reviewed the PSIEES cases in childhood reported in the literature.

Case. An 8.5-year-old boy was admitted to a local hospital with a one-month history of severe back and limb pain, and inability to move his lower limbs. Physical examination revealed paraparesis in the lower extremities. Spinal MRI revealed multiple intradural extramedullary masses at the L2-L3, L4-5 and L5-S1 levels. He underwent surgery and near total excision of all three masses were performed. Histopathological diagnosis of Ewing Sarcoma was confirmed with EWS-ERG gene rearrangement. The patient was treated according to EuroEwing chemotherapy protocol. A total dose of 4500 cGy radiotherapy was applied to the tumor location at L2-S1 paravertebral region. Eighteen months after the end of treatment, a mass in the left cerebellar hemisphere was determined. Gross total excision was performed. Histopathological examination of the tumor showed Ewing sarcoma. Radiological screening revealed isolated central nervous system recurrence. A total of 4500 cGy radiotherapy was applied. He is on a second-line treatment consisting of gemcitabine and docetaxel without any evidence of disease.

Conclusions. Ewing Sarcoma with spinal intradural region in childhood is very rare. We could only find 17 pediatric cases reported in the literature. Neurological findings occur earlier in tumors of this region. The prognosis is worse than other extraosseous Ewing sarcoma.

Key words: Ewing family of tumors, extraosseous Ewing sarcoma, intradural extramedullary tumors, cord compression.

Ewing's sarcoma (ES) family of tumors including Ewing sarcoma of bone, extraosseous Ewing sarcoma (EOES), primitive neuroectodermal tumors (PNET), Ewing sarcoma of the chest wall (Askin tumor) and atypical ES are highly aggressive mesenchymal neoplasms which originate from mesenchymal progenitor cells and which share specific chromosomal translocation (21;22)(q22;q12) resulting in a fusion of EWSR1 gene product with a member of the ETS family of transcription factors.[1] Ewing's sarcoma family of tumors account for 1.5-2% all childhood cancers. Ewing sarcoma of bone is the second most common malignant bone tumor in childhood that arises from long, short or flat bones.[1,2] In children, primary extraosseous manifestation has been reported in 15-20% of cases while approximately half of the cases are located in the extraskeletal regions in adults.[3] Extraosseous ES is more frequently seen in adolescent males. It is

✉ Eren Müngen
mungeneren@gmail.com

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more likely to originate from soft tissue of the thorax and abdomen (43%), extremities (26%) and pelvis (14%). The paravertebral region is a relatively rare location for EOES and constitutes approximately 7% of them.[4] In paravertebral space, tumors almost always arise from extradural paraspinal soft tissue. Primary spinal intradural-extramedullary ES family of tumor (PSIEES) is extremely rare.[4,5]

In this study, we present a rare pediatric case with PSIEES-confirmed with its characteristic translocation of EWS-ERG and relapsed disease in cerebellum which make this case very interesting. We also reviewed the pediatric PSIEES cases reported in the literature.

Case Report

An 8.5-year-old previously healthy boy was admitted to a local hospital with one month history of severe back and limb pain, and inability to move his lower limbs for the last few days. Physical examination revealed paraparesis in the lower extremities. Spinal MRI revealed multiple intradural extramedullary masses homogenously enhancing with intravenously administered Gadolinium-based contrast material at the L2-L3 level (3.8x1.6x1.2 cm), L4-5 level (2.6x0.9x1.1 cm) and L5-S1 level (1.1x0.4x0.7 cm) (Fig. 1). Metastatic workup with thoracic CT and PET-CT were performed for metastatic screening and found to be normal. He underwent surgery and near total excision of all three masses with L4-7 laminectomy was performed in the local hospital. After surgery, his symptoms improved and he was referred to our clinic.

Histopathologically, the tumor was composed of uniform small round cells with round nuclei containing fine chromatin and scanty eosinophilic cytoplasm. Neoplastic cells were found strongly positive for CD99, patchy and moderately positive for synaptophysin. These cells were negative for GFAP, NSE, inhibin, Pan-CK, EMA, desmin, LCA, S100 and WT1. The Ki-67 proliferation index was greater than 90%. Loss of INI1 expression was not detected. In

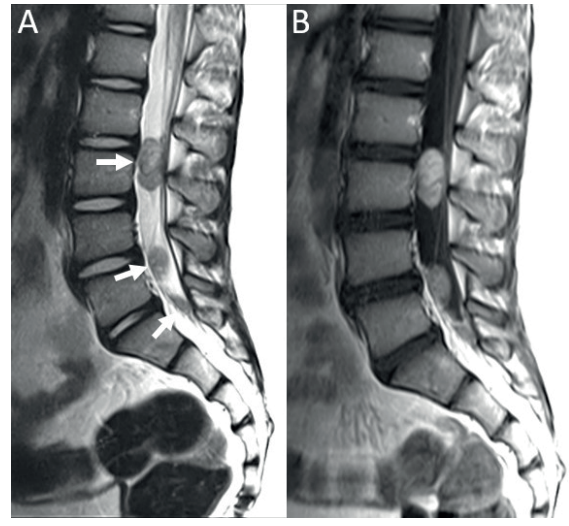

Fig. 1. Lumbar spinal MRI shows multiple intradural masses, which are T2 isointense to the spinal cord (arrows, sagittal T2-weighted image, A) and enhancing homogenously following gadolinium based contrast material administration (sagittal T1-weighted postcontrast image).

tumor tissue with RT-PCR, the fusion product related to EWS-ERG gene rearrangement was detected.

The patient was treated according to EuroEwing protocol. After six courses of vinkristin, ifosfamide, doxorubicin and etoposide (VIDE) treatment, a total dose of 4500 cGy radiotherapy was applied to the tumor location at L2-S1 paravertebral region. Then he received six cycles of VAC regimen (vincristine, actinomycin-D and cyclophosphamide). After the end of treatment the patient remained in remission for 18 months.

Three years after his first diagnosis, the patient came back with persistent headache for two weeks. Cranial MRI revealed a mass (4x4.5 cm) consisting of cystic and solid components in the left cerebellar hemisphere (Fig. 2). Spinal MRI was normal. Radiological screening revealed that it was isolated central nervous system recurrence. Gross total excision of the mass was performed.

The histopathological findings of the cerebellar mass were identical to those of the previous

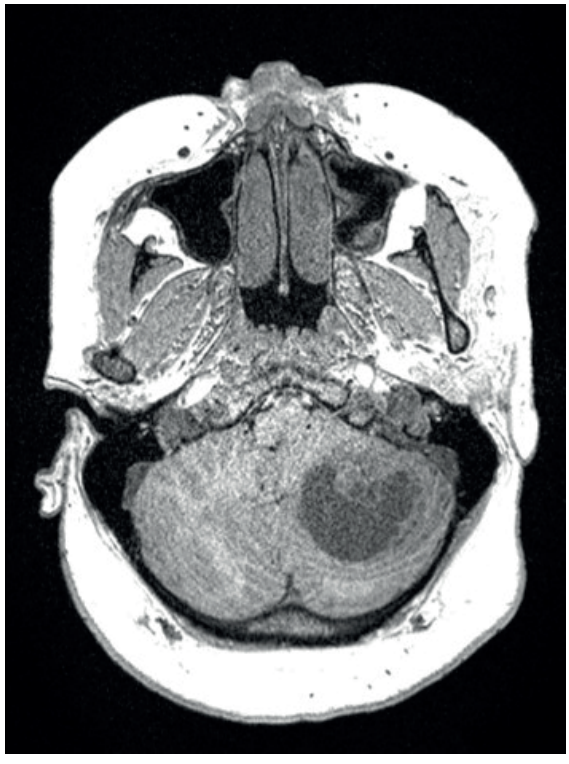

Fig. 2. Axial T1-weighted image shows a cystic mass with solid component in the left cerebellar hemisphere compressing the brainstem.

spinal intradural mass. The histopathological diagnosis of Ewing sarcoma was confirmed again with the presence of EWS-ERG fusion.

A total of 4500 cGy radiotherapy was applied to the cerebellar mass. He is on a second-line treatment consisting of gemcitabine and docetaxel without any evidence of disease.

The parents and patient were informed about the purpose of the case report presentation and informed consent was obtained.

