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Spectrum of Mutations of Thalassemia among Couples from Izeh City, Khuzestan Province, Iran

Azam Khedri[1], Fatemeh Asadi[2*], Seyedeh Moloud Rasouli Ghahfarokhi[3], Narges Obeidi[4,5], Frough Talebi[6], Sahar Moghbelinejad[7]

[1]Department of Clinical Biochemistry, Faculty of Medicine, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran
[2]Department of Molecular Genetics, Science and Research Branch, Islamic Azad University, Fars, Iran
[3]School of Nursing and Midwifery, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Department of Hematology, School of Para Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
[5]Blood Transfusion Organization, Bushehr, Iran
[6]Department of Midwifery, Faculty of Nursing and Midwifery, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
[7]Department of Genetics, Qazvin University of Medical Sciences, Qazvin, Iran

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**Corresponding author:*
Fatemeh Asadi
Department of Molecular Genetics, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran
Tel: +98 71 43311172
Fax: +98 71 43112201
Email: Fatemehasadi@miau.ac.ir

ABSTRACT

Background: Hemoglobinopathies are inherited blood disorders with an autosomal recessive pattern. We aimed to evaluate the frequency of mutations of thalassemia and hemoglobinopathies among couples referred to health centers of Izeh in Khuzestan Province, Iran.
Methods: This cross-sectional study was performed on 150 couples referred to Izeh Health Centers in 2015-2018. DNA was isolated from peripheral venous blood samples and then the *HBB* gene was analyzed by using Sanger sequencing. For molecular analysis of α-globin gene, multiplex Gap-PCR and ARMS-PCR was performed to identify mutations of α-thalassemia.
Results: DNA analysis revealed 13 different mutations for beta thalassemia in studied couples. Three mutations including 36/37 (- T), IVS-II-1 (G>A) and IVS-I-110 (G>A) accounted for 20.7, 19.3 and 13.3% of beta thalassemia mutations, respectively. For alpha thalassemia; α3.7 (49.5%), -- MED (19.1 %) and -α4.2 (3.1%) were identified as the most common mutations.
Conclusion: Considering common alpha and beta mutations of this geographic area of Iran could be useful concerning genetic counselling in of the population where the rate of consanguineous marriage is high.

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Introduction

Hemoglobinopathies are inherited disorders of hemoglobin all over the world. They are classified into thalassemia syndromes (α- and β thalassemia) and structural Hb variants resulting from mutations in the globin genes causing alterations in quantity and quality of globin chains.

Thalassemia is more prevalent in the Mediterranean region, North and West Africa, the Middle East, Indian subcontinent, southern Far East and southeastern Asia[1] β-thalassemia is commonly observed in people of Eastern Mediterranean region. Iran is one of the countries in which the frequency of β-thalassemia mutations is high with approximately more than two million β-thalassemia carriers and 25000 β thalassemia major (β-TM) patients .[1] The highest prevalence of thalassemia mutations (about 10 %) has been reported from Northern and Southern regions of Iran. Its prevalence in other regions has been estimated to be 4-8%.[2] Mutations in the *β*-globin (*HBB*) gene results in decreased or absent synthesis of β-globin chains.[3]

The *HBB* gene is located on a short arm of chromosome 11(11p15.5) and contains three exons with a length of 1600 base pairs.[4] The majority of β-thalassemia mutations result from point mutations; however, deletions have also been reported.[5] More than 400 β-thalassemia mutations

have been recognized but population studies show that 40 of these alleles are responsible for the most cases of β-thalassemia in the world.[6, 7] The most common mutations leading to α-thalassemia syndromes are deletional variants, while point mutations and small insertion/deletions may rarely occur. More than 750 different variants of α-globin genes have been reported worldwide. A significant aspect of alpha thalassemia in the Mediterranean population (including Iran) is the heterogeneity of the mutations, in particular, non-deletional mutations.[2] Structural hemoglobin variants result from point mutations in the α- or β-globin genes. The most widespread variants in the world are Hb S, Hb C, Hb E, and Hb D-Punjab.[3, 4]

Khuzestan province with approximately 4.000.000 inhabitants located in the southwest Iran is a mixture of different ethnic populations (Arab, Persian, Bakhtiari, Qashqai, and Lor) due to migrations and is a reservoir of various mutations of thalassemia and other hemoglobinopathies.[8] Izeh city (with dominant population of Bakhtiari) which is located in the North of Khuzestan province is the prominent ethnic population with a high frequency of consanguineous marriage. In the present study we aimed to investigate mutations of thalassemia and hemoglobinopathies among couples referred to health centers in Izeh, Khuzestan, Iran.

