Original Article

The Spectrum of Mutations in 100 Thalassemic Carriers Referred to Ghaem Hospital of Mashhad

Hamzehloei T PhD[1], Mohajer Tehran F PhD[1]

1- Department of Genetic, Medical school, Mashhad University of Medical Sciences

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Abstract

Background

Thalassemia is common in the Iranian population, and it must be considered in the differential diagnosis of the microcytic hypochromic anemia. The molecular analysis of β-thalassemia is necessary for prenatal molecular diagnosis. A-thalassemia caused by loss of function of either one of the two duplicated α-globin genes or in less frequent non deletion mutations mostly located in the α2-globin gene.

Materials and Methods

DNA were extracted from 100 whole blood using salting out method. The PCR was performed in two segments for entire β-globin gene and the α1 and α2-globin genes separately. Direct sequencing was carried out. The Gap-PCR was performed using published primers.

Results

Clinical application of DNA analysis on thalassemic patients showed 42 persons have various β-thalassemia mutations, 48 persons with αα/-α3.7 deletion and 8 persons with non deletion mutations of α1 and α2-globin genes. These mutations determined by direct sequencing of entire β-globin, α1 and α2-globin genes and Gap-PCR for detection of deletions. Thirteen different β-thalassemia alleles were identified, the most common being IVS I-5(G>C) and CAP+1 (A>C). The most α –globin mutation being αα/-α3.7 deletion.

Conclusions

The frequency of mutations in North-east of Iran shows that these mutations are not the same as frequent mutation in other province of Iran. Feature study could determine molecular analysis of frequent mutations, which is useful for differentiating mild from severe alleles. In addition, mutation definition in carriers should be necessary for prenatal testing and genetic counseling.

Key words

Thalassemia, Mutation, Prenatal Diagnosis

Corresponding Author
Hamzehloei T, Genetics Department, Medical School, Mashhad University of Medical Sciences, E-mail: hamzehloiet@mums.ac.ir

Introduction

Thalassemia is a group of genetic disorders characterized by a decreased or absent synthesis of globin, while hemoglobinpathies are results of structural defects of the globin chains (1). Both thalassemia and hemoglobinopathies are caused by mutations of the globin genes. Interactions of these mutant alleles result in several thalassemia syndromes with heterogeneous clinical severity; and very common in several developing countries ranging from the Mediterranean, Middle East and South Asia (1). Depending on the affected globin gene, thalassemia is divided into two major types: α-thalassemia and β-thalassemia. α-thalassemia is usually caused by either the deletion of one α-globin gene or both linked α-globin genes on chromosome 16 (1). More than 20 deletions in the α-globin gene cluster have been described (2). Non-deletion defects in the α-globin genes are relatively less observed. Although α-thalassemia is found throughout the world, its distribution varies greatly among different populations. The spectrum of molecular defects is might be ethnic-specific. Clinical phenotype of the carriers varies according to the number of affected genes. Carriers of three a-globin genes (-a/aa) present with no detectable red blood cell abnormalities or globin-chain imbalance, while carriers of two functioning a-genes (- -/aa or -a/-a) have mild microcytic, hypochromic anemia with normal hemoglobin A2 levels. Carriers of only one functioning a-gene (-a/- -) present with hemoglobin H disease, which is characterized by severe anemia with markedly unbalanced globin-chain synthesis ratios (mean, 0.43) (3). Inheritance of no functional a-globlin genes (- -1- -) is usually incompatible with life and leads to Hb Bart's hydrops fetalis. By contrast, the majority of molecular defects found in β-thalassemias are point mutations resulting from single nucleotide substitutions and small numbers of nucleotide insertions or deletions of the β-globin gene on chromosome 11 (4). More than 200 different β-thalassemia mutations have been characterized to date. While the mutations are often population-specific, for the large country such as Iran with different ethnic groups, a number of unknown mutations observed in clinics are not uncommon. Thus, the screening programs for heterozygotes should be established for each province. Many of the heterozygote couples, instead of taking the risk of having a severely affected, homozygous child would either interrupt or avoid any pregnancy. Detection of the β-globin gene mutation is necessary for a definitive diagnosis and management plan, including early prenatal diagnosis (PND) of b-thalassemias.

Materials and Methods

Patients

The patients were couples with hemolytic anamia referred to Mashhad's Ghaem hospital for molecular diagnosis of thalassemia. A diagnosis of beta-thalassemia trait was made if the blood count and morphology showed red blood cell microcytosis (MCV <80 f l), mean corpuscular Hb (MCH) values (MCH <27), mutant Hb bands were absent, and Hb A2 was >3.5%. A diagnosis of alpha-thalassemia trait was made if the blood count and morphology showed red blood cell microcytosis (MCV <80 f l), mean corpuscular Hb (MCH) values (MCH <27), a normal alkaline electrophoretic strip, and a normal Hb A2 quantitation was detected. The detection of abnormal Hbs was carried out by high- performance liquid chromate-graphy (HPLC).

