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Thalassemia Gene Mutations in Kohgiluyeh and Boyer-Ahmad Province

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ABSTRACT

Background: Thalassemia is the most common hereditary anemia which has a relatively high prevalence in Iran. In most cases, more than 300 mutations have been identified, which affect genes of alpha and beta globin chains and lead to lack of production or reduction of chains. Iran's population is composed of different ethnic groups, thus, determining the frequency and distribution of these mutations is essential in different parts of the country. We aimed to assess Thalassemia gene mutations in Kohgiluyeh and Boyer-Ahmad province.

Methods: In this cross-sectional study, 656 couples were selected and their Genomic DNA was extracted by DNA extraction kit method and tested using multiplex gap-polymerase chain reaction (gap-PCR), amplification refractory mutation system-PCR (ARMS-PCR), and DNA sequencing. Finally all data were analyzed using the SPSS version 17 software.

Results: More than 13 mutations were found on α-globin genes. Based on gene frequency, the most common mutant allele was –α3.7/αα (rightward) (71.3%) followed by the two gene deletion –α3.7/–α3.7 (2.5%). Other common mutations were polyA2 (2.1%), αcodon 19α/αα (1.7%), –α3.7/αα/–α3.7/αα (1.5%), – (α) 20.5 (0.6%), α–5 nt/αα (0.5%), and other mutations. In this study, more than 21 mutations were identified on beta thalassemia gene. The most common mutation was CD36- /37 (-T) (19.8%). Other common reported mutations included IVSII-1 (G>A) (9.5%), IVS I-110 (G>A) (4.7%), IVSII-745 (C>G) (4.4%), codon 82/83(-G) (3.7%), FSC 8/9 (+G) (1.7%), Codon19(1.5%), 25 bp deletion (beta0) (1.5%), IVS-I-116 (T>G) (1.4%), IVSI-6 (G>C) (1.1%), codon 5 (-CT) (0.9%), codon 88 (-C) (0.5%), and IVSI-1(G>A) (0.3%).

Conclusion: The frequencies of these mutations were different in various parts of the country. Therefore, defining thalassemia mutations is necessary to establish prenatal diagnosis programs leading to lower medical cost in Kohgiluyeh and Boyerahmad province.

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Introduction

Thalassemia is the most common hereditary anemia worldwide which is inherited as autosomal recessive.[1, 2] Thalassemias are named by reference to the affected globin chain. Alpha-thalassemia occurs when a genetic mutation leads to reduced synthesis of one or more of the four α-globin genes.[3, 4] Alpha-thalassemia has two carrier states: Alpha^{o}-thalassemia generally results from deletion or dysfunction of two α-globin genes, in cis (--/αα) and Alpha^{+}-thalassemia usually results from deletion or dysfunction of one α-globin gene.[5] In beta thalassemia, mutations either result in reduction (β+) or absent (β^{o}) synthesis of β-globin chains of hemoglobin tetramer.[6]

Thalassemia is commonly found in Africa, Middle

East, India, Southeast Asia, southern China and the Mediterranean region.[7] Among the eastern Mediterranean countries, the highest carrier frequency is reported from Cyprus (14%), Sardinia (10.3%), and Southeast Asia.[3] Iran is one of the countries in which thalassemia is prevalent. A higher prevalence of minor beta thalassemia has been expected in Iran and more than two million carriers of beta-thalassemia live in Iran.[8-11] Previous studies have estimated the prevalence of the β-thalassemia trait of more than 10% around the Caspian Sea and Persian Gulf.[12, 13] According to the World Health Organization, about 4% of Iran's population are carriers of the thalassemia genes.[11]

There are more than 300 mutations are responsible for different phenotypes of thalassemia. These include replacement, frame shift, insertion and deletion type mutations in the normal gene. Although the common mutations of the globin genes which result in the phenotype of thalassemia major have been characterized worldwide,[14, 15] there are a subset of common mutations in each ethnic group that the disease is prevalent.[16-19] For example, 10.5% of Kelantan Malaysian and 2.4% of Kelantan Chinese individuals carry α3.7 deletion.[20] One study revealed that the incidence of -α3.7/αα (rightward deletion) and -α4.2/αα (leftward deletion) traits were 12.0% and 4.3%, respectively, in southern Thailand.[21]

Previous studies have shown that –α3.7, polyadenylation signal (polyA2) site, – –MED and –α4.2 are the most common α gene mutations in Iranian patients.[22] The most identified mutations reside in southern and northern regions (more than 10 %), while other regions have a frequency of 8-10%.[23] For instance, a high rate of β-thalassemia mutation has been reported in South-East province (Sistan and Baluchestan).[24] Mutations in codons 36-37, β-globin IVS I-1, β-globin IVS I-110 and β-globin IVS II-1 account for more than 70% of the β-thalassemia alleles in Iranian populations.[25] In another study from Iran, β-globin IVSII-1 (G>A) and IVSI-5 (G>C) were the most frequent mutations detected in Iranian population.[26, 27]

Despite the abundance of the studies from different parts of Iran, there is limited published information about the pattern of thalassemia mutations in Kohgiluyeh and Boyer-Ahmad province. We aimed to assess the frequency of α and β globin gene mutations in Kohgiluyeh and Boyer-Ahmad province.