Materials and Methods

This descriptive study included 150 couples who were randomly selected from Izeh health centers during 2015 to 2018.

Complete blood count test (CBC), Hb electrophoresis and measurement of Hb A2 level, measurement of serum iron level, ferritin, and total iron binding capacity (TIBC) was performed and individuals with iron deficiency anemia were excluded from the study. Quantification of Hb A2, Hb F, Hb A and other Hb variants was performed using Sebia Minicap system (France) according to the manufacturer's instructions. The Minicap system uses the principle of capillary electrophoresis in free solution and automatically produces the electropherogram. Individuals with an elevated Hb A2 levels (>3.5%) were classified as of β-thalassemia.[5]

Genetic Analysis

Five ml of whole blood was collected into EDTA vacuum tubes (Becton Dickinson) for detection of mutations of thalassemia and hemoglobinopathies. Genomic DNA was extracted from leukocytes by conventional salting out method and then Sanger sequencing was performed to identify different types of mutations in *HBB* gene. Amplification and sequencing of the entire *β*-globin gene was performed by using two pairs of primers; the sequence of primers was selected according to the previous publications.[6] Polymerase chain reaction (PCR) was carried out in a 25 µl reaction mixture containing 50 ng of genomic DNA, 0.2 mM of each deoxynucleotide, 1.5 mM of $MgCl_2$, 1 x PCR Buffer, 0.2 mM of each primer, one unit of Taq-DNA polymerase and distilled water. After incubation at 94°C for 3 min, the amplification program was followed by 30 cycles at 94°C for 45 seconds, 65 °C for 45 seconds (annealing), 1.5 min at 72 °C (extension), and the final extension at 72°C for 7 min. After electrophoresis of the PCR products on a 1% agarose gel, sequencing of the PCR products was performed using an ABI 3730 xl DNA Sequencer (Applied Biosystems Inc, Foster City, CA). For molecular analysis of α-globin gene, multiplex Gap-PCR was used first to evaluate common α-thalassemia mutations and deletions in Iran (-- MED, -a $^{3.7}$, -α $^{20.5}$ and –a $^{4.2}$). If any mutation was not detected, ARMS-PCR was performed to screen for -α CD19, - α CS and α 5nt deletion.

Statistical Analysis

Data was analyzed with SPSS software (version 18.0), using descriptive statistical tests (mean, SD, and frequency for quantitative data including Hb, MCV and MCH and Hb A2.

Results

Among 150 couples, 102 couples (68%) had low MCV and MCH levels (MCV= 77.0±1.6 and MCH= 22.8. ±1.9) along with Hb A2 ≥3.5% and thus were identified as β-thalassemia carriers.

The couples with normal blood indices or those who had low MCV (<80 Fl) and MCH (<27 pg) with normal levels Hb A2 (Hb A2: 2.3±0.5) were considered as carriers for other hemoglobinopathies or other cases of thalassaemia such as α- thalassaemia. It was found that 64 of 300 (21.3%) of individuals were heterozygous for Hb S, Hb D, Hb C and or co-inheritance of an Hb variant and β-thalassemia (Table 1). Mean values of red cell parameters and hemoglobin electrophoresis were listed in Table 2.

Mutation analysis revealed 13 different types mutations in the *HBB* gene. Three alleles; CD 36/37 (- T), IVS-II-1 (G>A) and IVS-I-110 (G>A) with a frequency of 20.7%, 19.3% and 13.3%, respectively were the most common. Other mutations such as +20 (C>T) in the *5'* untranslated region, -28 (A>C), and IVS- II-745 (C>G) were also observed.

