Study of type and frequency of alfa-thalassemia mutations in a cohort of 3,823 patients from Isfahan Province, Iran

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ABSTRACT

Introduction: Alpha-thalassemia (α-thalassemia) is caused by a range of mutations in the α-globin gene resulting in the complete reduction or absence of α-globin chain production.

Material and methods: This study assessed the presence of α-thalassemia in 3,823 patients referred to Al-Zahra Hospital, Isfahan, Iran during a 10-year period (from 2012 to 2022). These patients experienced anaemia for more than ten years but had not the full indication for ß-thalassemia or iron deficiency.

Results: Based on the present assessment, 3,483 cases out of 3,823 suspicious cases had an α-Thalassemia-involved mutation (91.1%). According to the results, the most common detected mutation in the α-thalassemia carriers of Isfahan province was $-\alpha^{3.7}$ with a frequency of 81.58% (3,119 individuals), followed by α^{5nt} (–TGAGG) (3.71% in total or 39.01% between 364 patients), polyadenylation signal mutations (polyA2) (14.28% between 364 patients), $\alpha^{codon\ 19}$ (GCG4GC–, a2) (11.53%), $-\alpha^{3.7}/-\alpha^{3.7}$ (11.53%), $-\alpha^{20.5}$ (7.69%), Hb Constant Spring [Hb CS, a142, Stop>Gln; Hb A2: c.427T4C] (5.7%), $\alpha^{4.2}$ (5.49) and $--^{MED}$ (4.67%).

Conclusion: The results of this investigation may be valuable for designing a program for carrier screening, premarital genetic counselling, and prenatal diagnosis in the Isfahan province.

Key words: thalassemia, alpha-thalassemia, screening, prenatal diagnosis (PND), genetic counselling

INTRODUCTION

Thalassemia is an inherited group of blood disorders that is caused by the unusual production and formation of globin chains (structural units forming haemoglobin protein). Based on the disease-involved globin chain, thalassemia is categorized into two α- and β-thalassemia [1]. Alpha thalassemia (α-thalassemia) has been reported to be the most common monogenic disorder, which affects about 5% of the world's population [2]. Depending on the number of affected globin genes, the clinical symptoms of α-thalassemia place in range from almost asymptomatic anaemia to fatal haemolytic anaemia. More than 80 different genetic mutations have been described to be involved in α-thalassemia, whereas most of them are deletions [3, 4].

Depending on the number of mutated α-globin genes, α-thalassemia can be classified as α^+ and α^0 in which, α^+ patients experience a reduction in the α-globin synthesis, while α^0 patients have no α-globin production (α-thalassemia major). Patients with α-thalassemia major (α^0/α^0) usually die in the uterus or shortly after birth. In contrast, patients with alpha thalassemia minor (α^0/α^+) may develop mild to moderate anaemia and rarely need blood transfusions [5].

Studies have shown a unique mutational profile in different populations. In other words, in each population, few mutations are highly frequent, while the others have a low allelic frequency [6]. In this case, identification of the most frequent α-globin mutation in different populations and/or regions may improve prenatal diagnosis

Figure 1. A map of Iran showing the Isfahan province and others discussed in this study. West Azer: West Azerbaijan, Ham: Hamadan, Chah: Chaharmahal & Bakhtiari, Kohg: Kohgiulyeh & Boyer-Ahmad

(PND) and carrier screening processes [7]. This especially is worthwhile for the countries placed in the "thalassemia belt". The "thalassemia belt" consisted of countries with a high prevalence of thalassemia including Iran [7, 8]. To prevent and control severe thalassemia, the Ministry of Health of Iran has implemented a thalassemia screening program to diagnose thalassemia carrier parents for more than 20 years [9]. Previous studies have reported a broad spectrum of α-thalassemia mutations in different parts of Iran (especially Isfahan), and findings have shown that $-\alpha^{3.7}$ (rightward deletion), α^{5nt} (–TGAGG), $\alpha^{4.2}$ (leftward), the polyadenylation signal (polyA2) site (AATAAA4AATGAA), and $--^{MED}$ are the most common mutations found among Iranian patients from different origins [7, 10, 11]. As more than 5 million people live in Isfahan Province, and this Province has a heterogeneous population in the central region of Iran (Figure 1), it seems worthy to evaluate the frequency of these mutations in a regional platform.

