

Abnormal hemoglobins associated with the beta-globin gene in Antalya province, Turkey

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Aim: Abnormal hemoglobins are the most common hemoglobinopathies after beta-thalassemia in the world. More than 40 abnormal hemoglobin variants have been reported in the Turkish population. Therefore, it is one of the target areas for carrier screening. In our study, we aimed to screen the abnormal hemoglobins that cause clinical thalassemia in the Antalya population.

Materials and methods: The present study identified the abnormal hemoglobins associated with the beta-globin gene using different molecular genetic techniques following high performance liquid chromatography (HPLC) results. We studied 972 postnatal and 361 prenatal cases (total: 1333 cases, 2666 chromosomes) with the disorder from 1998 up to July 2008. DNA extraction from peripheral blood, chorionic villi samples (CVS), amniotic cells, and cord blood samples was carried out using standard procedures. Following polymerase chain reaction (PCR) and amplification of the beta-globin gene, allele refractory mutation system (ARMS), reverse dot blot hybridization (RDBH), Nanochip, and DNA sequencing were performed to identify the mutations. Variable number of tandem repeats (VNTR) analysis was used for elimination of maternal contamination in prenatal diagnosis. We identified and characterized abnormal hemoglobin variants with novel and rare beta-thalassemic mutations.

Results: Seven different abnormal hemoglobins were found in Antalya province, Turkey, namely Hb Antalya, Hb Tyne, HbS, Hb G-Coushatta, HbE, Hb Knossos, and Hb D-Los Angeles.

Conclusion: Some of the abnormal hemoglobin variants found are unstable and some of them cannot be detected by simple electrophoretic examinations. Our findings suggest that abnormal hemoglobins are more frequent than expected among the other abnormal hemoglobins found in Turkey, and it is important both to perform prenatal diagnosis and to give genetic counseling for abnormal hemoglobins to families at risk for thalassemia.

Key words: Abnormal hemoglobins, Antalya, beta-thalassemia, Turkish population

Antalya ilinde beta-globin geni ile ilişkili anormal hemoglobinler

Amaç: Anormal hemoglobinler dünyada beta-talasemiden sonra en sık görülen hemoglobinopatilerdir. Türk toplumunda 40'dan fazla anormal hemoglobin varyantı bildirilmiştir. Bu yüzden de taşıyıcı taramaları hedef alanlardan biridir. Çalışmamızda, Antalya toplumunda klinik olarak talasemi fenotipine neden olan anormal hemoglobinleri taramayı amaçladık.

Yöntem ve gereç: Bu çalışma, yüksek performanslı sıvı kromatografisinin sonuçlarını takiben farklı moleküler genetik teknikler kullanarak, beta-globin geni ile ilişkili anormal hemoglobinleri tanımladı. Çalışma, 1998- Temmuz 2008 tarihleri arasında 972 doğum sonrası ve 361 doğum öncesi olmak üzere 1333 olguda, 2666 kromozom incelenerek gerçekleştirildi. Genomik DNA periferik kan, koryon biyopsi, amniyotik hücreler, ve kordon kanlarından standart yöntemlerle elde edildi. Beta-globin geninin polimeraz zincir reaksiyonu (PCR) ile çoğaltılmasını takiben, alelik mutasyonu yansıtan sistem (ARMS), ters dot blot hibridizasyon (RDBH), Nanoçip ve DNA dizileme yöntemleriyle mutasyonlar tanımlandı. Doğum öncesi tanıda belirlenen mutasyonlarda, anneye ait kontaminasyonu dışlamak için değişik sayıda dizi tekrarları

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(VNTR) yöntemi kullanıldı. Biz, yeni ve nadir mutasyonlarla ilişkili anormal hemoglobin varyantlarını tanımladık ve karakterize ettik.

Bulgular: Çalışmamızda ilk defa ve nadir görülen yedi farklı anormal hemoglobin varyantı; Hb Antalya, Hb Tyne, HbS, Hb G-Coushatta, HbE, Hb Knossos ve Hb D-Los Angeles tespit edildi.