Discussion

Childhood tumors located in the perispinal region present with symptoms of spinal cord compression. In addition to intradural tumors, extradural paraspinal tumors may also cause spinal cord compression by extending through the neural foramina to the epidural region. In childhood, most common extradural tumors are neuroblastoma, ES and soft tissue sarcomas. Intradural extramedullary tumors consist of meningiomas, neurofibromas, schwannomas and malignant peripheral nerve sheath tumors. Ewing sarcomas causing spinal cord compression arise either from vertebrae or the extradural paravertebral region. The clinical possibility of ES is extremely low for intradural extramedullary tumors.[6,7] Ewing sarcoma rarely originates from the intradural region and constitutes only 2,9% of all perispinal ES in adults.[4] Less than 50 cases with PSIEES have been reported in the literature.[5]

In the literature, we could find only 17 pediatric cases (<18 years old) with primary spinal intradural ES family of tumors.[4,8-20] Histopathological diagnosis were EOES in ten and PNET in seven. We analyzed the clinical features of 18 cases in total, including our case (Table I). There were twelve boys and six girls (M/F=2). Their ages ranged from 5 to 17 years old with a median age of 14 years. The duration of complaints ranged from 20 days to one year. The most common complaints were back pain (n:7), leg pain (n:2), back and extremity pain (n:3), and motor weakness (n:5). Physical examination findings were not reported in four of the 18 cases. Among the remaining 14, loss of strength in the limbs was found in seven cases, sensory loss at limbs in eight cases, and reflex changes at lower extremities in one case. In seventeen of the 18 cases, there was a single mass which was most frequently located in the lumbosacral and thoracic regions, respectively. Interestingly, our case had three separate masses close to each other. None of the reviewed cases including ours had metastasis at diagnosis.

Molecular study was reported in four of 18 patients. EWSR1-FLI1 was found in two patients and EWS-ERG fusion transcripts were found in our case. In the fourth case the type of the translocation was not given. Gross total or subtotal resection was performed in all cases. There was no information about chemotherapy and radiotherapy in third and fourth cases, respectively. Nine cases received chemotherapy and eight received radiotherapy. One case died within one month after the operation. Thirteen

Table I. Clinical characteristics, treatment and outcome of patients with primary spinal, intradural-extramedullary Ewing Sarcoma.

Case	Author	Age, Gender	Symptom	Symptom duration	Physical Examination	Location	Surgery	CT	RT	Outcome
1	Hisaoka, 1997	14, M	BP, LP	3 mo	NA	L	GTR	NA	NA	Awod, 3 mo
2	Uesaka, 2003	11, F	LW	1 mo	SD, MD	C7-T1	STR	NA	NA	NA
3	Harimaya, 2003	14, M	BP	3 mo	NA	L1-L2	GTR	ICaE	No	Awod, 67 mo
4	Woestenborghs, 2005	11, M	LW	NA	MD	C4-T2	STR	VDC/IE	NA	NA
5	Perry, 2007	16, F	BP, LW	3 mo	MD, SD	L2-S1	STR	VDC/IE	4500cGy	Awod, 5 mo
6	Klimo, 2009	10, M	LP	sev.mo	SD	L4	STR	VDC/IE	5040cGy	Awod, 12 mo
7	Duan, 2011	8, M	BP, LW	1 mo	SD, MD	L2-L4	GTR	CT(+)*	3000cGy	Awod, 12 mo
8	Yan, 2011	10, M	NP	20 d	SD, MD	C2-C3	GTR	No	No	Died, 2 mo
9	Wu, 2013	15, F	BP, UBI	20 d	NA	T2-L5	Sy(+)*	No	No	R 6 mo, NA
10	Zhao, 2014	14, M	LP	12 mo	SD	L2-S1	STR	IDC	5000cGy	Awod, 12 mo
11	Kartal, 2016	5, M	BP	1 mo	I-DTRs, PR	T4-T7	GTR	NA	NA	NA
12	Chen, 2017	14, F	BP, LW	12 mo	MD, SD	T5-T10	Sy(+)*	No	5000cGy	R 36 mo,
13	Scantland, 2018	14, F	BP	6 mo	NA	L2-L3	GTR	VDC/IE	5042cGy	Awod, 84 mo
14	Yi, 2018	Mean 17 3M/1F	BP, LP	NA	SD	1 C	GTR or STR	VAC or VIDE	3000-5000cGy	NA
15										
16						1 T				
17						2 S				
18	Current Case	8,5 M	BP, LP	1 mo	MD	L2-S1	GTR	EuroEwing99	4500cGy	R 36 mo, Awod, 42 mo

M: male, F: female, d: day, mo: month, sev: several, C: cervival, T: thoracic, L: lumbar, S: sacral, BP: back pain, NP: neck pain, LP: Limb pain, LW: limb weakness, UBI: urinary bowel incontinence, GTR: gross total resection, STR: subtotal resection, R: resection, NA: Not available, I-DTRs: increase deep tendon reflexes, PR: pathologic reflex, SD: sensorial deficit, MD: motor deficit, CT: chemotherapy, RT: radiotherapy,Sy: surgery *: detail of the CT or surgery not known, VDC/IE: vincristin+doxorubicin+cyclophosphamide/ifosfamid+etoposide, VAC: vincristin+ actinomycin D+ cyclophosphamide, ICaE: ifosfamide+carboplatin+ etoposide, IDC: ifosfamid+ doxorubicin+ cyclophosphamide, cGy: centigray, R: relaps, Awod: alive without disease.

cases were alive without disease at a median of 12 (3-27) months. Two of 17 cases reviewed had relapsed disease at 6 and 36 months, respectively. Relapse site was not reported at the first case while local and systemic (lymph nodes, bones, bone marrow and leptomeninges) relapse was observed in the second case. Our case was also alive without disease at 42 months after a cerebellar relapse at 36 months after diagnosis, and his treatment is continuing.

There are some striking differences between ESB, EOES and PSIEES. When we consider the results of two major studies that compare the clinical features of ESB and EOES, there are no meaningful differences in age or sex between these two groups. In childhood both are seen more frequently in adolescence and slightly more in males.[21,22] In contrast, PSIEES is seen at a younger age (median 11 years) with an obvious male preponderance (M/F=4).

Clinical characteristics and outcome are different in ESB and EOES. Tumors are more likely to be smaller than 8 cm and more commonly arise from axial locations in ESB. There were no differences in metastatic status between ESB and EOES. In both groups 30% of patients had metastatic disease. In ES located in the paraspinal space, extra or intradural regions, the close proximity of the tumoral mass to the adjacent neural elements leads to early neurological symptoms. Thus, diagnosis usually takes place before the tumor reaches a large volume, and before the systematic spread of the disease.[23] Brain metastasis has been reported in 8-10% of cases in various series involving ESB while in paravertebral extradural ES, this was 3.3%.[24,25] In cases with PSIEES, central nervous system metastasis was not reported except for a few cases with medulla spinalis metastasis.[26,27] The majority of brain metastases in ES present with a single mass generally located in the parietal or frontal lobe, or with diffuse multiple nodules.[24,25] We could not find any reported cases with ES metastasis to the cerebellum at diagnosis or at relapse in English literature.

The management of ES requires a multidisciplinary approach, including surgical resection, adjuvant chemotherapy, and radiotherapy.[4,16,23] Complete resection of the tumor has been reported to have a positive effect on prognosis and survival. However, due to the location of the tumor near the spinal canal and its close proximity to the adjacent neural elements, gross total tumor resection may not always be possible. Furthermore, it may not be possible to ensure that the tumor is not disseminated through the cerebrospinal fluid after an aggressive surgery. Chemotherapy and radiotherapy appear to be main treatment modalities in PSIEES.[13,19] In our case, despite the combination of surgical treatment, chemotherapy and radiotherapy, brain metastasis appeared after a while.

Although morphological and genomic features of EOES are indistinguishable from classical ESB, a better outcome has been reported in EOES than that of ESB.[5] Both overall and event free survival are significantly better in EOES than ESB (85% vs 78% for OS and 76% vs 69% for EFS). However, prognosis of EOES located in the perispinal region is worse than in other extraosseous locations. It was reported in adult cases with PSIEES that the OS rate was 40% with a median 14 months and the overall recurrence rate was 46%.[21,22] In pediatric PSIEES, only nine of 18 reviewed cases were alive without disease at a median of 12 months. Although recurrence was reported in only three of the cases, it must be considered that the follow up time was short.

The spinal intradural region is an extremely uncommon location for ES in children. Although early diagnosis is often possible thanks to the early appearance of neurological symptoms due to proximity of the tumor to the spinal cord, the outcome of the patients tends to be worse than ES of other sites.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EM, NK; data collection: EM, KKO, FS; analysis and interpretation of results: EM, NK, TK, KKO, FS,

BY; draft manuscript preparation: EM, NK, TK. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Case Report

The rare reason of pain in hip girdle: Mucolipidosis type 3 gamma

Abdulkerim Kolkıran[1], Beren Karaosmanoğlu[2], Zihni Ekim Taşkıran[2], Pelin Özlem Şimşek Kiper[1], Gülen Eda Utine[1]

[1]*Division of Pediatric Genetics, Department of Pediatrics and* [2]*Department of Medical Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey.*

ABSTRACT

Background. Mucolipidosis type 3 gamma (ML–IIIγ) is an autosomal recessive, rare and slowly progressive lysosomal storage disease. Short stature, restricted joint mobility, thick skin, and flat face with mildly coarse features are major clinical findings. It usually manifests in the third year. With advancing age, claw hand deformities, carpal tunnel syndrome, and scoliosis may develop. Morbidity is determined mainly by skeletal involvement. N–acetyl glucosamine–1 phospotransferase enzyme is composed of 2α, 2β and 2γ subunits. The active enzyme is essential in the transport of hydrolases to the lysosomes, via addition of mannose-6-phosphate in the Golgi apparatus. *GNPTG* gene encodes the γ2 subunits, and biallelic mutations cause ML–IIIγ.