Materials and Methods

This study was conducted on 656 couples getting married (age range 18-33 years old), who were suspected of being thalassemia carriers due to RBC indices. The subjects were referred to the genetic center at the central clinic in Shiraz (Southern Iran) for premarital diagnosis test, 2013-2017. All subjects had a mean cell volume (MCV) of <80 fL, mean cell Hb (MCH) of <27 pg, A2>3.4% and normal electrophoretic pattern and serum ferritin level. Hb variants were determined by high-performance liquid chromatography (Bio-Rad, USA). 10 mL of blood (with EDTA as anticoagulant) was drawn from each subject and DNA was extracted from peripheral blood cells using the QIAamp DNA Blood Mini Kit, QIAGEN. Alpha and Beta-Thalassemia genotypes were identified by molecular methods using multiplex gap-polymerase chain reaction (gap-PCR) and DNA sequencing (ABI PRISM Big Dye Primer Cycle Sequencing).[28-31]

The method of amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and genomic sequencing were used for detection of β-Thalassemia genotypes. Gap-PCR approach followed by multiplex-PCR and reverse hybridization test strips (Vienna Lab Diagnostics, Vienna, Austria) were used for detection of α- Thalassemia genotypes.[32]

All data were analyzed using SPSS version 17 software and statistical analysis were conducted by analysis of variance (ANOVA) and chi-Square test at the significance level less than 0.05.

Results

Age range of the patients were 18-33 years old (26±7 years). 223(34.4%) (27 homozygotes, 4 compound heterozygous, 186 heterozygotes and 8 Unknown) out of 656 patients were found to be α-thalassemia carriers. At least 13 different gene mutations were identified in these subjects. As shown in Table 1, the –α3.7/αα deletion was the most common mutation (22.8%) found in 223 subjects with carrier state for α mutations (Compound heterozygous mutations were identified by letters and slash).

The remaining mutations identified in our samples were −α3.7/−α3.7 (2.5%), polyA2 (AATAAA>AATGAA) (2.1%), α codon 19 (GCG > GC–, α2) (1.7%), –α3.7/αα/–α3.7/αα (1.5%), – (α) 20.5 (0.6%), α−5 nt/αα (–TGAGG) (0.5%), –α3.7/αα -αα/αα (0.3%), polyA1 (AATAAA>AATAAG) (0. 27 homozygotes 3%), –α4.2/–α4.2 (0.2%), αCSα/αα (TAA>CAA) (0.2%), –α3.7/–α4.2 (0.2%),–α3.7/αα / αcodon 19 (0.2%) and other mutations (1.3%).

Among 433 individuals, 345 (52.94%) (151 homozygotes, 9 compound heterozygous, 175 heterozygotes and 33 Unknown) were found to be β-thalassemia carriers. As shown in Table 2, More than 13 mutations were found on β-globin genes.

The most common mutation was CD36/37 (-T) in 19.8%. Other common reported mutations for β-globin genes included IVS II-1 (G>A) (9.5%), IVS I-110 (G>A) (4.7%), IVS II-745 (C>G) (4.4%), codon 82/83(-G) (3.7%), FSC 8/9 (+G) (1.7%), codon19 (1.5%), 25 bp deletion (beta0) (1.5%), IVS I-116 (T>G) (1.4%), IVS I-6 (G>C) (1.1%), codon 5 (-CT) (0.9%), codon 88 (-C) (0.5%), IVS I-1(G>A) (0.3%), IVS I-10 (0.3%), IVS I-6 (T>C)/Codon 82/83 (−G) (0.3%), FSC 36/37 (–T)/IVSII-745 (C>G) (0.3%), IVSII-1 (G>A)/IVSII-745 (C>G) (0.2%), IVS II-1 (G>A)/ IVS I-6 (G>C) (0.2%), FSC 36/37 (–T) /IVS I-110 (G>A) (0.2%), FSC 8/9 +G/ codon 5 (-CT) (0.2%), FSC 8/9 +G/ IVSI-25bp del (0.2%).

