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The Effect of Xmn1 Gene Polymorphism on Blood Transfusion Dependency and Hemoglobin Concentration among Iranian Thalassemia Patients with IVSII-1 Mutation

Mozhgan Hashemieh MD [1], Azita Azarkeivan MD [2,*], Hossein Najmabadi PhD [3], Kourosh Sheibani MD MS [4]

1. Department of Pediatric Hematology Oncology, Imam Hossein Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Iranian Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Thalassemia Clinic, Tehran, Iran.
3. Kariminejad-Najmabadi Genetics Center, Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.
4. Basir Eye Health Research Center, Tehran, Iran.
*Corresponding author: Dr Azita Azarkeivan, Iranian Blood Transfusion Organization, Milad Tower, Tehran, Iran.. Email: azazarkeivan@yahoo.com. Orchid ID: 0000-0001-5026-0175

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Abstract

Background: One of the most important phenotypic modifying factors for thalassemia is the presence of Xmn1 polymorphism. This retrospective study was performed to investigate the overall prevalence of Xmn1 polymorphism among Iranian β-thalassemia patients with homozygote IVSII-1mutation and to assess the relationship between Xmn1 polymorphism with patients' hemoglobin levels and the response to hydroxyurea (Hu) therapy.

Materials and Methods: The present cross sectional study included 112 β-thalassemia patients with homozygote IVSII-1 mutation. Laboratory investigations included complete blood count and routine hematological indices were measured by Sysmex K1000 (Japan) blood auto analyzer. To find the state of Xmn1 polymorphism, blood samples were collected from patients using EDTA containers for genomic DNA analysis. DNA extraction and amplification-refractory mutation to determine the Xmn-1 polymorphism were performed.

Results: In total, 206 thalassemia patients including 112 patients diagnosed as thalassemia major and 94 patients diagnosed as thalassemia intermediate entered the study. The mean age at the start of transfusion was 5 ± 6.4 years old, and all of the patients received hydroxyurea. Twenty eight patients (14%) did not show any Xmn 1 polymorphisms (- / -), and 178 patients (86%) showed polymorphism either in one loci (- / +, 44 patients, 21.3%) or both loci (+ / +, 134 patients, 65%). Patients with Xmn1 polymorphism showed significantly higher age at diagnosis (p=0.002), higher age at start of transfusion (p=0.001), higher hemoglobin levels after treatment with hydroxyurea (p=0.005), and lower transfusion dependency (P=0.044).

Conclusion: The presence of Xmn1 polymorphism led to a delay in onset of blood transfusions, higher hemoglobin levels, better response to hydroxyurea treatment and milder phenotypic presentation among thalassemia patients with IVSII-1 mutation.

Keywords: Blood Transfusion, Hemoglobin, Polymorphism, Thalassemia.

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Introduction

Beta thalassemia is the most common form of chronic hemolytic anemia due to impaired globin chain synthesis which has carrier frequencies of 1 to 20% in Mediterranean region, Africa, and Southeast Asia, representing a major public health problem in these regions (1). Each year, at least 40,000 people worldwide are born with β - thalassemia including 23,000 people with β-thalassemia major (2). Iran is located on thalassemia belt and β - thalassemia is the most common genetic disorder in our country (3). According to available statistics, 25,000 β thalassemia major patients

and about two million carriers of this disease live in Iran (4). The spread of this disease varies among different regions of Iran, but it is most prevalent around the Caspian Sea and the Persian Gulf (5). The severity of β-thalassemia symptoms ranges from transfusion-dependent anemia to milder conditions observed among thalassemia intermediate and minor patients (2). One of the clinical challenges for the management of β-thalassemia is to identify the phenotype of patients as early as possible especially in those patients who are between the transfusion-dependant thalassemia major and the non transfusion dependant thalassemia intermedia (6).

