



# Molecular Analysis of $\beta$ -Globin Mutations Among $\beta$ -Thalassemia Patients in Hamadan

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## Abstract

**Background:**  $\beta$ -Thalassemia ( $\beta$ T) is one of the most common genetic diseases. The specific mutation profile of that region can be identified by determining the specific mutations of each region and ethnicity.

**Objectives:** This study investigated the  $\beta$ -globin mutations in patients with  $\beta$ T in Hamadan.

**Methods:** This cross-sectional study was performed on 47  $\beta$ T carriers. In the present study, the polymerase chain reaction (PCR)-sequencing technique was used to confirm  $\beta$ T carriers, and data were analyzed with SPSS-16 at a 95% confidence level.

**Results:** In general, 164 individuals (81 men and 83 women) suspected of having thalassemia were examined, where 28.7 % (n=47) of them were identified by PCR-sequencing with  $\beta$ T carriers (48.8% male and 53.2% females). Hemoglobin beta (HB $\beta$ ): c.251 del, HB $\beta$ : c.27dupG, and HB $\beta$ : c.92+5G>A mutations had the greatest effect on mean corpuscular volume (MCV) reduction, mean corpuscular HB (MCH) reduction, and HbA2 increment, respectively. The most common mutation in both males and females was the same (HB $\beta$ : c.315+1G>A).

**Conclusion:** According to the results, the most common mutations in the diagnosis of  $\beta$ T in Hamadan were serially HB $\beta$ : c.315+1G>A mutation and HB $\beta$ : c.25-26del, HB $\beta$ : c.112del, HB $\beta$ : c.20A>T, HB $\beta$ : 92+6T>C, and HB $\beta$ : c.316-106C>G.

**Keywords:** Mutation,  $\beta$ -Globin,  $\beta$ -Thalassemia, Genetics

## Background

$\beta$ -Thalassemia ( $\beta$ T) is the most common single-gene disorder worldwide, which is inherited in an autosomal recessive manner (1). The hemoglobin beta (HB $\beta$ ) gene contains three exons that are located on 11p15.5. Decreased synthesis in  $\beta$ -chain production following mutations in the  $\beta$ -globin gene causes an imbalance in the ratio of alpha- and  $\beta$ -chains that precipitate extra thalassemia  $\beta$ -chains (2).

It is mostly caused by point mutations while less frequently by a deletion in the  $\beta$ -globin gene. More than 300 mutations affecting the  $\beta$ -globin gene have been identified across the world. These mutations suppress the syntheses of globin chains partly or completely (3). Thalassemia is more prevalent in the Middle East, North and West Africa, southern Far East and southeastern Asia, and the Mediterranean region (4).

Iran is located on the thalassemia belt. The frequency of  $\beta$ T mutations in the Northern and Southern of Iran is high with a carrier rate of approximately 10% while it has been reported to be 4-8% in other regions (5). Hamadan has a mixture of different ethnic populations including Turk, Persian, Lor, and Lak. The type and prevalence of mutations might be different according to demographic

characteristics and ethnicity. A significant aspect of  $\beta$ T is the heterogeneity of the mutations.

Given that the Iranian population is composed of a mixture of different ethnic groups, the specific mutation profile of a region can be identified by determining the specific mutations of that region and ethnicity (6). Accordingly, it seems that the determination of the distribution and frequency of  $\beta$ -globin mutations in various regions of Iran is an urgent necessity. Because of the high carrier rate of  $\beta$ T in certain populations and the availability of genetic counseling and prenatal diagnosis, population screening is an important option in several at-risk populations. Population screening, along with genetic counseling is useful by allowing high-risk couples to make informed decisions on their reproductive choices. Therefore, the prevention of the disease and the success of prenatal diagnosis require adequate knowledge of the spectrum of  $\beta$ T mutations. Hence, the present study sought to investigate the mutations of  $\beta$ T in Hamadan, Iran.

