

## Prevalence of Hemoglobin Mutations and Hemoglobinopathies in Masjed Soleiman County, Southeastern Iran

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**ABSTRACT**

**Background and Objectives:** Hemoglobinopathies are characterized by defects in the synthesis of globin chains of hemoglobin (Hb). The purpose of the present study was to evaluate mutations associated with thalassemia and other hemoglobinopathies in Masjed Soleiman County, Iran.

**Methods:** This descriptive study was carried out on 456 individuals suspected of having hemoglobinopathies who were referred to health centers of the Masjed Soleiman Country in 2015-2017. Blood samples were collected in EDTA tubes. Complete blood count test was performed and red blood cell indices were determined. Level of Hb variants was measured using capillary electrophoresis. Reverse dot-blot, gap-polymerase chain reaction and Sanger sequencing were carried out to detect mutations.

**Results:** We found that 17.7% of the subjects were heterozygous for  $\beta$ -thalassemia. Frequency of mutations 36/37 (-T), IVS-II-1 (G>A) and IVS-I-110 (G>A) in the  $\beta$ -globin gene was 26.7%, 22% and 16.27%, respectively. In addition, 9.5% of the subjects contained Hb S, Hb D and Hb C, while 1.1% of the subjects showed co-inheritance of an Hb variant and  $\beta$ -thalassemia. In subjects with  $\alpha$ -thalassemia, the  $-\alpha^{3.7}$  (57.1%),  $-\alpha^{MED}$  (17.4%),  $-\alpha^{1.2}$  (3.1%) and  $-\alpha^{20.5}$  (1.5%) deletions were found as the most prevalent mutations.

**Conclusion:** In addition to the high prevalence of  $\beta$ -thalassemia and HBB gene mutations, we detected variants Hb S, Hb D, Hb C and co-inheritance of an Hb variants and  $\beta$ -thalassemia in individuals living in the Masjed Soleiman Country. We also identified four mutations in the  $\alpha$ -globin gene. These results can be useful for genetic counseling in this population.

**KEYWORD:** Hemoglobinopathies,  $\beta$ -Thalassemia,  $\alpha$ -Thalassemia, mutation, HB variant.

## INTRODUCTION

Hemoglobinopathies are among the most prevalent inherited disorders around the world. The hemoglobin (Hb) molecule in humans is made up of two pairs of unlike globin chains designated as  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  (1). Hb disorders are classified into thalassemia syndromes ( $\alpha$ - and  $\beta$ -thalassemia) and structural Hb variants. Approximately 7% of the world's population carry an abnormal Hb gene, and about 300,000-500,000 infants are born with clinically significant Hb disorders every year (2). Thalassemia is more prevalent in the Mediterranean region, North and West Africa, the Middle East, the Indian subcontinent, southern Far East and southeastern Asia. Iran is a high-incidence region for  $\beta$ -thalassemia, but the incidence rates vary from one area to another. For instance, the prevalence of  $\beta$ -thalassemia trait is above 10% near the Caspian Sea and the Persian Gulf and about 4-8% in other areas of Iran (3).  $\beta$ -thalassemia results from mutations in the *HBB* gene cluster which is located on the short arm of chromosome.

More than 300  $\beta$ -thalassemia-causing mutations and 1,000 Hb variants have been identified ([http://globin.cse.psu.edu/hbvar/men\\_u.html](http://globin.cse.psu.edu/hbvar/men_u.html)). In Iran, there are about 25,000  $\beta$ -thalassemia patients and 2 million carriers (4). In addition, 50 different mutations have been detected in different populations of Iran. According to studies, the prevalence of  $\beta$ -thalassemia is higher in the northern parts of Iran (4, 5). Abnormal manufacturing or absent of  $\alpha$ -globin chains causes  $\alpha$ -thalassemia. The  $\alpha$ -globin gene loci are duplicated ( $\alpha\alpha/\alpha\alpha$ ).  $\alpha$ -thalassemia carriers are individuals without the two  $\alpha$ -globin genes ( $--/\alpha\alpha$  or  $-\alpha/-\alpha$ ) that have smaller red blood cell (RBCs) and a lower RBC count (mild anemia). However, loss of three  $\alpha$ -globin genes ( $--/-\alpha$ ) results in Hb H disease. Hb Bart's hydrops fetalis syndrome is caused by deletion of all four  $\alpha$ -globin genes (6).