There has been progress in predicting phenotype from genotype among thalassemia patients indicating that variable phenotypes may occur based on different β-globin gene mutations (6). One of the most important phenotypic modifying factors for thalassemia is the presence of Xmn1 polymorphism (7). The association of Xmn1 has been shown to correlate with increased hemoglobin (Hb) production in studies conducted in different countries as well as in Iran (6-9). Xmn-1 polymorphism resulted from a C > T base substitution at the-158 position of Gγ globin (HBG2) gene (8). This polymorphism resides in close proximity to locus control region of β-globin gene (β-LCR), which controls differential expression of β-like globin genes throughout the life (10). In the present study, the aim was to investigate the overall prevalence of Xmn1 polymorphism among Iranian β-thalassemia patients with homozygote IVSII-1mutation, and to assess the relationship between Xmn1 polymorphism with patients' hemoglobin levels and the response to hydroxyurea (Hu) therapy. Hydroxyurea increases the production of fetal hemoglobin (HbF) via reactivation of gamma genes (11). It also suppresses the β globin expression through its cytotoxic effects, which may ameliorate the effects of the alpha/non alpha chain imbalance (12). In this study, only homozygote IVSII-1 patients were included to exclude the effect of alpha-globin gene mutations and other beta globin gene mutations.

Materials and Methods

This was a retrospective study which included 206 β-thalassemia patients with homozygote IVSII-1 mutation. All patients were regularly followed up at Zafar Thalassemia Clinic, Tehran, Iran. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (1397/5/110741), according to the tenants of declaration of Helsinki, regarding the human studies. All patients' medical history and clinical examination results were extracted from their files. Laboratory investigations included complete blood count and routine hematological indices measured by Sysmex K1000 (Japan) blood auto analyzer.

Detection of Xmn1 polymorphism

Blood samples were collected from patients into EDTA containers for genomic DNA analysis. DNA extraction and amplification-refractory mutation to determine the Xmn-1 polymorphism were performed as previously described by Miri et al.(8). In summary, amplification-refractory mutation system PCR (CinnaGen Company, Karaj-Iran) and TaqDNA polymerase (CinnaGen Company,

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Karaj-Iran)) were conducted to determine the Xmn-1 polymorphism as previously described (8).

Statistical analysis

To present data, mean, standard deviation, median, and range were used. To compare the groups, t-test, Mann-Whitney test, one way analysis of Variance, Kruskal Wallis test, Chi-square, and Fisher exact test were utilized. Some statistics were reported with their 95% confidence interval. All statistical analysis was performed by SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.). P-values less than 0.05 were considered statistically significant.

Results

In total, two hundred and six thalassemia patients including 112 females and 96 males were included in the present study. These patients were classified as thalassemia major and intermedia, based on their hematological and clinical phenotype. Patients were from various provinces of Iran and all of them had homozygous form of IVSII-1 mutation. Participants in the present study consisted of 112 patients diagnosed as thalassemia major and 94 patients diagnosed as thalassemia intermediate patients. The mean age at the start of transfusion was 5 ± 6.4 years old, and all of patients received hydroxyurea. The baseline demographic and clinical characteristics are illustrated in Table I. The patients were divided into three major groups based on Xmn1 polymorphism including Xmn-1 +/+, -/+, and -/- groups. Out of 206 patients, 28 patients (14%) did not show any polymorphism (- / -), and 178 patients (86%) showed polymorphism either in one loci (-/+, 44 patients, 21.3%) or both loci (+/+, 134 patients, 65%). There was a statistically significant difference between two sexes regarding the Xmn1 polymorphism, while female patients showing higher percent of polymorphism (p=0.012) (Table II). No statistically significant difference was observed between patients with thalassemia major or those with thalassemia intermedia regarding the Xmn1 polymorphism (p=0.133) (Table II). Table III shows the age at diagnosis, age at start of transfusion, splenectomy, hemoglobin levels, and transfusion frequency related to the presence or absence of Xmn1 polymorphism. There was statistically significant relation between age at diagnosis and Xmn1 polymorphism status indicating lower age at diagnosis for patients without polymorphism compared to patients with polymorphism (p=0.002). The age at start of transfusion was also significantly lower among patients without Xmn1 polymorphism (p=0.001). There was a statistically significant difference between patients with Xmn1 polymorphism and those patients without polymorphism in terms of hemoglobin levels after treatment with hydroxyurea indicating higher levels of hemoglobin among patients with polymorphism (p=0.005). There was also statistically significant difference between different Xmn1 polymorphism groups regarding the transfusion dependency indicating a higher percentage of transfusion dependency (0.044) among patients without Xmn1 polymorphism compared to those patients with Xmn1 polymorphism.

