IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

The Effect of Xmn -1 Polymorphism and Coinheritance of Alpha Mutations on Age at First Blood Transfusion in Iranian Patients with Homozygote IVSI-5 Mutation

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Received: 13, July, 2020
Accepted: 01, May, 2021

ABSTRACT

Background: Thalassemia syndromes are the most prevalent hereditary hemoglobinopathies in the world. Iran is located on the thalassemia belt. In this study, the effect of Xmn -1 polymorphism and coinheritance of alpha mutations on age at first transfusion and also transfusion interval in Iranian thalassemic patients with homozygous IVSI-5 mutation were assessed.

Materials and Methods: In this retrospective cross-sectional study 154 transfusion dependent thalassemia (TDT) patients (140 patients with β-thalassemia major and 14 cases with β-thalassemia intermedia) who were homozygote of IVSI-5 mutation have been participated. Blood samples were collected from participants using EDTA containers for genomic DNA analysis. DNA extraction and amplification-refractory mutation to determine the Xmn -1 polymorphism were performed. Multiplex PCR was performed to identify alpha globin deletions.

Results: The mean age of participants was 29±7, 58 of them were male and 96 were female. A significant relation between presence of Xmn -1 polymorphism and age at receiving first transfusion was detected. Coinheritance of alpha thalassemia mutation does not have significant effect on age at first transfusion or transfusion interval.

Conclusion: Presence of Xmn -1 polymorphism can delay the onset of transfusion in patients with homozygote IVSI-5 mutation.

Keywords: Thalassemia; Mutation; XmN-1 polymorphism; Transfusion

INTRODUCTION

Thalassemia syndromes are the most common hereditary autosomal recessive disorder in the world. Complete absence or reduction of β chain synthesis is the hallmark of β-thalassemia syndromes[1]. Each year at least 40,000 people with β-thalassemia are born worldwide with highest incidence in Mediterranean, the Middle East, North America and South East Asia regions [2]. Iran is located on thalassemia belt, so in our country, this genetic disorder is a major public health problem[3].

Approximately 3% of the world's population are carriers of β-thalassemia mutations[4]. At present, more than 300 mutations in β globin gene have been detected worldwide. The majority of these mutations are point mutations, but deletions have been reported[5]. Some of these mutations result in deficient synthesis of β chain (β^+ thal), and others lead to complete absence of β globin (β^0 thal). Therefore, the clinical manifestations of this disease can vary from asymptomatic cases to severe forms of thalassemia such as homozygous or double heterozygous variants, which require chronic repeated blood transfusions in order to survive[6]. In Iran, multiple various ethnic groups are living, so the frequency and distribution of mutations in the different parts of our country vary[4]. Najmabadi et al. in a 10-year study on 1217 β – thalassemia chromosomes of 164 affected patients and 889 unrelated carriers have been reported IVS II-I (G > A) was the most common mutation (34%) in Iran and the frequency of this mutation is higher in northern parts of Iran. The second prevalent mutation is IVS I – 5 (G > C) mutation (7.55%), and codon 8/9 (+G) and IVS I – 110 (G > A) were the other most common mutations[4]. Also, other studies have shown that IVS II – 1 and IVS I – 5 are the two common β gene mutations in Iran[5,7,8]. In a recent survey about different β globin gene mutations in Iran, other relatively prevalent mutations include frame shift (FS) mutations in codon 8/9 (+G), IVS I – 110 (G>A), FSC 36/37 (-T), IVS I – I (G>A), IVS I (-25 bp), and codon 44 (-C)[5]. Various forms of β thalassemia result from single base substitution within the sequence of the IVS – I donor site. In IVS I – 5 mutations, substitution of the G at position 5 of IVS – I by C occurs[9]. It is well established that some modifying factors can ameliorate phenotypic expression of β thalassemia homozygotes. Xmn–1 polymorphism is a documented factor, which increases fetal hemoglobin synthesis[10-14]. Xmn -1polymorphism is defined as a C – T substitution at position – 158 of the G gamma globin gene. This genetic variation leads to up regulation of globin gene expression in adulthood, and hence compensates the decreased level of β globin chain. The presence of Xmn -1polymorphism results in lesser imbalanced synthesis of α and β globin chains. This polymorphism resides in close proximity to locus control region (LCR) of β globin genes throughout life[15].