Structural Hb variants result from point mutations in the  $\alpha$ - or  $\beta$ -globin genes. The most widespread variants in the world are Hb S, Hb C, Hb E and Hb D-Punjab (7).

Different ethnic groups and tribes live in the Khuzestan Province, Iran. It has been reported that the prevalence of  $\alpha$ - and  $\beta$ -thalassemia as well as Hb S, Hb C and Hb D is high in this province (8). Bakhtiari people are the

predominant ethnic group that resides in the Masjed Soleiman County in the Khuzestan Province, and consanguineous marriage is a common custom among this ethnic group. In this study, we aimed to evaluate the prevalence of hemoglobinopathies in the Masjed Soleiman, Iran.

## MATERIALS AND METHODS

The study included 456 individuals suspected of having anemia and hemoglobinopathies who were referred to health centers of Masjed Soleiman in 2015-2017. Blood samples were collected in EDTA tubes. Screening for hemoglobinopathies included complete blood count, Hb electrophoresis and measurement of Hb A2 level. The standard diagnostic marker for  $\beta$ -thalassemia is elevation of the Hb A2 level (3.5%). Complete blood count was carried out with an automated hematology analyzer (Sysmex KX-21 N, Kobe, Japan). Quantification of Hb A2, Hb F, Hb A and other Hb variants was performed using Sebia Minicap (France) according to the manufacturer's instructions. The Minicap system uses the principle of capillary electrophoresis in free solution and automatically produces one electropherogram (9).

Genomic DNA was extracted from whole blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Reverse dot-blot hybridization analysis was performed using  $\beta$ -Globin Strip Assay SEA™ (ViennaLab Diagnostics, Vienna, Austria) according to the manufacturer's instructions to screen for common  $\beta$ -globin gene mutations. The results were then validated with amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) (10). For this purpose, primers were designed according to previous studies (4, 11). In patients without mutation, Sanger sequencing was performed to identify undetected mutations. DNA was amplified by PCR using specific primers as described by Galehdari et al., and PCR products were sequenced with an ABI 3730 DNA analyzer (Applied Biosystems Inc., CA, USA) (12). Individuals with low mean corpuscular volume (MCV < 80.0 fL), mean cell Hb (MCH) of less than 27.0 pg, normal Hb A2 and normal iron levels were suspected to be  $\alpha$ -thalassemia carriers.

Multiplex Gap-PCR was used to screen for  $\alpha$ -thalassemia mutations and common deletions in Iran ( $--^{MED}$ ,  $-a^{3.7}$ ,  $-a^{20.5}$  and  $-a^{4.2}$ )(13, 14). For subjects without mutation in the multiplex Gap-PCR, ARMS-PCR was first performed to screen  $-a^{CD19}$ ,  $-a^{CS}$  and  $-a^{5nt}$  deletion. If no mutation was detected, DNA sequencing was done to identify unknown mutations. Statistical analysis was performed using SPSS software (version 18.0).

## RESULTS

Among the 456 subjects, 81 subjects (17.7%) had low MCV and MCH levels and Hb A2 of less than 3.5%, and thus were identified as  $\beta$ -thalassemia carriers. It was found that 8.4% of individuals were heterozygous for Hb S, Hb D, Hb C and Hb Barts. In addition, 1.1% of the subjects showed co-inheritance of an Hb variant and  $\beta$ -thalassemia. Table 1 represents the mean values of RBC parameters and table 2 show

the distribution of Hb variants.

