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CASE REPORT

Is genetic counseling important in hemoglobin H disease?

Hemoglobin H hastalığında genetik danışmanlık önemli midir?

Yusuf Döğüş[1], Petek Çürük[1], Mehmet Akif Çürük[1]

[1]Çukurova University Medical Faculty Department of Biochemistry Adana, Turkey

Abstract

Alpha thalassemia is a genetic disease characterized by insufficient expression or definite absence of the α-globin chain. Three large deletions (thal-1; 26.5 kb or MedII, 20.5 kb and 17.4 kb or MedI) and two small deletions (thal-2; 4.2 kb and 3.7 kb) have been characterized in our country. In addition, two different PolyA mutations (PA1: AATAAA>AATAAG and PA2: AATAAA>AATGA) on the α2-globin gene (αα/α^{PA}α), 5nt deletion (αα/α^{5nt}α), and unstable Hb variant (CD 59; GGC→GAC) synthesized by the α1-globin gene (αα/αα^{CD59}) have been reported. More than ten different combinations of α-thal-1 and α-thal-2 (--/-α) or HbH genotypes with point mutations (--/α^{PA}α or --/αα^{CD59}) were determined. In this study, which was carried out in Çukurova region, it is aimed to emphasize the importance of giving genetic counseling to families with alpha thalassemia carriers and to determine genotype combinations. DNA was isolated from blood samples taken from 5 children and their families who were admitted to Çukurova University Balcalı Hospital and diagnosed with severe anemia (Hb <9, MCV <70) as a result of blood count HbA2 values were measured by HPLC. Gene deletions were determined by multiplex PCR. When two families with two children are compared, the fact that the second child of the family who receives genetic counseling service is a carrier and the second child of the family who does not receive genetic counseling service is patient highlights the importance of genetic counseling service.
Keywords: HbH, alpha thalassemia, gene deletion

Öz

Alfa talasemi, α-globin zincirinin yetersiz ekspresyonu veya kesin yokluğu ile karakterize edilen genetik bir hastalıktır. Ülkemizde üç büyük delesyon (tal-1; 26.5 kb veya MedII, 20.5 kb ve 17.4 kb veya MedI) ve iki küçük delesyon (tal-2; 4.2 kb ve 3.7 kb) karakterize edilmiştir. Ayrıca α2-globin geninde (αα/α^{PA}α), 5nt delesyonunda (αα/α^{5nt}α) ve iki farklı PolyA mutasyonu (PA1: AATAAA>AATAAG ve PA2: AATAAA>AATGA) ve α1-globin geni (αα/ααα^{CD59}) tarafından sentezlenen kararsız Hb varyantı (CD 59; GGC→GAC) bildirilmiştir. α-thal-1 ve α-thal-2 (--/-α) veya nokta mutasyonlu (-/α^{PA}α veya --/ααα^{CD59}) HbH genotiplerinin ondan fazla farklı kombinasyonu belirlenmiştir. Çukurova bölgesinde yapılan bu çalışmada alfa talasemi taşıyıcısı ailelere genetik danışmanlık verilmesinin öneminin vurgulanması ve genotip kombinasyonlarının belirlenmesi amaçlanmaktadır. Çukurova Üniversitesi Balcalı Hastanesi'ne başvuran ve kan sayımı sonucunda ağır anemi (Hb <9, MCV <70) tanısı alan 5 çocuk ve ailesinden alınan kan örneklerinden DNA izole edildi. HbA2 değerleri HPLC ile ölçüldü. Multipleks PCR ile gen delesyonları belirlendi. İki çocuklu iki aile karşılaştırıldığında, genetik danışmanlık hizmeti alan ailenin ikinci çocuğunun taşıyıcı, genetik danışmanlık hizmeti almayan ailenin ikinci çocuğunun ise hasta olması genetik danışma hizmetinin önemini vurgulamaktadır.

Anahtar kelimeler: HbH, alfa talasemi, gen delesyonu

INTRODUCTION

Hemoglobinopathies caused by structural changes in the polypeptide chains of the hemoglobin molecule or synthesis disorders are the most common inherited blood diseases in the world. Hemoglobinopathies are divided into two main classes abnormal hemoglobins and thalassemias. Abnormal hemoglobins are formed as a result of the displacement of amino acids in the normal hemoglobin chain, various fusions, and mutations. Thalassemia occurs when the globin chains in the structure of the hemoglobin molecule cannot be

Address for Correspondence: Yusuf Döğüş, Çukurova University Medical Faculty Department of Biochemistry Adana, Turkey E-mail: ydogus@cu.edu.tr
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synthesized as a result of mutations or are synthesized insufficiently[1].

