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Contents

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Editorial

Dear Trends in Pediatrics readers,

Trends in Pediatrics (TP) family continues to grow with your interest. On behalf of the editorial board, we're happy to announce the publication of the third issue.

In the current issue, there are seven articles, including four original articles. One of the articles is about Beta Globin Gene Mutations in Aydın Province. The other interesting article is related to Evaluation of Clinical Characteristics and Treatment Outcomes of Graves' Disease in Children and Adolescents. Also, Ventriculoperitoneal shunt infection in pediatric age, and Age at Onset of Menarche and Puberty of Girls in Aydin Region and the Factors Affecting Them are the other interesting articles. Moreover, there is an excellent review about Thalassemia.

In line with our ambitious goals, we invite all researchers dealing with pediatric patients to involve in the upcoming issues. We need your valuable ideas, so we kindly request you to send comments through our website www.trendspediatrics.com

Sincerely yours,

Serkan Fazlı Çelik

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Review

Thalassemia

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ABSTRACT

Defects in protein structure or synthesis of hemoglobin are called hemoglobinopathies. Thalassemia is the most common hemoglobinopathy, and it is estimated that 5% of the world population carries at least one variant allele of thalassemia. The thalassemias can be classified as alpha or beta thalassemias. Beta thalassemia may present as silent carriers with normal hematological parameters, while beta thalassemia carriers have hypochromic microcytic anemia, associated with a high HbA2. However, patients with beta thalassemia intermedia and beta thalassemia major need transfusion intermittently or regularly and they are called non-transfusion dependent thalassemias or transfusion-dependent thalassemias, respectively. This review focuses on pathophysiology, clinical, laboratory features of thalassemias along with their treatment and follow-up.

INTRODUCTION

Hemoglobin is a tetrameric protein that contains two pairs of globin chains. The defects in protein structure or synthesis of hemoglobin are called hemoglobinopathies. To date, more than 800 hemoglobin variants have been identified.[1] Two gene clusters are responsible for hemoglobin production; the alpha (α) gene cluster consists of zeta (ζ), alpha 1 (α1), and alpha 2 (α2) genes located on chromosome 16.[2,3] The beta (β) gene cluster consists of epsilon (ε), gamma 1 (γ1), gamma 2 (γ2), delta (δ) and beta (β) genes located on chromosome 11.[2,3] The embryonic hemoglobins Gower-1 (2 ε2), Gower-2 (α2 ε2) and Portland (ζ2 γ2) are formed starting from the 8th week of fetal life. HbF (α2 γ2), which is the main Hb of fetal life, is noticed from the 9th week on, while HbA (α2 β2), which is the main Hb of adulthood, gradually prevails in comparison to HbF. HbA2 (α2 δ2) is synthesized in scant amounts after birth. The final Hb pattern is obtained at least six months after birth and consists of 95% HbA, 3.5% HbA2, and <2.5% HbF.[4]

The thalassemia comes from the Greek word “thalassa” which means sea, and –emia originates from a Latin word that means blood. The disease was first described in the Mediterranean population and was referred to as Mediterranean anemia. Thalassemia is the most common hemoglobinopathy, and it is estimated that 5% of the world population carries at least one variant allele of thalassemia.[5] Although thalassemias often show an autosomal recessive inheritance pattern, some types of thalassemias have rare dominant inheritance pattern.[5] The thalassemias can be classified as alpha

or beta thalassemias since the primary globin type of adults is HbA (α2 β2).

PATHOPHYSIOLOGY

Thalassemias are a group of diseases in which the the balance between alpha globin, and beta globin is impaired. This imbalance is caused by a decrease in the production of one or more globin genes. The reduced globin chain causes unpairing of the remaining globins, which are precipitated in erythrocyte precursors (ineffective erythropoiesis) or destroyed in the circulation (hemolysis) ultimately.[4] As a result, patients have varying degrees of anemia and extramedullary hematopoiesis. While beta thalassemias often develop with point mutations, alpha thalassemias mostly develop with deletional mutations.[6,7] If the mutation is a "missense" mutation in beta thalassemias, this causes decreased beta globin chain synthesis called β+. In "nonsense" mutations, β0 is a phenotype in which there is no beta globin synthesis. If one of the two beta globin genes carries a mutation, these individuals are called thalassemia carriers or thalassemia minors, who are usually clinically asymptomatic. Individuals with a homozygous or compound heterozygote thalassemia mutation in the beta globin gene may present with either thalassemia major or thalassemia intermedia phenotype. To distinguish between thalassemia major and intermedia, several clinical and laboratory findings should be considered (Table 1).

BETA THALASSEMIAS

Most commonly seen in the Mediterranean region, they are also found in Asia and Africa regions close to the equator. As with sickle cell anemia and erythrocyte G6PD enzyme deficiency, thalassemias are common in areas where malaria was once endemic.[8] Carriers of thalassemia are more resistant to malaria than normal individuals, and malaria infection has a milder clinical course in these carriers. Approximately 150 million people who are carriers of thallassemia live in the Mediterranean countries, the Arabian peninsula, the Middle East countries, the west of Africa, Iran, Pakistan, Afghanistan, India, and Southeast Asia. Today, due to global migration and ethnic interactions, β thalassemia can be encountered in all parts of the world. The frequency of β thalassemia carriers in Turkey is 2.1%, and this rate rises to 13% in some of the provinces in the Mediterranean region.[9,10]

Beta thalassemia patients may present as silent carriers with normal hematological parameters and normal Hb A2 levels with several mutations, such as -101 promoter mutation.[9,10] These individuals can not be distinguished by routine screening. Beta thalassemia carriers have hypochromic microcytic anemia, associated commonly with a high HbA2 (3.5-8%), HbA2 and HbF (5-20%) levels.[4] An erythrocytosis (RBC> $5x10^{12}$/L), microcytosis, normal RDW are common for beta thalassemia carriers, but also iron deficiency anemia, alpha thalassemia trait, and chronic disease anemia should be considered for differential diagnosis.

In beta thalassemia intermedia (BTI) patients do not need a regular transfusion regimen. They need less than five transfusions within a year and are called non-transfusion dependent thalassemias (NTDT). They show decreased hemoglobin, erythrocyte count, erythrocyte indices (MCV, MCH, MCHC), and increased RDW. In peripheral smear, severe

Table 1. Clinical and laboratory differences between thalassemia major and thalassemia intermedia

	β- thalassemia major	β- thalassemia intermedia
Onset (year)	<2	>2
Hemoglobin (gr/dL)	<7	8-10
Hepatosplenomegaly	Severe	Mild to moderate
HbF (%)	>50	10-50

HbF: Fetal hemoglobin

hypochromia, microcytosis, anisocytosis, poikilocytosis, target cells, polychromasia, basophilic stippling, and normoblasts are observed. A slight increase in reticulocyte level (2-4%) can be observed. In hemoglobin electrophoresis, HbA decreases (10-20%), HbF (70-80%) and HbA2 increases.[4] The mother and father should also be screened for thalassemia carrier state with complete blood count and hemoglobin electrophoresis, but in rare instances, molecular diagnosis may be required.

The patients with beta thalassemia major (BTM) are transfusion-dependent. Laboratory features of BTM are similar to BTI. However, since HbA synthesis is far less compared to BTI, HbA is not seen in hemoglobin electrophoresis, and HbF is more than 80% of total hemoglobin.

BETA THALASSEMIA MAJOR

Patients with homozygous β0-thalassemia often present with severe anemia due to inadequate HbA synthesis in the first 3 to 4th months of life. However, depending on the type of mutation and HbF production, the need for transfusion may be delayed up to 2 years of age. In patients who do not receive adequate transfusion therapy, growth retardation, hepatosplenomegaly, hypersplenism, bone changes due to bone marrow enlargement, thalassemic face (maxillary hyperplasia, frontal bossing, depressed nasal bridge) develop.[4] Transfusion therapy aims to prevent these changes due to ineffective erythropoiesis.

At the onset, to differentiate thalassemia major and intermedia transfusion should be deferred until Hb level drops below 7 gr/dL. However, in patients with growth retardation, thalassemic facial changes, and progressive splenomegaly, transfusion therapy should be initiated earlier. It is necessary to evaluate Hb electrophoresis, Rh and Kell subgroups, viral serology (CMV, HIV, Hepatitis B, HCV) tests before transfusion. Contemporary transfusion programs recommend that a lower hemoglobin limit of 9-9.5 gr/dL should be set before transfusions.[5]. Since a Hb decrease of 1 gr/dL per week is expected in patients, regular transfusions should be done every 3 to 4 weeks. A subgroup-appropriate, leucodepleted erythrocyte suspensions should be preferred. Alloimmunization, transfusion-related viral infections, transfusion reactions, and annual transfusion rates should be monitored in these patients.

In transfusion-dependent thalassemias, iron overload inevitably will develop due to iron gained from transfusions and increased gastrointestinal iron absorption, which is responsible for morbidity and mortality. Iron accumulates primarily in the liver, followed by endocrine organs and the heart. Hypothyroidism, hypogonadotropic hypogonadism, growth hormone deficiency, hypoparathyroidism, and diabetes mellitus may develop due to iron overload.[11] Besides, iron overload in the heart, causing arrhythmias and heart failure, can be fatal. Chelation therapy is necessary to prevent these complications, and iron overload should be regularly monitored for adequate chelation. Monitoring of serum ferritin, measured at least 3- month intervals, facilitates chelation management, but it does not reflect tissue iron status properly. Today, T2* and R2* MR imaging has become the standard for indirect, quantitative measurement of iron accumulation in heart and liver.[12] It is recommended to start the follow-up with MRIs after the age of 8-10 and once a year henceforth.

Iron chelation should be initiated after 10 to 15 transfusions or when serum ferritin rises above 1,000 ng/mL.[13] However, iron chelators are not approved for use under two years of age. Information on three different iron chelators is shown in Table 2. These drugs can be used alone or together with deferiprone to reduce cardiac iron load. Ideally, serum ferritin level is desired to be between 500-1000 ng/mL. When the serum ferritin level decreases below 500 ng/mL in transfusion dependent patients, it is recommended to suspend chelator therapy.[13-15]

Splenectomy should be recommended in cases of hypersplenism, with signs such as the mass effect of the spleen, also in cytopenias or increased annual

Table 2. Iron chelators

	Deferoxamine	Deferiprone	Deferasirox
Administration	iv, sc, 8-12 h/day, 5-7 day/week	Oral tablets, suspension 3 times daily	Oral dispersible and film-coated tablet, Once daily
Dose	25-60 mg/kg/d	75-100 mg/kg/d	20-40 mg/kg/d (dispersible) 14-28 mg/kg/d (film-coated)
Excretion	Urine, feces	Urine	Feces
Adverse reactions	Local reactions, retinal toxicity, ototoxicity, bone toxicity	Agranulocytosis, arthralgia	Gastrointestinal disturbances, increase in hepatic transaminase levels , rash, mild creatinine increase, ophthalmologic toxicity, ototoxicity
Monitoring parameters	Eye examination and hearing test once a year	Complete blood count, ALT monitoring	Serum creatinine, ALT, total and direct bilirubin once a month
Specifications	Low adherence	Effective in reducing cardiac iron	High adherence

iv: Intravenous, sc: Subcutaneous, ALT: Alanine aminotransferase

need for blood transfusion (> 200 mL/kg) in patients with thalassemia.[5,16] Splenectomy is often not recommended before the age of 5, as the risk of sepsis is high. Apart from the risk of infection after splenectomy, the risk of thrombosis and pulmonary hypertension also increases.[16] Because of the possibility of pneumococcal sepsis, polyvalent pneumococcal vaccine and then lifelong prophylactic penicillin is recommended for at least 3-4 weeks before splenectomy.

It has been 30 years since the first hematopoietic stem cell trasplantation (HSCT) performed for thalassemia major patients. Today, allogeneic transplantation is a curative method and standard clinical practice in patients with thalassemia major. Therefore, all patients with thalassemia major should be screened for family donors, and HSCT should be recommended before organ damage due to development of iron overload. In Turkey, a study conducted with 245 children with thalassemia major, disease-free survival was 68%, overall survival 85%, and transplant-related mortality 7.7%.[17] According to Pesaro experience, hepatomegaly, portal fibrosis, and insufficient iron chelation are independent adverse risk factors for transplantation.[18]

Gene therapy seems to be an alternative, curative treatment option in patients with thalassemia major who do not have suitable HSCT donors. Autologous HSCT is performed after correction of beta-globin mutation mediated by lentiviral vectors or gene editing.[19] In addition, activation of HbF synthesis by these methods alleviates the clinical signs of beta thalassemia. Initial results are exciting, and permanent increase in Hb, decrease in the need for transfusion, and improvement in quality of life have been reported.

There is an increase in GDF11 production as a result of intramedullary apoptosis and ineffective erythropoiesis. GDF11 is a “transforming growth factor β ligand” and inhibits the differentiation of erythroid precursors. More effective erythropoiesis can be achieved with new treatment strategies based on the principle of binding to GDF11. For this purpose, there are two molecules developed as luspatercept (ACE-536) and sotatercept (ACE-011). Luspatercept prevents GDF11 from binding to its receptor. Its subcutaneous administration every three weeks has been recommended. According to the results of the Phase II study, in the thalassemia major group, a reduction of more than 33% in need

for blood transfusion was observed in 83% of the patients. On the other hand, in the non-transfusion dependent thalassemia group, Hb levels increased by more than 1 gr/dL in 78% and 1.5 gr/dL in 56% of the patients. The results are similar in the Luspatercept Phase III study (BELIEVE study).[20]

BETA THALASSEMIA INTERMEDIA

Patients with BTI are not transfusion dependent; however, they may need a transfusion during infections, inflammation, and pregnancy due to increased hemolysis. Hypochromia and microcytic anemia are present, and Hb levels are between 6-10 gr/dL. Medullary expansion in bone marrow, hepatosplenomegaly, hypersplenism, extramedullary hematopoiesis, pulmonary hypertension, leg ulcers, thrombosis, and growth retardation can be seen in patients with beta thalassemia intermedia.[21] Hemosiderosis may develop due to increased gastrointestinal iron absorption, especially in the liver. In these patients, it is more challenging to monitor iron overload by serum ferritin since iron accumulation is mainly in hepatocytes compared to macrophages. On the other hand, with or without hepatitis C infection, the risk of hepatocellular cancer is higher in patients with beta thalassemia intermedia than patients with thalassemia major. Regular transfusion may also be required in patients with TI when growth retardation, exercise intolerance, hypersplenism, bone changes, and extramedullary hematopoiesis develop.[21]

ALPHA (α) THALASSEMIAS

Alpha thalassemias are more common, especially in Southern China, Malaysia, and Thailand. The milder phenotypes are also found in people with African origin. The risk of hydrops fetalis is higher in Asians since alpha thalassemia-1 carriership (i.e., presence of 2 alpha globin gene deletions in the αα/- in cis position) are more prevalent in Asians.

In silent alpha carrier state (alpha thalassemia-2), Hb Barts is detected at a rate of 2-5% in the cord blood in the neonatal period, which disappears after the first three months. Detection of these individuals outside the neonatal period is only possible by performing in vitro Hb chain synthesis or molecular tests.

In severe alpha carrier state or alpha thalassemia-1, hematological features are similar to beta thalassemia carriers. MCV is low, and RBC is often high. However, HbA2 levels are normal or lower than normal. In peripheral blood smear, erythrocytes have hypochromia, microcytosis, anisocytosis, poikilocytosis, polychromasia, and basophilic stippling. Hb Barts is seen in 5 to 10% of newborns which disappears after six months of life. A definitive diagnosis is made by in vitro Hb chain synthesis and DNA studies.[22-24]

Patients with HbH disease may present with hemoglobin between 8-10 gr/dL and hypochromia, microcytosis, anisocytosis, poikilocytosis, polychromasia, and target cells in peripheral blood smears. Inclusion bodies are seen on the erythrocytes after incubation of erythrocytes with bright cresyl blue.[25] In 20-40% newborns Hb Barts is detected in Hb electrophoresis, replaced by HbH in 5-30% of the cases in time. A definitive diagnosis should be made based on a decrease in α chain synthesis and DNA studies. Acquired HbH disease has also been reported secondary to myeloproliferative and myelodysplastic diseases.[26]

HbH disease presents with hypochromia and microcytic moderate to severe anemia. Splenomegaly, scleral icterus, and gallstones can be observed in patients. Since they do not show symptoms except during infection, inflammation, or pregnancy, It may not be diagnosed until the second decade. HbH patients should be monitored for growth, osteopenia, and iron accumulation. Also, folic acid supplementation should be initiated.[23,24]

ERADICATION AND PRENATAL DIAGNOSIS

In Turkey, pre-marital screening has been carried out since the 2000s, and as of November 2018, pre-marital screening was extended to all over the country. Prenatal diagnosis was made for the first

time in 1975 by fetal blood sampling between 18-22 weeks of gestation by in vitro hemoglobin chain synthesis and measurement of α/β globin ratio. Today, thalassemia mutations are analyzed by isolating DNA from fetal chorionic villus samples.