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Table I: Demographic data of patients

Variable		Total
Type of Thalassemia	TM	94 (45.6)
	I	112 (54.4)
Sex	Male	94 (45.6)
	Female	112 (54.4)
Height	Mean ± SD	159 ± 13
	Median (range)	160 (99 to 183)
Weight	Mean ± SD	51 ± 10
	Median (range)	52 (15 to 74)
Age at Diagnosis	Mean ± SD	4.2 ± 5.1
	Median (range)	3 (0.3 to 30)
Age at start of Transfusion (Years)	Mean ± SD	5 ± 6.4
	Median (range)	3 (0.3 to 40)
Dose of Hydroxyurea	Mean ± SD	7.8 ± 2.2
	Median (range)	7 (2 to 14)
Ferritin Before Hydroxyurea	Mean ± SD	1565 ± 2036
	Median (range)	1100 (100 to 23834)
Hb Before Hydroxyurea	Mean ± SD	9.2 ± 1.2
	Median (range)	9.1 (6.6 to 14)
Splenectomy	Yes	119 (58.0)
	No	86 (42.0)
Age of Splenectomy (Years)	Mean ± SD	12 ± 7
	Median (range)	10 (1 to 36)

Table II: Drug usage, age, sex, and type of thalassemia distribution related to Xmn1 polymorphism

Variable		Total	Xmn 1 polymorphism			P
			- / -	- / +	+ / +	
Sex	Male	94 (45.6)	19 (67.9)	23 (52.3)	52 (38.8)	0.012*
	Female	112 (54.4)	9 (32.1)	21 (47.7)	82 (61.2)	
Type of Thalassemia	TM	94 (45.6)	17 (60.7)	22 (50.0)	55 (41.0)	0.133*
	TI	112 (54.4)	11 (39.3)	22 (50.0)	79 (59.0)	
Height		159 ± 13	164 ± 9	160 ± 12	158 ± 13	0.148†
		160 (99 to 183)	165 (143 to 180)	163 (130 to 178)	160 (99 to 183)	
Weight		51 ± 10	56 ± 8	51 ± 10	50 ± 10	0.032†
		52 (15 to 74)	56 (38 to 68)	53 (28 to 74)	51 (15 to 69)	
Dose of Hydroxyurea		7.8 ± 2.2	7.9 ± 1.4	7.7 ± 1.9	7.8 ± 2.5	0.943†
		7 (2 to 14)	7 (7 to 10)	7 (5 to 14)	7 (2 to 14)	

*† Based on One-way Analysis of Variance. , * Based on Chi-Square test., TM: Thalassemia Major ,TI: Thalassemia intermedia*

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Table III: Age at diagnosis, age at start of transfusion, splenectomy, hemoglobin levels, and transfusion frequency related to Xmn1 polymorphism

		Total	Polymorphism Xmn1			P
			- / -	- / +	+ / +	
Age at Diagnosis		4.2 ± 5.1	2 ± 2.1	3.8 ± 4.5	4.8 ± 5.6	0.002‡
		3 (0.3 to 30)	1 (0.5 to 8)	1.8 (0.5 to 20)	3 (0.3 to 30)	
Age at start of Transfusion		5 ± 6.4	2.4 ± 2.4	3.8 ± 4.5	5.9 ± 7.2	0.001‡
		3 (0.3 to 40)	1 (0.5 to 10)	1.8 (0.5 to 20)	4 (0.3 to 40)	
Splenectomy	Yes	119 (58.0)	16 (57.1)	29 (65.9)	74 (55.6)	0.486*
	No	86 (42.0)	12 (42.9)	15 (34.1)	59 (44.4)	
Age of splenectomy		12 ± 7	9 ± 5	13 ± 7	13 ± 8	0.135†
		10 (1 to 36)	8 (1 to 22)	10 (5 to 36)	11 (2 to 35)	
Hemoglobin	Before	9.2 ± 1.2	8.9 ± 0.9	9.3 ± 1.2	9.2 ± 1.2	0.326†
	After	9.4 ± 1.4	8.8 ± 0.8	9.9 ± 1.7	9.3 ± 1.3	0.005†
Transfusion	Dependency	98 (100%)	19 (19.4%)	21 (21.4%)	58 (59.2%)	0.044‡
	Independency	107 (100%)	9 (8.4%)	22 (20.6%)	76 (71.0%)	
Annual Transfusion (N/Year)	Before	13 ± 5	14 ± 5	13 ± 6	13 ± 6	0.773†
	After	5 ± 5	4 ± 4	4 ± 3	5 ± 5	0.158†
Ferritin	Before	1565 ± 2036	1286 ± 877	1668 ± 1433	1596 ± 2367	0.18†
	After	1421 ± 1460	1144 ± 628	1592 ± 1355	1423 ± 1608	0.456†

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Discussion

Beta thalassemia is the most common form of chronic hemolytic anemia due to impaired globin chain synthesis which has carrier frequencies of 1 to 20% in Mediterranean region, Africa, and Southeast Asia, representing a major public health problem in these regions (1). Each year, at least 40,000 people worldwide are born with β - thalassemia including 23,000 people with β-thalassemia major (2). Iran is located on thalassemia belt and β - thalassemia is the most common genetic disorder in our country (3).