Regarding theses, the present study aims to explore the α-thalassemia mutation spectrum and their hematological indices of people of childbearing age referred to Alzahra University Hospital, the central academic hospital in Isfahan.

MATERIAL AND METHODS

Editorial policies and ethical considerations

The study was approved by the Ethics Committee of Isfahan University of Medical Sciences. A written informed consent form was completed by the participants before sampling or their inclusion in the study.

Patients

The characteristics of all patients who were suspected of thalassemia (by examining blood indices) and were of reproductive age were evaluated for 10 years (2012–2021). All thalassemia carriers were diagnosed based on their blood indices, including hemoglobin (Hb) < 12 g/dL, mean cell volume (MCV) < 80.0 fL, mean corpuscular hemoglobin (MCH) < 27.0 pg, and normal Hb A2 levels.

Table 1. Allele frequency of α-thalassemia mutations in different regions of Iran

Region	$-\alpha^{3.7}$	α^{polyA2}	$--^{MED}$	$-\alpha^{4.2}$	$\alpha^{codon19}$ (GCG4GC–) (α2)	$-\alpha^{HbCS}$ α	$-\alpha^{20.5}$	$\alpha^{-5nt}\alpha$	IVS-I-1 (–5nt)	Ref.
Khuzestan [%]	70.8	0.70	9.80	1.40	8.40	NR	0.70	2.1	NR	[14]
Mazandaran [%]	49.53	15.19	5.84	8.76	3.04	NR	NR	NR	5.49	[15]
Kerman [%]	83.80	0.50	0.30	3.70	5.70	NR	NR	5.70	NR	[16]
Hamadan [%]	61.04	5.52	6.17	4.87	2.27	NR	3.57	10.06	NR	[17]
Lorestan [%]	56.35	9.39	6.08	1.66	NR	NR	0.55	15.47	NR	[18]
Hormozgan [%]	79.10	NR	NR	1.70	12.20	NR	NR	4.30	NR	[19]
Golestan [%]	54.30	3.70	16.00	8.60	NR	NR	NR	2.5	NR	[20]
Khorasan [%]	66.30	2.90	3.80	8.60	NR	1.90	NR	7.7	NR	[20]
Shiraz [%]	43.84	2.45	1.26	2.32	2.33	NR	0.44	NR	4.91	[21]
Kohgiulyeh & Boyer-Ahmad [%]	84.02	5.74	NR	1.23	4.92	NR	1.64	1.23	NR	[22]
Gilan [%]	37.00	11.10	11.10	3.70	NR	7.40	NR	11.10	NR	[20]
Ilam [%]	66.23	3.95	NR	8.33	NR	NR	0.88	10.09	NR	[23]
Kurdistan [%]	70.32	7.74	1.94	6.45	0.65	NR	NR	1.29	NR	[24]
Azerbaijan [%]	76.81	1.07	4.29	4.96	NR	NR	3.89	1.61	NR	[25]
Fars [%]	68.66	4.20	2.13	3.81	3.92	NR	0.75	8.26	NR	[21]
Sistan & Baluchestan [%]	76.52	NR	0.30	2.44	3.96	NR	NR	16.77	NR	[26]
Isfahan [%]	70.78	4.21	0.97	4.85	1.62	NR	3.88	8.74	NR	[7]
Tabriz [%]	81.50	1.10	5.10	5.20	NR	NR	4.40	1.70	NR	[25]
Tehran [%]	57.80	4.04	8.09	4.04	1.70	NR	5.80	14.40	NR	[27]
Iran (in total) [%]	67.10	6.78	3.60	4.68	2.60	NR	0.97	5.99	NR	[28]

NR — not reported

Exclusion criteria included patients with confirmed β-thalassemia, iron deficiency anaemia, and/or unavailability of genetic documents.