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Original Article

Evaluation of Beta Globin Gene Mutations in Beta Thalassemia Carrier Children in Aydın Province and its Environment

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ABSTRACT

Objective: *Beta thalassemia carriers (BTC) in Turkey is observed with a frequency of 2.1%, and it is the most common cause of anemia after iron deficiency. There are few studies showing the effect of genotype on phenotype in beta thalassemia carrying children. The aim of this study is to determine the mutation diversity of these children in and around Aydın and evaluate the effects of these mutations on complete blood count parameters and hemoglobin electrophoresis.*

Methods: *This study included mutation analysis of 65 patients who were diagnosed as BTC in Adnan Menderes University, Faculty of Medicine, Department of Child Health and Diseases, Pediatric Hematology Outpatient Clinic between 01.01.2014 and 01.08.2019. Complete blood count, hemoglobin electrophoresis and mutation analysis results were obtained from the computer data system and patient files. Research data were evaluated by using SPSS 21.0 statistics program.*

Results: *The study population with a mean age 8.34±4.94 years consisted of 39 (60.0%) male, and 26 (40.0%) female patients. When full blood count parameters were analyzed, 87.7% of the patients had anemia, 100% microcytosis and high red cell distribution width (RDW), 49.2% hypochromia and 87.7% increased red blood cell (RBC) counts. RDW level was ≥16% in 66.2% of the cases. Seventeen different mutations were detected. The mutations most frequently occurred in "intron 1" gene region (66.1%). The most common mutations were heterozygous IVS I-110 mutation in 44.11%, heterozygous IVS I-1 G>A mutation in 8.8%, heterozygous IVS I-6 T>C mutation in 7.5% and IVS II-745 mutation in 7.5% of the patients. RDW level was ≥16% in 66.2% of the cases. Seventeen different mutations were detected. The mutations most frequently occurred in "intron 1" (66.1%) gene region. Most commonly IVS I-110 (44.11%), heterozygous IVS I-1 G>A (8.8%) heterozygous IVS I-6 T>C (7.5%) and IVS II-745 (7.5%) mutations were observed. In patients with IVS I-110 mutation, average values for Hb (10.55 gr/dL), MCV (58.44 fL), RDW (16.51%), RBC (5680x10^9/L), HbA2 (4.77%), HbF (2.34%) were as indicated. Mutations detected in 12 patients with HbF level above 5% including: cases with IVS I-110 (n=5) mutations, heterozygous IVS I-6 T>C, Codon 39 C>T IVS I-116, c.25-26 del AA (plys9Valfs), c.27dupG (pSer10valfs*14), c316-373 (IVS II-478 C>A, and -87 C>T mutations. Mentzer index was calculated as >13 in six patients (9.2%). The mutations seen in these patients were IVS-I 110, c.27dupG (p.Ser10valfs*14), c316-373 (IVS II-478 C>A heterozygotes, and -87 C>T heterozygotes. There were four patients (6.1%) with a RDW index of >220. Two of these patients had c.27dupG (p.Ser10valfs*14) and others had heterozygous c316-373 (IVS II-478 C>A and -87 C>T mutations. Mutations detected in four patients with HbF levels in the range of 9.48-15.67 and the patients had heterozygous IVS I-116 T>G, IVS I-110 G>A, c.25-26 del AA (p.lys59valfs), and c27 dupG (pSer10 valfs*14) mutations, and three of these mutations carrying β° mutation type were located in exon 1 and one of them carrying β⁺ mutation type (IVS I-110) in intron 1.*

Conclusion: *The same mutations detected in patients with beta thalassemia carriers have different effects on complete blood count parameters. HbA2 and HbF levels which suggests that these mutations are not effective on the phenotype alone and there may be additional factors which should be clarified. We think that there may be BTC in cases with low RBC, Mentzer index of ≥13 and RDW index of ≥220, HbA2 <3.5 and studying the mutation analysis of these patients will contribute significantly to the literature.*

INTRODUCTION

Thalassemia is a hemoglobin (Hb) disorder characterized by a decrease in the synthesis of one or more globin chains.[1] The disorders which occur due to this decrease or inability to make the globin chain are classified as alpha (α) and beta (β) thalassemias.[2]

It is the single most common gene disease in the world, characterized with hemolytic anemia, microcytosis and shows autosomal recessive transition. There are approximately 270 million thalassemia carriers in the world.[1]

Generally, beta thalassemia is classified in two types as β^o and β^+ thalassemia. Rarely, a form that is lighter than these two alleles is also shown as β^{++} and causes minimal deficiency in β chain production. Beta thalassemia carriers (BTC) are clinically asymptomatic. Studies conducted all around Turkey reveal that an average of 2.1% of the population is BTC and this rate extends to 10% in some regions.[3,4] Heterozygotes can be diverse. It has been shown that individuals with type β^o thalassemia have a lower erythrocyte volume (MCV) than those with type β^+.[5] In cases with heterozygous thalassemia, HbF levels, like HbA2, differ according to the mutation types. And HbF values were found to be higher in studies conducted in the β^o group.

Complete blood count and peripheral blood smear examination are guiding, simple and fast terminating tests particularly in the initial evaluation of patients with anemia, in the differential diagnosis and in the selection of tests to be requested later. Additionally, hematological tests such as erythrocyte indices, hemoglobin electrophoresis are also carried out.[1] In addition, MCV, mean corpuscular hemoglobin (MCH) per erythrocyte and Hb are useful variables for screening hemoglobinopathy. For the diagnosis of thalassemia, results of electrophoresis and Hb values, types of Hb and abnormal Hb should be revealed.[2]

Beta globin is a relatively small and structurally simple gene. It is located in the globin gene cluster on the short branch of the 11th chromosome (11p 15.5). Mutations in the globin gene on the developmental process to Hb have the potential to result in β-thalassemia, sickle cell anemia, or formation of an abnormal Hb.[6] Detection of β globin gene mutations is required for early prenatal diagnosis of β-thalassemia or identification and treatment projects of carriers of heterozygous mutations.[2] In BTC, the effect of genotype on phenotype is considered inevitable. Since the carriers in the society have normal appearance, they cannot be detected unless thalassemia tests are performed. In areas where the risk of thalassemia is high, the carriers should be identified with community screening.

The aim of this study is to determine the mutation diversity in children with BTC status in Aydın and its surroundings and to evaluate the effects of these mutations on hemoglobin electrophoresis.

MATERIALS and METHODS

In this retrospective study, 65 patients without iron deficiency accepted as BTC whose diagnoses were confirmed by mutation analysis in Adnan Menderes University Faculty of Medicine Department of Child Health and Diseases, Pediatric Hematology Outpatient Clinic between January 2014 and August 2019 were included.

Ethics committee approval was obtained from Adnan Menderes University Medical Ethics Committee. Complete blood count parameters and HbA2 level at the time of diagnosis of these cases were obtained from file scans and computer data system. Parameters determined by whole blood count analysis were calculated with Mentzer index [MCV/red blood cell (RBC)] and red cell distribution width (RDW) index (MCVxRDW/RBC) formulas. Mentzer index of <13, RDW index of <220 were interpreted in favor of thalassemia carrier status. For HbF values, <1% was considered normal, 1-5% slightly high and >5% high.[7] Research data were evaluated by using SPSS 21.0 statistics program. The fitness of continuous variables to normal distribution was investigated using the Kolmogorov-Smirnov test. For the descriptive statistics of the study, the data fitting the normal distribution, were expressed as mean and standard deviation, and those that did not fit the normal distribution as median, minimum and maximum values. In the study, chi-square test was used to show whether there is a difference between

categorical variables. ANOVA was used to compare the parametric properties of continuous variables in independent groups, and the Kruskal- Wallis Test was used to compare those that do not have the parametric properties of continuous variables in independent groups. P value less than 0.05 was accepted as the level of statistical significance.

RESULTS

Of the 65 patients included in this study, 39 (60.0%) were male, 26 (40.0%) were female and the mean age was 8.34±4.94 years (range 1.0-18.0). Mean values for Hb 10.55±1.07 gr/dL (range, 8.00-13.10), RBC 5580±538x10^9/L (range, 3300-6670), MCV 59.38±5.48 fL (range, 47.50-74.60), MCH 18.77±1.96 pg (range, 14.10-24.70), MCHC 31.85±0.94 pg (range, 29.6-33.8), platelet (plt) count (387.58±120.04x10^9/L) (range, 215.0-787.0), hematocrit (Hct) 33.00±3.31% (range, 27.0-43.4), RDW 16.95±2.63% (range, 14.0-32.9), HbA2 4.75±0.86% (range, 2.42-6.70), HbF 2.80±3.28% (range, 0.00-15.67) were as indicated. Complete blood count parameters and results of electropheresis are presented in Table 1.

Seventeen different mutations were detected in the patients. The four most common ones were heterozygous IVS I-110 G>A (44.11%), IVS I-1 G>A (8.82%), IVS I-6 T>C (7.35%) mutations and IVS II-745 mutation (7.35%). The distribution and frequency of all detected mutations are reflected in Table 2.

Table 1. Complete blood count parameters and HbA2 and HbF levels

	n	Mean	Median	Minimum	Maximum	SS*
Hb (gr/dL)	65	10.55	10.60	8.00	13.10	1.07
RBC (10^9/L)	65	5580	5640	3300	6670	538
MCV (fL)	65	59.38	59.00	47.50	74.60	5.48
MCH (pg)	65	18.77	18.55	14.10	24.70	1.96
MCHC (gr/dL)	65	31.85	31.95	29.60	33.80	.94
Platelet (10^9/L)	65	387.58	364.50	215.00	787.00	120.04
Hct (%)	65	33.00	33.20	27.00	43.40	3.31
RDW (%)	65	16.95	16.25	14.00	32.90	2.63
HbA2 (%)	65	4.75	4.73	2.42	6.70	.86
HbF (%)	65	2.80	1.80	.00	15.67	3.28

**SD: Standard Deviation*

Table 2. Mutation distribution and frequency

Mutasyon	n	%
Heterozygous IVS I-110 G>A mutation	30	44.11
Heterozygous IVS I-1 G>A mutation	6	8.82
Heterozygous IVS I-6 T>C mutation	5	7.35
IVS II-745	5	7.35
Heterozygous c.31 C>T mutation	3	4.41
Heterozygous Codon 39 C>T mutation	3	4.41
Heterozygous IVS II-1 G>A mutation	2	2.94
c.27 dupG (p.Ser10 valfs*14)	2	2.94
Heterozygous c.316-373 (IVS II-478 C>A mutation)	2	2.94
Heterozygous IVS I-116 T>G mutation	2	2.94
Heterozygous c.25-26 del AA (p.lys9Valfs) mutation	2	2.94
c.135 del C (Codon 44(-C))	1	1.47
Heterozygous IVS II 81 C>T mutation	1	1.47
Heterozygous c.-29 G>A mutation	1	1.47
c.112 delT (p.Trp38Glysfs)	1	1.47
Heterozygous c.17-18 del CT mutation	1	1.47
Heterozygous -87 C>T mutation	1	1.47

**SD: Standard Deviation*

Table 3. Mutations and gene regions

Mutation	n	%	Mutation Region
Heterozygous IVS I-110 G>A mutation	30	69.7	Intron 1
Heterozygous IVS I-1 G>A mutation	6	13.9	
Heterozygous IVS I-6 T>C mutation	5	11.6	
Heterozygous IVS I-116 T>G mutation	2	4.6	
Total	43	100.0	
Mutation	**n**	**%**	**Mutation Region**
IVS II-745	5	50.0	Intron 2
Heterozygous IVS II-1 G>A mutation	2	20.0	
Heterozygous c.316-373 (IVS II-478 C>A mutation)	2	20.0	
Heterozygous IVS II 81 C>T mutation	1	10.0	
Total	10	100.0	
Mutation	**n**	**%**	**Mutation Region**
Heterozygous c.31 C>T mutation	3	30.0	Exon 1
Heterozygous c.25-26 del AA (p.lys9Valfs) mutation	2	20.0	
c.27 dupG (p.Ser10 valfs*14)	2	20.0	
Heterozygous c.-29 G>A mutation	1	10.0	
Heterozygous c.17-18 del CT mutation	1	10.0	
Heterozygous -87 C>T mutation	1	10.0	
Total	10	100.0	
Mutation	**n**	**%**	**Mutation Region**
Heterozygous Codon 39 C>T mutation	3	60.0	Exon 2
c.112 delT (p.Trp38Glysfs)	1	20.0	
c.135 del C (Codon 44(-C))	1	20.0	
Total	5	100.0	

IVS II-745 mutation was detected in five patients. Three of these patients were accompanied by the heterozygous c.31 C>T mutation. Therefore, although 65 patients were included in this study, 68 mutations were detected.

Mean Hb level of the patients was 10.55±1.09 gr/dL (range: 8.0-13.0) in those with a heterozygous IVS I-110 G>A mutation; 10.17±0.81 gr/dL (range, 9.0-11.3) for heterozygous IVS I-1 G>A mutation; 10.96±0.55 gr/dL (range, 10.2-11.5) for heterozygous IVS I-6 T>C mutation and 10.34±0.69 gr/dL (range, 9.5-11.1) for IVS II-745 mutation. There was no statistically significant difference between mean Hb levels among patients (p=0.408).

Mean Hct level of the patients was 33.11±3.53 (range, 27.00-43.40) in those with heterozygous IVS I-110 G>A mutation; 31.62±2.50 (range, 27.70-35.20) in those with IVS I-1 G>A, 33.84±2.64 (range, 31.00-36.50) in IVS I-6 T>C patients with heterozygous mutation; 32.24±2.99 (range, 28.00-35.50) in IVS II-745 heterozygous mutation. There was no statistically significant difference between mean Hct levels (p=0.544).

Mean RBC level of the patients was 5680±3840x10^9/L (range, 4880-6520) in those with heterozygous IVS I-110 G>A mutation; 5780±2960x10^9/L (range, 5560-6360) in heterozygous IVS I-1 G>A, mutation; 5400±2450x10^9/L (range, 5050-5660) in heterozygous IVS I-6 T>C mutation; 5460±4880x10^9/L (range, 4830-6010), and in heterozygous IVS II-745 mutation. There was no statistically significant difference between mean RBC levels (p=0.170).

Mean RDW level of the patients was 16.51±1.29 (range, 14.90-19.60) in those with heterozygous IVS I-110 G>A mutation; 16.88±1.51 (range, 15.40-19.40) in heterozygous IV mutation; 15.50±1.18 (range, 14.10-17.10) in heterozygous IVS I-6 T>C mutation and 16.25±1.16 (range, 15.00-17.80) in IVS II-745 mutation. There was no statistically significant difference between mean RDW values (p=0.326).

Mean HbA2 level of the patients was 4.77±0.71 (range, 3.70-6.70) in those with a heterozygous IVS I-110 G>A mutation; 5.02±0.98 (range, 3.50-6.10) in heterozygous IVS I-1 G>A mutation, 4.39±0.71 (range, 3.40-5.24) in heterozygous IVS I-6 T>C mutation and 5.00±0.73 (range, 4.40-6.10) in IVS II-745 mutation. There was no statistically significant difference between mean HbA2 values (p=0.424).

Mean HbF level of the patients was 2.34±2.94 (range, 0.00-13.32) in those with heterozygous IVS I-110 G>A mutation; 1.35±1.48 (range, 0.00-3.70), 1.83±3.00 (range 0.00-6.90) in heterozygous IVS I-1 G>A mutation; 1.83±3.00 (range 0.00-6.90) in heterozygous IVS I-6 T>C mutation and 2.44±1.66 (range, 0.00-4.00) in IVS II-745 mutation. There was no statistically significant difference between mean HbF values (p=0.726).

When 68 mutations determined in 65 patients were examined according to RDW indexes, it was >220 in four mutations (5.88%) and ≤220 in 64 mutations (94.11%).

