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Original Article

Thalassemic Mutations in Southern Iran

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Abstract

Background: Approximately 180 mutations have been described in β-thalassemia worldwide with specific spectrum in each ethnic population. This study determines the spectrum and the frequency of β-thalassemia mutations in patients with β-thalassemia trait and sickle cell-β-thalassemia.

Methods: Fifteen compound heterozygous sickle cell thalassemia (SCT) and 23 β-thalassemia trait patients were studied using reverse dot blot, denaturing gradient gel electrophoresis and direct genomic sequencing.

Results: We detected distinct β-thalassemia alleles in 15 compound heterozygous of SCT and 23 β-thalassemia trait patients. The most common mutation was IVSII-1(G→A), found in 15 of the 38 thalassemia chromosomes. IVSII.1 (G→A) mutation is a single nucleotide change of G to A at intervening sequence 2 position 1 of β-globin gene, detected in 11 out of 23 chromosomes in A/β-thalassemic patients and in four out of 15 chromosomes of SCT patients. This mutation constituted about 39% of the mutations in both groups. The -25bp 3´IVSI, deletion of 25 base pairs from 3' end of intervening sequence 1 of β-globin gene, was found to be the second prevalent mutation among all chromosomes.

Conclusion: Defining thalassemia mutations are necessary to establish prenatal diagnosis programs leading to lower medical cost. Amongst 10 different types of mutation detected in β-thalassemic patients from South of Iran, two mutations of IVSII-1(G→A) and -25bp 3´IVSI were the most predominant β-thalassemic alleles.

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Keywords ● β-thalassemia ● mutations ● sickle cell ● heterogenous ● Iran

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Introduction

Beta-thalassemia is a heterogeneous inherited disorder of β-globin synthesis. Approximately 180 mutations have been described in β-thalassemia wordwide.[1] β-thalassemia may arise from either deletions of large portions of the β-globin gene cluster or from mutations involving single base substitution or small deletions or insertions within or 5' to β-globin gene.[2] Previous surveys have demonstrated a specific spectrum of mutations in each ethnic population.[3] Some of these mutations, generally two or three, are found with high prevalence while others are rare, but the aggregation of both types involve a large numbers of patients.[3]

β-thalassemia is the most common single gene disorder in Iran and more than 25,000 affected individuals have been reported.[4] High prevalence of β-thalassemia (around 10%) occurs in North, close to the Caspian sea, and South of Iran close to the Persian Gulf.[4] The prevalence of β-thalassemia alleles in other parts of the country has been estimated to be 4-8%.[4] Karimi and colleagues performed a screening study on high school boys which was done in the Fars Province, South of Iran, and showed that the frequency of minor and major β-thalassemia was 6.9% and 0.72%, respectively.[5]

The population of Iran consists of multiethnic groups; therefore, one expects to find different distributions of β-thalassemia mutations in various parts of the country. Depending on the techniques used for the determination of mutations, between 4-17 β-thalassemia mutations have been reported in major or thalassemia intermedia patients in Iran.[6-8]

To establish a program for carrier screening, genetic counseling and the offer of prenatal diagnosis in our country defining the spectrum of β-thalassemia mutation in different areas of Iran is necessary. This study reports distinct β-thalassemia alleles in 15 compound heterozygous (Sickle-cell-thalassemia) and 23 β-thalassemia trait patients using reverse dot blot (RDB), denaturing gradient gel electrophoresis (DGGE), and direct genomic sequencing.

Patients and Methods

From 18 Sickle-Cell-Thalassemia (SCT) and 36 β-thalassemia minor patients who were analyzed, the type of mutation was detected in 15 SCT patients, nine females and six males, and 23 β-thalassemia minor, 14 females and nine males, 6-46 yrs-old from South of Iran. All patients had been admitted in the Hematology Center of Shiraz University of Medical Sciences. The type of mutation was not identified in three SCT and 13 β-thalassemia patients.

Complete blood counts were carried out using an automated cell counter. Fetal hemoglobin (HbF) and hemoglobin A2 (α2δ2) were determined by alkaline denaturation and DEAE-52 column elution methods respectively.[9,10] Sickle cell preparation test as well as Hb electrophoresis on cellulose acetate at alkaline and acid pH were used for diagnosis of sickle cell genotype /phenotype.[11]

From EDTA treated venous blood, DNA was prepared using the phenol-chloroform extraction method with minor modifications and kept at -20ºC until analyzed.[12] Initially, Polymerase Chain Reaction-Reverse Dot blot (PCR-RDB) technique, using strips containing probes were used for screening β-thalassemia mutations for the most common Mediterranean and Asian Indian β-thalassemia alleles that had previously been reported for Iranian population.[6-8] PCR-RDB technique has the capacity to screen several known mutations in a single hybridization reaction.[13,14]