Table 1: Distribution of haemoglobin variants among all couples

Haemoglobin variant	Number	% in 300 cases
Hb S trait (Hb AS)	20	6.6
Hb S/Beta thalassemia	4	1.33
Hb C trait (Hb AC)	16	5.33
Hb C/Beta thalassemia	7	2.33
Hb D trait (Hb AD)	13	4.33
HBE trait (Hb AE)	4	1.33
Total	64	21.33

Table 2: Mean values (mean±SD) of red cell parameters and hemoglobin electrophoresis

Variant	Frequency of patients	MCV	MCH	HbA	HbA2	HbF	Other
Hb S carrier	24(8.0%)	80.5±5.2	27.3.±5.9	82.2±4.8	3.3±.6	0.75±0.8	13.5±3.7
Hb D carrier	13(4.33%)	82.7±2.6	28.4.±3.2	79.7±1.6	2.4±.1.6	1.2±0.2	16.2±3.8
Hb C carrier	23(7.66%)	80.3±1.6	27.1.±2.5	74.6±5.7	2.2±1.8	1.7±0.3	21±10.5
Hb E	4 (1.33%)	81.5±3.6	22.2.±1.5	80.4±7.2	2.1±.5	0.5±0.9	15.3±7.6

Table 3: Summary of frequencies of Mutant alleles in all β-thalassemia subjects

n	Mutation	Type	Number of Mutant alleles	Origin	Frequency of allele
1	CD 36/37(-T)	β^0	62	Kurdish, Iranian	20.66%
2	IVS-II-1 (G>A)	β^0	58	Mediterranean	19.33%
3	IVS-I-110 (G>A)	β^+	40	Mediterranean	13.33%
4	IVS- II-745 (C>G)	β^+	34	Mediterranean	11.33%
5	5UTR+20(C>T)	β^+	32	Iranian	10.66%
6	-28 (A>C)	β^+	28	Kurdish	9.33
7	CD 82/83 (-G)	β^0	12	Azerbaijani	4.0
8	CD8/9 (+G)	β^0	8	Asian, Indian	2.66%
9	IVS-I (-del 25nt)	β^0	6	Med East	2%
10	CD8 (-AA)	β^0	6	Mediterranean	2%
11	IVS-I-5 (G>C)	β^0	6	Mediterranean	2%
12	IVSI-1 (G>A)	β^0	4	Mediterranean	1.33%
13	CD39 (C>T)	β^0	4	Mediterranean	1.33 %
	Total		300		100%

The three common deletions of α thalassemia were -α3.7 (49.5%), --MED (19.1%) and -α4.2 (3.1%). The results of allele frequency of thalassaemic subjects are summarized in Table 2.

It was found that 21.3% of individuals were heterozygous for Hb S, Hb D, Hb C and or co-inheritance of an Hb variant and β-thalassemia (Table 3). The genetic analysis for these Hb variants revealed mutations in the β-globin gene, HBB: c.20A>T), HBB: c.67G>C and HBB: c.19G>A corresponding to Hb S, Hb D and Hb C, respectively.

Discussion

Thalassemia syndromes are common genetic disorders with a great variety of different mutations in Iranian population. Recent studies have indicated the presence of more than 50 mutations in the *β*-globin gene in most provinces of Iran.[7, 8] In a study from Iran in Khuzestan province; Galehdari *et al* in 2010 found 42 different mutations associated with β-thalassemia and identified eight common β-globin variants, namely, Hb S [β6(A3)Glu→Val], Hb C [β6(A3)Glu→Lys], Hb D-Punjab [β121(GH4)Glu→Gln] and Hb O-Arab [β121 (GH4) Glu→Lys]. The distribution of thalassemia mutations in their study was typical of a heterogenous population with three mutations as the most common including [codons 36/37 (-T), IVS-II-1 (G>A) and IVS-I-110 (G>A)] at a frequency of 20.5, 20.0 and 14.2%, respectively.[9] In the present study, 13 *β* mutations were found which the most common mutations were similar to the abovementioned report.