When mutations with low RDW indexes were examined, two (50%) were c.27 dupG (p. Ser10 valfs*14) mutation, one was (25%) c.316-373 (heterozygous IVS II-478 C>A) mutation and one (25%) was identified as a heterozygous -87 C>T mutation. None of these patients had iron deficiency anemia. The RDW indexes of both patients (100%) with c.27 dupG (p.Ser10 valfs*14) mutation were >220. Four most common mutations among all were heterozygous IVS I-110 G>A (n=30), IVS I-1 G>A (n=6), IVS I-6 T>C (n=5) mutations and RDW index was ≤220 in all patients with IVS II-745 (n=5) mutations. When the relationship of mutations with HbF was examined, HbF value was zero in 21 mutations (30.88%), between 0-1 in 3 mutations (4.41%), between 1-5 in 32 mutations (47.05%) and greater than five in 12 mutations (17.64%). When the most frequently seen heterozygous IVS I-110 G>A mutation (n=30) was evaluated, the most common HbF values were between 1-5 (43.33%). Among the three most common mutations, HbF values detected in patients with heterozygous IVS I-1 G>A (n=6) and IVS II-745 (n=5) mutations were mostly between 1-5 (50%, 80%, respectively) whereas HbF values in 5 cases with heterozygous IVS I-6 T>C mutation were mostly zero (60%).

As for the relationship of mutations with the MI, four most common mutations were heterozygous IVS I-110 G>A (n=30), IVS I-1 G>A (n=6), and IVS I-6 T>C (n=5) mutations and mean Mentzer indexes of IVS II-745 (n=5) were 10.34±1.18, 9.46±0.82, 11.8±0.41 and 11.37±1.73, respectively. The mutations with mean MI value above 13 were heterozygous IVS I-110+-87 C>T (16.94%), IVS II-81 C>T (14.49%), c.316-373 (IVS II-478 C>A) (13.52±1.65) and c.27 dupG (p.Ser10 valfs*14) (14.72±3.22) mutations. Sixty-one (89.7%) of all mutations were detected in Aydın province. Five of them were in Muğla, one was in İzmir and one in Denizli.

When the gene regions of 68 mutations were analyzed, 43 (63.3%) were detected in intron 1, 10 (15.87%) in intron 2 region, 10 (15.87%) in exon 1 and five (7.93%) in exon 2 regions. The most common mutation was heterozygous IVS I-110 G>A mutation (n=30), followed by heterozygous IVS I-1 G>A (n=6) mutations and IVS I-6 T>C mutations (n=5) were detected in intron 1 region. Exon 2 (n=5; 7.93%) was determined as the least common gene mutation region and 60% of the mutations in this region are heterozygous Codon 39 C>T mutations.

DISCUSSION

BTC is the most common single gene disease in the world, and 4.5% of the world population carries a mutation in the globin chain.[8,9] Due to its geographical location, Turkey has been affected by a large number of societies and mutations of different types have been detected.[10,11] Since the carrier rate of thalassemia is 5.1% in Aydın, evaluation of mutation diversity in this province and determination of the frequent mutations have a vital importance for public health.[10] Hb as one of the complete blood count parameters, may be normal or low in individuals with BTC.[12] Hb level over 8 gr/dL is generally observed in thalassemia carriers. In cases with lower Hb levels, investigation of additional factors contributing to anemia is recommended.[13] The patients without iron deficiency had Hb levels between 8-13 gr/dL and 87.7% of them had anemia. Hb levels of >8 gr/dL suggested that there is no additional factor contributing to anemia. MCV level lower than normal

is an indicator of microcytosis. Thalassemia trait is the most common cause of microcytic anemia after iron deficiency anemia. Different MCV levels have been reported in BTC as follows: 60-79 fL [Aksoy et al.[14]], 59-83fL [Arpacı et al.[15]], 60-71 fL [Tanriverdi et al.[16]], 65-82 fL [Topal[17]], and 56-71 fL [Evrensel[18]]. In this study, MCV levels ranged between 47.5-74.6 fL which were found to be lower than normal in all the patients.

RDW level is increased in iron deficiency anemia and normal or slightly higher in BTC.[19] It has been stated that RDW is a more sensitive parameter in the diagnosis of mild iron deficiency anemia than the saturations of serum iron, ferritin and transferrin.[12] It is stated that iron deficiency is the most common reason of the rise in RDW. RDW may increase in thalassemia, other hemoglobinopathies and other conditions causing microcytosis.[20] Cook et al.[21] accepted ferritin as a more sensitive test compared to RDW in iron deficiency anemia and specified that it is an important parameter especially in the diagnosis of those who did not receive iron treatment priorly. In this study, normal values of RDW were not detected in patients without iron deficiency. The fact that RDW of 22% were slightly higher (<16.0) and RDW of 66.2% was higher (≥16) suggests that this parameter is not an essential parameter in differential diagnosis.

RBC counts are often increased in thalassemia carriers. RBC levels may be low, normal or high in iron deficiency anemia.[22] In their study conducted in 1999 on 195 patients with iron deficiency anemia and 463 cases with beta thalassemia carriers, Madan et al.[23] reported that RBC increased in the group with thalassemia carriers, but they did not find any significant difference. In this study, RBC counts were increased in 87.7%, and decreased in12.3% of the patients. Mutations detected in patients with low RBC counts were as follows: heterozygous c27dupG, IVS I-110 G>A IVS I-6 T>C, IVS II-745, IVS I-116 T>G and c.316-373 (IVSII-478 C>A mutations. In the literature, we did not find any study based on mutation examination in patients with low RBC counts. We think that patients with low RBC counts may also have thalassemia trait, and studying the mutation analysis of these patients having thalassemia trait will make a significant contribution to the literature.

In addition to RDW, RBC, MCV values, Mentzer and RDW indexes are frequently used in the differential diagnosis of BTC and iron deficiency anemia.[22] Mentzer index was found to be 95% sensitive in terms of differentiating beta thalassemia carrier status.[24] While MI is below 13 in BTC, this value is ≥13 in iron deficiency anemia. Oğuz et al.[22] reported MI levels between 7.00-12.20 in pediatric BTC. Rahim et al.[24] stated that they diagnosed BTC in 55 patients with MI <13 and 4 patients with ≥13 whereas Demir et al.[19] submitted that MI was not sensitive and specific in the differential diagnosis. MI level in our patients was between 8.30-17.0 and there were 6 patients (≥13) whose MI did not suggest the presence of a thalassemia trait. The mutations we detected in these patients were heterozygous IVS-I 110, c.27dupG (p.Ser10valfs*14), c316-373 (IVS II-478 C>A, and -87 C>T mutations. In the literature, no study was found examining the relation between BTC mutations with the MI, RDW index and RBC which are among the most reliable parameters used in the differentiation between iron deficiency anemia and BTC status. Demir et al.[19] suggested that RDW index is the second most effective parameter in the differential diagnosis of iron deficiency anemia and BTC in children. Vehapoğlu et al.[25] found RDW index <220 in 83% of patients with BTC. Examination of RDW indexes based on mutations in this study, revealed RDW index as <220 in 94.1% of the patients. There were 4 patients whose RDW indexes did not suggest the presence of a thalassemia trait (RDW index ≥220). HbA2 level >3.5% in Hb electrophoresis is diagnostic in cases where thalassemia carrier status is considered based on complete blood count parameters. HbA2 values were between 4.5-5.8% in Oğuz et al's study[22], 1.5-5.6% in Evrensel's study, and 3.8-7.5% in Mumbai and 3.5-7.5% in Delhi in Madan et al's study.[23] Mean HbA2 value of the patients was 3.7% in the studies by Topal[17] and Öney et al.[1] where they found the smallest HbA2 value as 3.77%. In this study, the lowest, and the highest HbA2 values were 2.42% and 6.7%, respectively. The HbA2 level was ≥%3.5 in 93.8% of the patients, and <%3.5 in 6.1% of the patients. In the literature, complete blood count index suggesting BTC, with normal MCV and HbA2 level in mutations such as IVS I-6 T>C, codon 27 G>T, LCR deletion in silent BTC have been reported. In the study of Galanello et al.[26], -101 C>T, IVS I-6 T>C

mutations were more frequently associated with normal or borderline HbA2 values. Decreased production of delta globin chains may cause normalization of HbA2 levels.[41] In this study, delta gene mutation was not examined and there were no children with iron deficiency anemia. The mutations we detected in patients with normal HbA2 levels were heterozygous HbA2 value -87 C>T, IVS II 81 C>T, IVS I-6 T>C, c316-373 (heterozygous IVS II-478 C>A) mutations. After investigating the presence of delta mutations, it was thought that whether these mutations progressed with normal HbA2 levels could be interpreted more accurately.

We had mean HbA2 level of 4.77±0.71 in patients with heterozygous IVS I-110 G>A mutation, 5.02±0.98 in heterozygous IVS I-1 G>A mutation, 4.39±0.71 in heterozygous IVS I-6 T>C mutation and 5.00±0.73 in heterozygous IVS II-745 mutation. There was no statistically significant difference between mean HbA2 values (p=0.424).

Approximately half of the BTC have normal HbF levels, whereas in the other half HbF levels are slightly increased.[13] Higher HbF can be seen in cases in the presence of promoter region mutation, alpha gene triplication and delta gene mutation.[27] Öney et al[1] found mean HbF levels of 84 BTC as 2.64% (range 0-8.5%) and suggested that this may be caused by β° mutation type. Macaulay[27] suggested in his study that very few cases had HbF values between 4-15%, most of which may be related to the transport of the hereditary persistent HbF gene. In this study, HbF value was normal in 35.3%, slightly high in 46.1% (1-5%), and high in 18.4% (>5%) of the cases. In 40% of the patients with, heterozygous IVSI-110 G>A mutation, HbF was between 1-5% and >5 in 41% of the patients. None of the patients with IVS I-1 G>A mutations had HbF values above 5%. Unlike our study, Kutlar et al.[28] detected high HbF values in IVS I-1 and IVS I-II. The highest HbF levels in our study were 9.48%, 9.7%, 13.32%, 15.67 %, and mutations detected in these individuals were heterozygous IVS I-116 T>G, c.25-26 del AA (p.lys59valfs), IVS I-110 G>A mutations, and nonsense mutation of c.27 dupG (p.Ser10 valfs*14) respectively. Three of these mutations had a β° mutation type, three were in exon 1, one in heterozygous IVS I-110 G>A mutation was located in intron 1 as the β^{+} mutation type. This situation in thalassemia carriers with high HbF may be related to mutation types.[28,29] These suggest that the HbF level may be associated with the mutation type in some of the BTC patients. The results found in those with the most common IVS-I 110 mutations also mark the effect of other factors.

There are many studies on β thalassemia mutations in the world. In a study by Talmaci et al.[30] on the Romanian population, IVS I-110 was the most common mutation with 31.25%, followed by Codon 39 and IVS II 745 mutations. In a study by Makhoul et al.[31] in Lebanon, IVS I-110 mutation was found in 34.2%, IVSI-1 in 15%, IVS I-6 in 14.4% , and Codon 29 in 9.6% their study population.

Even though there are differences in the percentages of patients involved, the IVS I-110 mutation is most frequently seen in the neighboring countries like Greece, Macedonia, Bulgaria, and Syria. While the most common mutation in Italy is CD39, it is IVS II-1 in Iran and Azerbaijan. In Azerbaijan, the determined IVS I-110 ratio is close to IVSII-1.[31] Tadmouri et al.[32] detected 31 different mutations, most frequently IVS I-110, in 795 cases in Istanbul, Adana, and Antakya. Other mutations observed are IVS I-6, Cd8, IVSII-745, IVS I-1, IVS II-1 Cd39, -30, Cd5 and -28 in order of decreasing frequency. According to the study of Tadmouri[27], in the Central Anatolia Region most frequently (52.3%) IVS1-110 mutation is seen. Topal et al.[17] reported the incidence rates of IVS I-110 mutation as 63.7% in Antakya, 68.3% in Kayseri and 46.7% in İzmir. The incidence rates of other mutations found in order of decreasing frequency were 18.2%, for IVS I-1, Cd9, IVS I-6, and 6% for IVS2-1. In Kayseri, incidence rates for IVS I-110 (68.3%), CD8 (19.5%), IVS I-10 (46.7%) were as indicated. In İzmir, IVS I-110 mutations were seen more frequently (46.7%) followed by Cd-30 mutations (13.3%) (14,17). In the study conducted by Öner et al in Turkey, common mutation types in order of decreasing frequency were IVS I-110 (42.5%), IVS I-6 (18%), IVSII-1 (11.5%) Cd8 (7.14%) Cd 39 (6%), IVSII-745 (4.4%), IVS I-1 (2.5%, -30%, and 2.2 Cd 5 1.1%. While Atalay et al. reported incidence rates of different mutations as follows: IVS I-110 (35.9%), IVSI-6 (21.6%), IVS I-1 (13.0%), IVSII-745 (3.6%), Cd8 (2.2%), and IVS II-1 (1.4%).[31] In this study, we detected 17 different mutations. The most frequent ones in order of

decreasing frequency were IVS I-110 (46.1%), IVS I-1 (12.2%), IVS I-6 (9.8%) and IVSII-745 (7.3%). Referring to other mutations, we identified greater regional differences which are compatible with the most common mutation study in Turkey in general. We detected IVS II-745 mutation in 7.3% of the patients, which is an important Mediterranean mutation. In addition, we did not come across a new region-specific mutation during the study.

The 70.8% of the mutations detected in this study were localized in intronic regions. The IVS I-110 is the most common mutation in Turkish population, and creates an exon binding region within introns of the first exon, where it will adhere to the second exon and causes adherence to the area during the formation of RNA.[34] In a study conducted by Baysal et al.[35] in the Turkish Republic of Cyprus, in patients with IVS I-110 mutation, levels of Hb (10.05-13.65 gr/dL), MCV (62.6-76.4 fL), HbA2 (4.15-5.15%), and HbF (0.05-2.35%) had been reported as indicated. In patients with IVS-I-110 mutation, Talmaci et al.[30] stated levels of Hb (11.5-13.5 gr/dL), MCV (63.3-73.4 fL), HbA2 (2.5-5.9%), and HbF (0-1.7%) as indicated. In this study, levels of Hb (8.0-13.0 gr/dL), MCV (47.5-72.0 fL), HbA2 (3.7-6.7%), HbF (0.05-2.35%) were measured as indicated. These findings have suggested that the factors other the phenotype are taking part in determining the phenotypic characteristics.

Heterozygous IVS II-1 (G>A) mutation is an intronic region mutation and in the study of Hattori et al.[36], levels of Hb (9.3-12.6 gr/dL), MCV (63.4-80.4 fL), HbA2 (4.2-5.6%), and HbF (0.3-1.2%) had been reported as indicated. This mutation is most often seen in Turkey and then Yemen. We found the incidence in the patients to be 2.94%. In these cases mean Hb (10.1-13.1 gr/dL), MCV (59.7-50.9 fL), HbA2 (5.85-5.90%), and HbF (2.54-4.49%) levels were determined as indicated. Heterozygous IVS I-6 (T>C) mutation is also an intronic region mutation. In the study of Orkin et al.[37], in carriers of this mutation, levels of Hb (9.55-14.35 gr/dL), MCV (64.7-77.3 fL), HbA2 (3.35-4.45%), and HbF (0.1-2.2%) had been reported as indicated. In our study, in these cases mean levels of Hb (10.2-11.5 gr/dL), MCV (61.70-65.10 fL), HbA2 (3.4-5.24%), and HbF (0-6.9%) were also determined. Heterozygous IVS II-745 (C>G) mutation is also an intronic region mutation. In the study of Orkin et al.[37], levels of Hb (9.95-13.05 gr/dL), MCV (64.9-76.5 fL), HbA2 (4.4-5.4%), HbF (0.4-2.2%) had been reported as indicated. We detected IVS II-745 mutation in five patients. In these patients levels of Hb (9.5-11.1 gr/dL), MCV (55.3-64.9 fL), HbA2 (4.4-6.1%), HbF (0.0-4.0%) were determined. Three of these patients were accompanied by the heterozygous c31 C>T mutation. We did not find any significant difference in the erythrocyte indicators of these three cases in which intron and exon mutations were seen in combination. One of the first mutations identified and studied extensively is (CAG-TAG) in codon 39.[38] This is the second most frequent mutation that causes beta thalassemia in the Mediterranean population and accounts for the majority of β-thalassemia cases in Sardinia.[38] In this study, we detected this mutation, which constituted 60% of mutations in exon 2, in three patients.

In conclusion, 17 different mutations were detected in BTC children in and around Aydın province. Four most common mutations were heterozygous IVS I-110 G>A, IVS I-1 G>A, IVS I-6 T>C and IVS II-745 mutations. It was thought that these mutations are not solely responsible for the effects of mutations on complete blood count parameters and hemoglobin electrophoresis but other factors are also effective on these parameters. Heterozygous IVS I-6 (T>C), c.316-373 (IVS II-478 C>A), -87 C>T, IVS II 81 C>T mutations were observed in patients with normal HbA2 levels. Mutations of β° thalassemia in exon 1 can progress with high HbF level. It was thought that further studies on mutation analysis performed in patients with normal HbA2, and increased HbF levels (>5) will contribute to the literature.

