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Alpha Thalassemia Allelic Frequency in Lebanon

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Background. Hemoglobinopathies are the most common reported monogenic disorders worldwide. It is well established that Mediterranean and Arab countries are high risk areas for thalassemia in general, and for alpha thalassemia in particular. Reports of alpha thalassemia gene mutations from the Lebanese population are limited. ***Procedure.*** We investigated the spectrum of alpha thalassemia mutations in a sample of 70 unrelated Lebanese families. Six different mutations of alpha thalassemia gene were identified. ***Results.*** The most prevalent mutations were the single gene deletion $-\alpha^{3.7}$ (43%) and the non-gene deletion α2 IVS1 [–5nt] (37%). The double deletional determinant $-^{MED}$ was detected only in 14% of thalassemic chromosomes. ***Conclusion.*** We determined the mutational spectrum of alpha thalassemia which might be used in the future for molecular investigations of the disease in susceptible patients in our population. Pediatr Blood Cancer 2015;62:120–122.

Key words: alpha thalassemia; hemoglobinopathies; Lebanon; mutational spectrum

INTRODUCTION

Alpha thalassemia is one of the most common single gene disorders worldwide [1–5] significantly occurring in ethnic groups tracing their origins to countries that border the Mediterranean Sea, the Middle East, and Southeast Asia [6,7]. It is characterized by reduction or absence of the alpha globin chains due to deletion or mutation in alpha globin genes. The phenotype of individuals with deletional alpha thalassemia is diverse and depends on the number and the type of genes that have been deleted or mutated [3,8] ranging from mild microcytic hypochromic anemia to hemolytic anemia which is the most severe type occurring in utero and causing hydrops in addition to demise in the fetus [4,5,9,10].

Carrier frequency of alpha thalassemia reportedly exceeds that of beta thalassemia [11]. Regional published studies show a substantial incidence of alpha globin gene defect in United Arab Emirates (16.5%), Oman (38.6%), and Saudi Arabia (50%) [9,11,12].

Most widely described mutations are single gene deletions $-\alpha^{3.7}$ and the $-\alpha^{4.2}$ which in addition to α2 IVS1 [–5nt], anti-3.7 gene triplication, $-^{MED}$ and $-^{20.5}$ double gene deletions are the mostly detected mutations in the Mediterranean region [2,13–15]. Point mutations within the alpha globin genes are less frequently reported [2,16,17] with the most commonly reported from Saudi population is $\alpha^{PA\ Saudi}$ and in the Mediterranean population α^{PA} [2,15].

While several studies in the Lebanese population have been carried out to assess the incidence and spectrum of beta thalassemia [18,19] mutations, only one has been conducted on alpha thalassemia [20]. The following is a retrospective observational study aiming at determining the spectrum of alpha thalassemia gene mutations in Lebanon.

METHODS

This retrospective study was carried out in the Medical Genetics Laboratory of the Pathology and Laboratory Medicine Department at the American University of Beirut Medical Center (AUBMC). Archived genetic results of patients who were referred for molecular analysis of HBA1 and HBA2 genes as an evaluation of anemia between the years 2009 and 2012 in the Medical Genetics Unit at AUBMC were reviewed. The genetic analysis of these patients were combined to the results of our previous report [21] which aimed at developing and validating a reverse-hybridization assay (Alpha-Globin Strip Assay) for the rapid and simultaneous detection of alpha thalassemia in a sample of 34 unrelated Lebanese families.

DNA Extraction

Genomic DNA was extracted and purified from whole blood sample using QIAamp DNA Mini Kit (QIAGEN GmbH, Germany) and as described by the manufacturer. DNA analysis for the identification of the following 21 most common α-globin point mutations and deletions reported worldwide: $-\alpha^{3.7}$ and $-\alpha^{4.2}$ single gene deletions, $-^{MED}$, $-^{SEA}$, $-^{THAI}$, $-^{FIL}$, and $-^{20.5}$ double gene deletions, anti-3.7 triplication, two point mutations in the alpha 1 gene α1 cd 14 and α1 cd 59, 11 point mutations in the alpha 2 α2 init cd, α2 cd 19, α2 IVS1 [–5nt], α2 cd 59, α2 cd 125, α2 cd 142 (Hb Constant Spring), α2 cd 142 (Hb Icaria), α2 cd 142 (Hb Pakse), α2 cd 142 (Hb Koya Dora), α2 poly A1 [AATAAA > AATAAG] and α2 poly A2 [AATAAA > AATGAA] was performed by means of Polymerase Chain Reaction (PCR) followed by Reverse-Hybridization techniques are given below.