Case. A previously healthy 14-year-old male patient had leg pain after the age of nine, and was admitted with short stature, mild coarse face, pectus deformity, digital stiffness, scoliosis, genu valgum and mitral valve prolapse. He did not have intellectual disability or corneal clouding. Radiographs showed irregularities in the acetabular roof and proximal epiphyses of the femur and irregularities in the end plates of vertebral bodies. A novel homozygous missense variant in the exon 5 of *GNPTG*, c.316G>T, confirmed the diagnosis of ML–IIIγ. Juvenile idiopathic arthritis (JIA), progressive pseudorheumatoid dysplasia (PPRD), ML–II, ML–IIIαβ, galactosialidosis and mucopolysaccharidosis should be considered in the differential diagnosis.

Conclusions. ML–IIIγ should be kept in mind in populations with high consanguineous marriage rates or with possible founder effect, in patients with short stature and skeletal destruction. Genetic tests should be planned for a definitive diagnosis.

Key words: Mucolipidosis type 3 gamma (MLIIIγ), *GNTPG* gene, Genetic skeletal disorder, painful hip girdle.

Mucolipidosis type 3 gamma (MLIIIγ) is a rare, slowly progressive lysosomal storage disease reported for the first time by Maroteaux and Lamy in 1966.[1-5] It is inherited in an autosomal recessive (AR) manner.[1-5] Stiffness in the finger joints usually begins in early childhood, whereas short stature, scoliosis and skeletal deformities are observed as a result of large joint involvement with advancing age.[5]

N–acetyl glucosamine–1 phospotransferase (GlcNAc–PTase) enzyme is a 540–kDa weight heterohexameric polypeptide composed of 2α, 2β and 2γ subunits.[1-9] Membrane dependent enzyme precursors α2β2 subunits and γ2 subunits are combined and carried to the Golgi apparatus.[4] Here, the α2β2 subunits in the precursor enzyme complex are activated with cleavage by site–1 protease (S1P).[4] The active enzyme plays an essential role in the transport of hydrolases to the lysosomes by modifying them with addition of mannose-6-phosphate (M6P) residues in the Golgi apparatus.[2-6] Hydrolases that cannot be modified with M6P as a result of GlcNAc–PTase enzyme deficiency cannot be transported to lysosomes.[1,4,6] Hence, non-digestible macromolecules accumulate in lysosomes, as granular intracytoplasmic inclusion bodies that can be

✉ Abdulkerim Kolkıran
kolkiran@hotmail.com

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seen microscopically.[1,4,6] These hydrolases that cannot be transported to lysosomes are secreted into the intercellular spaces.[1,4]

The *GNPTAB* gene encodes α/β subunits and the *GNPTG* gene encodes γ2 subunits.[1,2,4,5,7] Homozygous or compound heterozygous mutations in the *GNPTG* gene, which produce GlcNAc–PTase enzyme γ subunit deficiency, cause MLIIIγ disease.[1]

We report a 14-year-old male patient presenting with the complaints of short stature, scoliosis and hip joint damage.

Case Report

A 14-year-old male patient was consulted to the genetics department. He had bilateral Perthes disease sequela and scoliosis. He was the third live born to his 25-year-old mother following the third pregnancy, with an uneventful pregnancy at term, with a birth weight of 2600 g, by spontaneous vaginal birth. The parents were consanguineous (first cousins). Two healthy male children, now aged 18 and 15 years old, were born from the first and second pregnancies. The fourth pregnancy of the mother resulted in miscarriage in the 20th week of pregnancy. The grandson of the paternal uncle had similar findings in the hip joint. The pedigree of our patient is shown in Figure 1.

Bilateral Perthes sequelae and scoliosis were detected and femoral osteotomy operation was performed at the age of ten years when he was first referred due to in-toeing and bilateral leg pain. Stiffness of finger joints began at that age. All developmental steps were normal and there was no intellectual disability.

On physical examination, his weight was 39 kg (3rd–10th centile), height was 145 cm (<3rd centile) and head circumference was 54 cm (25th–50th centile). Arm span was 154 cm. The upper to lower segment ratio was calculated

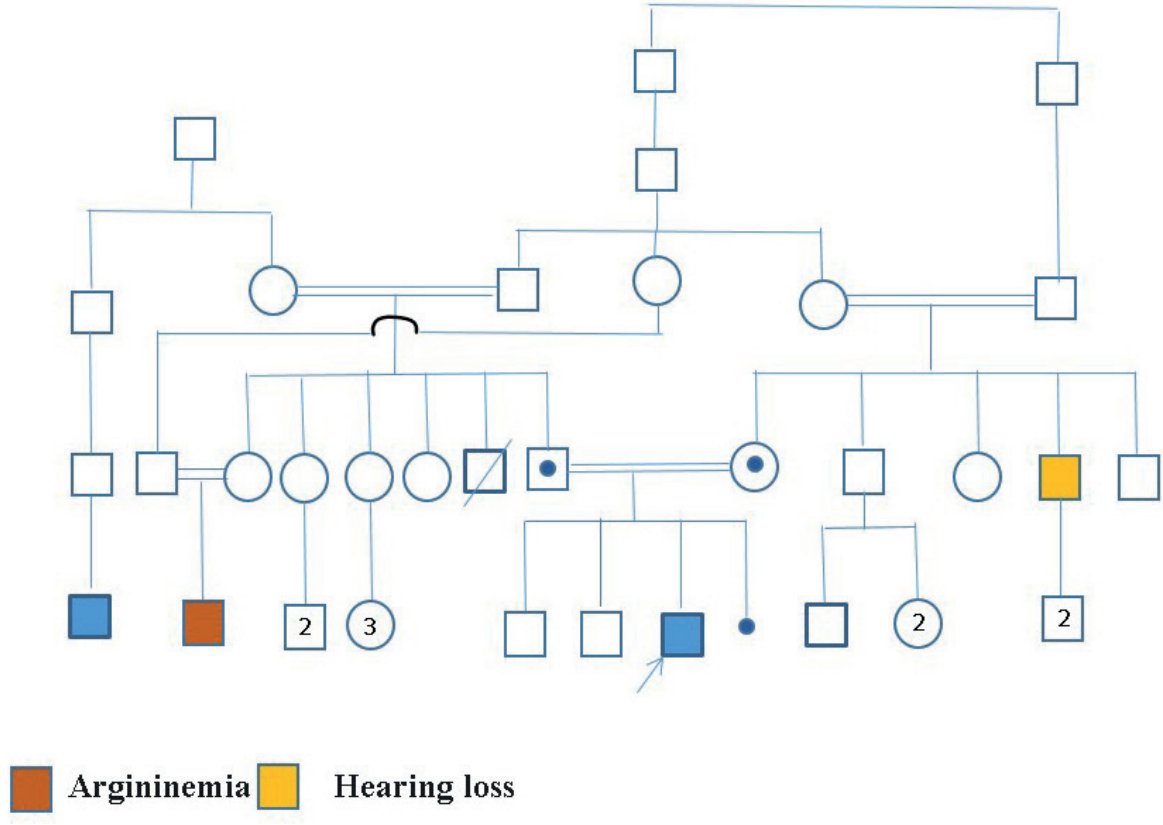

Fig. 1. Pedigrees of the patient.

as 0.85 (< –2 SD).[10] Narrow forehead, deep set eyes, bulbous nose tip, dental crowding, short and webbed neck, mild coarse face, pectus carinatum superiorly and excavatum inferiorly, scoliosis, accentuated thoracic kyphosis and lumbar lordosis, stiffness of the fingers, genu valgum and pes planus were noted. Acute phase reactants were all normal. Diseases such as progressive pseudorheumatoid dysplasia (PPRD) and juvenile idiopathic arthritis (JIA) were reviewed and excluded in differential diagnosis. Informed consent was received from the family for this publication.

Genetic analysis

Genetic analysis was performed after informed consent was taken from the parents. The study protocol was approved by the Hacettepe University Ethics Committee (GO 15–530/25). DNA samples of the patient were subjected to whole exome sequencing (WES) analysis at the Hacettepe University Faculty of Medicine, Pediatric Genetics Laboratory, Ankara (Turkey). Exome libraries were prepared by using the Ion AmpliSeq Exome RDY Kit, and sequencing was performed by Ion Proton System (Thermo Fisher Scientific). Homozygosity mapping and bioinformatics analysis were performed as outlined previously.[11] Segregation analysis was performed from DNA samples taken from the parents.