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Original Article

Evaluation of Clinical Characteristics and Treatment Outcomes of Graves' Disease in Children and Adolescents

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ABSTRACT

Objective: *The most common cause of hyperthyroidism in childhood and adolescence is Graves' disease. In this study, we aimed to evaluate the demographic characteristics, clinical and laboratory findings, treatment processes, and remission outcomes in patients followed up with the diagnosis of Graves' disease.*
Methods: *Medical records of 44 patients who were diagnosed with Graves' disease in the period between 1999-2018 in our clinic, were retrospectively reviewed. Patients were included in the analysis according to risk groups (low, medium, and high) for relapses.*
Results: *The median age of the patients was 13.2 years (9.2-15 years) and 35 (79.5%) of them were females. The most common complaints at the first admission were palpitations (52.3%) and sweating (52.3%), and the most common physical examination finding was tachycardia (43.1%) followed by goiter (40.9%). Propylthiouracil was started in 23 (52.3%) and methimazole in 21 (47.7%) patients. In the clinical follow-up, five patients (11.4%) achieved remission while relapses occurred in none of the patients. Among the patients who did not achieve remission, total thyroidectomy or radioactive iodine treatment were applied to 10 (25.6%) and four (10.2%) patients, respectively. As for the risk groups, 10 patients (35.7%) were in the low-risk, 17 (60.7%) in the moderate-risk, and one patient (3.6%) in the high-risk group. Remission occurred in two patients (20%) in the low-risk and in three patients (17.6%) in the moderate-risk group with a median time to remission being 25 and 18 months, respectively.*
Conclusion: *In this study, remission rates were found to be low in pediatric Graves' disease in accordance with the literature. We showed that long-term anti-thyroid therapy can be used to increase remission rates and alternative treatment options can be preferred in patients who are non-adherent to treatment and who do not achieve remission.*

INTRODUCTION

Hyperthyroidism is a metabolic disorder characterized by increased synthesis and release of thyroid hormones.[1] The most common cause of hyperthyroidism in childhood and adolescence is Graves' disease, accounting for 10-15% of cases with thyroid diseases in this age group. The incidence of pediatric Graves' disease is approximately 1/10.000. Graves' disease is rare under the age of five and the peak incidence of the disease occurs in the age range between 11-15 years. The incidence of Graves' disease is 6-8 times higher in girls.[2] Thyrotropin-stimulating hormone (TSH) receptor antibody (TRAb) is generally found positive at the time of diagnosis and this finding is helpful for the diagnosis.[1]

Anti-thyroid drugs (ATD), surgery, and radioactive iodine (RAI) are current treatment options in Graves' disease.[3] The effectiveness of these three modes of treatment differs depending on the development of relapse and in association with emergent side effects in the short and long term. Although the ideal mode of treatment for pediatric hyperthyroidism is debatable, medical treatment is recommended as the first-line option.[4] The indications for RAI or

surgical treatment in Graves' disease include development of relapse after adequate ATD, non-adherence to medications, and ATD-related toxicity.[5]

The aim of this study was to evaluate the demographic and clinical characteristics, laboratory findings, treatment processes, and remission outcomes in children and adolescents; who were followed up with Graves' disease in our clinic.

MATERIALS and METHODS

The medical records of 66 patients who were followed up with the diagnosis of hyperthyroidism in our pediatric endocrinology clinic in the period between 1999 and 2018, were retrospectively reviewed. Patients' gender, age, complaints at the first admission, family histories of thyroid diseases, physical examination findings, laboratory findings, results of imaging studies, treatment methods, and treatment responses were recorded. After excluding patients with incomplete medical records, the study was conducted on 44 patients.

Anthropometric measurements

Height was measured with the Harpenden stadiometer with an accuracy of 0.1 cm, and body weight was measured using a scale (SECA, Hamburg, Germany) with a sensitivity of 0.1 kg while wearing light clothing. Standard deviation (SD) scores for weight, height, and body mass index (BMI) were calculated using the online calculator designed for pediatric endocrinologists (Child Metrics)[6], based on the reference values created for the Turkish population by Neyzi et al.[7] The pubertal stage was determined according to Tanner.[8]

Diagnosis and descriptions

As for hormonal evaluations, the levels of serum free T3 (fT3), free T4 (fT4), and TSH at the time of diagnosis were studied via the chemiluminescent microparticle immunological assay method using a Beckman Coulter Dxl800 brand device. The reference values for thyroid hormones as fT3, fT4, and TSH were 2.5-3.9 pmol/l, 0.5-1.51 pmol/l, and 0.38-5.33 µIU/ml, respectively. TRAb, as one of the thyroid autoantibodies, was measured via immunological assay method using an Immunotech Beckman Coulter A15728 brand device. The reference ranges for TRAb levels for the years between 1999 and 2009 were as follows: 0-9 U/l: negative, 9-14 U/l: borderline, and >14 U/l: positive. The reference ranges for TRAb levels for the years between 2009 and 2018 were as follows: 0-0.1 U/l: negative, 0.1-1.5 U/l: borderline, and > 1.5 U/l: positive.

The diagnosis of hyperthyroidism was made in the presence of increased serum fT3 and/or fT4 levels accompanied by TSH suppression (<0.38 µIU/ml) and clinical signs of hyperthyroidism. Patients; who had clinical and laboratory findings of hyperthyroidism (TRAb positive or negative) and who received ATD for at least six months, were considered to have Graves' disease.

Remission was defined as being in a state of clinical and biochemical euthyroidism for at least one year after the discontinuation of ATD and as the maintenance of the euthyroid state without relapses during the clinical follow-up period. Relapse was defined as the occurrence of TSH suppression accompanied by increased fT4 and/or fT3 levels and positive TRAb levels in the follow-up of previously remitted patients. Failure to achieve remission was defined as the continuation of ATD therapy, resumption of treatment due to maintenance of the euthyroid status for less than one year after discontinuation of ATD, discontinuation of ATD but the maintenance of the euthyroid status for less than a year in the study period/inadequate follow-up records, and in whom received RAI or undergone surgery.

The final clinical conditions of the patients were examined by classifying them into three groups based on the duration of ATD therapy as less than 1 year, for 1-2 years, and more than 2 years, and on the outcomes as achieving remission, the occurrence of relapses, and failure to achieve remission. In addition; according to the study by Kaguelidou et al.[9], the patients were divided into risk groups based on the clinical and laboratory findings, demographic characteristics at diagnosis, and the estimated duration of ATD treatment. The patients were scored according to five criteria including ethnicity, age at diagnosis, the fT4 level at diagnosis, the TRAb level at diagnosis, and the duration of treatment. Based on the scores; the patients were classified as Group

A (low-risk group, 0-3 points), Group B (moderate-risk group, 4-7 points), and Group C (high-risk group, 8-11 points). Then, remission and relapse states were examined by the groups.

Ethics

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Local Ethics Committee (Ethics approval number: 2017/18-13), and an informed written consent form was not obtained due to the retrospective nature of the study.

Statistical Analysis

Statistical analyses were performed using the SPSS 24.0 software (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). The homogeneous distribution of the data was evaluated by the Kolmogorov-Smirnov test. For descriptive statistics, numbers and percentages were used for categorical variables; mean±standard deviation for numerical data conforming to the normal distribution parameters, and the median values (25p-75p) were used for numerical data not conforming to the normal distribution parameters.

RESULTS

The median age of the patients was 13.2 years (9.2-15 years) and 35 (79.5%) of them were females. Twenty-three of the patients (52.3%) were pubertal and the frequency of thyroid disease in the family history was 75%. Weight, height, and BMI SD scores were -0.17 [(-1.2)-(+1.2)], 0.17 [(-1.2)-(+1.2)], and -0.24 [(-1.3)-(+0.5)], respectively. The most common complaints were palpitations and sweating, and the most common physical examination findings were tachycardia and goiter. The complaints and clinical findings of the patients are summarized in Table 1. Laboratory and imaging findings of the patients at the time of diagnosis are presented in Table 2.

Propylthiouracil (PTU) and methimazole (MTZ) treatments were initiated in 23 (52.3%) and 21 (47.7%) patients, respectively. Propranolol was given additionally in 33 patients (75%) due to concurrent tachycardia. L-thyroxine was added (block and replace treatment model) to the treatment in 30 (68.1%) patients due to high TSH levels detected during the clinical follow-up. Side effects associated with ATD were 4-5 times elevated transaminase levels in two (4.5%) and leukopenia in one patient (2.3%).

The median duration of the follow-up of the patients was 32 months (22-55 months). Five patients (11.4%) achieved remission during the clinical follow-up period and the median time to remission was 22 months (11.0-26.5 months). No relapses occurred in any of the remitted patients during the follow-up.

Table 1. The complaints and clinical findings of the patients at the time of diagnosis (n=44)

Complaints [n (%)]		Clinical findings [n (%)]	
Palpitations	23 (52.3)	Tachycardia	19 (43.1)
Sweating	23 (52.3)	Goiter	18 (40.9)
Weight loss	17 (38.6)	Hypertension	9 (20.5)
Tremor	15 (34.1)	Exophthalmus	7 (15.9)
Irritability	13 (29.5)	Pre-hypertension	1 (2.3)

Table 2. Laboratory and imaging results of the patients at the time of diagnosis (n=44)

Laboratory results		Thyroid ultrasonography [n (%)]	
TRAb positive [n (%)]	37 (84)	Heterogeneity	20 (45.4)
TSH (µIU/mL)	0.01 (0.0045-0.017)	Hypoechogenicity	15 (34.1)
fT3 (ng/dL)	13.0 (7.9-21.3)	Pseudonodular pattern	8 (18.2)
fT4 (ng/dL)	3.8 (2.9-5.6)	Diffuse hyperplasia	5 (11.4)
		Normal	4 (9.1)
		Nodule	3 (6.8)

Data are e given as median values (25p-75p).

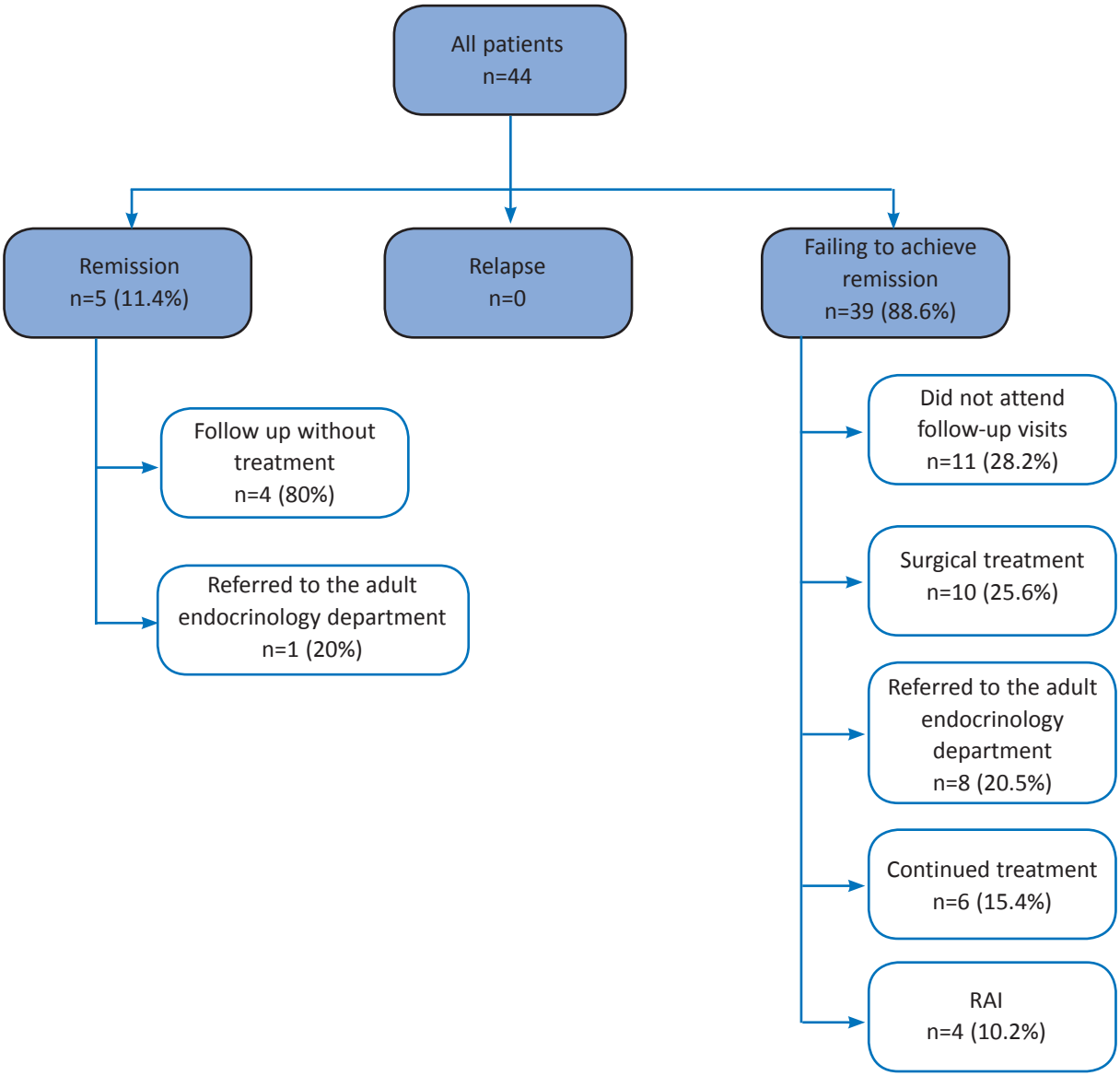

Figure 1. Final status of the patients when classified according to the remission and relapse rates (n=44)

The final status of the patients who failed to achieve remission are shown in Figure 1.

When the final status of the patients was classified according to the duration of medical treatment, two out of three patients who received treatment for less than one year and two out of 25 patients who received treatment for more than two years were found to be in remission (Figure 2).

When 28 patients with no missing data were evaluated by the risk groups; it was found that 10 patients (35.7%) were in the low-risk group, 17 patients (60.7%) in the moderate-risk group, and one patient (3.6%) in the high-risk group. In the low-risk group, two patients (20%) achieved remission; the median time to remission was 25 months, and none of the patients relapsed. In the moderate-risk group, three patients achieved remission (17.6%); the median time to remission was 18 months, and none of the patients relapsed. There was only one patient in the high-risk group; however, the final status of the patient could not be assessed because this patient did not attend follow-up visits (Table 3).