The severity of β-thalassemia symptoms ranges from transfusion-dependent anemia to milder conditions observed among thalassemia intermediate and minor patients (2).

One of the clinical challenges for the management of β-thalassemia is to identify the phenotype of patients as early as possible especially in those patients who are between the transfusion-dependant thalassemia major and the non transfusion dependant thalassemia intermedia (6).

There has been progress in predicting phenotype from genotype among thalassemia patients indicating that variable phenotypes may occur based on different β-globin gene mutations (6).

Presence of Xmn1 polymorphism has been reported to be one of the main phenotypic modifying factors of β-thalassemia (6-9). In the present study the aim was to investigate the overall prevalence of Xmn1 polymorphism among Iranian β-thalassemia patients with homozygote IVSII-1 mutation, and to assess the relationship between Xmn1 polymorphism with patients' hemoglobin levels and the response to hydroxyurea (Hu) therapy. In the present study the overall frequency of Xmn1 gene polymorphism was (86%), which happened either in one loci (- / +, 21%) or both loci (+/+, 65%).

In comparison in a study by Said et al., among Egyptian patients with thalassemia major or intermedia patients only 8% of patients showed Xmn1 polymorphism, which was significantly lower than the present study (6). In another study by Miri-Moghaddam et al., in South East of Iran, overall, Xmn-1 polymorphism was observed in 62% of patients which is more similar to the present study findings. It seems that the Xmn1 polymorphism has a variable geographical distribution among patients from different countries (8).

The frequency of positive heterozygote Xmn1 gene polymorphism in thalassemia major and intermedia has been previously compared among Egyptian patients with β-thalassemia (13,14). These studies showed a frequency of 9% polymorphism in β-thalassemia intermedia patients and 4% in β-thalassemia major patients (13,14). The present study findings were in line with these previous findings indicating no statistically significant difference regarding the frequency of positive Xmn1 gene polymorphism between patients with thalassemia intermediate or thalassemia major.

In the present study significant differences in transfusion dependency was observed indicating that Xmn1 positive patients were statistically less transfusion dependent, and the hemoglobin level after treatment with hydroxyurea was higher among patients with Xmn1 polymorphism compared to those patients without polymorphism. This was in line with earlier studies which have shown the

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presence of the Xmn1 polymorphism might contribute to higher hemoglobin levels among thalassemia patients (6-9).

In addition, patients with Xmn1 polymorphism in the present study were more frequently transfusion independent and had an older age at first transfusions.

We also observed that Xmn1 polymorphism had statistically significant effect on the response to hydroxyurea treatment. This was in concordance with previous studies that reported correlation between the presences of Xmn1 polymorphism and response to hydroxyurea therapy in β-thalassemia patients (15-18). For example Bradai et al., in a study including nine patients with thalassemia intermedia and 45 patients with thalassemia major (TM) found that Xmn1-/- was associated with a worse response to hydroxyurea treatment. However, other researchers have reported that the response to hydroxyurea was not significantly correlated with the presence of Xmn1 polymorphism (19,20). For example Koren et al., in a study on 18 beta-thalassemia patients treated with HU found no correlation between response to therapy and the presence of Xmn1 polymorphism, which might be attributed to their relatively small sample size (19). Also in another study by Dixit et al., including thirty-seven patients with beta-thalassemia intermedia no association between response to hydroxyurea and Xmn1 polymorphism was detected (20).

Some shortcomings of the present study are the retrospective nature of the study as well as the relatively low number of patients in thalassemia major and intermediate groups, which might make the results regarding the absence of Xmn1polymorphisem difference between these two groups of patients less reliable.

Conclusion

In conclusion the presence of Xmn1 polymorphism might lead to a delay in onset of blood transfusions, higher hemoglobin levels, better response to hydroxyurea treatment and milder phenotypic presentation among thalassemia patients with IVSII-1 mutation.

Conflict of interest

There is no conflict of interest.

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