Genetic analysis of the *HBB* gene revealed eight mutations among which 36/37 ( $-T$ ), IVS-II-1 ( $G>A$ ) and IVS-I-110 ( $G>A$ ) with respective frequencies of 26.7%, 22% and 16.27% were the most common mutations. Other frequent mutations were IVS-II-745( $C>G$ ), 5UTR+20( $C>$ ), CD82/83( $-G$ ), Fr 8/9 ( $+G$ ) and CD39 ( $C>T$ ). Frequency of mutant *HBB* alleles is presented in table 3. Mutations found in the *HBB* gene for Hb S, Hb D and Hb C were c.20  $A>T$ , c.67  $G>C$  and c.19 $G>A$ , respectively.

Moreover, 63 subjects (13.8%) were suspected cases of  $\alpha$ -thalassemia. The  $-a^{3.7}$  (57.1%),  $--^{MED}$  (17.4%),  $-a^{4.2}$  (3.1) and  $-a^{20.5}$  (1.5%) deletions were also detected in the suspected cases (figure 1), and no mutation was found in 12 cases. Table 4 shows the frequency of  $\beta$ -thalassemia mutations in different cities of the Khuzestan Province, Iran.

Table 1- Mean ( $\pm$  SD) values of RBC parameters and Hb fractions in variants detected by capillary zone electrophoresis

Variant	Frequency (%)	MCV (fL)	MCH (pg)	Hb A (%)	Hb A2 (%)	Hb F (%)	Other (%)
Hb S carrier	17(3.7%)	81.3 $\pm$ 7.6	26.8 $\pm$ 4.8	81.2 $\pm$ 4.8	2.3 $\pm$ 0.6	1 $\pm$ 0.4	15.5 $\pm$ 4.8
Hb D carrier	11(2.4%)	83.3 $\pm$ 5.6	27.5 $\pm$ 2.9	78.7 $\pm$ 1.6	1.9 $\pm$ 1.3	3.2 $\pm$ 0.2	16.2 $\pm$ 3.8
Hb C carrier	9(1.9%)	82.3 $\pm$ 9.6	29.2 $\pm$ 3.8	73.2 $\pm$ 5.7	3.1 $\pm$ 1.1	2.7 $\pm$ 0.7	21 $\pm$ 0.5
Hb Barts	2 (4%)	72.5 $\pm$ 3.6	22.2 $\pm$ 1.5	77.4 $\pm$ 7.2	2.1 $\pm$ 0.5	-	20.5 $\pm$ 7.6

Table 2- Distribution of Hb variants

Hb variant	Number of hemoglobinopathy cases (%)	Percent among all studied cases
Hb S trait (HbAS)	17 (38%)	3.7%
Hb S- $\beta$ -thalassemia	3 (6.9%)	0.65%
Hb C trait (HbAC)	9 (20%)	1.9%
Hb D trait (HbAD)	11 (25%)	2.4%
Hb C- $\beta$ -thalassemia	2 (4.5%)	0.44%
Hb Barts	2 (4.5%)	0.44%
Total	44 (100%)	9.5%

Figure 1- Multiplex-Gap PCR analysis of  $\alpha$ -globin gene on agarose gel electrophoresis. Lane 1 (left to right): DNA marker, lane 2: normal  $\alpha$ -globin gene (control), lane 3:  $-a^{3.7}$  deletion (2022 bp), lane 4:  $-a^{4.2}$  (1628 bp) and lane 5:  $-^{MED}$  (807 bp).