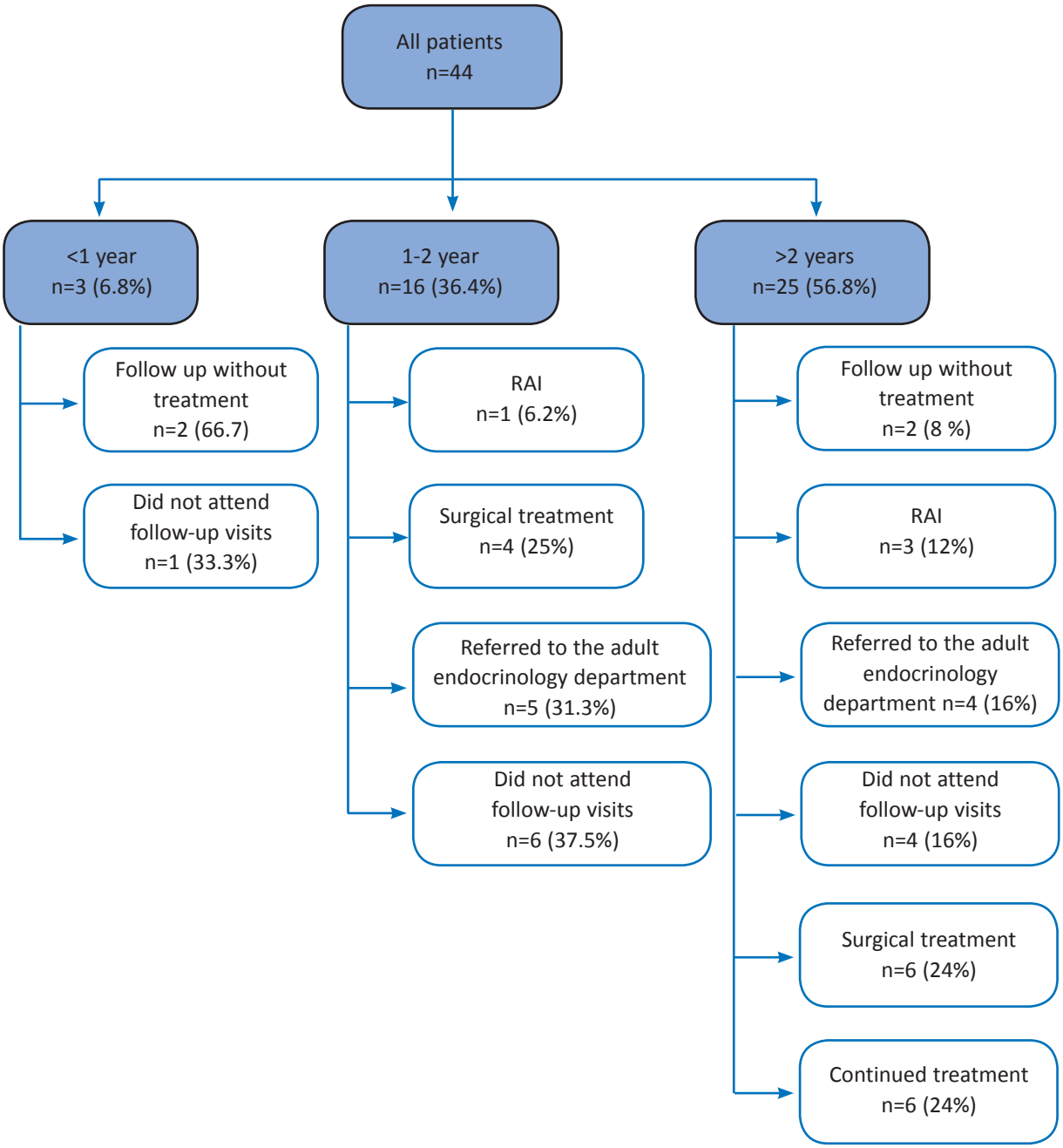

Figure 2. Final status of the patients when classified according to the duration of medical treatment (n=44)

DISCUSSION

In this study, we presented children and adolescents with Graves' disease with a female/male ratio of 4/1. The majority (52.3%) of our patients were pubertal at admission and the median age at diagnosis was 13.2 years (9.2-15.0). More than half of the patients presented with palpitations. These results were all in accordance with the previous studies.[12,10-18] It has been reported that Graves' ophthalmopathy is an inflammatory disease of the orbital tissues with prevalence rates ranging from 17% to 86% in children and adolescents with Graves' disease.[19-22] In recent studies from our country, the incidence of ophthalmopathy has been reported in the range of 10-24%.[12,13] We found that ophthalmopathy was

Table 3. Risk groups of the patients and clinical follow-up results (n=28)

Risk group	n (%)	Clinical follow-up results	n (%)
Low	10 (35.7)	Remission (%) Relapse (%) Median time to remission (month)	2 (20) - 25
Moderate	17 (60.7)	Remission (%) Relapse (%) Median time to remission (month)	3 (17.6) 0 18
High	1 (3.6)	Remission (%) Relapse (%) Median time to remission (m)	- - -

present in approximately one-sixth (15.9%) of the patients, as reported in the literature. Since the diagnosis of ophthalmopathy is usually based on the physical examination, the varying frequencies in the literature may be associated with individual descriptions of clinicians.

TRAb is specific for Graves' disease. TRAb positivity is detected in more than 95% of untreated newly diagnosed patients with Graves' disease.[23] Some studies demonstrated the frequency of TRAb positivity in a range of 68-92%.[12,13] The prognostic value of pretreatment TRAb levels has been shown in recent studies.[24,25] In the study conducted by Kaguelidou et al.[26], it was found that TRAb levels in children with a diagnosis of Graves' disease were significantly higher in five-year-old or younger patients at the time of diagnosis compared to those older than five years of age, and in patients with severe baseline symptoms compared to those without. In our study, TRAb was positive in 84% of the patients. Although the cause of hyperthyroidism in Graves' disease is antibodies that stimulate the thyroid gland, the TRAb levels were reportedly very low or undetectable in a small number of patients.[27] Theoretically, TRAb should not be negative in Graves' disease but the low sensitivity of laboratory methods may be responsible for false-negative results.

Treatment options for Graves' disease in children include ATD, RAI, and surgery. Each of these treatment approaches is associated with specific risks.[13,17,28] ATD is the first-line treatment option preferred in children in most countries.[29] However, ATD is associated with a variety of minor and major adverse effects. Fumarola et al.[30] associated the side effects of MTZ therapy with the dose of the medication but they reported that the adverse effects of PTU treatment were independent of dosage. Rivkees et al.[31] showed liver failure due to PTU in 14 children. Hepatic failure due to PTU is rapidly progressive and usually irreversible. In 2009, the United States Food and Drug Administration (FDA) recommended that PTU should not be used in children.[32,33] In another study, MTZ-associated adverse effects were found in 19% of the patients. The most common side effects were reported as skin rash and urticaria, while neutropenia and lymphopenia were found in low frequencies as 1-2%.[34] Rabon et al.[11] demonstrated that 21% of patients using MTZ developed adverse effects, including skin rash, arthralgia, increased transaminase levels, and neutropenia in order of decreasing frequency. Bayramoğlu et al.[14] reported that 22.9% of the patients using MTZ had side effects. They reported that 4.1% of the patients had a severe skin reaction that caused the discontinuation of MTZ therapy, 10.4% had increased levels of transaminases, and 8.2% had neutropenia.[14] Tunç et al.[13] reported that ATD-associated side effects occurred in two patients (4.4%) receiving PTU. Of these patients, one had increased levels of transaminases and the other developed skin rash.[13] In this study, we found that ATD was administered to all Graves' disease patients as the first-line treatment. As for the physicians' preferences for selecting an ATD, it was observed that treatment with PTU was started in more than half of the patients. We interpreted that this finding has resulted from reviewing patient data of previous years for the study but such adverse effect studies have become

more common recently. Adverse effects observed in our study were slight increases in transaminase levels in two and mild leukopenia in one patient. One patient with leukopenia and one patient with increased transaminase levels were both using PTU; which was replaced by MTZ. The other patient with increased transaminase levels was using MTZ. Although antithyroid therapy with MTZ was considered safe in pediatric patients, these patients should be carefully monitored for the potential adverse effects.

When remission cannot be achieved via medical treatment or when medication-associated serious side effects develop, other alternative treatment methods, which are RAI and surgery, should be considered. The American Thyroid Association recommends that radical treatment options should be considered in cases where remission cannot be achieved after two years of ATD treatment in children.[35] Gastaldi et al.[36] reported that 55% of the patients failed to achieve remission continued ATD treatment for more than two years, 25% underwent surgery, and 19% underwent RAI. Esen et al.[12] showed that 14.9% and 12.8% of the patients; who developed relapses after the discontinuation of ATD, underwent RAI and surgery, respectively. Another study reported that 47% of the patients receiving ATD developed relapses after drug discontinuation and 19% and 9.5% of these patients underwent surgery and RAI, respectively.[13] In this study; when the patients were examined in three groups according to the duration of ATD therapy, it was found that surgery (22.7%) was preferred over RAI (9.1%) as a second-line therapy option in non-remitters consisting of patients who received ATD for 1-2 years (n=16:36.4%) and more than two years (n=25:56.8%).

There is no consensus on the optimal duration of ATD treatment to ensure long-term remission in children. Tunç et al.[13] emphasized that radical treatment can be delayed even if remission is not achieved after two years of ATD therapy. They reported remission and relapse rates as 53.4% and 46%, respectively. They also noted that the total duration of ATD treatment is longer in remitted patients (42.14±14.35 months) which was an important predictor for the likelihood of long-term remission.[13] Esen et al.[12] found the cumulative remission rate as 17.6% and the median duration of treatment as 22.8 months (0.3-127) in patients who received ATD as the first-line therapy. In our study, the remission rate was 11.5% and none of the patients developed a relapse. The median length of ATD therapy before remission was 22 months (11.0-26.5). In a multicenter prospective study by Kaguelidou et al.[26]; 154 children diagnosed with Graves' disease were followed up for the development of relapses during the 1st and 2nd year after 24±3 months of MTZ treatment and it was reported that the relapse rates were 59% and 68% in the first and second years after discontinuation of ATD, respectively. The point to be noted here is the different definitions of remission and relapse among studies which can explain the different remission and relapse rates in our study and in other studies in the literature.

There are several studies investigating the predictive factors for remission or relapses and examining the necessity of early-stage radical treatment. Kaguelidou et al.[9], classified children with Graves' disease into prognostic risk groups based on the clinical and laboratory findings, demographic characteristics, and the estimated duration of ATD therapy. Patients were categorized into three groups as Group A (low risk), Group B (moderate risk), and Group C (high risk). The relapse rates in the two years after discontinuation of medical therapy were 46%, 77%, and 98% in Groups A, B, and C, respectively.[9] Leger et al.[37] reported that a total of two years of ATD therapy in children was associated with increased remission rates. Ohye et al.[38] reported a relationship between cumulative remission rates and the duration of treatment. Bayramoğlu et al.[14] reported remission in 24% of the patients who received treatment for more than two years; they reported that lower fT4 values and longer durations of MTZ therapy were associated with remission, but male gender was a factor for increased risk of relapses. Mussa et al.[39] showed that low TRAb levels at the diagnosis and throughout ATD therapy were associated with remission. Gestaldi et al.[36] suggested that a high TRAb value was a predictive factor for the development of relapses. In this study, we divided patients into risk groups according to the criteria used in the study

by Kaguelidou et al.[9] and found remission in two patients (20%) in the low-risk group and three patients (17.6%) in the moderate-risk group. Since the patient in the high-risk group did not attend follow-up visits, no conclusions could be drawn about the final status of the patient. Also, none of our patients developed relapses. We found that remission rates were lower in pediatric Graves' patients but the relapse rate was lower in remitted patients. The low relapse rate in our patients was thought to be associated with the heterogeneous follow-up periods of our patients receiving ATD treatment. Also, a two-year follow-up period of remitted patients might be considered as a cause of insufficient data collection. However, the median lengths of ATD use before remission were 25 and 18 months in patients in the low and high-risk groups, respectively. Consistent with the literature data, this finding can be interpreted as indicating a low risk for relapse in patients who received long-term treatment with ATD.

CONCLUSION

In conclusion, we found that children and adolescents with Graves' disease had low remission rates similar to the previous reports and that medical treatment-associated complications were low and transient. Besides, long-term ATD therapy can be used to increase remission rates and that alternative treatment options can be preferred in patients, who are non-adherent to treatment and who do not achieve remission.

Ethics Committee Approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Local Ethics Committee (Ethics approval number: 2017/18-13).

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Original Article

Ventriculoperitoneal Shunt Infections in a Tertiary Center: 3 Years Experience

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ABSTRACT

Objective: *Ventriculo-peritoneal shunt infection is the most important complication of shunt applications. In pediatric age, shunt infections are associated with shunt dysfunction, requirement for shunt revision, neurodevelopmental delay, prolonged hospital stay, and high treatment costs. In this study, we aimed to evaluate the characteristics of shunt infections of our patients and to compare the differences between early and late infections, infections caused by staphylococci and other strains and infection that did and did not recur.*

Methods: *In this retrospective study, shunt infections treated in the Pediatric Infection Clinic of Hamidiye Etfal Training and Research Hospital between July 2008 and July 2011 were evaluated.*

Results: *Forty-seven shunt infections in 42 patients were evaluated. Congenital anomalies were the most common etiology of hydrocephalus and fever was the most common symptom of the patients. Higher rates of early shunt infections, shunt infections in early childhood and infections caused by staphylococci species were observed. Patients with infections caused by staphylococci species received shorter duration of antibiotherapy (p=0.024). Infections that recurred in the six months of follow-up had higher rates of positive blood cultures (p=0.022). There was no statistically significant difference between early and late-term shunt infections.*

Conclusion: *Shunt infections were evaluated in different aspects in our study. Direct colonization of the shunt catheter still seems to be most important cause for the shunt infections because early infections and infections caused by skin flora were more common in our patient group.*

INTRODUCTION

Ventriculoperitoneal shunt (VPS) is the most common treatment for hydrocephalus, but despite the improvements in surgical techniques and infection control, complications still remain a problem.[1] Among the complications of VPS application, VPS infections are of serious importance due to shunt dysfunction requiring revision[2] and shunt infections are also associated with an increase in the duration and cost of hospitalization, reduced intellectual performance, neurological dysfunction in children who already have risk of neurodevelopmental problems due to hydrocephalus.[1,2]

Infection rates after VPS applications are reported between 5% and 18%, and the recurrence rate of VPS infection increases up to 26%. Risk of shunt infection has a negative correlation with age. There is also an increased risk in premature babies, in patients who underwent shunt surgery with the indication of post-hemorrhagic hydrocephalus, in patients with history of shunt infection or cerebrospinal fluid (CSF) leakage and intraoperative use of neuro-endoscope. The risk of reinfection is reported to be increased in patients with shunt applied before the sixth months of age, in males, and in patients with intracranial hemorrhage.[3]

Coagulase-negative staphylococci which originate from the skin flora are the most common microorganisms that cause VPS infection. In addition,

gram-negative microorganisms originating from the abdomen can cause shunt infections and fungal infections which can also be seen in patients who receive long-term antibiotherapy.[4,5]

There are differences between centers in terms of the management of shunt infections. Combination of temporary removal of the shunt, application of extra-ventricular drainage (EVD) after removal of the shunt, and intraventricular antibiotherapy are also frequently applied treatment methods in addition to systemic antibiotherapy.[1,3]

In this retrospective study, we aimed to investigate the clinical characteristics of the patients diagnosed as VPS infection and to compare early and late shunt infections, infections caused by staphylococci and other microorganisms and infections that did and did not recur.

MATERIALS and METHODS

Patient Selection and Data Collection

In this study, patients who were hospitalized in the Pediatric Infectious Diseases Clinic of a tertiary center between July 2008 and July 2011 with the diagnosis of VPS infection were evaluated retrospectively. Ethics committee approval was not obtained as it was not obligatory for retrospective studies in 2011. Demographic data and clinical features were recorded from the medical records of the patients.

Diagnosis and treatment protocol for VPS infection

The diagnosis of VPS infection was made by evaluating the patient's history and physical examination findings together with the results of laboratory tests. At least one of the following criteria were accepted as shunt infection; (1) Positive CSF culture, (2) increased leukocyte count (WBC $>10^6/mm^3$), increased CSF protein level (protein> 40 mg/dL) and/or decreased CSF glucose value (CSF glucose <simultaneous blood glucose/2) with the presence of axillary fever higher than 38°C without any other focus), (3) abscess observed at the distal end of the shunt catheter or signs of peritonitis, (4) purulent discharge or signs of local infection at the shunt site.

By examining the patient records; absence of any symptoms of shunt infection and fever (axillary temperature >38°C) observed in the last 48 hours, two consecutive sterile CSF cultures, CSF glucose >20 mg/dL, CSF protein <200 mg/dL, and lack of growth of microorganisms in CSF gram staining were considered successful treatment.[8-10]

Categorization of shunt infections

The patients were grouped as early and late shunt infection, taking into account the time from insertion of shunt to the onset of shunt infection. Shunt infections within and after the first six months following the operation were defined as "early and late shunt infections", respectively. Shunt infections of the patients were evaluated in terms of microorganisms grown in CSF cultures by dividing them into two groups as infections caused by staphylococci and the other microorganisms. The patients were also divided into two groups according to recurrence of the shunt infection in the 6 months of follow-up after the treatment. All these groups were compared in terms of clinical and laboratory characteristics, costs and duration of hospitalization.

Statistical Analysis

SPSS 19.0 (Statistical Package for the Social Sciences) program was used for statistical analysis. Mean, median, standard deviation, ratios and first and third quartiles were used in the descriptive statistics of the data. Mann-Whitney test was used to compare the means between the groups. Wilcoxon test was used for repeated measurements within the group. In the analysis of proportional data, Fischer test was used when chi-square test conditions could not be achieved.

RESULTS

Forty-seven cases of VPS infections diagnosed in 42 patients were included in our study. In 42 cases, the etiologies of the hydrocephalus were spina bifida in 17 (40%), germinal matrix/intraventricular hemorrhage (IVH) due to prematurity in 11 (26%), congenital hydrocephalus in 7 (16%), bacterial meningitis in 3 (7%), encephalocele in 1 (2%) intracranial hemorrhage developed after the neonatal period in 3 (7%) patients. Fever (71%), nausea and vomiting (42%), and change in consciousness (16.6%) were the most common

Table 1. Demographic and clinical features of the patients
*(Values in normal distribution are given as mean±standard deviation. Values not normally distributed are given as median and quartile 1-3 and marked with *. Abbreviations: GM-IVH: germinal matrix -intraventricular hemorrhage)*

Age, months	5* (3-15)
0-6	54.7 (23)
6-12	16.6 (7)
12-24	21.4 (9)
>24	7.1 (3)
Gender n (%)	
Male	15 (35)
Female	27 (55)
Etiologies of Hydrocephalus n, (%)	
Spina Bifida	17 (26)
GM-IVH	11 (26)
Congenital hydrocephalus	7 (16)
Encephalocele	3 (7)
Intracranial hemorrhage	3 (7)
Meningitis	1 (2)
Time between shunt operation and onset of infection, days	172 * (20-220)
Early/Late infection n, (%)	
Early	34/47 (72)
Late	13/47 (28)
Complaints on admission %, (n)	
Fever	71.4 (30)
Nausea/Vomiting	42.8 (18),
Change in conscience	16.6 (7)
Seizure	14.2 (6)
Headache	11.9 (5)
Rubor at the site of shunt	11.9 (5)
Restlessnes	7.1 (3)[d]
Recurrence in follow-up % (n)	40 (19)
Mortality	3 (7)

complaints and 42% (n=18) of the patients had two or more complaints on admission. The demographic data and clinical characteristics of the patients are summarized in Table 1, and their laboratory features in Table 2.