Sonuç: Sonuç olarak, anormal hemoglobinlerin bazıları stabil değildir ve basit elektroforetik incelemelerle tespit edilemezler. Türkiye'de tanımlanmış anormal hemoglobinlerden yedi tanesinin Antalya'da bulunması, beklenenden sık olduğunu göstermektedir. Anormal hemoglobinler talasemi riski olan ailelerde doğum öncesi tanı ve genetik danışmada oldukça önemlidirler.

Anahtar sözcükler: Anormal hemoglobinler, Antalya, beta-talasemi, Türk toplumu

Introduction

Thalassemia is the most common hemoglobinopathy, with a frequency of 12%, in Antalya province, which is located in the central part of the Mediterranean region in Turkey (1). Abnormal hemoglobins are the second most common hemoglobinopathies after beta-thalassemia in the Turkish population. Unstable hemoglobin disorders result from the presence of structurally abnormal hemoglobin variants with amino acid substitutions or deletions in globin chains. To date, more than 40 different unstable hemoglobin variants including alpha, beta, and gamma chains have been reported in the Turkish population (2). These hyperunstable variants offer important clues to identify structurally critical areas of the hemoglobin tetramer. HbS was reported for the first time by Aksoy et al. in Turkey (3). Other reports have shown the presence of several other abnormal hemoglobins in Turkey such as HbE, HbC, HbO Arab in homozygous, heterozygous, or compound heterozygous states (4-8). The aim of this study was to report the abnormal hemoglobins found in Antalya province, Turkey, and to emphasize the significance of their behavior in diagnosis.

Materials and methods

Patients diagnosed as carriers by Hb electrophoresis (HPLC), and pre- and postnatal patients at risk for thalassemia were screened for mutational analysis of the beta-globin gene. We studied 972 postnatal and 361 prenatal patients (total: 1333 patients, 2666 chromosomes) with the disorder from 1998 up to July 2008. DNA extraction from peripheral blood, chorionic villi samples (CVS), amniotic cells, and cord blood samples was carried out using standard procedures. Specific primer

sequences were used for amplification and sequencing of the β -globin gene (Table 1).

Following PCR amplification of the beta-globin gene, amplification refractory mutation system (ARMS) (9), reverse dot-blot hybridization (RDBH) [β -Globin Strip Assay Kit (Vienna Lab)], Nanochip (Nonogene Ltd., USA), and DNA sequencing (10) were performed to identify the mutations in the beta-globin gene. Variable number of tandem repeats (VNTR) analysis was used for elimination of maternal contamination in prenatal diagnosis as previously described (11). We identified and characterized abnormal hemoglobins with novel and rare beta-thalassemic mutations.

Results

Seven different abnormal hemoglobins were found in Antalya province in our study (Table 2). These were Hb Antalya, Hb Tyne, HbS, Hb G-Coushatta, HbE, Hb Knossos, and Hb D-Los Angeles, which were found in heterozygous or compound heterozygous states with other beta-thalassemic mutations.

Hb Antalya [Codons 3-5 (Leu-Thr-Pro/Ser-Asp-Ser)], a novel β -thalassemia mutation, was identified in a beta-thalassemia carrier with chronic microcytic anemia and in her mother. Hematological investigation of a 26-year-old woman due to her increased Hb A2 level (6.2%) led to identification of heterozygosity for a 9 bp (TCT GAC TCT) deletion/insertion at codons 3-5. As a result of these mutations, the orders of amino acids at codons 3-5 were changed from Leu-Thr-Pro to Ser-Asp-Ser. The whole frameshift was prevented by this rearrangement in the beta-globin gene. Physical examination was normal and her blood count showed

Table 1. Primer sequences used for amplification and sequencing of the β -globin gene.