$\beta$ T causes significant mortality in affected individuals, making prenatal diagnosis an important alternative for couples at risk of having  $\beta$ T major offspring. The prevention of the disease by genetic counseling and prenatal diagnosis plays a particularly important role in this part of the world

with limited resources for medical care.

## Methods

After approval by the Ethics Committee of Hamadan University of Medical Sciences, this cross-sectional study was performed on 47  $\beta$ T carriers who were from Hamadan during 2018-2021.

### Sample Preparation

Five milliliters of the whole blood was collected from the patients after obtaining a signed informed consent form. The complete blood count test and Hb electrophoresis were performed, and individuals with iron deficiency anemia were excluded from the study. According to the National Thalassemia Guideline, suspected individuals were referred for the detection of  $\beta$ T carriers by molecular genetics methods (7).

### Polymerase Chain Reaction

Genomic DNA was extracted from peripheral blood by the conventional salting-out method (8). The concentration of DNA was quantified using a spectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, DE, USA). The amplification and sequencing of the entire  $\beta$ -globin gene were performed using the following pairs of primers according to the previous publication (9):

HB $\beta$ 12R: 50 -CAC TGA CCT CCC ACA TTC CCT T-30;  
HB $\beta$ 12F: 50 -AAC TCC TAA GCC AGT GCC AGA AGA-30;  
and HB $\beta$  3R: 50 -GCA ATC ATT CGT CTG TTT CC-30;  
HB $\beta$  3F: 50 -ACT AAG CCA GTG CCA GAA GA-30.

The polymerase chain reaction (PCR) was performed in a 25  $\mu$ L reaction mixture containing 50 ng of genomic DNA, 12.5  $\mu$ L Master Mix (1X), 0.1 mM of each primer, one unit of Taq-DNA polymerase, and distilled water. After incubation at 94°C for 5 minutes, the amplification program was followed by 30 cycles at 94 °C for 45 seconds, 65°C for 1 minute (annealing), 30 seconds at 72°C (extension), and the final extension at 72°C for 7 minutes. After the electrophoresis of the PCR products on a 1% agarose gel, the sequencing of the PCR products was performed using an ABI 3730 xl DNA Sequencer (Applied Biosystems Inc, Foster City).

### Statistical Analysis

In the study, frequency and percentage, as well as mean

and standard deviation (SD) were applied to describe qualitative variables. Kolmogorov-Smirnov test was used to evaluate the normality of quantitative variables. Two independent sample *t* test was employed to compare the means. In this study, the significant level was less than 0.05, and data were analyzed by SPSS software version 16.

## Results

In general, 164 individuals (suspected of  $\beta$ T) were studied, including 81 (49.4%) men and 83 (50.6%) women. Further, 28.7% (n=47) of them, who were  $\beta$ T carriers including 22 48.8% men and 25 (53.2%) women, were diagnosed by the PCR-sequencing method. The mean ( $\pm$ SD) age of  $\beta$ T carriers was 26.84.7.21 years.

Table 1 summarizes data on blood indices in the carriers and non-carriers of  $\beta$ T. Based on the data, although the mean of the mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and HbA2 differed in men and women, these differences were extremely negligible and not statistically significant ( $P>0.05$ ).

In general, 14 different types of mutations were found among  $\beta$ T carriers (Table 2). Based on the results, the three most common mutations among the 47 carriers were HB $\beta$ : c.315+1G>A (32.4%), HB $\beta$ : c.112del (10.6%), and HB $\beta$ : c.25.26 del (10.6%), respectively. The most common mutations in women were HB $\beta$ : c.315+1G>A and HB $\beta$ : c.112del, respectively. For men, the most common mutation was HB $\beta$ : c.315+1G>A, and the two mutations HB $\beta$ : c.92+6T>C and HB $\beta$ : c.316.106C>G jointly ranked the second.

Table 3 presents the descriptive indices (mean and SD) of blood indices by the type of mutation in diagnosed  $\beta$ T carriers. The results demonstrated that the amount of the SD for mutations whose frequency was equal to one could not be calculated, and only the mean value was mentioned in this regard.