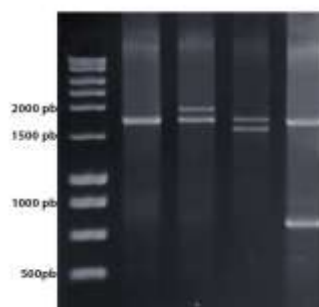


Table 3- Frequency of HBB gene mutations

Mutation	Type	Mutant allele	Origin of mutation	Frequency (%)
CDs 36/37 (-T)	$\beta^0$	23	Kurdish, Iranian	26.7
IVSII-1 (G>A)	$\beta^0$	19	Mediterranean	22
IVSI-110 (G>A)	$\beta^+$	14	Mediterranean	16.27
CD82/83 (-G)	$\beta^+$	11	Mediterranean	12.7
5UTR+20 (C>T)	$\beta^+$	7	Iranian	8.1
IVS II-745 (C>G)	$\beta^+$	6	Kurdish	6.97
Fr 8/9 (+G)	$\beta^0$	3	Asian, Indian	3.48
CD39 (C>T)	$\beta^0$	3	Mediterranean	3.48
<b>Total</b>		<b>86</b>		<b>100%</b>

Table 4-Percentage frequency distribution of mutations in  $\beta$ -thalassemia subjects in different cities of the Khuzestan Province, Iran

Mutation	Masjed Soleiman	Eizeh and Baq-Malek	Shadegan	Abadan	Shushtar	Ahvaz
CDs 36/37 (-T)	26.7%	22.70%	38.61%	15%	23.4%	20.54%
IVS-II-1 (G>A)	22%	19.23%	24.75%	25%	23.4%	20.01%
IVS-I-110(G>A)	16.27%	8.46%	5.94%	16%	10%	14.18%
CD 82/83 (-G)	12.7%	0.0	1.98%	0.0	9%	2.1%
IVS- II-745(C>G)	6.97%	11.53%	1.60%	0.0	0.9%	1.3%
5'UTR +20 (C>T)	8.1%	11.53%	0.0	0.0	0.9%	1%
CD 8/9 (+G)	3.48%	0.0%	1.98%	0.0	0.0	3%
CD39 (C>T)	3.48%	1.54%	0.99%	5%	9%	3.50%

## DISCUSSION

Hemoglobinopathies include thalassemia and structural variants of Hb (Hb S, Hb C and Hb E) that result from defects in the synthesis of the globin chains. Iran is a multi-ethnic state that shows heterogeneity in Hb variants and thalassemia mutations. The Khuzestan Province in southeast of Iran also encompasses various ethnic groups including Arab, Persian, Bakhtiari and Lurs, and has a high prevalence of  $\alpha$ - and  $\beta$ -thalassemia mutations (19). So far, 42 different  $\beta$ -thalassemia mutations and several common  $\beta$ -globin variants including, Hb S, Hb C Hb D-Punjab Hb O-Arab, Hb E and Hb J-Iran have been identified (4, 8, 12, 15, 16).

Hb S is produced by a point mutation in the *HBB* gene. Clinical features of Hb S- $\beta$  thalassemia are variable, ranging from a completely asymptomatic state to a severe disorder that is similar to homozygous sickle cell disease. The prevalence of this condition is high in south of Iran, particularly the Khuzestan Province (16).

Hb D is a  $\beta$ -chain variant that is mainly found in northwest India, Pakistan and Iran (17). It may be co-inherited with Hb S or  $\beta$ -thalassemia. The co-inheritance of  $\beta$ -thalassemia and Hb D can result in a slightly lower Hb level.

HbC is another variant in which lysine replaces glutamic acid in the sixth position of the  $\beta$ -globin chain. Individuals with Hb C trait (Hb AC) are phenotypically normal, while those with Hb C disease (Hb CC) may have a mild degree of hemolytic anemia and splenomegaly (18).

In the present study, 17.7% of the subjects were  $\beta$ -thalassemia carriers and had eight different types of  $\beta$ -thalassemia mutations. Among these mutations, frequency of CDs 36/37 (-T), IVS-II-1 (G>A) and IVS-I-110 (G>A) was higher. In addition, Hb S, Hb D and Hb C were identified as the most prevalent abnormal Hb variants. The genetic analysis of these variants revealed the following mutations in the *HBB* gene: c.20A>T (3.7%), c.67G>C (2.4%) and c.19G>A (1.9%) in Hb S, Hb D and Hb C, respectively. Moreover, co-inheritance of an Hb variant and  $\beta$ -thalassemia was found in 1.1% of the subjects.