Discussion

Thalassemia is the most common autosomal recessive disorder. Up to now, more than 200 mutations have been identified affecting both alfa and beta globin genes.[14] With the exception of a few deletions, most thalassemias are caused by point mutations.[33]

In this cross-sectional study, about 52.94% and 34.4% of

Table 1: Frequency of α-globin mutations for each genotype in patients from Kohgiluyeh and Boyer-Ahmad province, Iran

Genotype	no	Percent (%)
−α3.7/αα	149	22.8
−α3.7/−α3.7	16	2.5
αpoly A2α/αα (AATAAA>AATGAA)	14	2.1
αcodon 19α/αα (GCG>GC–)	11	1.7
−α3.7/αα/−α3.7/αα	10	1.5
−(α)20.5	4	0.6
α−5 nt/αα (–TGAGG)	3	0.5
−α3.7/αα/ αα /αα	2	0.3
αpoly A1α/αα (AAATA>AATAAG)	2	0.3
−α4.2/−α4.2	1	0.2
αCSα/αα(TAA>CAA)	1	0.2
−α3.7/−α4.2	1	0.2
−α3.7/αα /αcodon 19	1	0.2
Other mutations	8	1.3
Total	223	34.4

Table 2: Frequency of β-globin mutations for each genotype in patients from Kohgiluyeh and Boyer-Ahmad province, Iran

Genotype	no	Percent (%)
CD 36/37 (–T)	130	19.8
IVSII-1 (G>A)	62	9.5
IVS I-110 (G>A)	31	4.7
IVSII-745 (C>G)	29	4.4
codon 82/83(-G)	24	3.7
FSC 8/9 (+G)	11	1.7
codon 19	10	1.5
25 bp deletion (beta0)	10	1.5
IVSI-116 (T>G)	9	1.4
IVSI-6 (G>C)	7	1.1
codon 5 (-CT)	6	0.9
codon 88 (-C)	3	0.5
IVSI-1(G>A)	2	0.3
IVSı-10/n	2	0.3
IVSI-6 (T>C)/Codon 82/83 (−G)	2	0.3
FSC 36/37 (–T)/IVSII-745 (C>G)	2	0.3
IVSII-1 (G>A)/IVSII-745 (C>G)	1	0.2
IVSII-1 (G>A)/ IVSI-6 (G>C)	1	0.2
FSC 36/37 (–T) /IVS-I-110 (G>A)	1	0.2
FSC 8/9 +G/ codon 5 (-CT)	1	0.2
FSC 8/9 +G/)/ 25 bp deletion	1	0.2
Unknown	33	4.8
Normal	55	7.9
Total	433	65.6

subjects were carriers of β and α mutations, respectively. Although, we could not identify the type of β-Thalassemia mutation in 4.8% due to lack of informative data from gene analysis. Iran is a country that is located in a region with a high prevalence of thalassemia that is more prevalent in the north and south.[34] Similar to Garshasbi et al.[35] Mirbehbahani et al., in north,[12] Moghadam et al., in south,[30] a large number of Thalassemia mutation are beta-Thalassemia and alpha-Thalassemia is not as prevalent as β-Thalassemia.[35] The frequency of β-Thalassemia, however, is high and varies considerably between areas.[36] In general, β-Thalassemia mutations have been a reflection of people and area in correlation with migration and origin of ancestors.[27]

In this study, we detected more than 34 polymorphism in α and β-globin genes by molecular technics. Codon 36/37(T) mutation was found to be the most common mutation for β gene responsible for19.8% of β-thalassemia in Kohgiluyeh and Boyer-Ahmad Province. This result is in complete accordance with the data known about the provinces or cities neighboring Kohgiluyeh and Boyer-Ahmad Province. Kiani et al results showed that codons 36/37 (–T) mutation with a frequency of 33.8%, is the most common mutation in Lorestan Province.[37] In Doosti et al study, CD 36/37 (-T) with 40.24% were the most common mutation among beta-thalassemia carriers.[38] Codon 36/37 mutation with 20.5% has also been recently identified in patients from Masjed Solaiman, in

the province of Khuzestan, which borders Kohgiluyeh and Boyer-Ahmad.[39] codon 36/37 (–T) mutation with a frequency of 33.8% reported among the Lurs of western Iran as well as Jewish and Iranian Kurds.[40, 41] Similar incidence of this mutation in neighboring of Kohgiluyeh and Boyer-Ahmad Province may be due to high rates of work relationships, genetic admixture and high number of migrations.[42]

The IVSII-1 (G-A) mutation is the most prevalent variant of beta thalassemia in different regions of Iran,[43] While, in this study with 9.5% was the second most common mutated in Kohgiluyeh and Boyer-Ahmad. The frequency of this mutation decreases from north to the south of Iran.[44] The frequency in Golestan and Mazandaran provinces, is about 61% and 44.6% respectively, while the frequency of this mutation in Khuzestan and Hormozgan provinces in southwest of Iran is 20% and 9.6% respectively.[45, 46] Akhavan et al data revealed a higher haplotype heterogeneity in northing population.[45] This may be due to the difference of sample collection and diagnosis methods.