Table 2. Laboratory findings of the patients
(Abbreviations: WBC: white blood cell, Neut: Neutrophils, Hb: hemoglobin, CRP: C-reactive protein, CSF: cerebrospinal fluid)

	On Admission	On Discharge
WBC /uL	16214±9321	10230±2809
Neut /uL	9083±7654	4346±2001
Hb mg/dL	9.9±1.2	10.1±1.1
Platelet /uL	503500±218300	346000±51400
CRP mg/dL	9.7±11.5	0.4±0.15
CSF Glucose mg/dL	24±19.5	36.3±29.4
CSF protein mg/dL	375.9±547.7	84.8±63.4
CSF cell count / mm^3	901±2287	20.1±38.5

The median time from VPS insertion to the onset of infection was 78 days (Q1-Q3; 21-217). Twenty-nine (70%) patients were evaluated as "early shunt infection" due to the development of infection within the first 6 months. In comparison of the groups as early and late VPS infection, there was a significant difference between groups in terms of patients' ages. Patients with early shunt infection was significantly younger (p<0.001) than the patients with late VPS infection (Table 3).

Considering the CSF cultures of the patients, 81% of the patients had positive CSF culture and the CSF culture remained sterile in 8 (19%) patients. The majority of the shunt infections (59%, n=25) were caused by gram-positive bacteria, 26% (n=11) of them by gram-negative microorganisms (Figure 1). The duration of antibiotherapy was significantly shorter in "staphylococcal shunt infections" compared to shunt infections caused by other microorganisms (p=0.024) (Table 4).

Table 3: Comparison of early versus late ventriculoperitoneal shunt infections
*(Values in normal distribution are given as mean±standard deviation. Values not normally distributed are given as median and quartile 1-3 and marked with *. Abbreviations: TL: Turkish Lira, CRP: c-reactive protein, CSF: cerebrospinal fluid)*

	Early infection Mean±SD / n / (%)	Late infection Mean±SD / n / (%)	p
Age (months)	*4 (2-12)	*27 (14-31)	<0.001
Cost (TL)	8965±8906	26402±36175	0.064
Hospitalization duration (days)	33.10±17.79	39.25±27.07	0.856
Average duration of antibiotherapy (days)	27.27±12.90	30.58±19.46	0.944
Neutrophil count (/uL)	9173±8766	8821±5509	0.707
Hemoglobin (gr/dL)	9.70±1.07	10.47±1.42	0.084
CRP (mg/dL)	8.62±9.44	13.69±16.57	0.824
CSF Protein (mg/dL)	385.33±444.35	179.58±119.81	0.427
CSF Glucose (mg/dL)	21.97±17.61	30.33 ±21.98	0.283
Need for intraventricular antibiotherapy, n (%)	13 43.3%	2 16.7%	0.103

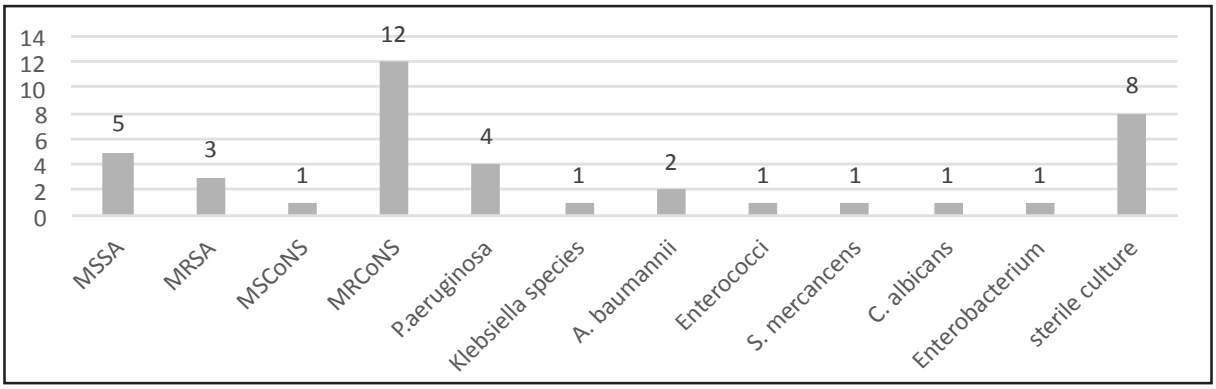

Figure 1. Microorganisms in cerebrospinal cultures
(MSSA: Methicillin- sensitive S. aureus, MRSA: Methicillin-resistant S. aureus, MSCoNS: Methicillin-sensitive coagulase-negative staphylococcus, MRCoNS: Methicillin- resistant coagulas- negative staphylococcus, P. Aeruginosa: Pseudomonas aeruginosa, A. baumannii: Acinetobacter baumanni, S. mercancens: Serratia mercancens, C. albicans: Candida albicans).

Table 4. Comparison of shunt infections caused by staphylocci versus other microorganisms

	Staphylococci Mean±SD / n / (%)	Other microorganisms Mean±SD / n / (%)	p
Cost (TL)	10359±10839	17209±28097	0.497
Hospitalization duration (days)	34.40±22.64	35.27±19.29	0.588
Average duration of antibiotherapy (days)	27.05±18.63	29.27±10.78	0.024
Neutrophil count (/uL)	9029.50±8334.87	9112.73±7695.52	0.910
Hemoglobin (gr/dL)	9.80±1.13	10.04±1.31	0.472
CRP (mg/dL)	7.08±9.55	12.78±13.37	0.166
CSF Protein (mg/dL)	199.70±155.04	441.86±496.45	0.262
CSF Glucose (mg/dL)	26.50±18.57	22.41±19.74	0.378
Need for intraventricular antibiotherapy, n (%)	13 (43.3)	2 (16.7)	0.103

Table 5. Comparison of ventriculoperitoneal shunt infections in terms of recurrence in 6 month follow-up
(EVD: Extra-ventricular drainage).

	Recurrence in 6 months follow-up		
	Yes	No	p
Duration of hospitalization (days)	41.1±39.2	35.9±20.8	0.831
Time between shunt application and onset of infection, days	*135.7±235.5	*274.9±408.4	0.738
Average duration of antibiotherapy (days)	32.3±24.0	27.4±12.6	0.536
Positive blood culture, n (%)	4 33.3%	1 3.6%	0.022
History of shunt infection, n (%)	6 50.0%	8 28.6%	0.193
Need for intratventricular antibiotherapy, n (%)	6 50.0%	9 32.1%	0.285

In the 6-month follow-up after the treatment, 40% (n=19) cases of the VPS infections recurred within 6 months. No statistically significant difference was observed between demographic data, clinical characteristics and laboratory values between the infection attacks that did, and did not recur. But blood culture positivity rates were significantly higher in the VPS infections that recurred (p=0.022) (Table 5).

DISCUSSION

Shunt infections still remain an important cause of morbidity and mortality in pediatric age.[1,11] Shunt infection is an important issue for the health system, considering the length of hospitalization, antibiotics applied and the need for shunt revision.[11] In our study, there was no significant difference between the subgroups of shunt infections in terms of

hospitalization cost.

The most common etiological factor in patients with shunt infection was reported as congenital anomalies.[1] In our study, the most common indication for shunt requirement was spina bifida, in accordance with the literature. When spina bifida, encephalocele and congenital hydrocephalus are evaluated together, it has been observed that congenital anomalies constitute 55% of our patients.

In our study, 71% of the patients were under one year of age, 55% of them were under six months of age and only 7% of them were older than two years of age. In the light of the recent literature, shunt infections are more common under 12 months of age, and it has been shown that the risk of shunt infection is three times higher in patients under 6 months of age.[12] Immaturity of the immune system has been considered as a reason for the increased risk of shunt infections in the early childhood. Morine et al.[13] also reported that the highest complication rate was in children under one year old. In this study, delayed wound healing, longer hospital stay and higher concentration of resistant microorganisms in the skin of infants were accused for higher rates of early shunt infections.

In our study, children under 2 years of age constituted 93% of all patients, consistent with the literature. There are two reasons for this result. Firstly, insertion of VPSs is generally required in a very early period of life. Congenital malformations, hemorrhagic disease of the newborn, germinal matrix-intraventricular hemorrhage due to prematurity, all of which constitute the majority of the indications for VPS applications. Even in the neonatal period shunting may be required.[14] Secondly according to the literature, VPS infections occur within the first 1-2 months after application of VPS[12,13], so that, it is thought that this is the reason why shunt infections were observed more frequently in early childhood.

According to data reported from Turkey[1], Portugal[14] and Korea[12], shunt infection most commonly develops in the first 1-3 months after the shunt procedure. In our study; 70% of shunt infections developed within the first six months in accordance with the literature, but there was no statistically significant difference between early and late shunt infections except the patients' ages. The patients diagnosed as early-onset shunt infection were significantly younger than the patients with late infection.

In previous studies, gram-positive bacteria and especially coagulase-negative staphylococci were reported as the most common cause of VPS infections.[1,12,14-16] Colonization of the shunt during application with direct skin flora is accused for the development of shunt infection. In our study, the most common strain in the CSF cultures of the patients was gram-positive microorganisms with a rate of 60%. In comparison of shunt infections caused by staphylococci and other strains, duration of antibiotic administration was found to be significantly shorter in infections due to *Staphylococci*. This result was thought to be due to microorganism resistance patterns in gram-negative and fungal infections leading to development of shunt infection, suggesting longer antibiotic administration in the treatment guidelines in infections caused by gram-negative strains.[16-18]

When the patients whose infections did, and did not recur in the follow-up after discharge were compared; the rate of microorganism growth in blood culture was significantly higher in patients with recurrence of shunt infection, than the group without. These data suggest that patients with positive blood culture had more severe and generalized infection[17], and the source of recurrent shunt infection in these patients was not only the colonization of the shunt catheter but also it may have had a different focus.

Our study should be interpreted within some limitations. Larger sample size would help to have more statistically significant results. Also a prospective design would provide a better follow up of the patients and chance to determine the risk factors for recurrence of the infection.

Despite advances in medical knowledge and practice, shunt infections continue to be an important complication in the treatment of hydrocephalus. Controversy still continues on the treatment management, risk factors and prevention of shunt infections.[18] Although antibiotic impregnated shunt applications are used to prevent shunt infections and

maximum infection control is applied during shunt application, the rates of shunt infection are not as low as desired.[19] It is thought that antenatal follow-ups and folic acid use to reduce anomalies such as spina bifida, which has an important place in the etiology of hydrocephalus[20], and practices to prevent germinal matrix-intraventricular bleeding in premature babies are considered to be of great importance since they will reduce the need for shunts in the society at the very beginning.[21]

Shunt infections, which were evaluated in many different aspects in our study, are one of the leading causes of central nervous system infections in childhood and, if not managed properly, may result in increased mortality and morbidity. Although shunt technology and intervention techniques have been developed in the last four decades, there is still no consensus on the prevention and management of shunt infections and their recurrence. Comprehensive studies on risk factors and treatment regimens to be carried out in cooperation with neurosurgeons and pediatricians are still required to establish a universal treatment protocol.

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Original Article

Age at Onset of Menarche and Puberty of Girls in Aydin Region and the Factors Affecting Them

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ABSTRACT

Objective: *In this study, we aimed to determine the range of mean age at onset of menarche (AOM) and puberty (AOP) of girls living in Aydin province and to determine the factors affecting the onset age of menarche.*

Method: *A total of 1891 girls aged between 8 and 16 years attending primary, secondary and high schools in Aydin province were planned to be included in the study. A questionnaire that was prepared in line with the literature was delivered to the parents in a closed envelope. The subjects who accepted to participate in the study were examined by an experienced physician by measuring height and weight and pubertal status was defined according to Tanner scale. BMI values were calculated.*

Results: *In total, 1520 female students were accepted to the study with the permission of their families. The mean AOM of participants was 12.11±1.32 years. The mean AOM was 13.12±1.46 years for their mothers, and 12.73±1.25 years for their sisters. June was the month that menarche occurred most frequently. We observed that the children living in rural areas had an earlier age of menarche. The mean age at onset of puberty was 9.71±1.46 years.*

Conclusion: *Our study is important in terms of being the first study conducted in Aydin province that determined the mean AOM and AOP of girls aged between 8 and 16. In our study, we showed that the age of menarche shifted to an early age, while the age of puberty did not shift. The age at onset of menarche and puberty were similar to the results obtained in other studies conducted in neighboring regions. We believe that larger scale studies may contribute to assess the actual mean age at menarche of girls living in Turkey.*

INTRODUCTION

The first menstruation, or menarche, is an important step of development and indicator of chronological age that shows biological maturity of a girl.[1,2] Previous studies have shown that the first menstrual experience is remembered negatively rather than being positive or neutral, which proves the importance of the first menstruation.[3] Menarche is one of the phases of sexual maturation in girls, and it is a physiological event that starts at about 8-9 years of age with changes in neuroendocrine system activity, followed by skeletal maturation, breast development, pubic hair growth, and that occurs within 1.3-1.5 years after rapid height spurt.[4] Studies have shown that many factors such as genetic factors, socio-economic status, nutrition and high-intensity physical activity can affect the age of menarche.[5]

Menarche follows changes called secondary sex characteristics. Normally, towards the end of adolescence, anovulatory and irregular cycles become menstrual, ovulatory and regular. As a result, complaints about the interval and length of the cycles are also reduced.[5,6] There is increasing evidence that the age at onset of menarche (AOM) has declined in recent years.[6,7] There is not enough information about what determines the age at menarche, and the underlying genetic mechanism is still not clear. The process of growth and development has accelerated and shifted to an earlier age. This is defined as the 'secular trend'. Malnutrition, chronic diseases, inadequate medical care, individual health,

genetic characteristics, race, environmental factors, and socio-economic and cultural status of the family may be effective on this shift.[7]

Menarche, as the beginning of the first menstrual period, is a very important turning point in a woman's life. This period is an important indicator of maturity in the evaluation of pubertal development.[8,9] The AOM is an indicator of the biological and social conditions of the society. It is an important event that occurs as a result of the sum of biological, environmental and social factors.[10] The AOM is significantly affected by genetic factors. In addition, it has been reported that it is highly related to the factors such as urbanization, socio-economic status, the number of children in families, nutrition, seasons, and physical activity.[11] Some researchers have reported that the AOM has declined in developed countries compared to previous years. Studies on the development of puberty in Turkish children are limited. In 1978, Neyzi et al. have reported the mean age at onset of puberty (AOP) in girls with higher socio-economic status living in Istanbul as 9.8±1.3 years, and the age of menarche as 12.4±0.1 years. In girls with lower socio-economic status, the age at menarche was 0.8-0.9 years later.[12] However, in a study by Ersoy et al., which has examined 1017 girls, no significant difference has been found between girls with higher and lower socio-economic status in terms of AOM (12.73±1.07 and 12.87±1.08 years).[13] In a study conducted in 2005, 3311 Turkish school-age children have been examined and it has been reported that breast development in girls started at 10.16 years, growth of pubic hair at 10.57 years, and menarche at 12.4 years.[14]

As the number of studies examining the age at onset of puberty and menarche in Turkish children and the factors affecting them are limited, it is not known exactly how many Turkish children have been affected by the shift of AOM and AOP, which is the secular trend. In this study, we aimed to determine the range of the mean age at onset of puberty and menarche in girls living in Aydin province and to determine the factors affecting the onset age of menarche.

MATERIALS and METHODS

Adnan Menderes University Ethics Committee approved the study. We first planned to include 1891 girls aged between 8 and 16 years who were attending primary, secondary and high schools in Aydin province, Turkey. The study was planned as a cross-sectional study, and it was conducted by using the multi-stage sampling method. Aydin province was divided into five regions (east, west, north, south and city center) by considering the socio-economic status and geographical settlement. One district from each region was selected by simple random sampling. One rural and one urban school (twenty-five schools in total) from the specified district were selected using simple random sampling. Students from the selected schools to be included in the study were stratified according to their grades. The students from each grade were determined from the student lists by using systematic sampling. The total number of students studying at the selected schools was 6028. The G-power program, a software that is used by the World Health Organization (WHO) to calculate the sample size for population-based researches, was used and the targeted sample size was calculated as 1891. After obtaining the necessary permissions for the study from the Office of the Governor and the Aydin Provincial Directorate of National Education, consent forms were obtained from the families of all children who participated in the study.