Genetic results

According to the NM_032520.4 coded transcript of the *GNPTG* in the NCBI database, a novel missense variant c.316G>T (p.Gly106Cys) was detected in exon 5. Parents were heterozygous for the same variant. According to the ACMG 2015 criteria, the variant is "disease causing". The variant is predicted as disease-causing according to *in silico* databases such as MutationTaster, and the CADD score is 32.

Discussion

Clinical manifestations of the extremely rare MLIIIγ disease is quite variable.[12] While very mildly affected cases present in later childhood with isolated hip dysplasia and some vertebral changes, in most of the cases MLIIIγ is a slowly progressive disease.[5] Increased stiffness of the joints suggestive of idiopathic arthritis is generally noted after the second year of life.[1,3,5,12] Although it is a multisystemic disease, skeletal involvement mainly determines morbidity.[5,8] Although destruction of the acetabulum and femoral head begin by 5 years of age, this usually begins in the adolescent period.[1,3] Skeleton findings progress as the age advances.[1]

In addition to dysostosis multiplex findings, progressive osteopenia, osteoarthritis and erosive bone lesions are seen as a result of cartilage and bone destruction in many joints including shoulder, elbow, hip and knee.[1,3,5,8] Scoliosis, claw hand deformity due to stiffness and contractures in joints and spinal cord compression can be seen particularly with advancing age.[1,8] Depending on these hip and leg pains, difficulty in squatting and a decrease in walking ability are observed.[1] Carpal tunnel syndrome is a common finding.[1,8] The radiographic findings of our patient are summarized in Figure 2.

Although the abnormal findings of hip joint in the previously reported patients generally started in late adolescent period, the present patient had an earlier (9 years old) onset of joint problems and walking difficulty.[3] Bilateral femoral osteotomy operation was performed at the age of ten years and mild dextroscoliosis, lumbar lordosis, and thoracic kyphosis were detected in the follow up. The patient had pectus carinatum and excavatum deformity.

Patients with the onset of stiffness of fingers at the age of 1.5 years are reported, nevertheless this usually begins in early childhood.[1,3,5] Nampoothiri et al.[1] reported that hand deformity started at the age of 10 years in Turkish patients. Onset of stiffness at the hand fingers at older ages is associated with a milder clinical course. In our patient, the stiffness of the hands began at the age of 12 years.[5]

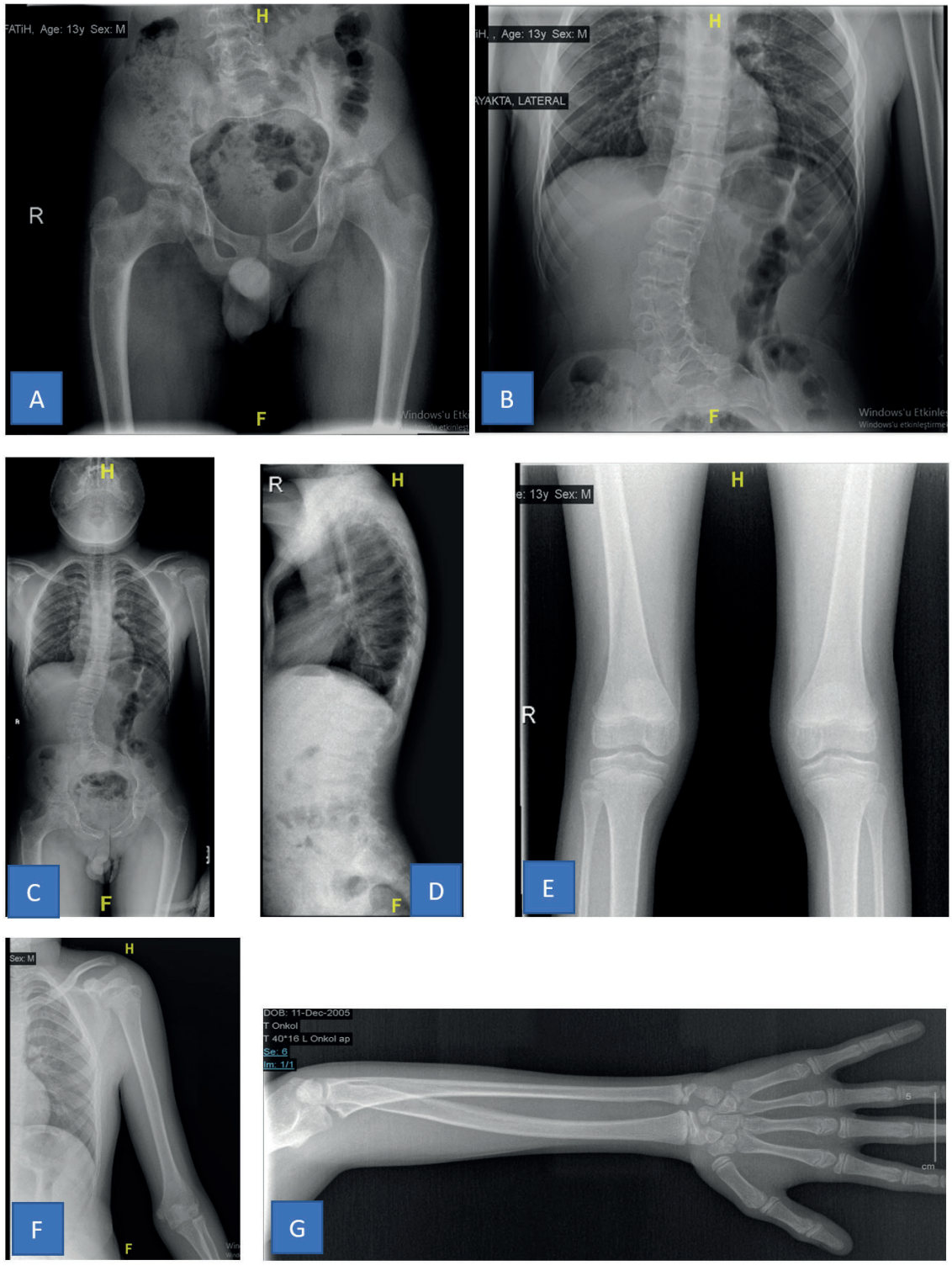

Fig. 2. Radiographs of the patient. **A)** Pelvic radiograph revealed irregularities in the acetabular roof, epiphyseal dysplasia of the proximal femora, coxa valga, and shallow acetabula. **B-C-D)** Mild scoliosis is seen on chest and abdomen radiography. Mildly flattened vertebral bodies with irregular upper and lower end plates and irregular narrowing of the intervertebral spaces and hypoplasia of the dorsal parts of the thoracic vertebral bodies and mild anterior hypoplasia is seen radiograph of spine. **E-F-G)** The long tubuler bones were normal.

Other clinical features of MLIIIγ include mild coarse facial appearance, short stature, corneal clouding, mild intellectual disability, restrictive lung disease, and heart valve anomalies.[1-3,5,6,8,9] Woody skin texture may occur that starts on the back of the hand and forearm and can be seen in the face over time.[1-3] Mild to moderate intellectual disability or learning disabilities may be seen in almost half of the MLIII patients.[12] Nevertheless, intellectual disability is usually seen in MLIIIαβ disease but not in MLIIIγ disease.[5]

There was no woody skin texture or scleroderma-like appearance on the skin of our patient. He had a mild coarse face appearance. Although he had pectus deformity, his lung functions were normal. Motor and language developmental steps were normal and he had no intellectual disability. There was no murmur on physical examination, but mild mitral valve prolapse was detected on echocardiography. There was no corneal clouding in the eye examination.

Besides clinical findings, increase in lysosomal hydrolases in plasma and decrease in intracellular enzyme level in fibroblast cultures support the diagnosis, but for the definitive diagnosis of MLIIIγ, a mutation in the *GNTPG* gene was detected and verified.[1,3,4,8,12] The segregation analysis revealed that the parents were heterozygous carriers. Genetic counseling was given accordingly.