For chromosomes in which PCR-RDB technique cannot detect any mutation, Denaturing Gradient Gel Electrophoresis (DGGE) technique can be used to detect single base changes and polymorphisms.[15] In brief the detection of 25bp deletion, in the 3´ end IVSI was done after observing abnormal framework of DGGE for G-fragment of β-gene and similarity of it to framework of a control sample with 25 bp deletion and the PCR-Electrophoresis based approach was used for final confirmation. Mutations in some chromosomes were determined by genomic sequencing using Sangers dideoxy chain termination method with Sequenase T7 DNA polymerase.[16]

Results

Hematological data for thalassemia trait and SCT patients are shown in Table 1. Ten different types of mutations which were found in patients are shown in Table 2. As shown, among the two groups, the most prevalent mutation was IVSII.1 (G→A), accounting for 47.8% mutations in β-thalassemia minor and 26.7% in SCT patients. This mutation constituted 39.5% of the mutations in both groups. The -25bp 3´ IVSI was found to be the second most prevalent mutation (18.4%) amongst all chromosomes. A rare mutation, IVSI (-1) Cd30 (G→A) was detected in only one thalassemia trait patient. The hematological characteristics of the patient in whom a new mutation was found, are: Hb 9.5g/dl; Hct 32%; HbA2 3.9%; HbF 2%; MCV 69.4fl; MCH 20.6pg; MCHC 29.7g/dl; and RBC 4.8×1012/l.

Table 1: Mean±SD of hematological aspects of patients with β-thalassemia minor (βTM) and Sickle Cell Thalassemia (SCT)

Data	βTM (n)	SCT (n)
Hb (g/dl)	11.9±1.5 (22)	10.0±1.4 (14)
HbS (%)	-	67.4±9.6 (15)
HbA_2 (%)	4.8±0.55(22)	4.4±0.87 (15)
HbF (%)	1.7±0.78(22)	12.1±5.8(15)
Hct (%)	38.9±5.1(22)	33.4±4.6(14)
MCV (fl)	64.3±5.5(22)	75.8±7.1(14)
MCH (pg)	19.7±1.5(22)	23.0±2.3(14)
MCHC (g/dl)	30.6±1.3(22)	30.4±2.0(14)
RBC ($\times 10^{12}$/l)	6.0±0.69(21)	4.4±0.55(14)

Table 2: β-thalassemia mutations

Mutation	type	n	%
β-Thalassemia trait			
IVSII.1(G:A)	β^0	11	47.8
-25bp 3´ IVSI	β^0	5	21.6
Fs8(-AA)	β^0	2	8.6
IVSII.745(C:G)	β^+	1	4.4
IVSI.5(G:C)	β^+	1	4.4
CD 30(G:A)	β^0	1	4.4
IVSI.110(G:A)	β^+	1	4.4
CD5(-CT)	β^0	1	4.4
Sickle cell thalassemia			
IVSII.1(G:A)	β^0	4	26.7
IVSI.1(G:A)	β^0	3	20.0
-25bp 3´ IVSI	β^0	2	13.3
Fs8(-AA)	β^0	2	13.3
IVSII.745(C:G)	β^+	2	13.3
CD 39(C:T)	β^0	1	6.7
IVSI.110(G:A)	β^+	1	6.7

Discussion

The high incidence of β-thalassemia alleles in Iran, which was estimated around 25,000 affected individuals and two million carriers of β-thalassemia, requires an extensive study for the type of β-globin gene mutations present. Defining thalassemia mutations in each geographical area is necessary to establish prenatal diagnosis programs to reduce medical care costs. A large majority of the thalassemia defects are due to point mutations affecting critical areas for the function of the β-globin. These mutations produce frame shifts and nonsense codons, defects in transcription, RNA processing, mRNA translation or they result in the synthesis of very unstable hemoglobin chains.[17]

The type of β-thalassemia mutation was determined in 15 out of 18 SCT patients which among them 80% of mutations were of β^0 mutations including IVSII position 1 G→A, IVSI position 1 G→A, IVSI 3'-end -25 bp, Fs8 (-AA) and Codon 39 C→T. Twenty percent of β-thalassemia chromosomes had a β^+ mutation (IVSII position 745 C→G, and IVSI position 110 (G→A). Generally, around 84% of β-thalassemia mutations in all of these patients were of β^0 mutations. IVSII-1 (G→A), found in 15 out of 38 thalassemia chromosomes (about 40%), was the most common mutation. This mutation was found in 11 out of 23 chromosomes (47.8%) in A/β-thalassemia patients and in four out of 15 (26.7%) chromosomes in SCT patients as well. The presence of IVSII-1 (G→A), as the most prevalent β-globin gene mutation, in homozygous or β-thalassemia intermedia patients from Southwest of Iran has previously been reported.[6,7]