Another factor that can ameliorate the severity of β thalassemia is the coinheritance of alpha mutations leading to a milder phenotype[16, 17]. Inactivation of only one alpha globin gene leads to decrease in severity of disease in carrier of mild beta thalassemia mutation, but the multiplication of α thalassemia can result in exaggeration of the phenotype in β thalassemia intermedia or thalassemia trait cases[18].

In this study, only patients with homozygote IVS I-5 mutation were included to evaluate the effect of Xmn -1polymorphism and coinheritance of α mutations on age receiving first transfusion and transfusion.

MATERIALS AND METHODS

This was a retrospective cross-sectional study which included 154 β thalassemia with homozygote IVS I – 5 mutations. All participants in this study were regularly followed up at Zafar thalassemia Clinic, Tehran, Iran. Also, this research was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR-SBMU.MSP.PEC.1398.832) according to the tenants of declaration of Helsinki, regarding the human studies. All patient's characteristics and laboratory data were extracted from their files.

Detection of Xmn -1polymorphism

Blood samples were collected with EDTA as anticoagulants. Genomic DNA was isolated from 5 – 10 ml blood according to standard protocols. After DNA extraction, amplification – refractory mutation to determine the Xmn-1 polymorphism was performed. In this research amplification – refractory mutation system PCR (Cinna Gen Company, Karaj – Iran) and Taq DNA polymerase (Cinna Gen Company, Karaj – Iran) were performed to determine the Xmn -1polymorphism as previously described [13].

Detection of α mutations

Multiplex PCR was performed to identify alpha globin detections (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -MED and the ααα anti -3.7 triplication [18].

Statistical analysis

To present data, we used mean, standard deviation, median and range. To compare the variables between the two groups, we used t-test, Mann-Whitney, ANOVA, Kruskal-Wallis test, Chi-Square and Fisher exact test. All statistical analyses were performed by SPSS version 22 (Armonk, Ny: IBM Corp). P-values less than 0.05 were considered statistically significant.

RESULTS

In total, 154 TDT (140 cases with β-TM and 14 patients with β-TI) have been participated in this study. The mean age of patients was 29±7 with a range of 8 to 47 years. Of whom (37.7%), 58 were male and 96 (62.3%) were female. Patients were from different provinces of Iran and all of them had homozygous of IVS I – 5 mutation. The demographic characteristics of cases entering the study are shown in Table 1.

No alpha mutation was detected in 109 of 154 patients participating gin this study, and 45 of patients had coinheritance of alpha mutations. The patients were classified into three major groups based on Xmn -1 polymorphism, including + / +, - / + and - / - groups. Out of 154 patients, 142 patients (92.2%) did not show any polymorphism, and 12 patients (7.8%) showed polymorphism either in one locus (- / +, 5 patients) or both loci (+ / +, 7 patients). The demographic characteristics of participants in this study according on Xmn -1polymorphism are shown in Table 2.

In our study, 83 of patients (53.9%) were splenectomized. The mean age of patients at first transfusion in their study was 34±8 months with a range of 2 to 240 months. Also, the mean time of transfusion interval was 23±8 days with a range of 10 to 90 days. Coinheritance of α mutations in these patients has no effect on age at first transfusion and transfusion interval (Table 3).

In this study, the age at first transfusion was also significantly lower among patients without Xmn -1 polymorphism (P value = 0.037). The mean age at first transfusion in patients without Xmn -1 polymorphism was 17±28 months and in patients with polymorphism was 55±65 months. However, there is no relation with the presence of Xmn -1 polymorphism and transfusion interval. The effect of Xmn -1 polymorphism presence on age at first transfusion and transfusion interval is illustrated in Table 4.