Alpha thalassemias are a common hereditary disease characterized by a decrease in the alpha globin chain involved in the structure of the hemoglobin molecule or not being synthesized at all. In healthy people, there are four genes (αα/αα), and two alpha globin genes (α1 and α2) on each chromosome[2]. Normal alpha genes (αα/αα), lack of one gene α^+ (-α/αα), missing two genes α^0 (--/αα), missing three genes (--/-α), and it is expressed as deficiency of four genes (--/--). The absence of one alpha globin gene (-α/αα) is known as a silent carrier. The absence of two alpha globin genes (--/αα) is referred to as alpha thalassemia trait or carrier status. The absence of three alpha globin genes (--/-α) leads to a condition known as hemoglobin H disease. Finally, the deficiency of all four alpha globin genes (--/--) results in a severe condition called hydrops fetalis. Hydropsy fetalis is incompatible with life. While deletional mutations often cause alpha thalassemia, there are also non-deletional mutation types[1-4]. Five different deletions [α-thal-1 (MED I:-17,4 kb, MED II:-26,5 kb, and 20,5 kb) and α-thal-2 (-3,7 kb and -4,2 kb)], two different poly A mutations, in an α2- globin gene 5 nt deletion and a point mutation in the α1- globin gene (codon 59: GGC → GAC) it is reported in Turkey[1,5].

Hemoglobin H (HbH) disease is characterized by the loss of three functional alpha-globin chains (--/-α) as a result of the coexistence of α^+ thalassemia and α^0 thalassemias resulting in a point mutation or small deletion or insertion of one of the alpha globin chains. In addition to the type that occurs only with deletions, there are less common HbH genotypes. These include co-inheritance of deletional (-) and non-deletional (α^T) disorders (--/-α^T) or homozygous ($\alpha\alpha^T$/ $\alpha\alpha^T$or$\alpha^T\alpha/\alpha^T\alpha$) of nondeletional disorder[6-9].

Patients with HbH mostly show the clinical picture of thalassemia intermedia. However, depending on the variety of mutations, the clinical course varies. These patients have microcytosis, and mild to moderate anemia with varying hemoglobin levels between 8-10 g/dL. Hypochromia is seen in the erythrocytes in the blood picture[10]. Apart from these, complications such as splenomegaly, gallstone formation, folic acid deficiency, and leg ulcers have been reported in individuals[11]. With these diseases, a blood transfusion may be required in individuals with HbH.

Genetic counseling was given to the families of 5 children diagnosed with hemoglobin H disease in this study, which was carried out in the Çukurova region in the south of Turkey. The purpose of the counseling was to inform families about Hb H disease and its effects on the health of their later children. The importance of providing genetic counseling to families against possible HbH disease is emphasized.

MATERIALS AND METHODS

The approval of the ethics committee of Çukurova University Faculty of Medicine was obtained. The study started with the approval of the Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Decision Number: 87, Date: 5.04.2019). Consent was obtained from all individuals participating in the study. The blood samples of the patients who applied to Çukurova University Balcalı Hospital were studied. Later, after finding siblings with hemoglobin H disease, the study was continued. Blood samples were taken from the families of children with hemoglobin H. The families were asked whether they received counseling services or not. No personal information about the patients was shared with anyone. Five mL of blood taken into tubes with EDTA was brought to the laboratory following the cold chain rules. Hemolysates were prepared and measurements were made to measure HbA2 values in the HPLC. DNA isolation was performed from the blood samples of the patients. Genomic DNA samples (Roche Cat No: 1179682801, USA) were obtained with a genomic DNA isolation kit. DNA samples were stored in a refrigerator (+4^{0}C) until the study day. Alpha thalassemia deletions were examined in DNA samples by single-tube multiplex PCR[12].

CASES

In this study, mutation types and hematological data of four families are given in Table 1. As a result of genetics counseling given to some families after their first child was sick, their second child was born as a carrier.

1st family; mother 32 years old (-$\alpha^{3,7}$/αα), father 37 years old (--^{20,5}/αα), first child 14 years old (-$\alpha^{3,7}$/--^{20,5}), second child 8 years old (-$\alpha^{3,7}$/αα). The family did not receive pre-marriage counseling. After learning that their first child had hemoglobin H, she received genetic counseling service against the

possibility of her second child having hemoglobin H. Their second child was born with the mother's mutation (-$\alpha^{3,7}/\alpha\alpha$).