This study indicated that, –α3.7/αα deletion with 22.8% was the most common mutation in α globin mutations. -α3.7 was the most common mutation (71.7%) in microcytic hypochromic anemia cases in two provinces of southern Iran: Fars & Kohgiluyeh and Boyerahmad.[47] Doosti et al, showed α3.7/αα with 55.99% was the most common mutation among alpha thalassemia carriers of Shadegan City, Southwest of Iran.[38] In Zandian et al study, the common α-globin gene mutation −α3.7 with 62.6% was investigated in Khuzestan Province.[48] karamzade et al, found that −α3.7 with a frequency of 70.7% was most common deletion in Esfahan.[49] In general, the -α3.7 gene deletion has a global distribution among all ethnic groups, especially prevalent in most tropical and subtropical populations studied.[32] As expected, like other reports from other parts of country,[22, 47, 50] alpha -thalassemia mutation spectrum among Lurs ethnic (Kohgiluyeh and Boyer-Ahmad Province) are similar to those reported from south neighboring provenances and other parts of Iran.

In present study −α3.7/−α3.7 with 2.5% was the second most frequent mutation in alpha-thalassemia patients of Kohgiluyeh and Boyer-Ahmad. In Similar observations, the results of first PND test in shadegan revealed −α3.7/−α3.7 with 12.5% was the second common among alpha-thalassemia carriers.[38] In Zandian study, −α3.7/−α3.7 single gene with 10.7% was the second most frequently identified variant in Khuzestan province.[48]

Like other reports from diverse parts of country and neighboring provinces, the prevalence of the −α3.7/−α3.7 genotype (26.9%) in two provinces of southern Iran: Fars & Kohgiluyeh and Boyer-Ahmad still is one of the main health problem.[47]

The other prevalence of α and b-globin gene mutations in our study, has been reported among common mutations in neighboring provinces or countries, although some mutations were rare. For example, in Najmabadi et al study, Six mutations including IVS I-110, IVS II-1, IVS I-1, IVS I-5, cd36/37 and IVS I-25 bp were among common mutations in southwest of Iran.[10] Moghadam et al, results showed that IVS II-I (G-A), Cd 36-37(-T), IVS I-5 (G>C), IVSI-25b Del (252–276), IVS I-110 (G>A) and C44 (_C), Fr 8-9(+G) and IVS II-745(C>G) were the most common mutations in Southern Iran.[30] Karamzade et al, founded -α4.2, polyA2, -α 20.5, Hb CS, polyA1, α codon 19, – –MED and α codon14 were most mutations among Isfahan alpha-thalassemia patients.[49] The other mutations identified in Faramarzi et al study, subjects were: a-5nt, polyA1, Hb CS, codon 142, a2 (3.7%), –a 4.2, --MED, –a codon 19 (GCG>GC–), –a 20.5, aaa anti3.7 triplication and other mutations.[47]

In this study, we evaluated a large group to detect the most prevalent of alpha and beta thalassemia mutations in Kohgiluyeh and Boyer-Ahmad region. As expected, like other reports from other parts of country and also from neighboring provinces, mutations have different frequencies in different parts.

Conclusion

A high rate of carrier state for thalassemia mutations in Kohgiluyeh and Boyer-Ahmad Province was observed. This high allelic diversity of thalassemia mutations in Kohgiluyeh and Boyer-Ahmad reflects the heterogeneity of this population in this geographical area. The difference in the prevalence of thalassemia mutations reported from different parts of the world may reflect the effect of different factors including cousin marriage, environmental factors, geographic region, race and ethnicity and detection methods.

In conclusion, although mutations for thalassemia are variable and heterogeneous among Iranian population, limited numbers of mutations are responsible for the majority of genetic defects in different areas of the country. This fact provides the possibility of planning efficient platforms for prenatal diagnosis of thalassemia using molecular based approaches in each region. In addition, timely genetic counseling and the availability of centers for prenatal diagnosis in early pregnancy is recommended in this area.

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Conflict of Interest: None declared.

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