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ORIGINAL ARTICLE

Molecular spectrum of α-thalassemia mutations in Erbil province of Iraqi Kurdistan

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Abstract

α-Thalassemia is a globally prevalent genetic disorder of hemoglobin (Hb) structure where the rate of α-globin chain synthesis is reduced or absent based on the underlying α-globin mutation(s). This study aimed to define the spectrum of α-globin gene mutations and assess their relative frequency within a group of α-thalassemia carriers. A total of 96 young subjects with unexplained hypochromia and microcytosis were recruited. They were referred from the premarital hemoglobinopathy screening and genetic counseling center in Erbil. All subjects were genetically tested for 21 common α-globin gene mutations using multiplex PCR and reverse hybridization. Six different α-globin gene mutations and nine different genotypes were detected in 84 carrier subjects. Their mean Hb was 12.9 (± 1.29) g/dL, of whom 49 subjects (58.3%) had a normal Hb level. The two most frequently encountered mutations were $-\alpha^{3.7}$ deletion (62.86%) and $\alpha 2^{-5nt}$ mutation (20%). Deletions were encountered in 71.43% of the mutated alleles. The most commonly observed genotype was $-\alpha^{3.7}/\alpha\alpha$ (46.43%), followed by $-\alpha^{3.7}/-\alpha^{3.7}$ and $\alpha^{-5nt}\alpha/\alpha\alpha$ genotypes (10.72% each). Carriers of $\alpha^{poly\text{-}A1}\alpha/\alpha\alpha$ and $-\alpha^{3.7}/-\alpha^{-5nt}\alpha$ genotypes showed significantly lower Hb, mean cell volume, and mean cell Hb values comparing to carriers of most other genotypes. In our population, the spectrum of α-globin mutations was confined to a limited number of mutations with deletions being mostly observed.

Keywords α-Thalassemia · $-\alpha^{3.7}$ · α-Globin genotype · Erbil · Iraqi Kurdistan

Introduction

α-Thalassemia is known to be the most common monogenic disorder in the world. It is commonly encountered in South-East Asia, Mediterranean and Middle-East areas, India, and Sub-Saharan Africa [1–7]. Unlike b-thalassemia, where point mutations are common, gene deletions are mostly the cause of a-thalassemia. Deletions may involve one (-α/αα) or both (--/αα) of the two duplicated α-globin genes (α1 and α2) located on chromosome 16. Point mutations of α-globin genes, which are also called non-deletion mutations, are also seen with less frequency [2, 8]. A normal person has four functioning copies of α-globin genes, two on each chromosome (αα/αα). The clinical phenotype of the carriers varies according to the number of affected genes. Individuals with single α-globin gene defect (-α/αα), called silent carriers, usually have normal hemoglobin (Hb) and red cell indices; less commonly they may present with mild reduction of the mean cell volume (MCV) and the mean cell hemoglobin (MCH). Those with two defected α-globin genes, (--/αα) or (-α/-α), known as a-thalassemia minor, show reduced MCV and MCH, with or without anemia. Remarkable imbalance of α:β globin chain will results when three α-globin genes are defected (--/-α) and will result in hemoglobin H disease [4]. The latter is the most symptomatic form of α-thalassemia that is compatible with life. It is characterized by moderate to severe anemia, usually requiring irregular transfusion. Inheritance of no functional α-globin genes (--/--) is not compatible with life and leads to Hb Bart's hydrops fetalis [9].

Although α-thalassemia is found throughout the world, its prevalence among different populations is variable [10]. In Iraqi Kurdistan, α-thalassemia is not common; its carrier rate

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is estimated at < 1%. β-Thalassemia is much more common in this region with a carrier rate of 8% [11]. Since 2009, the health authorities in Iraqi Kurdistan started the premarital hemoglobinopathy screening and genetic counseling program as an attempt to reduce the incidence of symptomatic thalassemia syndromes. In Erbil, a province with nearly two million people in northern Iraq, the genetic determinants of β-thalassemia have been previously explored [12, 13]. So far, no study has scrutinized the genetic basis of α-thalassemia in this province. Knowing the spectrum of α-globin mutations would be essential for this screening program to become more effective. This study aimed to uncover the range and frequency of α-globin mutations in a cohort of carrier subjects.

Patients and methods

This study was conducted in the genetic section of PAR hospital's lab from 2017 to the end of 2019 in Erbil, Iraqi Kurdistan. A total of 96 young individuals who were suspected to be α-thalassemia carriers participated in this study. They were referred for premarital testing to undertake α-globin gene analysis. Informed consent was obtained from the enrolled individuals. The study was approved by the Research Ethics Committee of Hawler Medical University, Erbil, Iraq.