Table 1: Demographic data of patients entering the study

Variable		Total
Age	Mean ± SD	29 ± 7
	Median (range)	30 (8 to 47)
Sex	Male	58 (37.7%)
	Female	96 (62.3%)
Place of birth	Hormozgan	52 (34.9%)
	Tehran	18 (12.1%)
	Kashan	1 (0.7%)
	Bushehr	3 (2.0%)
	Arak	0 (0.0%)
	Mazandaran	3 (2.0%)
	S&B	13 (8.7%)
	Chaharmahal	6 (4.0%)
	Esfahan	1 (0.7%)
	Ilam	3 (2.0%)
	Karaj	4 (2.7%)
	Golestan	10 (6.7%)
	Gilan	1 (0.7%)
	Khorasan	4 (2.7%)
	Zanjan	1 (0.7%)
	Ghom	3 (2.0%)
	Khuzestan	11 (7.4%)
	Kohkilouyeh	1 (0.7%)
	Kerman	9 (6.0%)
	Fars	1 (0.7%)
	Hamedan	1 (0.7%)
	yazd	1 (0.7%)
	Kermanshah	2 (1.3%)
ABO and Rh	O+	64 (41.6%)
	O-	5 (3.2%)
	A+	39 (25.3%)
	A-	3 (1.9%)
	B+	33 (21.4%)
	B-	5 (3.2%)
	AB+	4 (2.6%)
	AB-	1 (0.6%)
Type of thalassemia	Beta Thalassemia Major	140 (90.9%)
	Intermedia	14 (9.1%)

Table 2. Demographic data of patients entering the study based on Xmn 1 polymorphism

Variable		Xmn 1 polymorphism		
		-/-	+/-	+/+
Age	Mean ± SD	29 ± 7	31 ± 12	29 ± 5
	Median (range)	30 (8 to 47)	33 (15 to 47)	30 (20 to 34)
Sex	Male	56 (96.6%)	0 (0.0%)	2 (3.4%)
	Female	86 (89.6%)	5 (5.2%)	5 (5.2%)
Place of birth	Hormozgan	48 (92.3%)	2 (3.8%)	2 (3.8%)
	Tehran	15 (83.3%)	0 (0.0%)	3 (16.7%)
	Kashan	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Bushehr	3 (100.0%)	0 (0.0%)	0 (0.0%)
	Arak	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Mazandaran	3 (100.0%)	0 (0.0%)	0 (0.0%)
	S&B	12 (92.3%)	0 (0.0%)	1 (7.7%)
	Chaharmahal	6 (100.0%)	0 (0.0%)	0 (0.0%)
	Esfahan	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Ilam	3 (100.0%)	0 (0.0%)	0 (0.0%)
	Karaj	4 (100.0%)	0 (0.0%)	0 (0.0%)
	Golestan	10 (100.0%)	0 (0.0%)	0 (0.0%)
	Gilan	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Khorasan	4 (100.0%)	0 (0.0%)	0 (0.0%)
	Zanjan	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Ghom	2 (66.7%)	1 (33.3%)	0 (0.0%)
	Khuzestan	11 (100.0%)	0 (0.0%)	0 (0.0%)
	Kohkilouyeh	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Kerman	8 (88.9%)	1 (11.1%)	0 (0.0%)
	Fars	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Hamedan	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Yazd	1 (100.0%)	0 (0.0%)	0 (0.0%)
	Kermanshah	2 (100.0%)	0 (0.0%)	0 (0.0%)
ABO and Rh	O+	59 (92.2%)	3 (4.7%)	2 (3.1%)
	O-	5 (100.0%)	0 (0.0%)	0 (0.0%)
	A+	34 (87.2%)	1 (2.6%)	4 (10.3%)
	A-	2 (66.7%)	1 (33.3%)	0 (0.0%)
	B+	32 (97.0%)	0 (0.0%)	1 (3.0%)
	B-	5 (100.0%)	0 (0.0%)	0 (0.0%)
	AB+	4 (100.0%)	0 (0.0%)	0 (0.0%)
	AB-	1 (100.0%)	0 (0.0%)	0 (0.0%)
Type of thalassemia	Beta Thalassemia Major	134 (95.7%)	3 (2.1%)	3 (2.1%)
	Intermedia	8 (57.1%)	2 (14.3%)	4 (28.6%)