Primer Name	Primer Sequence
D-1 (R)	5'-TCTCCTTAAACCTGTCTTGT-3'
D-2 (R)	5'-CCCTTCCTATGACATGAACTTAACCAT-3'
D-3 (F)	5'-CAGTGTGGAAGTCTCAGG-3'
D-4 (F)	5'-GTCTGTGTGCTGGCCCATC-3'
D-5 (R)	5'-GGTCAATATGT-3'
D-6 (F)	5'-TCCTGATGCTGTTATGGG-3'
D-7 (F)	5'-ATACAATGTATCATGCCTCTTGCACC-3'
D-8 (R)	5'-GTATTTTCCCAAGGTTTGAAGTAGCTC-3'
D-9 (F)	5'-GACAAAGCTCTTCCACT-3'
D-10 (F)	5'-TGTGGAGCCACACCCTAGGGTTGG-3'
D-11 (F)	5'-CACAGTCTGCCTAGTACAT-3'

Table 2. Abnormal hemoglobins found in the Antalya population.

Abnormal Hemoglobins	Cases (n)	Chromosomes	Frequency (%)
Hb Antalya [FSC 3-5 (+T)(-C)]	2	2	0.07
Hb Tyne [Cod 5(C-T)]	3	3	0.11
Hb S [Cod 6 (G-T)]	190	255	9.0
Hb G Coushatta [Cod 22 (A-C)]	4	4	0.15
Hb E [Cod 26 (G-A)]	3	3	0.11
Hb Knossos [Cod 27 (G-T)]	3	3	0.11
Hb D Los Angeles [Cod 121(G-C)]	9	9	0.33

the following values: Hb 10.6 g/dL and MCV 63 fL. The peripheral blood smear was normal except for a mild hypochromia. The Hb A level was found to be 93.8%. The iron status and serum ferritin levels of the patient were 56 mg/dL and 42 ng/mL, respectively. The frequency of this abnormal hemoglobin was found to be 0.07% in our study.

We also determined the co-inheritance of Hb Tyne (codon 5 Pro \rightarrow Ser) and HbS (codon 6 Glu \rightarrow Val) in a 9-year-old girl diagnosed clinically with beta-thalassemia/HbS. The following measurements were taken on physical examination: weight: 14 kg, height: 102 cm, liver: 2 cm, spleen: 4 cm. Blood count had the following values: RBC: $2.11 \times 10^{12}/L$, Hb: 6.6 g/dL, MCV: 73.1 fL, MCH: 31.3 pg, HbA1: 42.5%, HbA2: 3.9%, HbF: 6.1%, HbS: 40.7%. The patient received 7-8 units of blood transfusion yearly. Two other patients were also found to be carriers for Hb Tyne. Its frequency was found to be 0.11% in this study.

In the present study, 190 patients were found to be associated with HbS. Of these, 50 patients had homozygous HbS, 95 had heterozygous HbS, and 45

had compound heterozygous for HbS and different beta-thalassaemic mutations. The frequency of HbS is 9% among 2666 chromosomes analysed in our study.

We found Hb G -Coushatta [Codon 22 (A-C)] at the first exon of β -globin gene of a pregnant woman. This woman had the following hematological values: Hb A1: 45.3%, Hb A2: 53.2%, Hb F: 0.7%, MCV: 92 fL, MCH: 30.9 pg, MCHC: 33.6 g/dL, RBC: $4.66 \times 10^{12}/L$. In conclusion, unstable Hb variants that can migrate like known variants (such as HbD, HbS, HbF) should be investigated by different molecular and biochemical techniques, especially in the prenatal diagnosis of beta-thalassemia. The fetus was prenatally heterozygous for this variant. The frequency of this abnormal hemoglobin was found to be 0.15%.

Three HbE carriers (0.11%) were found in this study. Of them, a proband who was a 2-year-old girl had the following hematological values: Hb A1: 75.1%, Hb E: 22.9%, Hb F: 2.0%, MCV: 56.3 fL, MCH: 17.8 pg, MCHC: 31.6 g/dL, RBC: $4.61 \times 10^{12}/L$.

We report the effect of compound heterozygosity of Hb Knossos and IVSII-745 on beta-thalassemia major phenotype. A 9-year-old male with beta-thalassemia major was referred to us for screening of beta-globulin gene mutations. On physical examination he displayed a typical beta-thalassemia major phenotype. He had been regularly receiving blood transfusion for 7 years. Hb was 8.4 g/L, MCV: 78.6 fL, HbA₂: 2.6%, HbF: 5.4%, and HbA₁: 92%. Two other patients also were found to be carriers for this variant, and its frequency was 0.11% overall.