Sample collection and DNA extraction

The study was approved by the Ethics Committee of Isfahan University of Medical Sciences. After obtaining the written informed consent, the blood sample of the patients was collected (10 mL). Afterwards, genomic DNA was extracted using the high salt extraction technique as explained by Miller et al. [12].

Polymerase chain reaction (PCR)

The multiplex polymerase chain reaction (multiplex-PCR) protocol, primer sequences designation, and PCR cycling conditions were set up based on Chong et al. [13] instructions. This method was used as a primary screening trial for common deletion mutations ($\alpha^{3.7}$, $\alpha^{4.2}$, $\alpha^{20.5}$, and $--^{MED}$) followed by reverse hybridization using the α-globin Strip-Assay (ViennnaLab Diagnostics, Vienna, Austria) to discover other mutations including codon 14 (G4A) (α1), codon 19 (–G) (α2), IVS-I, 5nt (–TGAGG) (α2), codon 142 (T4C) (α2), codon 59 (G4A) (α2), polyadenylation signal (polyA1) site (AATAAA4AATAAG) (α2), and polyA2 (AATAAA4AATGAA) (α2). These mutations were not covered by the primary polymerase chain reaction (PCR). The results of PCR were confirmed by the DNA Sanger sequencing technique.

Statistical analysis

The Statistical Package for Social Sciences; version 26 software (SPSS Inc, Chicago, IL, USA) is used for data analysis.

RESULTS

Patients demographics

We have screened 3,823 individuals (1,949 males and 1,874 females aged 18–45 years old) for the α-globin gene mutations, who were referred to the Genetics Laboratory of Alzahra Hospital, Isfahan, Iran from 2012 to 2022. For this purpose, the presence of 9 different α-thalassemia-related mutations was assessed using multiplex PCR and reverse hybridization methods. The mutations were selected based on the previous studies performed on the Iranian subpopulations. Results of the previous studies along with the allele frequency of different a-thalassemia mutations in Iranian sub-populations are summarized in Table 1 [14–28]. Also, 340 individuals were excluded as no α-globin gene mutation has been detected in them (8.9%). The lack of α-globin mutations in these subjects was confirmed using DNA sequencing.

Mutation analysis

Among all 9 studied mutations, the $\alpha^{3.7}/\alpha\alpha$ genotype was the most prevalent one (81.50%) (Table 2), while the other

Table 2. Overview of α-thalassemia mutation frequencies and mean (± standard deviation) of hematological values

Genotype	N	[%]	Hb [g/dL]	MCH [pg]	MCV [fL]	Hb A1 [%]	Hb A2 [%]
$\alpha^{3.7}/\alpha\alpha$	3,119	81.58	14.53 ± 2.53	25.58 ± 1.54	78.25 ± 1.50	97.43 ± 0.96	2.56 ± 0.96
Other mutations	364	9.52	13.38 ± 1.33	23.14 ± 2.15	73.23 ± 517	97.29 ± 0.87	2.48 ± 0.52
No mutation	340	8.90	13.5 ± 0.29	25.48 ± 0.30	76.45 ± 1.48	97.93 ± 0.98	2.06 ± 0.98

Hb — hemoglobin; MCH — mean corpuscular haemoglobin; MCV — mean cell volume

Table 3. Less frequent α-thalassemia mutations and mean (± standard deviation) of hematological values