Table 3. The effect of alpha mutation on age at start of transfusion, transfusion interval and splenectomy

Variable		Total	Mutation		P
			No	Yes	
Age at start of transfusion	Mean ± SD	34 ± 8	39 ± 23	15 ± 14	0.414‡
	Median (Range)	8 (2 to 240)	8 (2 to 240)	9 (3 to 60)	
Transfusion interval	Mean ± SD	23 ± 8	22 ± 6	24 ± 13	0.449†
	Median (Range)	21 (10 to 90)	21 (12 to 40)	20 (10 to 90)	
Splenectomy	Yes	83 (53.9%)	60 (55.6%)	23 (50.0%)	0.527*
	No	71 (46.1%)	48 (44.4%)	23 (50.0%)	

‡ Based on Mann-Whitney test; † Based on t-test; * Based on Chi-Square test.

Table 4: The effect of Xmn 1 polymorphism presence on age at start of transfusion, transfusion interval and splenectomy

Variable		Total	Polymorphism Xmn 1		P
			No	Yes	
Age at start of transfusion	Mean ± SD	8 ± 34	17 ± 28	55 ± 65	0.037‡
	Median (range)	8 (2 to 240)	8 (2 to 240)	36 (4 to 228)	
Transfusion interval	Mean ± SD	23 ± 8	23 ± 8	23 ± 7	0.941†
	Median (range)	21 (10 to 90)	21 (10 to 90)	21 (15 to 30)	
Splenectomy	Yes	83 (53.9%)	77 (54.2%)	6 (50.0%)	0.778*
	No	71 (46.1%)	65 (45.8%)	6 (50.0%)	

‡ Based on Mann-Whitney test; † Based on t-test; * Based on Chi-Square test

DISCUSSION

In the present study, the main goal was to assess the effect of Xmn -1 polymorphism and coinheritance of α mutation on the age at first transfusion and transfusion interval in Iranian thalassemia patients with homozygote IVS 1-5 mutation. The previous studies considering the effect of genetic factors including subtype of mutation and Xmn phenotypes on the course and outcome of thalassemia are rare, especially among the Iranian patient population. Since it has been established that the effect of these genetic factors on the course and outcome of the disease is different in various ethnicities, we conducted the present study among Iranian thalassemia patients to avoid some limitations of previous studies namely their relatively low number of participants. Our study population was from a referral clinic in Tehran city and patients were from different provinces, which are living in Tehran.

In multiple studies, it has been demonstrated that IVS 1-5 is the second most common mutation among various Iranian populations[5]. Moreover, IVS 1-5 is the most common documented β chain mutation in southern parts of Iran [19]. In our study, the place of birth in 52 patients (34.9%) was Hormozgan province, which is in line with other studies [19].

Furthermore, this mutation is prevalent in south-East and North-East regions of Iran[5]. In addition, IVS 1-5 is the most common β chain mutation with β° phenotype, in Pakistan, our neighbor country [20].

This mutation is prevalent in other neighboring countries such as India, Saudi Arabia, United Arab Emirate, Kuwait, Bahrain and Iraq [21-26].

In the present study, the majority of our patients had β-TM (90.9%), and only 14 patients (9.1%) had β-TI. This finding is similar to other studies, indicating that patients with homozygous IVS 1-5 mutation often have β-TM phenotype [5].