2nd family; mother 36 years old (-$\alpha^{3,7}/\alpha\alpha$), father 43 years old (--$^{20,5}/\alpha\alpha$), first child 14 years old (-$\alpha^{3,7}$/--^{20,5}), second child 5 years old (-$\alpha^{3,7}$/--^{20,5}). The family did not receive counseling before the marriage. After learning that their first child had hemoglobin H, she refused to receive counseling services in case her second child was sick. The second child carries the mother and father's mutation together (-$\alpha^{3,7}$/--^{20,5}).

3rd family; mother 26 years old (--$^{20,5}/\alpha\alpha$), father 29 years old (-$\alpha^{3,7}/\alpha\alpha$), first child 7 years old (--$^{20,5}/\alpha\alpha$), second child 7 years old (--$^{20,5}/\alpha\alpha$) the third child is 1 year old (-$\alpha^{3,7}$/--^{20,5}). She did not receive pre-marital genetic counseling. The first and second children are fraternal twins. Twins were born with a mother's mutation (--$^{20,5}/\alpha\alpha$). She did not receive genetic counseling after her twins were born. The family learned that they had alpha thalassemia carrier when their third child (-$\alpha^{3,7}$/--^{20,5}) was born.

4th family; mother is 25 years old (-$\alpha^{3,7}/\alpha\alpha$), the father is 30 years old (--$^{MedI}/\alpha\alpha$), and the child is 1 year old (--^{MedI}/-$\alpha^{3,7}$). After the family learned that their first child, who did not receive pre-marriage counseling, had hemoglobin H, they received genetic counseling service against the possibility of their future child having hemoglobin H.

Table 1. Hematological and mutation characteristics of family members

Family	Names	Sex-Age	RBC	HB	HCT	MCV	MCH	MCHC	HbA2	HbF	Mutations
1st family	Father	M-37	5.96	13.2	50.9	85.4	22.1	25.9	2.3	2.4	--$^{20.5}/\alpha\alpha$
	Mother	F-32	4.47	12.5	46.8	104.7	28.0	26.7	1.6	1.5	-$\alpha^{3.7}/\alpha\alpha$
	1st child	M-14	5.22	09.7	40.1	76.8	18.6	24.2	0.9	1.2	-$\alpha^{3.7}$/--$^{20.5}$
	2nd child	F-8	4.60	11.5	43.2	93.9	25.0	26.6	1.8	1.7	-$\alpha^{3.7}/\alpha\alpha$
2nd family	Father	M-43	5.25	11.0	41.4	78.9	21.0	26.6	2.5	1.3	--$^{20.5}/\alpha\alpha$
	Mother	F-36	5.26	13.5	46.2	87.8	25.7	29.2	2.2	0.7	-$\alpha^{3.7}/\alpha\alpha$
	1st child	M-14	5.87	09.5	36.4	62.0	16.2	26.1	1.7	0.9	-$\alpha^{3.7}$/--$^{20.5}$
	2nd child	F-5	5.14	08.8	33.1	64.4	17.1	26.6	3.3	2.1	-$\alpha^{3.7}$/--$^{20.5}$
3rd family	Father	M-29	5.11	14.0	42.5	83.2	27.4	32.9	1.9	1.3	-$\alpha^{3.7}/\alpha\alpha$
	Mother	F-26	5.78	10.1	36.0	62.3	17.5	28.1	1.5	1.1	--$^{20.5}/\alpha\alpha$
	1st child	M-7	5.47	10.9	36.5	66.7	19.9	29.9	2.0	0.8	--$^{20.5}/\alpha\alpha$
	1st child	M-7	5.41	10.8	35.8	66.2	20.0	30.2	1.8	0.9	--$^{20.5}/\alpha\alpha$
	2nd child	M-1	5.64	08.6	31.4	55.7	15.2	27.4	1.2	3.1	-$\alpha^{3.7}$/--$^{20.5}$
4th family	Father	M-30	6.11	13.7	48.3	79.1	22.4	28.4	1.7	1.5	--$^{MedI}/\alpha\alpha$
	Mother	F-25	5.20	13.3	44.3	85.2	23.6	30.0	1.8	1.1	-$\alpha^{3.7}/\alpha\alpha$
	1st child	F-1	5.27	08.9	34.5	65.5	16.9	25.8	2.1	2.4	--^{MedI}/-$\alpha^{3.7}$

DISCUSSION

Hemoglobin H disease is caused by defects in 3 out of 4 alpha genes found in healthy people. HbH (--/-α) is compatible with life. The clinical picture of this disease is generally similar to that of thalassemia intermedia. However, clinical signs differ from patient to patient. While some patients may need intermittent or frequent transfusions, some patients do not require blood transfusions[1-3].