Genotype	N	[%]	Hb [g/dL]	MCH [pg]	MCV [fL]	Hb A1 [%]	Hb A2 [%]
$\alpha^{5nt}/\alpha\alpha$	142	39.01	13.24 ± 0.83	22.72 ± 1.69	73.53 ± 3.39	97.43 ± 0.38	2.47 ± 0.31
$\alpha^{polyA2}/\alpha\alpha$	52	14.28	14.03 ± 1.49	24.50 ± 1.95	75.51 ± 6.34	97.40 ± 0.41	2.43 ± 0.32
$\alpha^{codon19}/\alpha\alpha$	42	11.53	13.47 ± 1.18	24.53 ± 1.55	76.00 ± 3.90	97.32 ± 0.46	2.54 ± 0.37
$-\alpha^{3.7}/-\alpha^{3.7}$	42	11.53	13.27 ± 1.86	22.70 ± 2.31	71.18 ± 5.78	96.78 ± 1.98	2.62 ± 0.86
$-\alpha^{20.5}/\alpha\alpha$	28	7.69	12.70 ± 1.58	20.40 ± 1.85	66.63 ± 4.83	97.01 ± 1.10	2.36 ± 0.80
$-\alpha^{CS}/\alpha\alpha$	21	5.7	13.69 ± 1.64	24.67 ± 1.43	75.57 ± 2.96	97.22 ± 0.30	2.22 ± 0.43
$-\alpha^{4.2}/\alpha\alpha$	20	5.49	13.52 ± 1.51	24.77 ± 1.00	75.82 ± 3.10	97.47 ± 0.21	2.54 ± 0.20
$--^{MED}/\alpha\alpha$	17	4.67	13.31 ± 1.32	20.90 ± 0.86	66.86 ± 2.89	97.31 ± 1.25	2.57 ± 1.10

Hb — hemoglobin; MCH — mean corpuscular haemoglobin; MCV — mean cell volume

mutations were only detected in 8.9% of the studied population. In the case of assessing the 8 remained mutations, the $\alpha^{5nt}/\alpha\alpha$ genotype was the most prevalent mutation in the α-thalassemia carriers (39.01%), followed by $\alpha^{polyA2}/\alpha\alpha$ (14.28%), as well as $\alpha^{codon\ 19}/\alpha\alpha$ and $-\alpha^{3.7}/-\alpha^{3.7}$ 4 with an equal percentage (11.53%), respectively. Other detected mutations were $-\alpha^{20.5}/\alpha\alpha$ (7.69%), Hb Constant Spring [Hb CS, α142, Stop>Gln; Hb A2: c.427T4C] (5.7%),$-\alpha^{4.2}/\alpha\alpha$ (5.49%), and $--^{MED}/\alpha\alpha$ (4.67%) (Table 3).

Analysis of the haematological indexes

To assess any probable relationship between α-thalassemia mutations and complete blood count (CBC) indexes, some factors were assessed including Hb, MCV, and MCH. In this case, there was no significant difference between Hb indexes, while people with $-\alpha^{20.5}/\alpha\alpha$ and $--^{MED}/\alpha\alpha$ showed a slightly decreased MCH, and an approximately 5-units decreased MCV (Table 3).

DISCUSSION

According to World Health Organization (WHO), Iran as a Middle Eastern country is located in the thalassemia belt [29]; therefore, to reduce the increasing number of thalassemia patients, a public premarital screening program has been established since 1991. However, because of the very heterogeneous nature of thalassemia, an important issue in the case of thalassemia prevention and screening is to know its mutational background. So far, numerous studies have been performed to identify the α-globin mutations in different Iranian sub-populations, and at least 42 types of α-thalassemia mutations have been reported [7, 19, 30–32]. Despite these actions, thalassemia is still one of the leading health problems in Iran [21, 33, 34]. As the Iranian population consisted of a broad range of sub-populations, it is crucial to clarify α-thalassemia mutations in different regions and subpopulations to make a better decision for disease screening, prevention, and management. So, the present study aimed to evaluate the α-globin mutations in the Isfahan Province, Iran.

In concordance with the other studies performed on the different Iranian sub-populations, the $-\alpha^{3.7}$ deletion was found to be the most prevalent α-thalassemia mutation in carrier individuals of Isfahan (81.58% of all 3,823 studied subjects) [16, 20, 22, 25, 28, 30, 31, 35–38]. The high prevalence of $\alpha^{3.7}$ deletion mutation may be due to the high consanguinity rate among the Iranian population.

Based on the present findings, the $-\alpha^{5nt}$ deletion (39.01% out of all 364 patients with mutations other than $-\alpha^{3.7}$) was the second most frequent mutation that is in concordance with the study performed on the neighbouring provinces including Hamadan (10.06%), Sistan and Baluchestan (16.77%), Lorestan (15.47%), Tehran (14.40%), Fars (8.26%), Kohgiluyeh and Boyer Ahmad (7.00%), and Kerman (5.7%) [7, 16, 18, 20, 26, 27, 30]. However, the reported allele frequencies in different provinces were different which may be due to the sub-population diversity (Table 1) [14–28].