Karimi and colleagues identified four different mutations (IVSII.1, IVSI.110, IVSI.1 and Fs 8/9) in β-thalassemia intermedia patients from South of Iran, indicating that IVSII.1 G→A as the most frequent mutation.[7] Although, they could not identify the type of β-thalassemia mutation in 61% of their patients, Merat and coworkers, on the other hand, found 10 different mutations among 17 β-thalassemia patients from the same region with high frequency (31%) of IVSII.1 G→A mutation followed by 15% IVSI.6 (T→C) mutation.[6] In the present study the frequency for IVSII.1 G→A mutation was 40% which was higher than what is reported in previous studies.[6,7] This is of interest because in previous reports, the 25 bp deletion 3´ IVSI was not found or only in one chromosome.[6,7] In contrast, in this study, this mutation was detected in 7 out of 38 chromosomes (18.4%) representing the second most prevalent mutation in this region. The presence of 25 bp deletion with high frequency of 36% was found in patients living in Bahrain,[18] could be attributed to gene-flow and genetic admixtures that might have occurred in this region.

The rare codon 30 [IVSI position -1 (G→A)] mutation found in only one patient, completely prevented mRNA splicing and could not produce mRNA is carriers of this mutation as previously reported in a Bulgarian family and a Turkish thalassemic patient.[1]

Conclusion

The information provided on the distribution and the frequency of β-thalassemia alleles in South of Iran is useful for physicians to establish specific therapeutic approaches and for genetic counselors to educate young couples and families to decide about their future life.

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References

1 Tadmouri GO, Bilenoglu O, Kantarci S, et al. A rare mutation [IVS-I-1 30 (G-A)] in a Turkish β- thlassemia major patient. *Am J Hematol* 2000; 63: 223-5.

2 Diaz-Chico JC, Yang KG, Yang KY, et al. The detection of β-globin gene mutations in β-thalassemia using oligonucleotide probes and amplified DNA. *Biochem Biophys Acta* 1988; 949: 43-8.

3 Weatherall DJ. The Thalassemias. In Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U, editors: Williams Hematology.6th ed. New York; McGraw-Hill; 2001. p. 547-80.

4 Najmabadi H, Teimourian Sh, Khatibi T, et al. Amplification refractory mutation system (ARMS) and reverse hybridization in the detection of beta thalassemia mutations. *Arch Irn Med* 2001; 4: 165-70.
5 Karimi M, Alavian Ghavanini A, Kadivar MR. Regional mapping of the gene frequency of β- thlassemia in Fars province, Iran during 1997-1998. *Iran J Med Sci* 2000; 25: 134-7.
6 Merat A, Haghshenass M, Pour ZM, et al. β-thalassemia in Southwestern Iran. *Hemoglobin* 1993; 17: 427-37.
7 Karimi M, Yarmohammadi H, Farjadian S, et al. Beta-thalassemia intermedia from southern Iran: IVSII-I(G:A) is the prevalent thalassemia intermedia allele. *Hemoglobin* 2002; 26: 147-54.
8 Najmabadi H, Karimi-Nejad R, Sahebjam S, et al. The β-thalassemia mutation spectrum in the Iranian population. *Hemoglobin* 2001; 25: 285-96.
9 Betke K, Marti HR, Schlict I. Estimation of small percentages of fetal haemoglobin. *Nature* 1959; 184: 1877-8.
10 Fairbanks VF, Klee GG. Biochemical aspects of hematology. In: Burtis CA, Ashwood ER, editors: Tietz text book of clinical chemistry. Philadelphia: W.B Saunders; 1994. p. 2041-2.
11 Schneider RG. Differentiation of electrophoretically similar Hbs- such as S, D, G and P; or A_2, C, E, and O by electrophoresis of the globin chains. *Clin Chem* 1974; 20: 1111-5.
12 Old JM, Higgs DR. Gene analysis. In Weatherall DJ, editor: Methods in Haematology. The thalassemias. Vol 6. Livingstone; 1983. p. 74-101.
13 Sutcharitchan P, Saiki R, Huisman TH, et al. Reverse dot-blot detection of the African-American β-thalassemia mutations. *Blood* 1995; 86:1580-5.
14 Cai SP, Wall J, Kan YW, Chehab FF. Reverse dot blot probes for the screening of β -thalassemia mutations in Asian and American blacks. *Hum Mutat* 1994; 3: 59-63.
15 Ghanem N, Girodon E, Vidaud M, et al. A comprehensive scanning method for rapid detection of β-globin gene mutations and polymorphisms. *Hum Mutat* 1992; 1: 229-39.
16 Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain termination inhibitors. *Proc Natl Acad Sci USA* 1977; 74: 5463-9.
17 Murru S, Loudianos G, Deiana M, et al. Molecular characterization of β–thalassemia intermedia in patients of Italian descent and identification of three novel β–thalassemia mutations. *Blood* 1991; 77: 1342-7.
18 Jassim N, Merghoub T, Pascaud O, et al. Molecular basis of β-thalassemia in Bahrain: An epicentre for a Middle East specific mutation. *Ann NY Acad Sci* 1998; 850: 407-9.