Three multiplex PCR reactions were performed for each sample. For each reaction we used 1.66 U Taq DNA Polymerase, 2 μl/primer pair and 10–20 ng DNA. The thermocycling program included a pre-PCR step at 95°C for 5 minutes, 3 cycles of denaturation at 97°C for 40 seconds, annealing at 64°C for 40 seconds and extension at 72°C for 90 seconds, 37 cycles of denaturation at 97°C for 40 seconds, annealing at 58°C for 40 seconds and extension at 72°C for 90 seconds, and a final extension step at 72°C for 5 minutes. PCR products were visualized on 3% agarose gel under UV light.

For mutational detection, 10 μl of each PCR product was denatured then hybridized to blotted alpha-globin membranes (Vienna lab, GmbH Vienna, Austria) as previously described [20].

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Conflict of interest: Nothing to declare.

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Data Analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 19. Sample characteristics were done using frequencies and percentages.

RESULTS

In this study, 148 patients were included. Among these, 70 unrelated Lebanese families from different communities and ethnic background carried abnormalities in the alpha globin gene. Twenty eight patients were found to be affected with alpha thalassemia, 15 of which (53%) were homozygous for one alpha thalassemia mutation and 13 were compound heterozygous. Forty two families were found to be carriers of alpha thalassemia mutations. Six mutations were identified. These mutations were the $-\alpha^{3,7}$ (43%) single gene deletion, α2 IVS1 [–5nt] (37%) mutation, $-^{MED}$ double gene deletion (14%), two point mutations α2 polyA1 (3%) and α2 poly A2 (2%), and one anti-3,7 gene triplication (1%) (Table I).

Out of the 28 affected families, 15 were Muslims (53%), eight were Christians (29%) and four were Druze (14%). The alpha globin mutations in among these ethnic groups is represented in Table II

DISCUSSION

Although thalassemia in the Mediterranean and the Middle East countries are reportedly high [3,22,23], accurate epidemiological data on its frequency and distribution in these regions lack. In Lebanon, only one study conducted in 2000 by Qatanani et al. [20], reported an incidence of 8.2% alpha gene mutations in patients with beta thalassemia intermedia.

This is the first study that aims at determining the spectrum of alpha thalassemia mutations in Lebanese anemic patients. Our findings reveal that the three most common mutations in Lebanese families are $-\alpha^{3,7}$ single gene deletion, α2 IVS1 [–5nt] and $-^{MED}$ double gene deletion accounting for 94% of alpha thalassemia alleles. Studies from the Mediterranean and Arab countries show that these mutations account for more than 68% of the alpha thalassemic alleles (Table I). The remaining 5% were nondeletional alleles and included α2 polyA1 and polyA2 mutations. The $\alpha^{3.7}$ determinant was the most common alpha thalassemic allele detected in our population (43%), which is consistent with the observations in other countries from the Middle East and the Mediterranean region, where this mutation is predominant (Table I).

A previous study on occurrence of alpha thalassemic allele in 73 patients with beta thalassemia from the South of Lebanon [20] detected only three mutations, including two of those detected in the current study: $-\alpha^{3,7}$ (1.4%), $-\alpha^{4.2}$ (4.2%), and $-^{MED}$ (1.4%). In contrast to studies from Bahrain (53%) and Saudi Arabia (32.8%), where, in general, the poly A1 mutation is the most and second most common mutation respectively [24,25], it only constituted 3% of our characterized alpha thalassemic alleles. The polyA2 mutation was documented in 2.0% of our alpha thalassemic alleles. This has been described in Iraq: 1%; Turkey: 4%; Iran: 0.5% [26–28] and was not detected in Israel, Jordan, UAE, Saudi Arabia, Algeria, and Oman (Table I). Anti-3.7 triplication, a sporadic mutation, without any hematological sign unless associated with beta globin mutation [2,26] was detected in only one of our patients, with an allele frequency of 1% which is similar to the Iranian population of the Kerman province (26%) and lower than that reported in Israel (3.2%) [29].