In another study performed by Hosseini Nezhad *et al*., the three most common mutations consisted of IVS-II-1 (G>A) (26%), IVS-I-1 (G>A) (16%), and IVS-I-110 (G>A) (14%).[10] These findings were also consistent with other studies from Khuzestan. In a study by Sayahi *et al*. on spectrum of thalassemia among voluntary couples in city of Shushtar, CD 36/37 (-T), IVS II-1 (G>A) and IVS I-110 (G>A) were identified as the most common mutations.[11]

Dehghani *et al*., reported two mutations; CD 36/37 *(-* T) (%13.9) and IVS- II-1 (G>A) (10.1%) as the most prevalent mutations of β-thalassemia from Ahwaz, the capital of Khuzestan province.[12]

In another study from Khuzestan province, the most common mutations were: IVSII-1(25%), IVSI-110 (16%), CD 36/37(15%), CD5(9%), IVSI-S(8%), IVSI-6(7%), IVSI-1(5%) and CD39(5%). IVSII-I was the most frequent mutation in this survey.[13]

Doosti *et al*. evaluated mutations of the alpha and beta-thalassemia among carriers of thalassemia referred for prenatal diagnosis in Shadegan City. CD36/37(-T) (38.61%), IVS- II-1 (G>A) (24.75%) and CD (-AA) (12.87%) were reported as the most common mutations.[14]

In another study from Khuzestan province, some different mutations were reported which included IVS-II-745(C>G), +20(C>T in the 5′ untranslated region) and IVS-II-745(C>G).[15]

Asadi and colleagues found the same mutations as our study from other cities of Khuzestan province.[16] Comparison of frequency of β-thalassemia mutations among different cities in Khuzestan province is shown in Table 4.

Our findings revealed some similarities and differences with other parts of Iran (Table 5). For example, the mutation in codons 36/37(-T) has been identified as the most common β-thalassemia mutation in the majority of Iran's provinces. It was found first in Lorestan and then in Isfahan with a frequency of 33% and 9.7%, respectively.[17] While its frequency is low in Hamedan and Hormozgan province.[8]

Table 4: Frequency of detected mutations in *β*-thalassemia subjects in comparison to other provinces of Iran

	Mutation	Izeh% (n=300)	Khuzestan% (n=1241)	Lorestan% (n=130)	Bushehr (%) (n 120)	Kermanshah% (n=370)
1	codons 36/37 (-T)	20.66	20.54	33.80	0.0	7.84
2	IVS-II-1 (G>A)	19.33	20.01	27.70	13.7	32.9
3	IVS-I-110(G>A)	13.33	14.18	11.50	5.9	8.38
4	IVS- II-745(C>G)	11.33	1.30	1.60	3.4	0.0
5	5'UTR +20 (C>T)	10.66	1	0.0	0.0	0.0
6	-28 (A>C)	9.33	0.82	0.0	0.0	0.0
7	CD 82/83 (-G)	4.0	2.10	0.0	0.0	1.08
8	CD 8/9 (+G)	2.66	3	10.80	8.8	13.51
9	IVSI (-del 25nt)	2	2.54	0.76	24.0	0.0
10	CD39 (C>T)	2	3.50%	0.0	3.4	2.97
11	IVS-I-1 (G>A)	2	3.7%	0.0	8.8	4.59
12	CD8 (-AA)	1.33	3.0%	0.0	0.0	5.94
13	IVS-I-5 (G>C)	1.33	5.18	4.70	7.8	2.16

Table 5: Frequency of detected mutations in *β*-thalassemia subjects in comparison to other cities in Khuzestan (All numbers are percentage)

Mutation	Izeh	Masjed soleiman	Izeh/Baq-Malek	Shadegan	Abadan	Shoushtar	Ahvaz
Codons 36/37 (T)	20.66	26.7	22.70	38.61	15	23.4	20.54
IVS-II-1 (G>A)	19.33	22	19.23	24.75	25	23.4	20.01
IVS-I-110(G>A)	13.33	16.27	8.46	5.94	16	10	14.18
CD 82/83 (-G)	11.33	12.7	0.0	1.98	0.0	9	2.10
IVS-II-745(C>G)	10.66	6.97	11.53	1.60	0.0	0.9	1.30
5'UTR +20 (C>T)	9.33	8.1	11.53	0.0	0.0	0.9	1
CD 8/9 (+G)	4.0	3.48	0.0	1.98	0.0	0.0	3
CD39 (C>T)	2.66	3.48	1.54	0.99	5	9	3.50%
IVS I (-del 25nt)	2	2	3.06	1.98	-	4.5	2.54
CD39 (C>T)	2	2	1.54	0.99	-	0.9	3.5
IVS-I-1 (G>A)	2	2	1.92	0.99	5	-	3.73
CD8 (-AA)	1.33	1.33	6.15	12.87	-	1.88	3.00
IVS-I-5 (G>C)	1.33	1.33	1.54	0.99	8	0.9	5.18