The included subjects had a mean cell volume (MCV) of < 80 fL, mean cell Hb (MCH) of < 27 pg, and normal adult electrophoretic pattern and normal iron studies. The Hb and red cell indices were analyzed by the automated hematology counter (Swelab Spanga, Sweden). The WHO international reference range of Hb was used to define anemia (13.5–17.5 g/dL in male and 12.0–15.5 g/dL in female). Hb analysis was performed by the automated capillary electrophoresis system (CapillaryS 2; Sebia, Paris, France). DNA was extracted from peripheral blood using Gentra Puregene blood kit (Qiagen, Germantown, MD, USA). α-globin genotyping was performed by a commercially available reverse dot blot assay (α-thalassemia Strip Assay—ViennaLab Diagnostics, Vienna, Austria) which could detect common 21 mutations seen in the Mediterranean area according to the manufacturer's instructions. These 21 α-globin mutations are as follows: two single gene deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$), five double gene deletions [$--^{MED}$, $--^{SEA}$, $--^{THAI}$, $--^{FIL}$, $-(\alpha)^{20.5}$ kb], the $\alpha\alpha\alpha^{anti\text{-}3.7}$ gene triplication, two point mutations on the α1 gene [codon 14 (TGG > TAG); codon 59 (GGC > GAC) (Hb Adana)], and 11 mutations on the α2 gene [initiation codon ATG > ACG; codon 19 (GCG > GC-), IVS-I(-5 nt) (-TGAGG); codon 59 (GGC > GAC); codon 125 (CTG > CCG) (Hb Quong Sze); Hb Constant Spring (HbCS) codon 142, Term → Gln, TAA > CAA; Hb Icaria, codon 142, Term → Lys, TAA > AAA; Hb Pakse', codon 142, Term → Tyr, TAA > TAT, Hb Koya Dora, codon 142, Term → Ser, TAA > TCA; polyadenylation signal site, poly-A1 (AATAAA > AATAAG); and poly-A2 (AATAAA > AATGAA).

Statistical analysis was performed using the statistical package for the social sciences (version 22.0). Data was described in number and percentage. Quantitative data were described using mean, standard deviation median, and range. One-way ANOVA and post hoc analysis were used to compare Hb value in the different genotypes. A P value of < 0.05 was considered statistically significant.

Results

Of the 96 participants, 84 were carriers of α-thalassemia mutations confirmed by the molecular method. The remaining 12 participants showed no mutations with the used method, and therefore, they were excluded from this analysis. The mean age of the enrolled subjects was 24.4 (± 3.77) years. Their mean Hb was 12.9 (± 1.29) ranging from 10.0 to 16.9 g/dL. Anemia was encountered in 35 subjects (41.7%); the remaining 49 subjects had normal Hb levels with low MCV and MCH. Table 1 illustrates the mean of different hematological parameters in the enrolled subjects.

Molecular testing revealed six different α-globin mutations in 84 samples involving 105 alleles. The $-\alpha^{3.7}$ deletion was the most frequently mutated allele (62.85%), followed by $\alpha 2^{-5nt}$ mutation (20%). The $--^{MED}$ double-gene deletion was the third most prevalent mutated allele (6.67%). Collectively, deletion mutations were observed in 71.43% of the mutated alleles, whereas non-deletion mutations were found in the remaining 28.57% of the characterized alleles. The allele frequencies of mutations are presented in Table 2.

The enrolled α-thalassemia carriers revealed nine different genotypes. Two-thirds of them, 56 subjects, had single-gene defect and the remaining 28 subjects had two defected genes. Genotypes with single-gene deletion were observed in 41 subjects; the remaining 15 subjects had single non-deletion mutation genotypes. Genotypes with two defected genes, on the other hand, were found in four

Table 1 The mean of hematological parameters in the enrolled subjects

Parameters	Mean (SD)	Median	Range
Hb	12.9 (1.29)	12.8	10.0–16.9
RBC	5.5 (0.45)	5.4	4.86–7.22
MCV	70.8 (5.07)	71.0	23.0–79.9
MCH	23.4 (1.60)	23.5	19.1–26.7
MCHC	33.0 (1.01)	33.0	30.6–36.1
HbA2	2.4 (0.18)	2.4	2.0–2.9