GlcNAc–PTase enzyme activity determines the severity and name of the disease.[6] If mutations in the *GNTPAB* gene cause complete absence of enzyme activity, it causes MLII disease, while in the presence of 10% and above enzyme activity occurs MLIIIαβ disease.[1,2,4,6] Mutations in the *GNTPG* gene cause MLIIIγ disease.[1,3,9] So far, 50 different mutations in the *GNTPG* gene have been reported in 79 patients.[4] These mutations occur 35% frameshift 23% nonsense and 23% splice site, 15% missense, 4% deletion duplication and insertions.[4] 23% of all mutations are intronic mutations.[4]

Owing to the initial joint findings, patients can be followed up with a diagnosis of JIA, one of the most common chronic diseases of childhood, before the establishment of the definite diagnosis.[3] Even a number of treatments such as, nonsteroidal anti-inflammatory drugs, systemic and intraarticular glucocorticoids and nonbiologic and biologic disease modifying antirheumatic drugs might have been tried before the patient's admission. Differential diagnosis can be made with high serum lysosomal enzyme activity, presence of dysostosis multiplex findings, lack of high serum acute phase reactants levels, autosomal recessive (AR) inheritance pattern and the detection of a pathogenic GNTPG gene variant.[3] PPRD resulting from WISP3 gene mutations should be considered in the differential diagnosis due to joint stiffness, kyphoscoliosis and claw hand deformity.[3] However, PPRD differs by the presence of milder vertebral changes, broad lower ilia, swollen ends of the short tubular bones, normal serum hydrolase levels, and no GNTPG gene mutation.[3,12] Mucolipidosis II, ML-IIIαβ, mucopolysaccharidosis types I, II, IV, VI, and VII, other genetic skeletal disorders including spondyloepiphyseal dysplasia congenita, Kniest dysplasia, X-linked spondyloepiphyseal dysplasia tarda, and Dyggve-Melchior-Clausen dysplasia should all be considered in the differential diagnosis.[3,12] Differential diagnosis is easier in ML-II disease, as it occurs due to the complete absence of GlcNAc-PTase enzyme and the clinical findings begin prenatally or at birth and result in demise in early childhood.[3] The clinical distinction between MLIIIγ and ML-IIIαβ is quite difficult, nevertheless it is generally accepted that MLIIIγ is a milder form of mucolipidosis compared to that of MLIIIαβ disease.[4,9] In addition, forearm hypertrophy and supination defect may be a distinctive feature in MLIIIγ disease.[1]

There is no cure for MLIIIγ yet.[3] Treatment is completely symptomatic and supportive.[3] Physical therapy can be applied for joint stiffness and contractures. Total hip replacement (THR) and knee replacement therapy can be applied since complaints about hip and knee joints and the associated findings increase with

advancing age.[1,3] Surgical correction may be required for carpal tunnel syndrome and claw hand deformities.[3] For scoliosis and spinal cord compression, supportive treatments and surgical corrections should be applied as necessary.[5] As the pain in the hip and knee can significantly decrease the quality of life, necessary treatments should be applied. Biphosphonate therapy can be given when necessary for osteoporosis.[3,5]

Despite being a rare disease, the prevalence of ML-IIIγ may be high especially in countries with high consanguineous marriage rates. For this reason, appropriate genetic tests should be planned in patients presenting with joint findings in the hips and fingers, coarse face, short stature, corneal clouding and scoliosis.

Author contribution

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

Ethical approval

Informed consent was received from the family for this publication. The study protocol was approved by the Hacettepe University Ethics Committee (GO 15–530/25).

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Conflict of interest

The authors declare no conflict of interest.

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Case Report

Mitochondrial trifunctional protein deficiency as a polyneuropathy etiology in childhood

Özlem Ünal Uzun[1], Büşra Çavdarlı[2], Selen Karalök[3]

[1]*Department of Pediatric Metabolic, Kocaeli University Faculty of Medicine, Kocaeli,* [2]*Medical Genetics, Ankara City Hospital, Ankara;*
[3]*Department of Pediatric Neurology, Akdeniz University Faculty of Medicine, Antalya, Turkey.*

ABSTRACT

Background. The mitochondrial trifunctional protein (MTP) is a multienzyme complex of the fatty acid beta-oxidation cycle. Mitochondrial trifunctional protein deficiency (MTPD), a rare condition that leads to failure of converting certain fats to energy is characterized by decreased activity of three enzymes in the enzyme complex. Signs and symptoms of MTPD may present during infancy or later in life; those that begin after infancy include hypotonia, muscle pain, rhabdomyolysis, and peripheral neuropathy. We report a Turkish boy diagnosed with MTPD after being investigated for polyneuropathy of unknown origin since infancy.

Case. A 5.5-year-old male patient was admitted to our clinic with complaints of weakness in the arms and legs, physical inactivity compared to his peers, fatigue, weakness and, difficulty in climbing stairs since infancy. Electroneuromyography (ENMG) analysis showed moderate symmetric distal sensorimotor and axonal neuropathy. On the background of chronic polyneuropathy, the patient had acute relapsing episodes with progressively worsening severity in the follow-up period until 12.5 years of age. Whole exome sequencing (WES) was performed in the patient and, revealed that the patient had a homozygous c.1390G>A (p.Gly464Ser) pathogenic variant of the *HADHB* gene. Although rhabdomyolysis is a well defined accompanying clinical feature of MTPD, it was not present in our patient who only had worsening muscle weakness during attacks.

Conclusion. On the background of chronic polyneuropathy and acute relapsing episodes triggered by fasting or illnesses and rhabdomyolysis physicians should suspect disorders of the fatty acid beta-oxidation cycle.

Key words: polyneuropathy, mitochondrial trifunctional protein deficiency, fatty acid oxidation disorder, *HADHB* gene.

The mitochondrial trifunctional protein (MTP) is a multienzyme complex of the fatty acid beta-oxidation cycle. It is composed of four alpha and four beta subunits as HADHA and HADHB. The three functions are 2-enoyl coenzyme A (CoA) hydratase (LCEH), long-chain 3-hydroxy acyl-coenzyme A dehydrogenase (LCHAD), and 3-ketoacyl CoA thiolase (LCTH).[1] The *HADHA* gene encodes the LCEH and LCHAD enzymes and the *HADHB* gene encodes the LCTH enzyme. The cytogenetic location of both genes is at 2p23.3. Mitochondrial trifunctional protein deficiency (MTPD) which is a rare condition that leads to failure of converting certain fats to energy is characterized by decreased activity of all three enzymes.

Signs and symptoms of MTPD may begin during infancy or later in life. Clinically, classic MTPD is classified into three main clinical phenotypes including the neonatal onset of a severe, lethal condition resulting in sudden unexplained infant death, the infantile-onset of a hepatic Reye-like syndrome, and the late-adolescent onset of primarily a skeletal myopathy.[2,3] Symptoms that occur during infancy are various and may include feeding difficulties, hypoglycemia, hypotonia, and liver problems, and these patients may present with heart problems, respiratory insufficiency, coma, and sudden death. Signs and symptoms

✉ Özlem Ünal Uzun
unalozlem@gmail.com

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of MTPD that may begin after infancy include hypotonia, muscle pain, rhabdomyolysis, and peripheral neuropathy. Symptoms can be triggered by periods of fasting or by illnesses such as viral infections.

Patients presenting with the prolonged, progressive course of the disease, which has been associated with myopathy, recurrent rhabdomyolysis, and sensorimotor axonal neuropathy tend to survive into adolescence and adulthood.[4] Here, we report a Turkish patient diagnosed with MTPD who had been under investigation for polyneuropathy of unknown origin since his infancy.

Case Report

A 5.5-year-old male patient was admitted to our clinic with complaints of weakness in the arms and legs, fatigue, physical inactivity compared to peers and, difficulty in climbing stairs since infancy. His prenatal follow-up was normal and he was born at term with a birth weight of 3160 g with an uneventful delivery. He was the third child of consanguineous Turkish parents, and his siblings were healthy. He had walked when he was one year old and started using words at 1.5 years old. However, a waddling gate was first noticed at three years of age.

On admission to our center, growth was found to be normal for age. He had mild kyphoscoliosis. Neurological examination revealed absence of deep tendon reflexes and mild waddling gait. Muscle strength was 4/5 in the proximal and distal assessment of all four limbs. Routine investigations including whole blood count, biochemical parameters, Creatine Kinase (CK) level and vitamin B_{12} level were within normal ranges. An inherited polyneuropathy was suggested in the patient. Metabolic investigations including serum and urine amino acids, serum ammonia level, lactic acid, and pyruvic acid analysis, tandem mass spectrometry, urine organic acid analysis, and very-long-chain fatty acid analysis were normal. Cranial magnetic resonance imaging (MRI) was normal and cardiology and ophthalmology referrals also found no remarkable findings.

In an electroneuromyography (ENMG) analysis performed before admission to our clinic, a sensorimotor neuropathy had been suspected. ENMG analysis was repeated in our center and it showed moderate symmetric distal sensorimotor and axonal neuropathy prominent in the lower extremity. Muscle biopsy showed prominent fiber type grouping which was consistent with secondary neurogenic changes. The patient was investigated for Charcot-Marie-Tooth disease (CMT), however, the molecular genetic analysis performed for peripheral myelin protein 22 (PMP22) gene mutation was found non-consistent with the diagnosis. Therefore the possibility of mitochondrial disease was suspected in the patient and multivitamin supplements were initiated, which yielded partial benefit.

