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**Research Article** 

# Association Between Serum Ferritin Level and Gene Mutations in Patients with Thalassemia Major and Intermediate

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## Abstract

**Background:** Beta-thalassemia is one of the most important health problems worldwide. Identifying common mutations as well as the relationship between serum ferritin level and gene mutations in patients with intermediate and major thalassemia can be helpful in preventive programs.

**Objectives:** The purpose of this study was to assess the association between serum ferritin level and gene mutations in patients with intermediate and major thalassemia.

**Methods:** This descriptive cross-sectional study was done using patients' medical records available in the hospital archives, and data of 132 patients with mutated genes referred to Ahvaz Shafa hospital from 2012-2017 were evaluated. In addition to determining the frequency of different gene mutations, the relationship between different gene mutations and serum ferritin levels, as well as hemoglobin levels before the first blood transfusion and the age of the patients (month) before the first blood transfusion, were investigated.

**Results:** There was no significant relationship between mutated genes and serum ferritin levels (P = 0.2). There was a significant difference between the types of gene mutations and the levels of hemoglobin before the first blood transfusion (P = 0.01). There was no significant difference between the types of gene mutations and the age of the patients before the first blood transfusion (P = 0.4). **Conclusions:** There was no association between the types of mutations and serum ferritin levels as an indicator of disease severity. Patients with hemoglobin levels of 6 to 7 mg/dl were found with a higher likelihood of receiving a blood transfusion, with a higher percentage having the IVSII-I gene mutation, but given that many mutations were random and we just had one or two patients with these mutations, it cannot be used as an indicator for disease severity. We need to conduct studies with a larger sample size to be used as an indicator for disease severity. Since the influence of the other accompanying genes and polymorphism plays an important role in the clinical manifestations of genetic diseases, it can be the reason for some negative results.

Keywords: Ferritin, Mutations, Thalassemia

## 1. Background

Thalassemia refers to a group of genetic disorders of globin-chain production, in which there is an imbalance between the alpha and beta-globin chain production. Beta-thalassemia syndrome results from a decrease in the beta-globin chain, which results in a relative excess of the alpha-globin chain (1). Beta-thalassemia is one of the most important health problems worldwide. The spread of the disorder varies around the world. The results of studies show that this disorder is more prevalent in the north and south of Iran (1, 2). Due to the types of mutations and their importance in different populations, it is important to study the genetic mutations of these mutations and lab-

oratory tests. The prevalence of beta thalassemia mutations in the world is not accidental, and each population and ethnic group has its own specific mutations. Prevention of beta-thalassemia requires a comprehensive examination of various molecular mutations and the association between these mutations and laboratory tests. The average probability of having a baby with beta-thalassemia in Iran is estimated to be one in 300 births, which is almost three times higher than the highest incidence of inherited diseases, such as Down syndrome (3, 4). Considering the different ethnicities in the Khuzestan province, it can be concluded that the diversity of mutations causing beta-thalassemia is also high. In some studies, the association between serum ferritin levels and gene mutations in some diseases, like hereditary hemochromatosis was in-

Copyright © 2021, Jundishapur Journal of Chronic Disease Care. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited. vestigated, but there is no study on this association in patients with thalassemia (5). To this end, this study was done to identify common mutations in beta-thalassemia individuals and the relationship between these mutations and the laboratory tests, which can help us to diagnose patients more quickly and take action when a genetic test is not possible.

## 2. Objectives

The purpose of this study was to assess the association between serum ferritin level and gene mutations in patients with intermediate and major thalassemia in order to determine the frequency of different gene mutations and the relationship between gene mutations and laboratory tests.