Table 2 Allele frequencies of the α-thalassemia mutations

Mutation	Frequency	%
$-\alpha^{3.7}$	66	62.86
$\alpha2^{-5nt}$	21	20.00
$--^{MED}$	7	6.67
$\alpha2^{poly-A1}$	6	5.71
$\alpha1^{Adana}$	3	2.86
$-\alpha^{4.2}$	2	1.90
Total	105	100

different patterns: inheritance of a large double-gene deletion, coinheritance of two single-gene deletions, coinheritance of a single-gene deletion with a non-deletion mutation, and coinheritance of two non-deletion mutations, detected respectively in 7, 9, 9, and 3 subjects. Table 3 presents the α-globin genotypes and hematological parameters of 84 α-thalassemia carrier individuals. Variations were observed in the Hb and red cell indices within the nine different genotypes. Carriers of $\alpha2^{poly-A1}$ mutation ($\alpha^{poly-A1}\alpha/\alpha\alpha$) and those with $-\alpha^{3.7}/-\alpha^{-5nt}\alpha$ genotype had significantly lower Hb, MCV, and MCH values comparing to the other genotypes. All subjects of the latter two genotypes were anemic (Tables 3 and 4).

Discussion

In clinical practice, effective discrimination of β-thalassemia carrier state and iron deficiency anemia is basically possible by the routine complete blood counts, Hb electrophoresis, and iron studies. However, the diagnosis of α-thalassemia remains presumptive awaiting confirmation by molecular testing. In this study, 96 subjects with a presumptive diagnosis of α-thalassemia carrier had premarital genetic testing for α-thalassemia. α-globin gene mutations were defined in 84 subjects who were tested for common 21 mutations. Multiplex PCR and reverse hybridization did not reveal any mutation in twelve other subjects who were either free of mutations, or possibly carried other rare mutations. These cases were not included in this analysis; however, the outcome of sequence analysis for these samples is not expected to significantly affect the results of this study.

In the current study, six distinct gene mutations were detected including two single gene deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$), one double-gene deletion ($--^{MED}$), and three non-deletion mutations ($\alpha2^{-5nt}$, $\alpha2^{ploy-A1}$, and $\alpha1^{Adana}$). The identified spectrum of mutations was limited; this is similar to results of previous studies by Al-Allawi et al., who reported comparable range of mutations in the two adjacent provinces of

Table 3 α-Globin genotypes and mean hematological values

Genotypes	n (%)	Hb	Anemia n (%)	RBCs	MCV	MCH
$-\alpha^{3.7}/\alpha\alpha$	39 (46.43)	13.2 (1.04)	9 (23.1)	5.5 (0.39)	73.9 (3.58)	24.2 (1.13)
$-\alpha^{3.7}/-\alpha^{3.7}$	9 (10.72)	12.1 (0.90)	9 (100)	5.2 (0.14)	68.6 (2.3)	23.2 (1.46)
$\alpha^{-5nt}\alpha/\alpha\alpha$	9 (10.72)	13.4 (1.81)	3 (33.3)	5.7 (0.49)	71.6 (3.54)	23.4 (1.23)
$--^{MED}/\alpha\alpha$	7 (8.33)	13.4 (0.98)	1 (14.3)	5.9 (0.52)	67.9 (1.84)	22.8 (1.04)
$\alpha^{poly-A1}\alpha/\alpha\alpha$	6 (7.14)	11.0 (0.68)	6 (100)	5.1 (0.16)	64.1 (4.04)	21.4 (1.57)
$-\alpha^{3.7}/-\alpha^{-5nt}\alpha$	6 (7.14)	11.7 (0.54)	6 (100)	5.4 (0.18)	64.2 (4.21)	21.6 (1.56)
$-\alpha^{3.7}/\alpha\alpha^{Adana}$	3 (3.57)	13.2 (0.68)	0	5.5 (0.05)	72.3 (2.48)	23.9 (1.33)
$\alpha^{-5nt}\alpha/\alpha^{-5nt}\alpha$	3 (3.57)	13.7 (0.97)	0	6.5 (0.59)	63.2 (2.29)	21.0 (0.45)
$-\alpha^{4.2}/\alpha\alpha$	2 (2.38)	13.6 (0.63)	1 (50.0)	5.3 (0.08)	75.8 (2.54)	25.7 (0.85)
Total	84		35 (41.7)			

Table 4 ANOVA post hoc test significance of correlation between different genotypes and Hb values