In a study by Galehdari et al. (2011) in the Khuzestan Province, the most frequent mutations were CD 36/37 (-T) (20.4%), IVS-II-1 (G>A) (20.0%) and IVS-I-110 (G>A) (14.2%), and Hb S, Hb D and Hb C were reported as the most prevalent variants (12). Another study in the Khuzestan Province reported CD 36/37 (-T) and IVS II-1 (G>A) as the most common mutations with frequency of 13.9% and 10.4%, respectively (20). Similar to our findings, in the mentioned study, 0.5% of subjects showed co-inheritance of HbS and  $\beta$ -thalassemia.

The CDs 36/37 mutation was reported as the most common mutation in the Lorestan (33.8%) and Isfahan (19.7%) provinces (21, 22), which is similar to our results. Studies in north and west of Iran reported IVS-II-I as the most common mutation (15, 23).

In our study, the most common mutations were CD 82/83(-G), 5UTR+20(C>T) and IVS II-745(C>G). However, these mutations have been rarely detected in other provinces of Iran. We identified the  $-\alpha^{3.7}$  deletion (57.1 %) as the most common mutation in the  $\alpha$ -globin gene, which is consistent with results of previous studies in Iran (25-27). Other mutations detected in our study included  $-\alpha^{MED}$  (17.4%),  $-\alpha^{4.2}$  (3.1%) and  $-\alpha^{20.5}$  (1.5%). Moreover, 12 subjects showed no mutation in the  $\alpha$ -globin gene.

The most common deletions observed in  $\alpha$ -

thalassemia patients included  $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $-\alpha^{MED}$  and  $-\alpha^{SEA}$  deletions (24). In Iran, the most common mutation are  $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $-\alpha^{20.5}$  and  $-\alpha^{MED}$  deletions (16, 28, 29).

In a study by Khosravi et al., the  $-\alpha^{3.7}$  and  $-\alpha^{MED}$  deletions were reported as the most common mutations in the Bakhtiari population of the Khuzestan Province (30). Hossieni Nejad et al. conducted a study on  $\alpha$ - and  $\beta$ -globin gene mutations in Abadan and Khorramshahr (Khuzestan Province) and reported IVSII-I as the most prevalent mutation (31), which is inconsistent with our findings. Similar to our study, a study in city of Shadegan (Khuzestan Province) detected CD 36/37(-T) (38.61%) and  $-\alpha^{3.7}/\alpha\alpha$  (52.99%) as the most common mutations among  $\beta$ -thalassemia and  $\alpha$ -thalassemia carriers, respectively (32). In study of Sayahi et al. on spectrum of thalassemia among voluntary couples in city of Shushtar, CD 36/37 (-T), IVSII-1 (G>A) and IVSI-110 (G>A) were identified as the most common mutations (33). In this study, we used capillary zone electrophoresis for the detection of hemoglobinopathies. Joshaghani et al. used the same technique for detection of Hb abnormalities in north of Iran, and identified the Hb S (55%), Hb H (27%), Hb Barts (41%), Hb D (4.68%) and Hb E (2.7%) variants (34). These findings are in line with our results except for the Hb E, which was not detected in our subjects.

## CONCLUSION

In addition to the high prevalence of  $\beta$ -thalassemia and *HBB* gene mutations, we detected Hb variants Hb S, Hb D, Hb C and co-inheritance of Hb variants and  $\beta$ -thalassemia in individuals from the Masjed Soleiman Country in the Khuzestan Province, Iran. We also identified four mutations in the  $\alpha$ -globin gene. These results can be useful for genetic counseling in this population.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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