At 6.5 years of age, he had complaints of walking deterioration and gait disturbance concurrent with an infection. On neurologic examination ataxia was noted in addition to the absence of deep tendon reflexes. Muscle enzymes including CK and lactate dehydrogenase, liver enzymes, renal functions, and electrolytes were checked for rhabdomyolysis and they were within normal ranges. He was investigated for causes of ataxia. Serum alpha-fetoprotein, vitamin A and vitamin E levels were normal. Hearing assessment, ophthalmologic evaluation, echocardiography, spinal MRI, and molecular genetic analysis performed to evaluate Friedreich ataxia were also normal. In the follow-up period, the gait disturbance complaint regressed spontaneously. Gait and walking disturbance attacks repeated during infectious illnesses at 9 and 11 years of age. In these two attacks, the severity of symptoms had progressed and he could not even stand and walk, even though muscle enzymes continued to be normal (CK levels were 123 and 140 U/L, respectively). Repeated cranial and spinal MRI studies were unremarkable. ENMG performed at 12 years of age showed chronic severe sensorimotor and axonal neuropathy. Although

gate disturbance regressed after the course of each attack, his walking became relatively worse at 12.5 years of age and the family declined to use any vitamin supplements.

Whole exome sequencing (WES) was performed in the patient and, revealed that the patient had a homozygous c.1390G>A (p.Gly464Ser) pathogenic variant of the *HADHB* gene compatible with MTPD. Parents were detected as heterozygous for the variant. After definitive diagnosis, all routine biochemical and metabolic investigations including serum ammonia level, lactic acid, and pyruvic acid analysis, tandem mass spectrometry, urine organic acid analysis, and ophthalmologic evaluation were performed once again and found normal. Enzyme analysis of the three enzymes forming the MTP could not be performed in the patient due to the fact that these tests can only be performed by very few laboratories related to technical difficulties and financial problems. A low-fat diet with restriction of long-chain fatty acid intake and substitution with medium-chain fatty acids (MCT oil) was commenced. At the last visit, he was 13.5 years old, and he had difficulty standing on his heels. However, his walking and balance were much better under diet and MCT oil treatment.

Informed consent was received from the family.

Evaluation of the Variant:

The c.1390G>A mutation, which was detected as homozygous in the *HADHB* gene in our patient, is a rare variant that has not been detected in healthy population scans previously performed. In the analysis of this variant with in-silico prediction programs, it was evaluated as a disease-causing variant. This variant, which has not been reported in the literature and online databases, is classified as a "variant of unknown significance" according to the American College of Medical Genetics and Genomics variant classification guidelines.[5] However, since our patient's clinical findings and clinical progression were compatible with MTPD, this variant was thought to be responsible for the patient's clinic.

The variant corresponds to the first nucleotide of the exon 16 in the genomic sequence. At the translation level, this variant could create a new possible splice site. The analyses according to "Human Splicing Finder", the c.1390G>A variant at the beginning of the coding region is estimated to create a new acceptor site (New site: +58.9).[6] If a new acceptor site has occurred, the structure of the protein may have changed.

The evaluation at the level of amino acid exchange; glycine at the position 464, is the first amino acid of the beta-strand of the protein. The variant is replaced by the C terminal of the Thiolase domain (Fig. 1a). The newly formed Serin amino acid has an extra-OH group that may cause the formation of a new hydrogen bond (Fig. 1b, c).[7] A new hydrogen bond may decrease the stability of the protein by changing the interatomic interactions, atomic fluctuation, and local flexibility (Fig. 1d,e,f).[8]

Polyphen-2 score: 1

PROVEAN: -5.403 (cutoff:-2,5)-deleterious

Discussion

Inborn errors of metabolism (IEMs) can present at any age from childhood to adulthood and may affect the peripheral nervous system, usually as part of a diffuse neurological or systemic clinical picture. Inherited metabolic neuropathies comprise a clinically, biochemically, and genetically heterogeneous group of diseases. Rarely, neuropathy can be the unique initial sign of an IEM.[9] Rare causes of metabolic neuropathy include fatty acid beta-oxidation disorders, mitochondrial disorders, porphyria, disorders of the lipid or glycolipid metabolism (eg. Refsum disease, Fabry disease, abetalipoproteinemia, Tangier disease). MTPD is a fatty acid beta-oxidation cycle disorder, and is characterized by a wide clinical spectrum ranging from severe neonatal manifestations including cardiomyopathy, hypoglycemia, metabolic acidosis, skeletal myopathy and neuropathy, liver disease, and death to a mild phenotype with peripheral polyneuropathy,

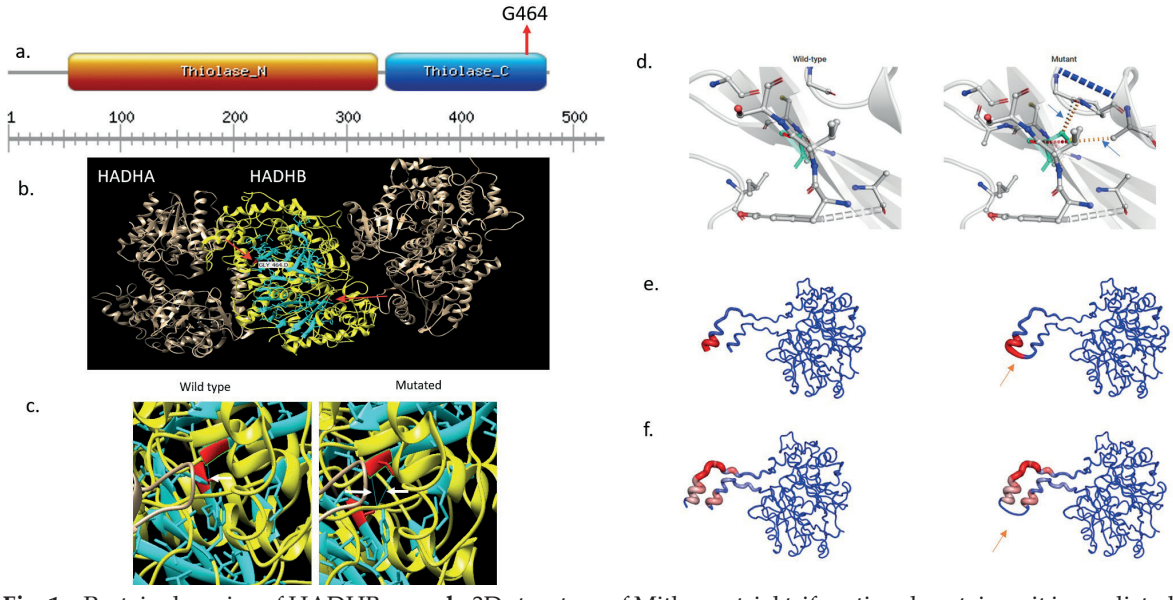

Fig. 1a. Protein domains of HADHB gene; **b.** 3D structure of Mithocontrial trifunctional protein; **c.** it is predicted that the mutated protein has an extra H bond **d.** Blue bars indicate possible new interatomic interactions, **e.** H bonds may provide change in the amplitude of absolute atomic action by decreasing atomic fluctuation, **f.** and local flexibility.

episodic rhabdomyolysis and pigmentary retinopathy.[10]

The most common motor symptom of peripheral neuropathy is weakness. It may present as clumsiness, difficulty in climbing stairs, or impaired fine motor skills. These motor symptoms were present in our patient since infancy. Ataxia is another motor symptom associated with neuropathy. Ataxia was a prominent symptom in our patient only during the course of deteriorating attacks that developed in association with an infection. Sensory symptoms, of which none were seen in our patient may include numbness, paresthesia, pain, or burning sensations.

Fu et al.[11] reported an 8-year-old girl with lower limb weakness since birth who was ultimately diagnosed with MTPD. Blood acylcarnitine analysis revealed slightly increased long-chain 3-OH-acylcarnitine levels; EMG suggested peripheral nerve injury; muscle biopsy confirmed a neurogenic lesion in muscle fibers, as shown by EMG. Analysis of the *HADHB* gene identified a homozygous missense mutation c.739C > T (p.R247C). They stated that neonatal-onset, as seen in their patient, has not been reported for the neuromyopathic phenotype of mitochondrial trifunctional protein deficiency.[9] Clinical findings of our patient were similar to their patient, but repeated acylcarnitine analysis including in deterioration attacks were normal in our patient.

Naiki et al.[12] reported peripheral polyneuropathy, rhabdomyolysis, and infantile-onset hypoparathyroidism in two siblings with *HADHB* gene mutations. Recently, van Vliet et al.[13] reported a 20-year-old woman who had axonal motor-sensory polyneuropathy of unknown origin since childhood. She presented with progressive dyspnea, and increased muscle weakness which had been preceded by an infectious disease. Laboratory testing showed rhabdomyolysis and hypocalcemia with low parathyroid levels. Sequence analysis of the *HADHB* gene showed two heterozygous variants. The authors emphasized the importance of performing metabolic screening when patients are most symptomatic, since normal results could be inconclusive in the absence of metabolic stress.[11] Serum calcium,

phosphorus, and magnesium levels were normal in our patient, and rhabdomyolysis was not seen, however, he experienced deterioration attacks concurrent with infectious illnesses.