A 40-item questionnaire form, which was prepared in line with the literature, was delivered to the parents of the students in a closed envelope. The questionnaire form was consisted of questions such as socio-demographic information about the student and the family, the educational status of the parents, status of social security and monthly income, the AOM of the student, mother and sister, presence of bad habits such as alcohol or cigarette, exercise status, use of mass media, and factors related to puberty. Children with chronic diseases and those on chronic medication were excluded from the study.

An experienced physician who was knowledgeable about anthropometric methods measured the height and weights of the children using the same devices. Body mass index (BMI) (kg/m^2) was calculated for each and every child. The BMI and percentile curves for Turkish children were taken as reference. Moreover, the pubertal development of all cases was

visually determined according to Tanner classification by a female doctor alone, without completely undressing the student. All examinations were performed in a closed and separate room, which was deemed appropriate by the school administration. Girls with Tanner stage 1 breast development were considered prepubertal, and those with Tanner stage 2 breast development and above were considered pubertal.

Statistical analysis

Statistical analysis was performed using the Windows based SPSS 16.0 program. Kolmogorov-Smirnov test was used to determine whether the quantitative variables were normally distributed. The groups were compared using Mann Whitney U or Kruskal Wallis tests in terms of quantitative variables. Paired comparison tests were performed to determine the groups that showed difference after Kruskal Wallis analysis. Descriptive statistics were given as standard mean±standard deviation for all quantitative variables in order to fit the representation commonly used in the literature. For the analysis of categorical variables, Chi-square test was used, and descriptive statistics were given as frequency and percentage. Spearman correlation analysis was used to analyze the relationship between quantitative variables. Values of $p<0.05$ were considered statistically significant.

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RESULTS

A total of 1520 female students between the ages of 8 and 16, attending primary and high schools, were included in the study with their parents' permission. The mean age of 1520 children was 12.73±2.65 years. 133 children had stage 1 thelarche, the others were stage 2 or further. The mean AOP was 9.71±1.46 years, while the mean age at onset of pubic hair development was 10.71±2.16 years (Table 1). The BMI of the children in the stage 2 thelarche group, which we consider as the age at onset of puberty, was 17.40±2.76 kg/m^2, and the BMI of the prepubertal group was 15.85±2.27 kg/m^2 (p=0.008).

The AOP in children consuming fast-food was 9.66±1.53 years, and in those who did not consume fast-food was 10±0.93 years. The difference between them was statistically significant (p=0.032).

The comparison of physical exercise status showed that girls who did not exercise regularly entered puberty at a statistically earlier age than those who exercised regularly (p<0.001) (Table 2).

Among all participants, 874 children stated that they had menarche, and the mean AOM was 12.11±1.32 years. The mean menarcheal age of mothers of children who had menarche was 13.12±1.46 years. The mean AOM of the children was significantly lower than the mean AOM of their mothers (p<0.05). The mean AOM of the older sisters of 318 girls who experienced menarche was 12.73±1.25 years. The mean AOM of the children was significantly lower than the mean AOM of their sisters (p<0.05). June was the month that menarche occurred most frequently. Looking at the seasonality, summer was the season with highest rate of experiencing menarche (39.06%). The mean AOM of children living in rural areas was significantly earlier (11.75±1.21) than the children living in urban areas (12.15±1.32) (p=0.006).

Table 1. The mean age values of the children according to stages of thelarche and pubarche

	Age of thelarche (mean±sd)	Age of pubarche (mean±sd)
Stage 1	9.13±1.32	9.43±1.34
Stage 2	9.71±1.46	10.71±2.16
Stage 3	11.60±2.40	12.81±2.34
Stage 4	13.70±1.94	14.48±1.44
Stage 5	14.70±1.08	14.63±1.20
Total		1520

Table 2. Age of onset of puberty in children who exercising regularly and in those who do not exercise regularly

Status of regular exercise	Age of onset of puberty (mean±sd)	n	p
Doing exercise	9.96±1.40	50	<0.001
Not doing exercise	9.61±1.48	127	
Total		177	

When we compared the mean AOM and mother's education level, we found that children of illiterate mothers experienced menarche at an earlier age than children whose mothers were primary school graduates (p=0.046) and secondary school graduates (p=0.042) (p=0.003). The comparison between mean AOM and the social security status of the mother showed that the children whose mother had a social security coverage and whose socioeconomic status was better experienced their menarche at an earlier time in (p=0.002). When AOM and maternal marital status were compared, we showed that children of married mothers had their menstruation earlier than children of divorced mothers (p=0.005) and children of separated parents (p=0.003).

The comparison between AOM and delivery method demonstrated that the AOM of children born by cesarean section was earlier than those born by normal spontaneous vaginal delivery (p<0.001). When AOM and time of birth were compared in all groups, we found that children born postmature and premature experienced menarche at an earlier age (p=0.004 and p=0.017, respectively) than children who were born on normal time.

DISCUSSION

Since many years, it is well known that the growth-development process and the age of puberty are significantly affected by genetic and ethnic characteristics, as well as environmental factors such as geographical location, nutritional status and life style. Since the middle of the 19th century, the living conditions of people living in industrialized countries have improved and towards the end of the century, due to advancements in knowledge on nutrition and protection against infectious diseases, the children have been better nourished, protected from infections and ultimately improved. As this was a prominent development seen in the 20th century, it was called the secular trend, and it has affected the pubertal development as well as growth.[15]

Studies have shown that the mean AOM has declined in developed countries, and this decline has also been observed in developing countries in recent years, as well.[16] Environmental changes, better nutrition, and consequently physical development likely have led to this change. The decline in age of menarche in the last century is accepted as a positive indicator of the health status of the population.[17]

The most comprehensive study conducted in the United States of America (USA) is a cross-sectional study by Herman-Giddens et al.[18], involving 17,077 girls aged between 3-12 years. In this study, it has been shown that the AOP of African-American girls was earlier than the age of white girls, and according to previous USA data, puberty had shifted to younger ages.[19]

In studies conducted in Turkey, in 1975, Neyzi et al. have reported the age at onset of puberty as 9.8 years[20] and in 2011, Atay et al. have reported it as 9.65 years.[21] In our study, the AOP was 9.71±1.46 years. Although our result is similar to the Atay's study, it suggests the age of puberty has shifted to a younger age in the past 40 years. When we compared our results with the studies conducted in Europe, we found that the mean AOP was significantly lower.[22,23]

A recent study conducted in Europe in 2006, the age at onset of pubarche has been reported as 11.29±2.01 years.[23] In studies conducted in Turkey, in 1975, Neyzi et al. have reported the mean age at onset of pubic hair growth as 10.4 years[20], while Atay et al. have stated this age as 10.09 years.[21] In our study, the mean age at onset of pubarche was 10.71±2.16 years, and this result is similar to previous studies conducted in our country. We thought that the

reason for the lack of a significant change in the age at onset of pubarche over the years may be that other pathways and endocrine systems such as the adrenal gland might be effective in onset of pubarche other than the hypothalamic-pituitary-gonadal (HPG) axis.

A longitudinal study conducted in Sweden has shown that children with high BMI entered puberty at an earlier age.[24] In our study, BMI of girls with stage 2 thelarche was significantly higher than the prepubertal ones, and that the age at onset of puberty of the children consuming fast food was earlier than children who did not consume any fast-food. We already know that the increase in mean fat tissue in childhood has an effect on the onset of puberty, but consumption of fast food increases calorie density, resulting in greater energy intake.[25] Increased adipose tissue increases the aromatization of androgens to estrogens, and as BMI increases, the HPG axis is positively affected, thus shifting the age of puberty to an earlier time. In their study, Merzanich et al. have suggested that regular physical activities and active training increase energy expenditure, lower BMI and delay menarche.[26] In our study, we demonstrated that girls who do not exercise regularly enter puberty at a statistically earlier age than those who exercise regularly. Increased physical activity has an effect on menarche, changes in energy balance and body composition.[25,26] The increased physical activity decreases the body fat ratio and blood levels, and as a result, the menarche is delayed. This result suggests that the age at menarche can be delayed in case of regular physical activity.

In 1960s, Marshall and Tanner have reported the mean age of menarche as 13.5±0.1 years in girls living in England.[22] Wyshak et al. have shown that the AOM shifts 2-3 months earlier every 10 years in Europe.[16] In the study conducted by Neyzi et al. in 1975 in our country, the age of menarche has been found as 12.4±0.1 years. In their study, Neyzi et al. have also investigated the socio-economic status and they have found that as the socioeconomic status increased, the frequency of menarche in these age groups also increased.[20] In 2011, Atay et al. have reported the age of menarche as 12.74 years and it has been stated that the result of this study was almost the same with Neyzi's study, a study that was conducted 40 years ago.

In their study, Ersoy et al. have determined the mean age of menarche as 12.82±1.07 years.[13] In our study, mean age of menarche was 12.11±1.32 years. The results of our study are similar to previously conducted Turkish studies. It is clear that there may be regional differences in our country due to geographical, cultural and socio-economic diversifications. In addition, many immigrants are welcomed to our country from neighboring countries because of an ongoing civil war. Therefore, in order to find the true mean age of menarche in Turkey, similar studies should be conducted in other regions of Turkey.

The similarities between mother-daughter and sisters show the effect of hereditary factors on age of menarche. The similarity between sisters is generally more than the similarity between mother-daughter, and this situation reveals that the common interaction resulting from the shared family environment affects individuals from the same generation more than individuals belonging to different generations.[27]

In a study conducted by Al Alwan et al. in Saudi Arabia.[28], the mean AOM was 13.08±1.10 years, and the mother's menarcheal age was 13.67±1.40 years. It has been shown that girls experience menarche at an earlier age than their mothers. In our study, the mean AOM was significantly lower than the mean AOM of older sisters and mothers, which shows the effect of genetic factors on AOM. Considering the fact that AOM of children are significantly lower than the mean AOM of mothers and older sisters, we can suggest that the mean AOM of Turkish girls have shifted to a younger age. A similar result obtained in our study depends on the facts that the living conditions of children have gradually improved, they have got better health care services and their socio-economic status have converged. Due to the similar living conditions and genetic characteristics of the sisters, the mean AOM has come closer to each other and shifted to an earlier time than the menarcheal age of their mothers.

Many studies conducted in Europe have shown that menarche is associated with study tempo at school and holiday periods. The frequency of menarche increases during or with the onset of holidays.[29] The stress experienced due to school activities may have a negative effect on puberty and on the contrary,

puberty is positively affected during the holiday periods when students relax. Matchcock et al. have shown that the most frequent month for menarche occurrence was June (14.5%), followed by January (12%).[30] In our study, June (19%) and February (11%) were the months that menarche occurred most frequently. Throughout the year, summer was the season with most frequent menarches (39.06%). Considering that June is the month when summer holiday starts and February is the month when winter break starts, we can conclude that menarche frequency increases in holiday seasons and this may be a result of relaxation of children after intense school pace and exam stress.

Today, while the secular trend continues in developing countries, it has come to a halt in many developed countries.[31] There are differences between the rural and urban residents of the same country as well as between countries. In a study conducted in 2005 and covering different geographical regions of China where 92.757 girls between the ages of 9-18 were included, it has been shown that the AOM was 12.76 years in rural areas and 12.60 years in the city. The AOM has been found to be earlier for girls living in the city than in rural areas. Our study has demonstrated that the mean AOM of children living in rural areas was earlier than children living in the city. We can attribute this to the fact that the living conditions of rural and urban areas are getting close to each other, children continue their school education in larger schools, and grow up in the same environment.

In a study investigating the age of menarche in Poland and examining both retrospective and prospective data, age of delayed menarche was found to be associated with low educated parents (especially for mother) and poor performance at school.[32] In our study, the age of menarche was found to be earlier in the group whose mother did not attend any school. Considering the living conditions of Turkey, mother having the basic education and increased awareness should positively affect the children, leading to delayed menarche in children of these mothers.

In a study conducted about 20 years ago in high schools in Istanbul, the mean age at menarche was 12.58±1.04 years in the high socio-economic status group, 13.03±0.89 years in the middle socio-economic status group, and 13.33±1.10 years in the low socio-economic status group.[33] In our study, we demonstrated that the AOM was earlier in children with a higher family socioeconomic status. The AOM may have shifted to an earlier time because of the family's social security, economic freedom and better living standards. In addition, the increase in prevalence of obesity and BMI may also have contributed to this shift.

Toromanovic et al. have demonstrated that the age of menarche of girls with single or divorced mothers were earlier than girls with married mothers.[34] In a study by Kurdzielewicz, it has been shown that children with broken families had delayed menarchy.[32] It has been suggested that the high pressure in these families affect the HPG axis. In our study, the AOM was found later in children with separated parents. In a healthy and safe environment, where the children grow with their parents, hitting puberty and timely functioning of the HPG axis help menarche begin within the physiological period. We think that the stress experienced by children with separated parents puts pressure on the HPG axis, which may cause a delay of menarche.

Ruder et al. have compared the age at menarche and birth characteristics, and have found that a birth weight below 500 g was associated with delayed menarche (approximately 2.7 months).[35] In our study, children born at term experienced menarche at a later age than children born pre and post-term. We can suggest that the AOM of children who have completed intrauterine life in its normal process and physiology is later than the AOM of others. This situation can be interpreted as stimulation of the HPG axis during the growth of premature babies. In the future, more information can be obtained on this subject if studies are carried out to reveal the age at onset of menarche of premature babies.

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Case Report

Chryseobacterium indologenes as a Rare Pathogen of Bacteremia in Febrile Neutropenia

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ABSTRACT

Chryseobacterium indologenes (C. indologenes) is nonmotile, oxidase-, and indole-positive gram-negative aerobic bacillus. Immunosuppression, comorbidities, use of broad-spectrum antibiotics are known risk factors for C. indologenes-related infections. We report a neutropenic fever caused by C. indologenes in a 16-month-old boy who was treated due to the neuroblastoma. According to the antimicrobial susceptibility test result, he was treated with cephaperazone/sulbactam.

INTRODUCTION

Chryseobacterium indologenes (C. indologenes) is aerobic, non-fermentative, non-motile, oxidase- and indole- positive, Gram-negative bacillus.[1] *C. indologenes* is known to cause different types of infections such as bacteremia, pneumonia, meningitis and shunt infection, especially in patients hospitalized long-term with indwelling devices and using long-term broad-spectrum antibiotics. *C. indologens* can also be the cause of serious infections in immunocompromised patients. With increased use of colistin and tigecycline against carbapenem- resistant microorganisms such as *Acinetobacter baumannii, Escherichia coli* and *Klebsiella pneumoniae*, there is an increase in the incidence of infections associated with *Chryseobacterium* spp.[1,2] Herein, we describe a neuroblastoma patient with febrile neutropenia who had a bloodstream infection due to *C. indologenes* which was successfully treated with cephaperazone/sulbactam.[1,2]

CASE REPORT

A 16-month-old boy was admitted to our hospital with complaints of high fever, weakness and pallor. On physical examination, a 2 cm palpable mass on the right upper quadrant was detected. An adrenal mass was detected on the right side of abdomen on abdominal computed tomography. The patient was diagnosed with neuroblastoma and the neuroblastoma protocol of Turkish Pediatric Oncology Group,

including A9 (vincristine 1,5 mg/m^2 on days 1 and 5; dacarbasine 200 mg/m^2 on days 1-5; Ifosfamid 1500 mg/m^2 on days 1-5; adriamycin 30 mg/m^2 65 on days 4 and 5) and A11 (cyclophosphamide 300 mg/m^2 on days 1-5, etoposide 80 mg/m^2 on days 1-4; cisplatin 30 mg/m^2 on days 1-5) was started alternately for each 21 days.

Febrile neutropenia was diagnosed after the fourth course of treatment. The patient underwent a detailed physical examination and blood culture was obtained before starting antibiotic and cephaperazone/sulbactam monotherapy was begun. Vancomycin was added to the treatment after the fever persisted for 72 hours. On the fifth day of fever, *C. indoligenes* was isolated using the BACTEC 9120 system (Becton-Dickinson Diagnostic Systems, USA). Identification and antimicrobial susceptibility testing of the isolate was performed using the Vitek2® system (bio-Mérieux, France) according the recommendation of the Clinical and Laboratory Standards Institute.[3] According to the antibiotic susceptibility test result, treatment regimen was not changed as *C. indologens* were sensitive to cephaperazone/sulbactam. The patient became afebrile on the seventh day of cephaperazone/sulbactam treatment and treatment was completed before any complications developed.