Molecular Analysis

The genomic DNA isolated from peripheral blood cells by a salting out procedure (5). For identifying α-thalassemia genotype, seven common deletional mutations were

studied as previously described using Gap-PCR (6). The nondeletional mutations also ruled out using the entire α_1 and α_2 globin genes DNA sequencing (data was not shown and can be provided upon request).The three exons and intervening introns of the ß -globin gene was amplified using the Gene Amp PCR system 9700 (Applied Biosystems, CA), with a 30 cycling protocol which consisted of denaturation at 95°C for 30 s, 62°C for 1 min, and 72°C for 10 min. DNA was amplified in 25 µl containing 10 mM Tris (pH 8.3); 50 mM KCI; 1.5 mM MgC12; 200 pmol each of dATP, dCTP, dTTP, dGTP; 10 pmol each of primers (SEQ1F, SEQ1R and SEQ2F, SEQ2R) and 2.5 units of Taq DNA polymerase (Cinnagen). The DNA sequenced with the use of a primer set encompassing exons 1 and 2 (for fragment A: SEQ1F 5′-GGGCCAAGAGATATATCTTAG -3′, SEQ1R 5'-CAATGACATGAACTTAACCATAG -3′) and another encompassing exon 3 (fragment B: SEQ2F 5′-ATGTATCATGCCTCTTTGCACC -3′, SEQ2R 5′-GCACTGACCTCCCACATTCC -3′). The sequencing reactions were performed by using the dideoxy termination procedure of Sanger by an automated sequencer analyzer (ABI-3730XL Capillary, Applied Biosystem, USA) as per the manufacturer's instructions. The analysis was also conducted in the DNA of normal.

The nucleotide change was compared with the Database of Human Hemoglobin Variants and Thalassemias. The nucleotide numbering is based on GenBank accession number U01317.

Results

Among the 100 cases with haemolytic anamia referred to Ghaem hospital for molecular analysis, 42 individuals carried ß-globin mutations, 48 cases carried at least one α-globin gene deletion and 8 individuals presented a non-deletional mutation for either α_1 or α_2-globin genes. The ß-globin mutations were as follows; IVSI-5 (G>C) (40%), CAP+1 (A>C) (16%), IVSII-1 (G>A) (12%), codon 29 (C>T) (7%), beta 131Gln>His (5%), codon 37 (G>A) (5%), IVSI-110 (G>A) (5%), -88 C>A (5%), and the overall mutations, codon 8 (-AA), codon8/9 (+G), codon 37/38/39 (-7nts), codon 15 (G>A), and Codons 22/23/24 deletion of –AAGTTGG (2%). The most α-globin gene deletion was αα/-α3.7 (79%). Other α-globin gene deletions were αα/-α4.2, -α3.7/-α3.7 and α-4.2/-α3.7 were in small amounts. The point mutations in α-globin genes were IVS-I donor site (GAG GTG AGG->GAG G- - - - -); alpha2-globin gene, HBA2:c. [43T>A (or HBA1), Poly A (A->G); AATAAA->AATAAG of the alpha2 gene, and alpha1 108 (-C). In two individuals the mutation was not detected.

Table 1. Distribution of β-thalassemia mutations in west provinces of Iran compare with this study

	Kurdestan (%) (Ref: 16)	Lorestan (%) (Ref:17)	Eastern Azerbaijan (%)(Ref:18)	Western Azerbaijan (%) (Ref:19)	Khorasan Razavi (This study)
IVSI-5 (G>C)	(1.52)	(4.67)	(2.6)	(0.8)	40%
CAP+1 (A>C)	-	-	-	-	16%
IVSII-1 (G>A)	(31.8)	(27.7)	(32)	(50.7)	12%
codon 29 (C>T)	-	-	-	-	7%
IVSI-110 (G>A)	(1.52)	(11.5)	(16)	(5.3)	5%
codon8/9 (+G)	(13.6)	(10.76)	(18.67)	(16)	5%
codon 37 (G>A)	-	-	-	-	5%
-88 C>A	-	-	-	-	2%
codon 8 (-AA)	(6.06)	-	-	-	2%
codon 15 (G>A)	-	-	-	-	2%
Unknown	(21.12)	(7.63)	-	-	7%
Total	66	130	144	150	42

Discussion

Thalassemia is one of the most common single gene disorders in the middle-east population. The heterozygous carriers of β-thalassemia in Iran are about 4-10% among the different ethnic groups (7). Among some ethnic groups, for unknown reasons, the gene frequency has remained high and has clearly emerged as one of the most common public health problems in Iran. The World Health Organisation has highlighted the importance of characterization of the spectrum of β-thalassemia mutations as one of the ways for community control of β-thalassemia. Thus, characterisation of the patients in this study is essential for the patient management in this country. The distribution of β-thalassemia mutations among the Khorasan Razavi's population was different to the distribution of β-thalassemia mutations in other parts of the country specifically from the western provinces. This can be due to the number of refugees, from Afghanistan, and admixture with other populations who visited Mashhad due to a religious city such as some Arab population from Persian Gulf bordering countries. Our result showed IVSI-5 (G> C) as the most frequent mutation (40%). This type of gene defect was previously reported at high prevalence in Hormozgan (8), Kerman (9) and Sistan-va-Balochistan (10). These provinces are located in south and southeast of Iran. Also, IVSI-5 (G> C) was reported as the most frequent mutation in south of Iran (11), Baluchistan and Sindh provinces of Pakistan (12), and widespread in the neighboring countries: Western province of Saudi Arabia, Bahrain, Kuwait, UAE, and Oman have 22.5%, 16.2%, 18%, 55%, and 62% of IVSI-5 (G> C), respectively (13). These data show that IVSI-5 is the predominant mutation in the countries around Persian Gulf. But this mutation is not common in the west of Iran (Table 1). The CAP+1 (A>C) mutation which is second common mutation in our study is relatively high in north India and Pakistan (14) and in Arab population from Saudi Arabia, but is less common in other

Arab countries (13). This can be due to neibouring of Khorasan Razavi's province with Pakistan. The 3.7-kb deletion, which is also known as rightward a-thalassemia-2, is the result of homologous recombination between misaligned homologous Z regions, and is most prevalent in Mediterraneans and Africans (15), was also the most common mutation in our study.

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Conflict of Interest

The authors have no conflict of interest to discloser.

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