Yamamoto et al.[14] reported a 45-year-old man presenting with slowly progressive muscle weakness and sensory disturbances in his lower limbs and multiple episodes of exercise-induced severe muscle fatigue and brown urine during his childhood, which had disappeared by age 20. A nerve conduction study showed peripheral axonal neuropathy. Similar to our patient, they considered CMT disease, however, exome sequencing failed to identify a mutation in the known genes associated with CMT. Then, he recurrently developed severe rhabdomyolysis that required hospitalization. They re-examined exome sequencing and found a mutation in *HADHB*. This case of adult-diagnosed MTP deficiency was characterized by slowly progressive peripheral neuropathy masquerading CMT in addition to muscular symptoms.[14]

No pathogenic variant has been identified in exon 16 of the HADHB gene so far, which may be pathologic for the disease. However, it has been reported that mutations detected in the last exons of the gene may be presented with late-onset and milder clinical findings.[15]

The lack of enzyme level measurement is a limitation of this report. However using online modeling and prediction programs, we have been able to predict that the mutation identified herein can destabilize the structure of the protein. Although acylcarnitine levels were repeatedly normal in our patient, clinical findings may be the result of conformational changes in the protein by the Gly464Ser homozygous variant that may lead to a less functional protein. Nevertheless, functional studies are required to clearly understand the effect of the variants that have not any clinical significance yet.

In conclusion, it is evident that the differential diagnosis of isolated polyneuropathy in childhood may be challenging. Most peripheral nerve disorders in children are hereditary. Characterization of a peripheral nerve disorder involves a careful neurologic examination, family history, electrodiagnostic studies. Although rhabdomyolysis is a well-defined accompanying clinical feature of MTPD, muscle weakness that is getting worse in attacks in the absence of rhabdomyolysis was an interesting aspect of our patient. On the background of chronic polyneuropathy, acute relapsing episodes triggered by fasting or illnesses and rhabdomyolysis should suggest the fatty acid beta-oxidation cycle.

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Author contribution

OU and SK collected the data; OU wrote the manuscript; BC performed genetic analysis.

Conflict of interest

Authors declare no conflict of interest.

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Case Report

Covid-19 in a patient with Familial Hemophagocytic Lymphohistiocytosis in children

V. Hülya Üzel[1], Kamil Yılmaz[2]

[1]*Division of Pediatric Hematology,* [2]*Department of Pediatrics, Dicle University Faculty of Medicine, Diyarbakır, Turkey.*

ABSTRACT

Background. Based on the information obtained so far, COVID- 19 is relatively mild in children. We will present a 6-month-old male patient infected with COVID -19 in April 2020, while receiving HLH 2004 chemotherapy protocol with the diagnosis of familial (Genetic / Primary) Hemophagocytic Lymphohistiocytosis (HLH).

Case. Herein we present a case accompanied by a defective perforin gene defect in the primary HLH pathogenesis, Covid-19 infection with the presence of fever and hyperferritinemia, which was evaluated in favor of reactivation and the patient was given both the HLH-2004 chemotherapy protocol treatment and COVID -19 therapy as recommended by the guidelines. Our patient improved clinically and in terms of laboratory test results at the end of the 15th day of hospitalization and was discharged.

Conclusions. It should be remembered that COVID-19 can be seen with different clinical manifestations in the pediatric age group, and COVID-19 tests should be recommended, especially in children with immunosuppression and fever.

Key words: COVID-19, hemophagocytic lymphohistiocytosis, perforin.

The coronavirus disease 2019 (COVID-19), was declared as a public health emergency of international concern on January 30, 2019 and as a pandemic on March 11, 2020 by the World Health Organization (WHO).[1] According to the data obtained to date, the disease is milder in children.[2] Patients undergoing cancer treatment and those who have had cancer constitute a risky group in the COVID-19 pandemic.[3-5]

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening clinical syndrome characterized by an uncontrolled and ineffective immune response triggered mostly by infectious agents. HLH is generally classified as familial HLH (FHL), which occurs due to an underlying genetic defect, and secondary HLH that can develop due to a variety of acquired causes.[6] Viruses are the most important infectious agents that trigger HLH, and herpesviruses, especially Epstein Bar Virus (EBV) and Cytomegalo Virus (CMV) are frequently detected. Herein we will present a 6-month-old male patient who was diagnosed with FHL, who was not fully compatible with stem cell donors and was infected with COVID -19 in April 2020, while receiving the HLH 2004 chemotherapy protocol.

Case Report

The patient applied to our hospital at the age of about 2 months, with the symptoms of fever, restlessness and paleness, splenomegaly on physical examination and bicytopenia on her blood count. The patient was diagnosed with HLH due to the clinical, laboratory and bone marrow aspiration smear findings. The HLH 2004 chemotherapy treatment protocol was started. Viral and bacterial examinations (serology, culture) were negative. The marriage between mother and father was a first-degree consanguineous marriage and the genetic

✉ V. Hülya Üzel
drhulya-uzel@hotmail.com

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analysis of the patient revealed a PRF1 Exon 3 c.1122G> (p.Trp374Ter) rs104894176 mutation, which was found to be compatible with homozygous class 1 HLH.

The treatment of primary HLH was given to the patient. The patient, who did not have a fully compatible stem cell donor, recovered clinically and in terms of laboratory findings in the first month, applied with the complaint of fever and cough during the 12th week of the HLH protocol (17 April 2020). In his physical examination, his general condition was moderate, he was conscious, his body temperature was 38.5oC, respiratory rate was 48/min, pulse was 127/min, abdominal distension was present and rales were detected in the basal areas of the lungs. Other system examinations were found normal. Considering that hemophagocytic reactivation might occur, a laboratory examination was performed which showed the following: hemoglobin 10.5 g/dL, leukocyte count 15020/mm3, neutrophil: 6.5 uL, monocyte: 2.023 uL, lymphocyte: 6.06 uL, platelet count 440.6 / mm3, prothrombin time (PT) 16sec, partial thromboplastin time (aPTT) 31 sec, INR 1.34, serum triglyceride 519mg / dL, ferritin 795 ng / mL, fibrinogen 468mg / dL and lactic dehydrogenase (LDH): 357 U / L, C- reactive protein (CRP): 10.27 mg / L (0-0.5), Procalcitonin:> 100. Arterial blood gases, liver and kidney function tests, serum electrolytes, albumin, bilirubin and full urine examination were normal. Peripheral smear, showed 60% neutrophil, toxic granulation, 40% lymphocyte, platelets were sufficiently clustered and atypical cells were not observed.

Hyperferritinemia (795mg / dl), hypertriglyceridemia (519mg / dl) were detected in the patient. Bilateral perihilar infiltrations were seen on the chest x-ray of the patient, who had no contact history with a person diagnosed or suspected to have COVID-19 (Fig 1). Thorax CT revealed peripheral atelectasis and consolidation areas were observed in the posterior sections of the lower lobe of both lungs, and small peribronchial consolidation areas and nodular infiltrations were observed in the upper lobes in both lungs, and those findings were evaluated as possible COVID-19 (Fig. 2). HLH 2004 treatment protocol was continued considering the possible HLH reactivation in the patient with persistent fever and hyperferritinemia and hypertriglyceridemia. Cyclosporine, dexamethasone and intravenous immunoglobulin (IVIG) were given. An oro-nasopharyngeal swab sample was taken.

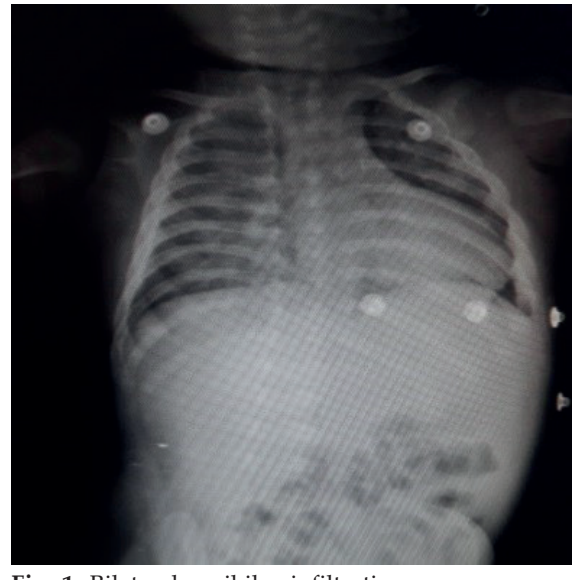

Fig. 1. Bilateral perihilar infiltrations were seen on the Chest x-ray.