## 3. Methods

The present descriptive cross-sectional study was conducted based on hospital information. After the approval of the study by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (Code: IR.AJUMS.REC.1398.297), the patients' medical records were used to collect required information from hospital archives. We reviewed all patients' records, and a checklist was used to collect demographics data, like the type of thalassemia, type of gene mutation, serum ferritin levels, age, sex, ethnicity, hemoglobin levels before first blood transfusion, and age of the patients (month) before first blood transfusion. The inclusion criteria were as follows: 1) all the patients older than one year of age; 2) all transfusion-dependent patients; 3) all the patients who were subjected to at least 10 blood transfusions; and 4) all the patients who had received first blood transfusion when they were younger than two years of age. By studying the files of 132 patients with mutated genes referring to Ahvaz Shafa Hospital from 2012-2017, the frequency of various mutations in the beta-globin gene and the relationship between serum ferritin and hemoglobin levels before first blood transfusion and various gene mutations were investigated. To analyze the data, Chi-squared test and analysis of variance (ANOVA) (or Kruskal-Wallis test) were used. All analyzes were performed using SPSS software version 20. The significance level was set at 0.05.

## 4. Results

Of the 132 patients studied, 56.8% cases (n: 75) were male and 43.2% cases (n: 57) were female. According to age, 2.3% of the patients aged 1-5 years, 10.6% aged 6-10

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years, 16.7% aged 11-15 years, 11.4% aged 16-20 years, and 59.1% aged over 20 years. The distribution of the studied patients based on ethnicity was as follows: 52.3% Arabs, 26.5% Fars, 12.9% Bakhtiari, and 8.3% Lor (Table 1). Also, 90.9% of the studied patients had major thalassemia, and 9.1% had intermediate thalassemia. The highest amount of ferritin level was more than 2500 mg/dl (50.8%), and its lowest amount was less than 500 mg/dl (7.6%) (Table 2). The highest value of the first hemoglobin level before the first blood transfusion was between 6 and 7 mg/dl (42.4%), and the lowest was less than 3 mg/dl (2.3%). The highest frequency of blood transfusion according to the time of the first blood transfusion was 5 to 10 months (46.2%), below 5 months (26.5%), 10 to 15 (15.9%), and over 15 months (11.4%) (Table 3). The highest percentage of mutated genes was as follows: IVSII-I / IVSII-I (28%), CD36-37 / CD36-37 (9.8%), CD8/CD8 (5.3%), and IVSI-110/IVSI-110 (4.5%), respectively. We investigated 58 types of phenotypes and 25 types of genotypes. The most mutated genes based on serum ferritin level were IVSII-I/IVSII-I (28%), CD36-37/CD36-37 (9.8%), CD8/CD8 (5.3%), and IVSI-110/IVSI-110 (4.5%). Using the Chi-squared test, there was no statistically significant difference between mutated genes and patients' blood ferritin levels (P = 0.2). This means that there is no relation between the types of mutations and the level of serum ferritin as an indicator of disease severity. The results showed that there was a significant difference between the types of gene mutations of the studied patients with the level of hemoglobin before first blood transfusion in different classes using the Chi-squared test (P = 0.01). Patients with hemoglobin levels of 6 to 7 mg/dl were found with a higher likelihood of receiving a blood transfusion, with a higher percentage of them having IVSII-I gene mutation. Generally, among the different ethnic groups, the Arabs ethnicity (52.3%) was the common ethnicity, and IVSII-I was the commonest gene mutation in Arab people. The results showed that there was no statistically significant difference between the type of gene mutations in the studied patients and the age (month) at the first blood transfusion using the Chi-squared test (P = 0.4).

# 5. Discussion

Studies have shown that the molecular basis of betathalassemia major and intermediate is very different in different parts of the world. Therefore, it is necessary for each country to determine its own pattern of mutations. Comparisons between different provinces of Iran show that the distribution of mutations varies considerably according to type and frequency (2). In the present study, in addition to determining the frequency of different gene mutations

Variable	No. (%)
Age	
1-5	3 (2.3)
6-10	14 (10.6)
11-15	22 (16.7)
16-20	15 (11.4)
< 20	78 (59.1)
Total	132 (100)
Sex	
Male	75 (56.8)
Female	57 (43.2)
Total	132 (100)
Ethnicity	
Lor	11 (8.3)
Arab	69 (52.3)
Fars	35 (26.5)
Bakhtiari	17 (12.9)
Total	132 (100)