In many published articles, it has been reported that Xmn -1 polymorphism is one of the most important modifying factors in phenotype of patients [11-13] .In our study, there was a significant relationship between the presence of Xmn -1 Polymorphism and age of onset of transfusion (P=0.037). In other words, patients with Xmn -1 polymorphism had an older age at first transfusion. Moreover, there was no significant relation between the presence of Xmn -1 polymorphism and transfusion interval. These findings are similar to those of previous studies on patients with homozygote IVS II-I mutation[27]. Also, our results are in line with other similar studies. Aditya in a study on 50 β-thalassemia major patients reported a positive correlation between the presence of Xmn -1 and age at first transfusion [10]. Sharma in another study on 130 patients with β-thalassemia major found that the presence of Xmn -1 polymorphism delays the age of receiving the first blood transfusion [28]. Nemati et al. in a study on 197 β-TM patients in Kermanshah province, Iran, reported that there is no significant correlation between the presence of Xmn -1 polymorphism and the age of receiving the first blood transfusion[29]. Furthermore, our results are not consistent with the results of Tantawy et al. who reported that the presence of Xmn -1 polymorphism was not associated with the age of receiving the first blood transfusion, fetal hemoglobin level and transfusion frequency [30]. The other results in this study were the absence of relation between the presence of Xmn -1 polymorphism and transfusion interval. Our results are similar to Oberoi who observed no significant correlation between the Xmn -1 polymorphism and age at onset of symptoms, age at diagnosis, transfusion frequency or the mean hemoglobin level [31]. On the other hand, Maryami et al. have shown that β°/β° patients with Xmn -1 polymorphism showed a significant increase in transfusion interval and require less blood transfusion[32]. Irshad et al. have demonstrated that the transfusion intervals in Xmn -1 (- / +) and Xmn -1 (+ / +) were approximately 30 days in comparison to 7-15 days in Xmn -1 (- / -) patients [33]. Neishabouri et al. in a large study on 362 patients with thalassemia major or intermedia from different provinces reported that Xmn -1 polymorphism alone could not predict the severity of disease[18].

Furthermore, Oberoi reported that Xmn -1 polymorphism could not be relied on in determining the course of disease in thalassemia intermedia [31]. The exact reason for these conflicting results regarding the effect of Xmn -1 polymorphism on disease course is not clear. The difference in sample size in different studies may be a contributing factor. Also, Xmn -1 polymorphism has various penetrations in different multiethnic populations in the world and the percentage of positive Xmn -1 polymorphism differs in different societies. Moreover, other mechanisms except Xmn -1 polymorphism could affect the phenotype and course of β-thalassemia. These genetic elements may be unrelated to the beta globin locus. These quantitative trait loci (QTLs) are on chromosome 8q, 6q23 and 2p15. Moreover, some transcription factors can influence the globin gene expression, including GATA-1, NEF-2 and EKLF. Alteration of these transcription factors has an important role in phenotype determination [34]. In our study, there was no relation between the presence of α mutation with age at first transfusion and transfusion interval. Unlike β-thalassemia mutations, more than 95% of detected α-thalassemia mutations results from deletion of one or both α-globin genes from chromosome 16. The most prevalent α^+-thalassemia single gene deletion defects are $-\alpha^{3.7}$

and –α [4.2 35]. Besides, the most common double gene deletions of α-thalassemia are South Asian (- - / SEA), Mediterranean (- - / MED) and Filipine (- - / FIL) variants. The –α[3.7] deletion has a global distribution in all parts of the world, but –α [4.2] deletion has been detected mostly in Southeast Asia [36]. Among 154 patients in this study, 45 patients had coinheritance of α mutation. The most common single gene deletion was –α [3.7], with 27 patients (17.5%) having heterozygous and 11 patients (7.1%) showing homozygous state, which is similar to Neishabouri results [37]. Also, in this study, the coinheritance of α thalassemia mutations in patients with homozygote IVS 1-5 did not have any effect on age at first transfusion or transfusion interval, showing a consistency with similar articles [17,18].

A limitation of the present study was the relatively high number of incomplete patients' records, which might affect the reliability of our findings. Moreover, this study involved the small sample size of patients with β-TI, which makes our results among this group of patients less reliable.

CONCLUSION

In patients with homozygous IVS 1-5 mutation, the presence of Xmn -1 polymorphism might delay the onset of transfusion, but coinheritance of α mutation has not any effect on onset or interval of blood transfusion. Larger population-based studies are necessary to document a relationship between β chain mutations and Xmn -1 polymorphism or coinheritance of α mutation.

CONFLICT OF INTEREST

The authors have no conflict of interest with the subject matter of the present study.

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