The results showed that the mean MCV in the HB $\beta$ : c.251 del mutation is the lowest compared to other mutations. In other words, this mutation has the greatest impact on MCV. Subsequently, the mean MCV was lower in HB $\beta$ : c.27dupG and HB $\beta$ : c.112 del mutations, respectively. The findings represented that the HB $\beta$ : c.20A> T mutation has a less effect on the MCV value compared to other mutations.

It was further found that the mean MCH in HB $\beta$ : c.27dupG mutation is the lowest compared to other mutations in  $\beta$ T carriers, indicating that the HB $\beta$ : c.27dupG

**Table 1.** Blood Indices in Positive and Negative Carriers of  $\beta$ T by Gender

Gender	$\beta$ T Carriers			$\beta$ T Non-carriers		
	MCV (Mean $\pm$ SD)	MCH (Mean $\pm$ SD)	HbA2 (Mean $\pm$ SD)	MCV (Mean $\pm$ SD)	MCH (Mean $\pm$ SD)	HbA2 (Mean $\pm$ SD)
Male	69.61 $\pm$ 7.03	22.91 $\pm$ 3.17	4.37 $\pm$ 1.71	78.30 $\pm$ 5.55	25.71 $\pm$ 2.23	2.53 $\pm$ 0.88
Female	70.77 $\pm$ 9.86	22.72 $\pm$ 3.54	3.80 $\pm$ 1.36	78.43 $\pm$ 4.83	25.47 $\pm$ 1.98	2.38 $\pm$ 0.62
<i>P</i> value	0.661	0.849	0.238	0.889	0.549	0.274

Note.  $\beta$ T:  $\beta$ -thalassemia; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; SD: Standard deviation.

**Table 2.** Frequency Distribution of Mutations in βT Carriers

Mutation	Male (n=22)	Female (n=25)	Total (n=47)
HBβ: c.25.26 del	2	3	5
HBβ: dup exon1	0	1	1
HBβ: c.283G>A	1	1	2
HBβ: c.315+1G>A	4	7	11
HBβ: c.135 del	1	2	3
HBβ: c.138c>A	1	0	1
HBβ: c.20 A>T	1	3	4
HBβ: c.27 dupG	1	1	2
HBβ: c.112 del	1	4	5
HBβ: c.251 del	1	0	1
HBβ: c.92+5G>C	2	1	3
HBβ: c.92+6T>C	3	1	4
HBβ: c.92+1G>A	1	0	1
HBβ: c.316.106C>G	3	1	4

Note. HBβ: Hemoglobin beta; βT: β-thalassemia.

**Table 3.** Mean and SD of the Blood Index According to the Type of Mutation in βT Carriers (n=47)

Mutation	MCV (Mean±SD)	MCH (Mean±SD)	HbA2 (Mean±SD)
HBβ: c.25-26 del	70.96±9.89	23.54±4.24	4.16±1.03
HBβ: dup exon1	79.20±0.0	24.40±0.0	1.60±0.0
HBβ: c.283G>A	79.30±2.069	26.75±1.34	1.80±0.01
HBβ: c.315 +1G>A	70.44±6.04	22.30±1.78	3.81±0.95
HBβ: c.135 del	66.73±4.18	23.07±3.35	4.00±0.0
HBβ: c.138 c>A	67.20±0.0	22.00±0.0	3.70±0.0
HBβ: c.20 A>T	89.17±4.61	30.33±0.45	2.13±0.51
HBβ: c.27 dup G	61.65±1.48	19.15±0.64	5.40±0.85
HBβ: c.112 del	63.65±3.75	19.85±0.92	5.05±1.34
HBβ: c.251 del	60.80±0.0	19.80±0.0	4.20±0.0
HBβ: c.92 +5G>C	66.73±1.92	21.83±0.59	5.80±1.59
HBβ: c.92 +6T>C	66.02±1.63	20.65±0.77	5.60±2.19
HBβ: c.92 +1G>A	74.90±0.0	25.20±0.0	4.90±0.0
HBβ: c.316-106 C>G	66.50±9.92	21.75±3.49	3.90±1.95