 
 Table 1. Frequency Distribution of Patients With Beta-Thalassemia Major and Intermediate According to Demographic Variables

Table 2. Frequency Distribution of Patients by Type of Thalassemia and Ferritin Level

Variable	No. (%)
Type of thalassemia	
Major	120 (90.9)
Intermediate	12 (9.1)
Total	132 (100)
Ferritin level	
> 500	10 (7.6)
500-1000	14 (10.6)
1000-2500	41 (31.1)
> 2500	67 (50.8)
total	132 (100)

in patients, the relationship between different gene mutations and serum ferritin levels, as well as the hemoglobin levels before the first blood transfusion and the age of the first blood transfusion was investigated. We tried to find whether the studied patients had lower hemoglobin level or if he or she had been subjected to blood transfusion at younger ages and also whether higher serum ferritin level is related to some types of gene mutations or not. It should be noted that high level of serum ferritin is a very common finding in patients with major thalassemia, which is caused by repetitive blood transfusion. Therefore, there 
 Table 3.
 Frequency Distribution of Patients in Terms of Hemoglobin Level and Age

 Before the First Blood Transfusion
 Patients

Variable	No. (%)	
Hemoglobin before the first blood transfusion(mg/dl)		
> 3	3 (2.3)	
3-4	6 (4.5)	
4-5	16 (12.1)	
5-6	45 (34.1)	
6-7	56 (42.4)	
> 7	6 (4.5)	
Total	132 (100.0)	
Age before the first blood transfusion(month)		
5>	35 (26.5)	
5 - 10	61 (46.2)	
10-15	21 (15.9)	
> 15	15 (11.4)	
Total	132 (100.0)	

is an indirect relationship between serum ferritin levels and gene mutations in patients with thalassemia. In the present study, the commonest mutated genes were IVSII-I /IVSII-I (28%), CD36-37/CD36-37 (9.8%), CD8/CD8 (5.3%), and IVSI-110 / IVSI-110 (4.5%), respectively. In a study conducted by Zandi et al. on beta-thalassemia carriers in Birjand, the IVS I-5 had the highest frequency (47.1%) and the frequency of the Codon44, Fr 8/9, IVS II-I, and Codon 37/38/39 mutations was as follows: 17.8, 8.8, 5.9, and 5.9%, and respectively.

Compared with the results of other studies, they concluded that the pattern of mutations obtained in Birjand differed significantly from those in northern and western Iran (6), but in our study, the most common mutation was IVSII-1. In the study by Mohammadi et al. on 116 patients with beta-thalassemia major from different parts of Khuzestan province, the most common mutations were Codon36/37 (14.7%), IVSI-110 mutation (14.2%), IVS11-1 (6.9%), Codon 8 (6.5%), Codon 5 (5.2%), other cases (31%), and unknown mutations (21.6%) (8). In the study by Galedari et al. on 202 patients with beta-thalassemia major in Ahvaz, a total of 29 mutations in the 404 studied alleles were found to be the most common mutations, including IVSI-110 (17.8%), CD 36-37 (16%), IVSI-5 (6.9%), and CD5 (5.2%), respectively (7). In the study by Sharifi et al., the commonest types of mutations in the beta-globin chain in suspicious couples with minor thalassemia in Ilam city were IVSII-1, IVSI- 5, IVSI-6, CD36/37, Fr8-9, and CD82/83 and IVSII-1 was the most common mutation among the other mutations (8). Hashemi et al. reported that IVSII-IG > A was the most common beta-thalassemia mutation in the northern provinces of