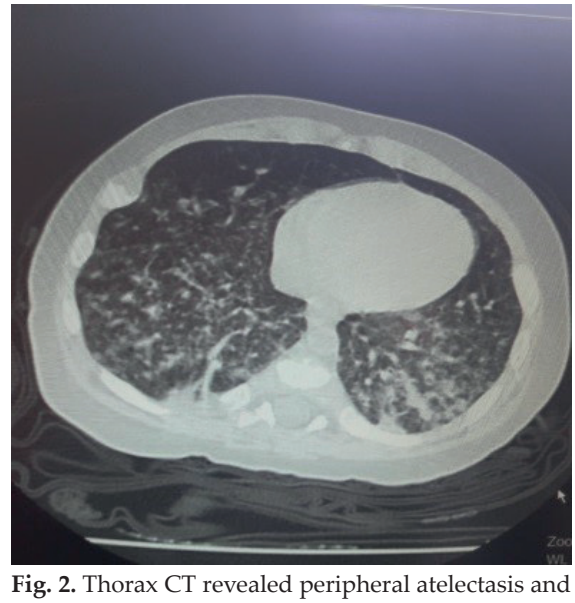

Fig. 2. Thorax CT revealed peripheral atelectasis and consolidation areas.

The patient who was positive for SARS CoV2 polymerase chain reaction (PCR) was hospitalized in the COVID-19 pediatrics clinic. The patient was treated according to the recommendation of guidelines for COVID-19 pneumonia and unclear coinfection.

Antimicrobial therapy, hydroxychloroquine, azithromycin, low molecular weight heparin (LMWH), HLH-2004 treatment protocol were administered. In blood gas analysis; PH: 7.47, PCO2: 29.3mmHg, 45.8mmHg, PO2, Lactate: 5.5mmol / L, cSO2: 83.2%. The patient was taken to the intensive care unit and oxygen support was given with a mask. When the patient's respiratory distress increased, the level of D-Dimer was studied and it was found that D-dimer level increased to 9.11, and a low-molecular-weight heparin (enoksoparin) single dose of 100 unit /kg /dose was started. Lopinavir / ritonavir combination (Kaledra tb) was given. On the fifth day of hospitalization, the patient with a hemoglobin level of 7.4 gr / dL was given cross-matched, irradiated erythrocyte support.

As the patient's symptoms regressed on the 7th day of hospitalization, he was transferred back to the pediatric ward. In addition to the HLH 2004 protocol, Azithromycin, hydroxychloroquine was given for five days, Meropenem, Amikacin and Lopinavir / ritonavir for ten days and low molecular weight heparin (LMWH) for fourteen days. No side effects were observed in the patient. On the 10th day, when the COVID -19 PCR test was negative, the patient was clinically and laboratory stable, and the HLH -2004 protocol was continued and he was discharged on the 15th day of his admission. Consent was received from the patient's family for this case report.

Discussion

Cancer patients who have been receiving active chemotherapy, any antibodies or targeted therapy or intensive radiotherapy in the past 6 months, are susceptible to infection due to immune system deficiency, and have a high risk of contact with infected or carrier individuals due to clinical controls, examinations and treatments.[1,3] Our patient was also a patient with familial HLH and was receiving chemotherapy treatment.

FHL is defined as a rare, autosomal recessively inherited immune regulation disorder characterized by uncontrolled T cell and macrophage activation and excessive cytokine release.[7] The first identified genetic defect is a mutation in the PRF1 gene (FHL-2).[8,9] The most common mutation is W374X, the exon three stop codon mutation. The genetic result of our patient was detected as PRF1 Exon 3 c.1122G> (p.Trp374Ter) rs104894176 homozygous class1 HLH (FHL-2). Today, the treatment that provides a cure in familial (primary) HLH is to perform hematopoietic stem cell transplantation (HSCT) after remission is achieved by chemotherapy.[9]

HLA appropriate donor was not found from our patient's family and relatives. The patient was in remission from the first month of treatment, clinically and in terms of laboratory findings. In the 12th week of his treatment, he was infected with COVID-19. In addition to the clinical deterioration in familial HLH, infections are known to lead to acquired HLH. The most important infectious agents leading to HLH are viruses. Especially herpesviruses, EBV, CMV are frequently detected.[4,8] In the course of sepsis developing due to infections, some patients may develop symptoms of macrophage activation syndrome (MAS) or, in other words, acquired (secondary) hemophagocytic lymphohistiocytosis (sHLH). It is thought that COVID-19 infection starts with type II pneumocyte damage and subsequently leads to the development of viral pneumonia and acute respiratory distress syndrome (ARDS), and these clinical manifestations may activate macrophage activation syndrome (MAS) and disseminated intravascular coagulation (DIC).[10-13]

It is extremely difficult to differentiate COVID-19 pneumonia and ARDS pathologically. On the

day of admission to the hospital, treatment was started for the coinfection of COVID-19 pneumonia / HLH reactivation based on the guidelines. High CRP and ferritin levels reported in many COVID-19 cases were also detected in our patient and were found to be compatible with the literature.[14] In the follow-up of our patient, the level of d-dimer gradually increased and he was taken to the intensive care unit because of respiratory distress. We added a single dose of LMWH to the treatment of the patient on the 3rd day of his hospitalization, considering that micro-thromboses may have started.

Although studies in children are very limited, recent studies have indicated the importance of viral load in COVID-19-pneumonia.[15-17] For this reason, it is very important to develop an effective antiviral which should be included in the treatment plan early. The necessity of applying the HLH 2004 protocol in the treatment of patients developing MAS or secondary HLH is especially important in intensive care patients.[15]

Both the HLH-2004 chemotherapy protocol treatment and COVID-19 treatment were administered to our patient as recommended by the guidelines. Our patient improved clinically and in terms of laboratory findings at the end of the 15-day hospitalization period and was discharged.

In conclusion, it should be remembered that COVID-19 can be seen with different clinical manifestations in the pediatric age group. We recommend performing COVID-19 testing especially in children who are under immunosuppressant treatment and have a fever. In this manuscript, we would like to state that the COVID-19 infection is among the factors that can cause HLH reactivation in familial HLH patients. In patients with high mortality, such as familial HLH, early detection and treatment of COVID -19 infection will increase chance of survival.

Author contribution

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

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Short and long term side effect of colistin treatment in preterm infants

Sadık Yurttutan[1], Burcu Cantay Atalay[2]

[1]*Division of Neonatology and* [2]*Department of Pediatrics, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Turkey.*

Dear Editor,

We read with interest the recently published article by Kaya Aksoy G et al.[1] in which the side effects associated with colistin use in premature infants were evaluated.

In this study, 47 premature infants were evaluated in terms of their serum creatinine, AST, ALT and electrolytes before and during colistin use; the relationship between these parameters and colistin use was investigated. Among the side effects, the researchers emphasized that acute kidney injury (AKI) was the most prevalent, occurring in 17% of cases. They also reported that AKI had a significant relationship to gestational age and aminoglycoside use. In the study, the notable electrolyte disorders that colistin use was associated with were hypomagnesemia, hypocalcemia and hypokalemia. This study found a link between hepatotoxicity and colistin use, which has not been mentioned as a side effect by similar studies conducted in the past. The study also reported that colistin less frequently caused AKI in premature newborns compared to pediatric patients, and that electrolyte disorders were more common in these newborns, indicating that they should be monitored for electrolyte imbalances, especially hypomagnesemia.

Recently, there has been an increase in the number of studies evaluating the effectiveness as well as the safety of colistin. These studies have primarily been conducted on term newborns or pediatric patients, rather than pre-terms. We would like to share the data of one of our largest studies conducted at the Kahramanmaraş Sütcü İmam University, Faculty of Medicine's Neonatal ICU, in which we evaluated the side effects and safety principles of colistin on 121 premature babies between 2014-2016. Similar to other studies conducted on infants. Our study reported nephrotoxicity to be the most common side effect (10.7%). Çakır U and colleagues' publication mentioned side effects of bartter-like syndrome, to which we observed tubulopathy in 3 patients in our study.[2] Similar to the results of Kaya Aksoy G and colleagues as well as Alan S and colleagues magnesium levels of the cases in our study decreased significantly after the use of colistin.[1,3] Prolonged intolerance of the gastrointestinal system was a side effect unprecedented in other studies but was observed in 9.1% of cases in our study, which diminished alongside the discontinuation of colistin. 10 patients developed cholestasis, and although the clinical signs and symptoms subsided, total resolve occurred only after discontinuation.

A study on very low birth weight infants reported that the rate of nephrotoxicity increased as birth weight decreased; however, our study did not find a statistically significant correlation between kidney failure and birth weight.[4]

It should be noted that preterm newborns taking colistin should not only be monitored for short term problems, but also for long term problems. In our study, which was a follow-up of our first study, we monitored the long term ototoxic effects of colistin treatment on preterm

✉ Sadık Yurttutan
dryurttutan@gmail.com

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infants; the hearing of 30 premature infants taking colistin was evaluated over a long period and 1 patient was discovered to have developed bilateral and 2 patients unilateral hearing loss. This is the first study reporting colistin associated hearing loss in premature infants.[5]

Preterm infants taking colistin treatment should be closely monitored for acute side effects (renal tubulopathy, hepatopathy, enteropathy) and should especially be followed up for ototoxicity in the long-term follow-up.

Key words: colistin, preterm, side effect.

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