HbD Los Angeles [Cod 121 (G-C) Glu-Gln] was found in only 9 patients (0.33%) in a heterozygous state among the 2666 chromosomes analyzed in our study.

The consanguinity rate was found to be 27% among the families with beta-thalassemia and sickle cell anemia in our study.

Discussion

The exact number of subjects having abnormal hemoglobins in Turkey is not known. However, 7 different abnormal hemoglobins were found in our study in Antalya alone. One of them, Hb Antalya [Codons 3-5 (Leu-Thr-Pro/Ser-Asp-Ser)], a novel beta-thalassemia mutation characterized by chronic microcytic anemia, has been identified by direct DNA sequencing of the beta-globin gene in Antalya, Turkey (12). Its frequency was 0.07% in our study.

HbS [Cod 6 (G-T) Glu-Val] is the most common abnormal hemoglobin in our study, as well as in Turkey generally. In various screening studies, the prevalence of HbS in this population was found to be between 3% and 47%. Although its overall frequency in Turkey is 0.3%, it is observed with a frequency of 3%-37% in Manavgat, Antalya (13). Haplotype analysis of HbS in Turkish patients has revealed haplotype 19 (Benin Type) (14). This haplotype is related to the severe clinical findings of sickle cell anemia. Hb Tyne [Cod 5 (C-T) Pro-Ser] firstly discovered in 2 unrelated English citizens by Langdown et al. in 1994 (15). This variant is reported for the first time as compound heterozygous with HbS associated with thalassaemic phenotype in this study (16). It is a very rare variant in the Turkish population.

Hb G-Coushatta [Cod 22 (A-C) Glu-Ala] was first described by El-Hashemite et al. (17). We determined four patients (0.15%) who carry this variant in our study. One of them had compound heterozygous [HbG /IVSII.1 (G-A)] and the beta-thalassaemic phenotype (18). The remaining 3 were carriers for this variant.

Hemoglobin E (HbE) [Cod 26 (G-A) Glu-Lys] is a variant hemoglobin with a mutation in the beta-globin gene causing substitution of glutamic acid for lysine at the 26th position of the beta-globin chain. It is the second most common abnormal hemoglobin after sickle cell hemoglobin (HbS) in the world. HbE is common in South-East Asia. This mutation produces the third most common abnormal hemoglobin in Turkey (13,19). The mutation creates an alternate splicing site within the first exon of the beta-globin gene. The beta chain of HbE is reduced. HbE carriers, heterozygous subjects, are clinically asymptomatic. All of our patients were heterozygous for this mutation.

Hb Knossos [Cod 27 (G-T) Ala-Ser] is a rare hemoglobin variant, which was first described in a Greek family causing silent beta(+) thalassemia (20). We found the combination of Hb Knossos [Cod 27 (G-T)] and IVSII-745 (C-G) in a Turkish patient with beta-thalassemia major. Hb Knossos are unstable and cannot be detected by simple electrophoretic examinations. If co-inherited with another thalassaemic mutation in a compound heterozygous state, it may lead to thalassemia major phenotype (21). It is most frequently seen in the Mediterranean region. Heterozygous inheritance of the mutation results in a mild β -thalassaemia phenotype, whereas homozygous inheritance leads to thalassemia intermedia. In addition, this result may provide important clues to identify critical amino acids responsible for stabilization of the hemoglobin tetramer.

HbD Los Angeles is the second most common abnormal hemoglobin after Hb S in Turkey. While its frequency is reported as 0.2% in Turkey (22), it was reported as 0.33% in our study. As a result, the frequencies of abnormal hemoglobins show differences in the populations according to region (23,24). Consanguineous marriages are also high (35.17%) in Antalya, and in its nearby towns. Therefore, the consanguinity rate was also found to

be high (27%) among the families with beta-thalassemia and sickle cell anemia in our study (1).

In conclusion, these findings suggest that abnormal hemoglobins are more frequent than expected, and are important both for the treatment of thalassemia and in genetic counseling to families at risk for thalassemia, especially in prenatal diagnosis

due to different clinical effects of abnormal hemoglobins.

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