DISCUSSION

Children treated with solid tumors or hematological malignancies have an increased risk of infections. In these patients, the incidence of bacteremia is still reported as 10-40%, and mortality rates reach to 9-24% due to serious complications.[4,5]

With the developing modern culture systems, previously unidentifiable microorganisms have become identifiable and in many infectious diseases, new microorganisms have been reported as causative agents. One of them is *C. indologenes*, a member of the *Chryseocabterium* spp. The SENTRY Antimicrobial Surveillance Program is a worldwide study that monitors the susceptibility and resistance of bacteria and fungi to antibiotics based on results from more than 119 sentry hospitals and laboratories in North America, Latin America, Europe and the Asia-Pacific region. According to the data of this study, which was carried out between 1997 and 2001, *Chryseobacterium* spp. appear to be a rare pathogen representing only 0.27% of non-fermentative Gram-negative bacilli and 0.03% of all bacterial isolates collected from adults and children. In addition, in this study, it was reported that *Chryseobacterium* spp. can only be isolated in 0.10% of the culture samples taken from the respiratory tract and 0.03% of the blood culture samples.[2,6]

Bacteremia due to *C. indologenes* is becoming increasingly common.[7] It has been reported that bacteremias due to *C. indologenes* are associated with nosocomial pneumonias, biliary tract infections, peritonitis, urinary tract infections, surgical wound infections, cellulitis, intravascular catheter-related bacteremia and primary bacteremia.[2,8] Also it has been reported that most cases with *C. indologenes* sepsis, have either severe underlying diseases such as malignancy or diabetes mellitus, either taking long-term broad-spectrum antibiotic therapy or using indwelling devices.[9,10]

C. indologenes has been rarely reported to be a causative agent in febrile neutropenia in children. In their studies evaluating *Flavobacteriaceae* bacteria in children, Cooper et al found that nine out of the 13 flavobacteiaceae growths in the last 20 years were *C. indologenes* [(11)]. Five of nine cases with growth of *C. indologenes* in their culture media were immunosuppressive patients (two patients with acute lymphoblastic leukemia and one patient each with hemophagocytic lymphohistiocytosis, aplastic anemia and acute myeloid leukemia) in that study. Although it was reported that three cases had neutropenic fever, it was thought that leukostasis in T-ALL patient with fever at the stage of diagnosis may be due to the presence of lymphoblasts in peripheral blood, although it was not mentioned in the article, a total of four cases had neutropenic fever. Only one of these five patients was not catheterized and diagnosed with febrile neutropenia. To our knowledge, *C. indologenes* were not identified as the causative agent of febrile neutropenia, except in the cases mentioned in this study. This shows us that *C. indologenes* are rarely seen among microorganisms that cause bacteremia in febrile neutropenia. In our case, *C. indologenes* was isolated from blood cultures, and our case had not central catheter,

which was among the risk factors for *C. indologenes* bacteremia.

An empirically effective drug selection in infections caused by *C. indologenes* is difficult due to the limited antimicrobial susceptibility of the organism. Studies have shown that *C.indologenes* is susceptible to piperacillin-tazobactam, piperacillin, cefoperazone, ceftazidime, cefepime, cefpiroma, minocycline, rifampicin, TMP-SMZ and new fluoroquinolones (garenoxacin, gatifloxacin, levofloxacin); conversely, resistant to extended- spectrum penicillins, first and second generation cephalosporins, carbapenems, ceftriaxone, aztreonam, ticarcillin clavulonate, chloramphenicol, erythromycin, clindamycin, aminoglycosides, tetracycline and teicoplanin. *C. indologens* can produce a variety of β-lactamase species, which contribute to multiple antibiotic resistances.[12]

We started the treatment with cefaperazone/sulbactam, which is an antipseudomonal antibiotic as empirically recommended in febrile neutropenia guidelines.[13] As our patient did not have a blood culture result at 72 hours and his fever continued, vancomycin, an antibiotic in the glycopeptide group, was added to the treatment as stated in the guidelines. On the 5th day of the treatment, the treatment was continued without antibiotic change due to bacterial sensitivity to cefaperazone/sulbactam according to the blood culture and antibiogram results and the treatment was successfully completed without any complications.

In our case, cephaperazone/sulbactam treatment, which was started empirically, was continued after the antibiogram showed its antimicrobial sensitivity and the treatment was successfully completed without any complication. We think that blood culture results should be monitored closely and treatment options should be adjusted according to antibiotic susceptibility in these patients.

In conclusion, this case shows us that although the majority of *C. indologenes* infections are associated with indwelling catheter use, non-catheter-related bacteremia can also be a causative factor, and *C. indologenes* should be considered as a possible cause of febrile neutropenia in children.

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Case Report

Ophthalmoplegia in the Acute Phase of Hemolytic Uremic Syndrome: A Case Report

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ABSTRACT

Hemolytic uremic syndrome (HUS) is a common form of thrombotic microangiopathies. Among its extrarenal complications, ocular involvement is very rare.
We present a patient with HUS, whose first symptom was isolated abduction deficits in the eyes. Lethargy was added during the clinical course, suggesting neurological involvement. Although conventional magnetic resonance imaging was normal, symmetric diffusion restriction was detected in bilateral putamen on diffusion-weighted images. Treatment with peritoneal dialysis, fresh frozen plasma infusions, and eculizumab was initiated. The patient responded well to the treatment and was discharged with excellent neurological, hematological, and ophthalmological outcomes. Nephrological follow-up is being continued due to proteinuria
To our knowledge, presenting with ophthalmoplegia in the acute phase of hemolytic uremic syndrome is very rare. The patient's ophthalmological and neurological symptoms improved after eculizumab treatment. We suggest that eculizumab is effective in the acute phase of HUS in cases of ophthalmological involvement.

INTRODUCTION

Hemolytic uremic syndrome (HUS) is a common form of thrombotic microangiopathy. It is characterized by hemolytic microangiopathic anemia, thrombocytopenia, and acute renal failure.[1,2] New researches on genetic mutations on the alternative pathway of the complement ensured a better understanding of the underlying causes.[3,4] As a result of these researches, the classification changed. The new classification is based on pathophysiological considerations and triggering factors.[3] The initial management of HUS is supportive, based on appropriate electrolyte and fluid management, erythrocyte, and platelet transfusions when indicated. Dialysis should be initiated in the presence of symptomatic uremia, azotemia, severe fluid overload, or electrolyte abnormality that is refractory to medical therapy. Plasmapheresis and eculizumab are other treatment options in atypical forms. Since the complement-regulated abnormalities play a key role in the mechanism of atypical-HUS, eculizumab is used as a highly effective therapy, which is a monoclonal C5-inhibitor antibody.[4]

The most common extrarenal complication is neurological involvement, and magnetic resonance imaging (MRI) is the most sensitive diagnostic technique especially in the diagnosis of non-hemorrhagic central nervous system (CNS) lesions. Main conventional-MRI findings in children are symmetric lesions of

basal ganglia (BG) or extensive cortical and/or subcortical lesions, which may be reversible or not.[5] Although features of conventional-MRI findings are well known, abnormalities of diffusion-weighted images (DWI) have been reported in only a few cases. Of the extrarenal complications, ocular involvement is infrequent. Previous ocular findings are retinal, choroidal, and vitreal hemorrhages and ischemic signs like cotton wool spots, retinal whitening, and non-perfusion zones.[6] In the literature, a case presented with isolated ophthalmoplegia in the acute phase and diffusion restriction in the BG without conventional-MRI abnormality has not been reported yet.

Herein we reported a four-year-old boy with atypical-HUS who responded well to eculizumab. The patient's first symptom was isolated ophthalmoplegia, and DWI revealed bilateral symmetric diffusion restriction in putamen without conventional-MRI abnormality.

CASE REPORT

The informed consent was taken from the parents. Four days before the presentation to our clinic, a four-year-old boy had been hospitalizing due to abdominal pain and vomiting in another hospital. He was referred with acute ophthalmoplegia. Physical examination revealed hypertension (130/90 mmHg), bilateral periorbital edema, and abduction deficits in the eyes (Figure 1a). Fundus examination revealed no abnormality. Laboratory investigations showed elevated blood urea nitrogen (BUN), creatinine and uric acid, proteinuria, hemolytic anemia, and thrombocytopenia (Table 1). Peripheral blood smear revealed schistocytes, fragmented erythrocytes, and thrombocytopenia. Hemolytic anemia, thrombocytopenia, acute renal injury indicated HUS. In 24-hour urine collection, nephrotic-range proteinuria (279 mg/m²/h) was detected. ADAMTS-13 activity was revealed higher than 10%, and the stool testings for *Escherichia coli (E.coli)* O157: H7 and polymerase chain reaction (PCR) for Shiga Toxin-producing E. Coli (STEC) were negative. Fresh frozen plasma (FFP), sulbactam-ampicillin, and allopurinol treatments were started. Short-acting and long-acting calcium-channel-blockers were administered due to severe hypertension. On the second day, lethargy was developed, and brain-MRI was performed. DWI revealed bilateral symmetric diffusion restriction in putamen compatible with cytotoxic edema (Figure 2a-b), while conventional-MRI and MRI-angiography showed no ab-

Figure 1. (a) Abduction defisits in the eyes, (b) Improvement of abduction deficit in the eyes after eculizumab treatment (Informed consent was taken from the guardian)

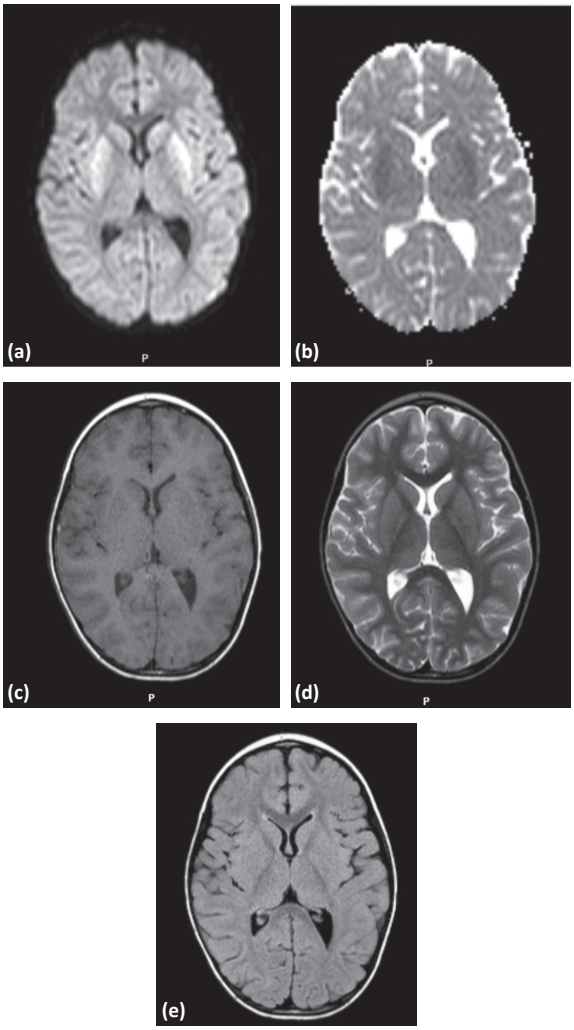

Figure 2. (a) Restricted diffusion of bilateral putamen as high signal on DW isotropic image and (b) low signal on ADC map (c) Normal conventional MRI findings, T_1 weighted axial images, (d) Normal T_2 weighted axial images, (e) Normal FLAIR-weighted images

Table 1. Laboratory results

	Laboratory results at the time of admission to the hospital	Laboratory results at the time of discharge
Hemoglobin	8,7 g/dL	9.4 g/dL
White blood cell	11.39x10³/uL	8.09x10³/uL
Platelets Count	48000/mm³	417000/mm³
Blood Urea nitrogen	62.15 mg/dL	35.51 mg/dL
Creatinine	3.28 mg/dL	0.65 mg/dL
Total bilirubin	2.77 mg/dL	0.57 mg/dL
Indirect bilirubin	2.11 mg/dL	0.43 mg/dL
Lactate dehydrogenase	2640 U/L	227 U/L
Uric acid	9.2 mg/dL	5 mg/dL
Alanine aminotransferase	154 U/L	15 U/L
Aspartate aminotransferase	67 U/L	30 U/L
C3	1.29 mg/dL	
Urine protein	+++	++
Urine hemoglobin	+	-

normality (Figure 2c-d-e). Given the negative results of stool PCR and culture, atypical-HUS was thought. Due to the neurological involvement of atypical-HUS, after vaccination of meningococcus, eculizumab was administered according to pediatric dose recommendations. Peritoneal dialysis (PD) was initiated on the day of 5, owing to anuria and progressive azotemia. The patient's ophthalmoplegia (Figure-1b) and neurological symptoms improved on the day of four, after eculizumab. After eculizumab, six times of erythrocyte transfusion, 15 days of FFP infusions, ten days of allopurinol, and 12 days of PD, the patient was discharged with normal hematological, ophthalmological, and neurological findings (Table 1). Nevertheless, since persistent proteinuria, nephrological follow-up is being continued.

DISCUSSION

The extrarenal complications of HUS involve the gastrointestinal system, extremities, heart, lung, eyes, and CNS. Neurological involvement is the most frequent extrarenal complication and occurs in about 20-50% of HUS patients. The pathophysiology of neurological involvement is unclear, though a toxin-mediated vasculopathy in small vessels is considered to be the postulated mechanism.[7] The most common signs of neurological involvement are seizures, visual disturbances, alterations of consciousness, hemiparesis, and brain-stem symptoms. The prognosis is variable, and predicting the neurologic outcomes is difficult in the acute phase. Clinical outcomes may be favorable even in patients with severe MRI findings, except for hemorrhagic lesions. Although computed tomography is a sensitive tool, especially for hemorrhagic lesions, it may be normal in the acute phase in cases of non-hemorrhagic lesions. MRI is more sensitive to the neurological involvement of HUS. Non-hemorrhagic conventional-MRI findings are symmetric BG involvement compatible with reversible edema, cortical/subcortical patchy lesions, and T2-hyperintensity lesions in white matter, especially in parieto-occipital areas, known as posterior reversible encephalopathy syndrome.[7,8] Previous studies on DWI suggested that DWI might ensure the early detection of imaging abnormalities in the neurological involvement of HUS. However, the presence of early diffusion abnormality findings was not valuable in predicting neurological outcomes.[9,10] The patient had symmetric diffusion restriction in bilateral putamen on DWI with normal conventional-MRI findings in the acute phase. To our knowledge, this was the first HUS patient who had normal conventional-MRI findings with symmetric diffusion restriction in BG. This finding may support the view of DWI might ensure the detection of the earliest non-hemorrhagic CNS lesions. It is known that early use of eculizumab improves neurological outcomes.[11] Maybe the administration of eculizumab therapy contributed to the favorable neurological outcomes.

Ocular involvement is a rare extrarenal complication, and it is reported in 4% of all pediatric HUS cases. Cases reported to date regarding ocular involvement consist of vitreous hemorrhage, retinal hemorrhage, retinal artery/vein occlusion, ischemic retinopathy, neovascularization, choroidal hemorrhage, and/or optic atrophy, premacular hemorrhage, and purt-

scher retinopathy. Only one case was reported with ophthalmoplegia who had also had optic disc edema and was seen after improvement of acute atypical-HUS. The probable mechanism was asserted as thrombotic microangiopathy, which affected the optic nerve head as papillitis, and inferior rectus muscle as ophthalmoplegia, and the patient's eye findings benefitted from intravenous dexamethasone.[12] Sturm et al.[13] reported their three cases with ocular involvement (purtscher retinopathy and intraretinal hemorrhages) of HUS that two of them developed vision loss during the follow-up. As we browsed through the treatments, all of them had PD, two of them had occlusion therapy for amblyopia, and one of them had multiple sessions of pan-retinal laser photocoagulation, which was not successful. One of them who had intraretinal hemorrhages, and was treated with the only dialysis was observed complete recovery in the visual functions. David et al.[14] presented a 23-year-old woman who had bilateral severe retinal detachment during the clinical course of HUS. Since treatments with plasmapheresis, hemodialysis, and systemic steroids provided partial systemic and ocular recovery, systemic weekly eculizumab was added, resulting in complete recovery. Interestingly, our patient's first symptom was ophthalmoplegia with normal fundoscopic examination findings and he responded well to eculizumab.

To our knowledge, presenting with isolated ophthalmoplegia in the acute phase of atypical-HUS is very rare. Neurological and ophthalmological findings were ameliorated after eculizumab therapy. The probable mechanisms of ophthalmoplegia are thrombotic microangiopathic involvement of external rectus muscles, abducens nerves, or brain-stem. We suggest that isolated ophthalmoplegia may be seen in the acute phase of HUS, and eculizumab may ensure favorable outcomes in both ocular and CNS involvement.

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