Iran (Gilan, Mazandaran, and Golestan). They stated that the distribution of mutations in the north was different from that in the northwest, south, or southeast of the country (9). Hosseini et al. assessed 707 patients with betathalassemia from Arabs, Bakhtiari, Fars, Kurd, and Lor ethnicities, and a total of 39 mutations were detected, and the Cd 36/37(-T) mutation with 156 cases, IVSII-1 (G > A) with 129 cases, and IVSI-110 (G > A) with 66 cases had the highest frequency (10). In the Omrani study, 150 chromosomes of 75 individuals with minor and beta-thalassemia major were studied to determine the types of mutations. Mutations of IVS II- I (G->A) with a frequency of 50.7%, Fr 8-9 (+G) with a frequency of 16%, Codon 44 (-C) with a frequency of 9.3%, IVSI-5 (G $\rightarrow$  C) and IVSI-6 (T  $\rightarrow$  C) each with a frequency of 8%, IVIS-110 (G $\rightarrow$ A) with a frequency of 5.3%, and finally Codon 30 with a frequency of 2.7% were reported. The above 7 mutations covered more than 95% of patients. Compared with the results of other studies, the frequency of mutations in other studies was different from other provinces and the Mediterranean region (11). Darabi et al. assessed 68 patients with the diagnosis of beta-thalassemia major in Kurdistan province and reported mutations of IVS-II-1 in 30 alleles (22.5%), Fr8-9 (+G) in 22 alleles (15.94%), IVSI-1 in 13 alleles (9.42%), and C36 /37 (-T) in 11 alleles (7.97%) as the most common mutations. The type of mutation remained unknown in 42 of the 138 alleles studied (12). Pour Feizi et al. investigated 100 patients with beta-thalassemia in East Azarbaijan and Ardabil provinces. The most common mutations observed in this study were CD36-37/IVSI-110 /CD8/CD5 mutations with the frequencies of 14.7%, 14.2%, 6.5%, and 5.2%, respectively (13). However, in our study, the IVSII-I /IVSII-I (28%), CD36-37/CD36-37 (9.8%), CD8/CD8 (5.3%), and IVSI-110 / IVSI-110 (4.5%) were the commonest mutated genes, respectively. Reasons for differences in the results of different studies can be related to the prevalence of consanguineous marriages in some ethnic groups and differences and genetic affinities in different ethnic groups. In the present study, in addition to determining the frequency of different gene mutations in patients, the relationship between different gene mutations and serum ferritin levels, as well as the hemoglobin levels before the first blood transfusion and the age at the first blood transfusion, were investigated to indicate whether the patient had lower hemoglobin levels or if he or she had received a blood transfusion at a younger age and also whether higher ferritin levels are associated with some types of gene mutations or not. Using the Chi-squired test, there was no statistically significant difference between mutated genes and patients' blood ferritin levels (P = 0.2). There was a significant difference between the different gene mutations of patients and the level of hemoglobin before the first blood transfusion in different classes (P =

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0.01). However, given that many mutations are random and we just had one or two patients with these mutations, it cannot be used as an indicator of disease severity. We need to conduct studies with a larger sample size to use it as an indicator for disease severity. There was no statistically significant difference between the types of gene mutations and the age of the patients receiving blood transfusion (P = 0.4). Because beta-thalassemia mutations are different in different Iranian ethnic groups, it is recommended that such studies be conducted separately in different ethnic groups and races.

## 5.1. Conclusion

In the present study, IVSII-I / IVSII-I (28%), D36-37/CD36-37 (9.8%), CD8/CD8 (5.3%), and IVSI-110 / IVSI-110 (4.5%) were the commonest mutated genes. There was no association between the types of gene mutations and serum ferritin levels as an indicator for disease severity. Patients with hemoglobin levels of 6 to 7 mg/dl were found with a higher likelihood of receiving a blood transfusion, with a higher percentage having the IVSII-I gene mutation, but given that many mutations were random, and we just had one or two patients with these mutations, it cannot be used as an indicator for disease severity. There is a need to conduct studies with a larger sample size. There was no statistically significant difference between the types of gene mutations and the age of the patients who received blood transfusion (P =0.4).

Since the influence of the other accompanying genes and polymorphism plays an important role in the clinical manifestations of genetic diseases, they can be considered as the reason for some negative results.

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#### Footnotes

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