The second common mutation in our study was IVS-II-1 (G>A), known as the Mediterranean type, found in countries such as Syria, Lebanon and Palestine.[18] This mutation is frequently observed in Iran with a frequency of %66 in the northern provinces (Mazandaran, Gilan, and Golestan),[19] 21% in the north western provinces (Azerbaijan and Ardabil),[2] 31.3% in Qazvin[20] and 25.61% in Hamedan.[6]

IVS-I-110 is the third most prevalent mutation in our study which has been found in North west of Iran with a frequency of 18%.[2] Interestingly, this mutation has not been reported in Hormozgan according to the literature.[8] IVS-I-110 has been also reported in countries such as Lebanon and Turkey with frequency of 33.0% and 39%, respectively.[21]

We also found less common mutations of IVS- II-745(C>G), +20(C>T in the 5′ untranslated region, -28 (A>C) with frequencies of 11.33, 10.66 and 9.33%, respectively.

The codons 8/9 (+G) is an Asian-Indian allele which was found in the most parts of Iran with a frequency of 13.5% in Kermanshah,[22] 15.7% in Kurdistan and West Azerbaijan,[23] and 14.5% in North-west of Iran;[2] whereas, it has a low frequency in Khuzestan (3%),[9] similar to the statistics of our study.

IVS-I-5 (G>C) which has been observed as the second most prevalent variant in Iran with a frequency of 69% in south Iran (Hormozgan) and 72% in south east of Iran,[8] was uncommon in our study and other reports from Khuzestan province (5%).

Other detected mutations were CD82/83(-G), IVSI (-del25nt), CD39 (C>T), IVSI-1 (G>A) and CD 8 (–AA), which accounted for 11.3% of mutant alleles. This finding was approximately similar to the study by Galehdari *et al.* which reported a frequency of 15.8% for these mutations (Table 3).[9]

The most prevalent deletions for α thalassemia were -α $^{3.7}$, -α $^{4.2}$, and -- MED. The deletions that most commonly lead to α-thalassemia whole over the world include; -$\alpha^{3.7}$, -$\alpha^{4.2}$, Mediterranean (--^{MED}) and Southeast Asian deletions (--^{SEA}). The most common deletions of α-thalassemia in the Iranian population are −$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$ and -- ^{MED}.[24-29]

In a study by Khosravi *et al.*, the -a $^{3.7}$ and -- ^{MED} deletions were reported as the most common in Bakhtiari population of the Khuzestan province.[30] Deletion of -α3.7 was reported with high prevalence (79.6%) among alpha thalassemia carriers in Shushtar city.[11]

Nezhad *et al.* showed that -α3.7/αα (50%), Med/αα^{thal} (12%), and -$\alpha^{4.2}$/αα (10%) were the most prevalent α deletional mutations in southwestern Iran.[10]

Meanwhile, -α3.7 (57.1 %), -- MED (17.4%), -α4.2 (3.1%) and -α20.5 (1.5%) are reported as common mutations of α thalassemia in Masjed Soleiman, Khuzestan.[31]

Conclusion

In this study, we found 13 different mutations in addition to previous ones in Khuzestan province. This could due to the dominance of specific mutations in certain geographic areas. 36/37 (- T), IVS-II-1 (G>A) and IVS-I-110 mutations of beta gene and also IVS-II-745 (C>G), *+20* (*C>T*) in the 5' untranslated region , and -28 (A>C, Codons 82/83 (–G) were reported with high frequency in Izeh city. These mutations in this area should be considered in genetic counselling and thalassemia prevention strategies.

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Conflict of Interest: None declared.

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