	$-\alpha^{3.7}/-\alpha^{3.7}$	$\alpha^{-5nt}\alpha/\alpha\alpha$	$--^{MED}/\alpha\alpha$	$\alpha^{poly-A1}\alpha/\alpha\alpha$	$-\alpha^{3.7}/-\alpha^{-5nt}\alpha$	$-\alpha^{3.7}/\alpha\alpha^{Adana}$	$\alpha^{-5nt}\alpha/\alpha^{-5nt}\alpha$	$-\alpha^{4.2}/\alpha\alpha$
$-\alpha^{3.7}/\alpha\alpha$	0.003	0.826	0.730	0.000	0.002	0.930	0.495	0.740
$-\alpha^{3.7}/-\alpha^{3.7}$		0.011	0.013	0.072	0.573	0.106	0.022	0.080
$\alpha^{-5nt}\alpha/\alpha\alpha$			0.905	0.000	0.005	0.841	0.622	0.839
$--^{MED}/\alpha\alpha$				0.000	0.006	0.779	0.697	0.902
$\alpha^{poly-A1}\alpha/\alpha\alpha$					0.253	0.005	0.001	0.005
$-\alpha^{3.7}/-\alpha^{-5nt}\alpha$						0.053	0.011	0.043
$-\alpha^{3.7}/\alpha\alpha^{Adana}$							0.572	0.749
$\alpha^{-5nt}\alpha/\alpha^{-5nt}\alpha$								0.853

Duhok and Sulaimaniyah in Iraqi Kurdistan [14, 15]. The worldwide prevalent -$\alpha^{-3.7}$ deletion comprised 62.86% of the mutated alleles and was detected in 57 (67.85%) of the carrier subjects. These results are very similar to what have been reported in the neighboring zone [2, 14–18]. The $\alpha2^{-5nt}$ non-deletion mutation at IVS-1 region of α2 gene was the second most common mutation found in 14.82% of the carriers and constituted 20% of the characterized alleles. The frequency of latter mutation was high comparing to previous studies results from Iraqi Kurdistan, south of Turkey, and west of Iran (2.9%, 6.7%, and 4.9% respectively) [15, 18, 19]. This mutation has been also reported at various rates in the Arabia area and Mediterranean region [20–25]. In fact, the high prevalence of the -$\alpha^{3.7}$deletion, and the other point mutations, do not impose a real risk among our population. This is because of the low prevalence rates of the large double-gene deletions. In the current study the --^{MED} deletion allele frequency was 6.67% and no allele with -$\alpha^{20.5}$ deletion was detected. Consequently, the inheritance of -$\alpha^{3.7}$ deletion will not lead to significant complication coupled with either -$\alpha^{3.7}$ or another point mutation.

The carrier subjects in this cohort displayed nine different α-globin genotypes. Deletion genotypes were detected in more than two-thirds (57 subjects, 67.9%). The Hb and red cell parameters revealed notable differences within the different genotypes. Individuals of certain number of genotypes were alike as they had comparable Hb, MCV, and MCH values. Carriers with non-deletion mutations had significantly lower Hb comparing to those with deletion genotypes. Majority of carriers with deletion genotypes, apart from those with -$\alpha^{3.7}$/-$\alpha^{3.7}$ genotype, had normal Hb values. Hence, premarital genetic testing for α-thalassemia should be considered for subjects with normal Hb values and borderline low MCV and MCH. There is no clear explanation why the $\alpha2^{poly\text{-}A1}$ mutation, which is known to be prevalent in the Arabian Peninsula, is associated with lower Hb comparing to the other point mutations. Homozygosity for the latter mutation is the main genotype causing HbH disease in Saudi Arabia, UAE, Kuwait, and Bahrain [16, 21, 22, 26]. The exact mechanism explaining how two mutations cause HbH phenotype is still puzzling. The most acceptable explanation is that poly-A signal mutation affects the α1 gene expression as well [27]. Hb Adana was the only detected α1 globin gene mutation in the enrolled subjects. It was observed in compound heterozygous state with -$\alpha^{3.7}$ deletion in three subjects. The hematological parameters of these three subjects did not differ from those who carried single-gene deletion. It appeared that $\alpha1^{Adana}$ mutation did not show remarkable influence when found in combination with -$\alpha^{3.7}$ deletion. This could be due to the fact that the level of transcription of the two α-globin genes is not equal; the α2 gene produces two to three times more α-globin than the α1 gene [1].

In conclusion, a limited range of α-globin gene mutations were detected in this cohort comparing to a relatively diverse spectrum of mutations reported in the Mediterranean and the Middle East regions. Deletion mutations were mostly encountered with the -$\alpha^{3.7}$ deletion being the most prevalent. These results will certainly contribute to improve the thalassemia carrier screening and genetic counseling program, and will be helpful for prenatal diagnosis.

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Data availability The author is ready to share the "encrypted" data whenever requested.

Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest in publishing this article.

Ethical approval This study was approved by the Research Ethics Committee of Hawler Medical University, Erbil, Iraq.

Consent to participate Informed consent was obtained from the enrolled individuals.

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