The $-\alpha^{polyA2}$ (14.28%), $-\alpha^{codon19}$ (11.53%), $-\alpha^{3.7}/-\alpha^{3.7}$ (11.53%), and $-\alpha^{20.5}$ (7.69%) mutations were the subsequent common mutations identified in this study, respectively. These study results are in concordance with the results obtained of $-\alpha^{polyA2}$ point mutation analysis in Mazandaran (15.19%), Gilan (11.10%), and Kurdistan (7.74%) provinces in which, $-\alpha^{polyA2}$ is also introduced as the third most frequent mutation [20, 24]. Contrary to this study, studies in the south of Iran have not reported this mutation [19, 26]. The frequency of $-\alpha^{codon19}$ (GCG4GC–) (α2) mutation and homozygous deletion of $-\alpha^{3.7}/-\alpha^{3.7}$ are equally reported as the fourth most frequent mutation (11.53%). In a similar

study previously conducted in Isfahan, the frequency of homozygous deletion of -$\alpha^{3.7}$/–$\alpha^{3.7}$ was reported to be 2.8% [7]. This difference may be due to the low sample size of the previous study. Deletion of –$\alpha^{codon19}$ (GCG4GC–) (α2) in Fars as a neighbouring province (3.92%) was also reported as the fourth mutation [21]. It is important to note that this mutation has not been reported in the studies performed in Lorestan, Golestan, and Azerbaijan [18, 20, 25].

The frequency of –$\alpha^{20.5}$ mutation in this study is reported to be 7.69%, while in many studies this mutation has not been observed or has been introduced as a rare mutation [14, 16, 18–21, 24, 26, 28]. However, the allele frequencies reported for the provinces of Tehran (5.80%), Azerbaijan (4.40%), and Isfahan (3.88%) were similar to the present study [7, 25]. Differences in the allele frequency percentages that are seen between these studies and the present one may be due to the number of participants and/or different studied sub-populations.

The – –^{MED} deletion mutation is prevalent among Iranians, Palestinians, and Arabs [4]. However, this mutation has the lowest frequency in the present study (4.67%) which is in concordance with the results obtained by Isfahan, Fars, Sistan & Baluchestan, and Kerman [7, 16, 21, 26]. According to the present results, the –$\alpha^{20.5}$ mutation (7.69%) was more prevalent than the – –^{MED} (4.67%) which is similar to the studies of Fars, Isfahan, and Tehran [7, 10, 21, 27], while in Mazandaran, Hamadan, Lorestan, Kohgiluyeh & Boyer Ahmad, Kurdistan, Gilan, Azerbaijan, and Khuzestan, the – –^{MED} mutation occurred more frequently than the –$\alpha^{20.5}$ mutation [4, 14, 17, 18, 20, 24, 25, 30, 31, 35, 38, 39], which may be due to the studied sub-populations.

The frequency of Hb Constant Spring mutation [Hb CS, α142, Stop>Gln; Hb A2: c.427T4C] in this study was 5.8%. It should be noted that this mutation has not been observed in the other studies, except in Gilan (7.40%) and Khorasan (1.90%) provinces [20].

We also found a probable relationship between –$\alpha^{20.5}$/αα as well as – –^{MED}/αα, and the MCV index. While the thalassemia itself may result in a reduction in the MCV index, it seems that the mentioned mutations may cause a more severe microcytosis in red blood cells (RBCs). This finding may be valuable in a faster screening of the carriers; however, it should be confirmed via further studies.

The results of this study indicated that there are a variety of α-thalassemia mutations in Isfahan Province, Iran. In conclusion, this study's results provide a better understanding of α-globin mutations and their distribution in Iran. Identification of the geographical distribution of α- and β-thalassemia allelic frequencies is vital for a better diagnosis and management of β-thalassemia carriers. Moreover, the results may also be useful for upgrading the genetic counselling and PND processes in the at-risk groups.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to patients' privacy or ethical restrictions.

Article information

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