HbH disease is suspected in case of unresponsiveness to iron replacement therapy and findings of microcytic anemia in complete blood counts and peripheral smears. In the first decade of the disease, most patients do not need erythrocyte transfusion. The diagnosis is made when 5-30% HbH is detected in Hb electrophoresis. Since the HbH molecule is unstable, the blood taken from the patient should be loaded on Hb electrophoresis within 30 minutes. Patients with HbH disease also have 20-40% Hb Barts in the evaluation of cord blood[13,14].

HbH disease is a severe form of anemia but is compatible with life. Regular blood transfusions are rarely required. Patients with HbH disease have severe hypochromia with low MCV and MCH values. Infection or treatment with oxidant drugs worsens anemia during pregnancy[15].

In this study; The first children of families number 1 and 2 were born with HbH, and after their child was born, they learned that they were also carriers of alpha thalassemia. The first family received genetic counseling. The second family did not receive genetic counseling. their second child; is a carrier in the first family. The second family had a child with HbH. The importance of getting genetic counseling in their second pregnancy is understood.

The presence of 12 different genotypes was determined in an individual with 32 HbH. The most common genotype among these genotypes is $(-\alpha^{3,7}/--^{20,5})$[1]. Another study helps detect genetic heterogeneity for Turkey in the south[16]. In this study; The genotype of four children with HbH is $(-\alpha^{3,7}/--^{20,5})$ and one $(--^{MedI}/-\alpha^{3,7})$.

In a study conducted by Shiqiang et al.; it has been found that patients with HbH disease in Guangxi have unique gene profiles. Deletion alpha thalassemias were found to be the most common in patients with 615 HbH. In addition, the rate of association of β-thalassemia with HbH disease was found to be high. The hematological data of HbH disease of different genotypes are very different and the hematological data of hemoglobin H disease of the same genotype have also changed significantly. However, when there is concomitant thalassemia, most patients have reduced symptoms due to the balancing of the α and β chains. Therefore, it was emphasized that these features should be considered in the clinical and prenatal diagnosis of anemia[17].

Hama et al. suggested that most patients with HbH disease do not need a lifelong blood transfusion. Some patients with Hb below 6 g/dL depend on blood transfusion. These patients require high-volume blood transfusion and iron chelation therapy. This is a portion of patients who initially should receive blood transfusions, and some may need blood transfusions later with anemia complications. Guangxi region, children with severe thalassemia prevention to reduce the birth of thalassemia pregnant women to provide free prenatal diagnosis and control plan has been developed. According to this control plan; Anemia, HbH is only one symptom of the disease. Each patient's age at the time of diagnosis to assess the overall clinical picture, transfusion requirements, and other factors should also be considered, such as jaundice and hepatosplenomegaly. HbH disease risk for all couples, in the prenatal diagnosis of the thalassemia prevention plan, and according to experts it is necessary to make genetic counseling[18].

Preimplantation genetic diagnosis (PGD) is widely used in many IVF (In Vitro Fertilization) centers worldwide in thalassemia. PGD enables couples with a genetic disease to have healthy children without exposing them to conditions such as invasive prenatal diagnosis and termination of pregnancy[19]. However, there are different approaches for prenatal diagnosis and families to be referred to PGD due to reasons such as the variety of mutations in α-thalassemia, and differences in gene expression. Many physicians consider the possibility of having a child with transfusion-dependent thalassemia as a criterion when recommending prenatal diagnosis and PGT to individuals[20]. Therefore, genetic counseling should be given to families who are likely to have children with HbH.

This work is done; Families who do not receive genetic counseling before marriage, learn that their children have HbH, which shows the situation of their other children and their future children after receiving genetic counseling. The importance of pre-marital genetic counseling is emphasized.

More comprehensive studies are needed to detail the relationship between α-globin gene mutations and phenotype to provide more accurate genetic counseling to individuals with α-thalassemia carriage. In this study, mutation types and hematological data of four families with children with hemoglobin H are given in Table 1. As a result of genetics given to some families after their first child was sick, their second child was born as a carrier. The importance of genetic counseling is emphasized. We aimed to contribute to the literature by sharing the data of these four families.

It seems inevitable that the rapid advances in molecular genetics in the last 10 years will make genetic testing an integral part of physicians' daily practice in the coming years. For this reason, cooperation between the physician who diagnoses and monitors the patient and the geneticist in the clinic is becoming increasingly important to provide accurate, reliable, and effective genetic counseling to

families. This study is expected to improve genetic counseling, raise awareness and improve premarital screening.

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