Note. βT: β-thalassemia; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; SD: Standard deviation; HBβ: Hemoglobin beta.

mutation has a greater effect on MCH in comparison with other mutations. Then, the mean MCH had the lowest values in the two mutations of HBβ: c.251 del and HBβ: c.112 del, respectively. The highest amount of MCH was observed in the HBβ mutation: c.20A> T. Overall, the two mutations of HBβ: c.251 del and HBβ: c.27dupG are the most effective mutations on MCV and MCH.

In the present study, according to the frequency of βT mutations (Table 2), the βT diagnosis profile can be classified into three categories. Therefore, the priority in the diagnosis of βT is the evaluation of HBβ: c.315+1G>A, and then the evaluation of HBβ: c.25-26del, HBβ: c.112del, HBβ: c.20A> T, HBβ: 92+6T>C, and HBβ: c.316-106C>G, and finally, assessment of other mutations.

Table 4 provides the laboratory variables of 164 individuals (47 and 117 βT and non-βT carriers, respectively). The results of the independent t-test showed that the means of variables such as Hb, fetal Hb, and red blood cell (RBC) were significantly different between βT carriers and non-carriers ( $P<0.05$ ). Furthermore, the results revealed that the means of HbF and RBC in βT carriers were higher compared to non-carriers while the mean Hb in βT carriers was lower in comparison with non-carriers.

**Discussion**

Iran is a multi-ethnic country, and the distribution and prevalence of βT mutations vary in different parts of this country. Therefore, it is necessary to study the specific mutations of each region and ethnicity. On the other hand, recognizing common βT mutations and examining them in the fetus can prevent the birth of a baby with major thalassemia. Moreover, the specific mutation profile of that region can be determined by evaluating the specific mutations of each region and ethnicity.

Some blood counts may be associated with βT. For example, in a study conducted by Afrouz et al on 111 marriage volunteers referring to health centers in Yasuj province, the mean of MCV and MCH in patients with βT was statistically significantly lower than this index in patients with αT (10).

Similarly, Madan et al found that Hb, MCV, and MCH were significantly lower ( $P <0.001$ ) in βT patients while HbA2 represented an increase in these patients (11).

The results of our study indicated a significant decrease in MCV, MCH, and Hb indices in βT carriers compared to non-carriers, which is in line with the results of Afrouz et al (10) and Madan et al (11).

In this study, there was a significant difference between the mean Hb, HbF, and RBC between βT carriers and non-carriers ( $P<0.05$ ). The means of HbF and RBC were higher in βT carriers compared to non-carriers while the mean of Hb was lower in carriers in comparison with non-carriers. In our study, there was a small difference between the three MCV, MCH, and HbA2 indices in men and women carrying βT, which was not statistically significant ( $P>0.05$ ).

In their study on the Iranian population in Chaharmahal-Bakhtiari and Isfahan provinces, Heidari Soureshjani et al found that the Fr36/37 (-T) mutations were 34 (26.35%) and 22 (32.35%), respectively. They had the highest frequency among the studied mutations and showed the

**Table 4.** Mean and SD of the Blood Indices of Individuals

Variables	βT Carriers	βT Non-carriers	P Value
Hb	13.14±1.68	14.07±1.67	0.002
HbF	1.05±0.356	0.72±0.43	<0.001
RBC	5.59±0.73	5.52±0.69	0.046

Note. Hb: Hemoglobin; HbF: Fetal hemoglobin; RBC: Red blood cell; SD: Standard deviation; βT: β-thalassemia.