Our results differ from those reported from other neighboring Mediterranean and Arab populations such as populations from Algeria, Greece, Cyprus, Turkey, Iraq, Jordan, and United Arab Emirates [2,15,30–33]. The α-thalassemia determinant $-^{20.5}$ which is believed to be quite frequent in among these populations, was not identified in our patients [Table I]. Likewise the $-\alpha^{4.2}$ single gene deletion which is commonly reported in Arab countries was absent in our population [2,15,30]. A possible explanation to the disparity between our results and those reported from other Arab studies may be due to the heterogeneous backgrounds of our families in contrast to the more pure Arab homogenous background [34].

Among population, mutation panels differ from one country to another and in between religious denominations. In the Israeli population, the mutations identified among the Druze are $-\alpha^{3,7}$ and $-^{Med}$, the most common mutations among Arabs (Christian and Muslim) are $-\alpha^{3,7}$ and α2 IVS1 [-5nt]. Among Jews, the most frequent determinant is $-\alpha^{3.7}$ [14].

In Lebanon, several ethnic and demographic groups compose the population and intermarriage is so far a rare occurrence. Mutations

TABLE I. The Frequency of Detectable α-Thalassemia Mutations in Some Middle Eastern and Mediterranean Populations

Country	% of most common α-thalassemia mutations in some Middle Eastern and Mediterranean countries								
	$-\alpha^{3.7}$	$-\alpha^{IVS\ I(-5nt)}$	$-^{MED}$	$-\alpha^{PA1}$	α^{PA2}	$-\alpha^{20.5}$	$-\alpha^{4.2}$	Other	Reference
Lebanon (140[a])	43	37	14	3	2	—	—	$\alpha\alpha\alpha^{anti\text{-}3.7}$ (1%)	This study
Israel (464[a])	51	11.1	10.1	—	—	—	2.2	$\alpha\alpha\alpha^{anti\text{-}3.7}$(3.2%); $-^{Yem}$ (1.6%)	[29]
Jordan (336[a])	45	27	4					$\alpha^{T\text{-}Saudi}$(23%)	[2]
Iran (736[a])	83.8	4.2	0.3	0.5	0.5	—	3.7		[26]
Iraq (104[a])	59.6	2.9	23.1	3.8	1	2.9	1.9	$\alpha^{CS}\alpha$ (1.9%); $\alpha\alpha^{Adana}$ (2.9%)	[27]
Algeria (306[a])	63	—	6.5	—	—	6.5	—	αNcoα (13%)	[35]
UAE (84[a])	28.2		0.7	47.4		9.0		$-^{SEA}$ (1.4%); $\alpha^{CS}\alpha$ (11.5%)	[36]
Turkey (225[a])	53.33	—	15.11	1.33	4.0	6.66	0.44	$\alpha\alpha\alpha^{anti\text{-}3.7}$(2.22%)	[28]
Cyprus (156[a])	37.2	8.3	37.8	3.8	2.6	8.3	—		[30]
Greece (150[a])	28.0	5.3	26.7	15.3	0.6	12.7	—	$\alpha^{CS}\alpha$ (0.6%)	[33]
Bahrain (97[a])	32.0	12.0	—	53.0	1.0	—	2.0		[24]
Saudi Arabia (66[a])	53.0	1.5	—	32.8	—	—	10.6		[25]
Oman (174[a])	41.7						1.2		[31]

[a]Number of chromosomes.

TABLE II. Alpha Globin Mutations in Different Ethnic Groups

	Muslim	Christian	Druze
Homozygous 3.7	5	2	2
Homozygous IVS1–5nt	4	1	0
Homozygous MED	0	1	0
Heterozygous 3.7/MED	3	1	0
Heterozygous 3.7/IVS1 [–5nt]	1	2	0
Heterozygous 3.7/polyA-1	0	0	1
Heterozygous MED/IVS1–5nt	2	0	1
Heterozygous polyA-1/IVS1–5nt	0	1	0
Total	15	8	4

in alpha globin genes have been detected in Muslims, Druze, and Christians with no significant ethnic variability (Table II).

CONCLUSION

In this study, we determined the most common alpha thalassemia mutations in the Lebanese population. Our findings highlight two mutations accounting for more than 80% of alpha globin mutations. This would eventually help to come up with a simplified and affordable mutation screening for our population. Apart from its scientific interest, this information provides a valuable impact both at the social and economic levels in a country where genetic testing are not commonly covered by third parties.

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