highest relation with MCV and HbA2 blood indices (12), which is in conformity with the results of our research. The Fr36/37 (-T) mutation (i.e., HB $\beta$ : c.112 del) was the third mutation to reduce the mean MCV. The first and second mutations in the current study were HB $\beta$ : c.251 del and HB $\beta$ : c.27 dupG, respectively. The mean of HbA2 was highest in HB $\beta$ : c.92+5G>A, HB $\beta$ : c.92+6T>C, and HB $\beta$ : c.27dupG mutations, respectively, compared to other mutations in  $\beta$ T carriers. The lowest HbA2 levels were observed in HB $\beta$ : dupexon1, HB $\beta$ : c.283G>A, and HB $\beta$ : c.20A>T mutations, respectively. Therefore, unlike the study by Heidari Soureshjani et al (12), the Fr36/37 (-T) mutation (i.e., HB $\beta$ : c.112 del) was not the most common mutation affecting HbA2 in this study although this mutation was the fourth mutation to increase the mean HbA2.

In the current study, the HB $\beta$ : c.27dupG mutation had the greatest effect on MCH while the HB $\beta$ : c.20A>T mutation represented the least effect on MCH. These results have not been reported in previous studies and are not comparable in this regard. In a study in mainland China by Lai et al, a meta-analysis of 16 articles (1981-2015) identified the CD41/42 mutation as the most common  $\beta$ -globin gene mutation while the most common mutation was HB $\beta$ : c.315+1 G>A in our study (13). According to the findings of Lama et al in Nepal (14), Mustafa et al in the Maldives (15), and Alaithan et al in Saudi Arabia (16), the most common mutation was IVS I-5 (G>C).

In their review article (2005-2014), Bazi and Miri-Moghaddam concluded that the IVS I-5 (G>C) and IVSII-1 (G>A) were introduced as the most common mutations (17). Likewise, Maryami et al demonstrated that IVSII-1 (G>A) and IVSI-5 were the most prevalent mutations among Iranian populations, respectively (1). Boonyawat et al in Thailand also indicated the above-mentioned mutation (IVSI-5) was the third most common mutation after mutations in codon 42.41 and codon 17 (AT), respectively (18).

Similar to the findings of previous studies (1,17,19,20), the results of our study revealed that the IVSII-1 mutation (G>A, namely, HB $\beta$ : c.315 + 1G>A) was the most common type in both females and males. Compared to the results of previous studies, the prevalence of the IVSI-5 (G>C) mutation (i.e., HB $\beta$ : c.92+ 5G>C) in our study was 6.4% and this mutation was not the most common mutation in Hamadan, which contradicts the findings of the above-mentioned studies. There are more similarities between our results and those of other studies in Iran in terms of the most common mutations causing  $\beta$ T compared to other regions.

Differences between our study and other studies suggested that the prevalence of thalassemia-causing mutations varies between regions and races. Genetic-ethnic heterogeneity in Iran has led to the emergence of different types of mutations in this gene. Comparisons between different provinces showed that the distribution of mutations considerably differs in type and frequency.

Approximately 60 different types of mutations have so far been reported in Iranian patients. The familiarity of diagnostic centers with Iranian mutations and their distributions can be effectively helpful in genetic diagnostics in different regions of Iran (21).

## Conclusion

According to the results related to the diagnosis of  $\beta$ T in Hamadan, the most common mutations were HB $\beta$ : c.315+1G>A, HB $\beta$ : c.25-26del, HB $\beta$ : c.112del, HB $\beta$ : c.20A>T, HB $\beta$ : 92+6T>C, and HB $\beta$ : c.316-106C>G, respectively. In this study, HB $\beta$ : c.251 del, HB $\beta$ : c.27dupG, and HB $\beta$ : c.92 + 5G>A had the greatest effects on decreasing MCV and MCH while increasing HbA2, respectively. Thus, these mutations should be considered in genetic counseling and thalassemia prevention strategies.

## Authors' Contributions

FB designed the experiments, supervised the work, interpreted the data, and produced and wrote the final draft manuscript. In addition, FR conducted the experimental work, analyzed the data, and wrote the draft manuscript. HR edited the manuscript. Finally, ARS analyzed the data and edited the manuscript.

## Conflict of Interest Disclosures

Authors declare no conflict of interests.

## Ethical Issues

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.228).

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