

Spectrum of Alpha Thalassemia Alleles in Kuwait

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Abstract

Background: The frequency of alpha thalassemia trait is about 40% in the Kuwaiti population, but there has been no comprehensive study of the prevalent alleles. This is a report of the patients who were referred for molecular diagnosis over a 20-year period. **Methods:** Blood samples from suspected cases were sent to the Hemoglobin Research Laboratory of the Department of Pediatrics, Kuwait University. A retrospective study of the molecular characterization of samples from 1994 to 2015 was carried out. The alpha globin genotypes were determined by a combination of PCR, allele-specific oligonucleotide hybridization and reverse dot-blot hybridization (Vienna Lab Strip Assay). **Results:** 400 samples were characterized and analyzed from individuals aged <1 month to 80 years, with a median of 6 years (~60% children and adolescents). Most (90.8%) were Kuwaiti nationals. The common genotype was homozygosity for the polyadenylation-1 mutation (α PA-1 α / α PA-1 α) in 33.3%, followed by heterozygosity ($\alpha\alpha$ / α PA-1 α) for the same mutation in 32.3%. The PA-1 was therefore the most frequent allele (0.733). The frequency of the α^0 , -MED was 0.19. Rare alleles that were found in very low frequencies included the α^0 (-FIL) in a Filipino child, Hb Constant Spring, Hb Adana, and Hb Icaria. **Conclusion:** There is a wide variety of alpha thalassemia alleles among Kuwaitis, but the nondeletional PA-1 is by far the most common cause of moderate to severe HbH disease phenotype. The α^0 (-MED) allele is also encountered, which has implications for pre-marital counselling especially the possibility of having babies with alpha thalassemia major (Barts hydrops fetalis).

Background

Alpha thalassemia is one of the most widespread genetic diseases worldwide with frequencies of the carrier state reaching up to 80–90% in some areas[1]. In tropical Africa it is about 25–30%[2], while in the Arabian Peninsula, it varies from a low of close to zero in the desert areas to as high as 60% in the agricultural zones of Eastern Saudi Arabia[3, 4].

The normal individual has a complement of four α -globin genes ($\alpha\alpha/\alpha\alpha$), any one of which can be absent, producing varying degrees of alpha thalassemia[1]. In α^0 alleles, both genes on a chromosome are deleted (-), while in α^+ , a variable portion of the α^2 or α^1 gene is deleted, resulting in reduction in the chain synthesized. The size of the deletion in α^0 alleles is variable and each is named for the part of the world where it is prevalent. Thus -MED, -FIL, -SEA are found in the Mediterranean, Philippines and South East Asia, respectively[5]. The α^+ deletions are designated by their sizes and the commonest is the $-\alpha^{-3.7\text{kb}}$ allele. One- or 2-gene deletions ($-\alpha/\alpha$, $-\alpha/-\alpha$, $-/\alpha\alpha$), produce alpha thal trait, usually associated with mild microcytic, hypochromic anemia. The loss of 3 genes ($-/-\alpha$) produces the classical HbH (β_4 tetramers) disease, which is characterized by moderate to severe anemia. The loss of all 4 genes ($-/-$) causes Hb Bart's (γ_4) hydrops fetalis, which is incompatible with life in the absence of early onset of chronic transfusion or stem cell transplantation[6].

Apart from deletions, there are a few point mutations that affect the transcriptional efficiency of the α globin genes and are designated $\alpha^T\alpha$ or $\alpha\alpha^T$ depending on whether the α^2 or α^1 gene is affected[7].

Collectively, these nondeletional α -thalassemia alleles commonly affect the promoter, the initiation codon (ATG), splicing signals (GT/AG), the termination codon (TAA), and the polyadenylation signal (AATAAA) of the affected gene. Of these, the most common are the $\alpha^{\text{IVSI}(-5\text{ nt})}$ (in Mediterraneans), polyadenylation site mutations α_2^{AATAAG} , α_2^{AATGAA} and $\alpha_2^{\text{AATA--}}$ (in the Mediterranean and Middle East)[7–11][71–74], termination codon mutations leading to elongated Hb variants, such as Hb Constant Spring (HbCS), Hb Icaria, Hb Koya Dora, Hb Seal Rock and Hb Pakse [12–14]. Heterozygotes for these alleles have a thalassemia minor phenotype, but homozygotes and compound heterozygotes often have HbH disease of varying severity and blood transfusion requirements.

Alpha thalassemia frequency is about 30–40% in Kuwait and HbH disease is mostly linked to the polyA ($\alpha^{\text{PA-1}}$) mutation[15, 16]. However, there has been no comprehensive study of the whole spectrum of alpha thalassemia alleles. In particular, it is important to establish the prevalence of the α^0 allele in the population to determine the chance of having the more severe alpha thalassemia major (Bart's hydrops fetalis). To this end, we have analyzed all cases of suspected α -thalassemia referred for molecular diagnosis over a 20-year period.

Methods

The hemoglobin research laboratory in the Department of Pediatrics, Kuwait University receives samples from the hematology clinics in Mubarak Al-Kabeer Hospital and some of the other government hospitals in Kuwait. The study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Kuwait University and the Ministry of Health, Kuwait. Informed, signed consent was obtained from the adult patients and from the parents or guardians of the minor patients in the study. Those between the ages of 7 and 18 years were also given the opportunity to affirm their participation. Patients were suspected to have some form of α -thalassemia if they have persistent microcytic, hypochromic anemia in the absence of iron deficiency and with normal hemoglobin electrophoresis. Some patients had H inclusions on cresyl blue staining of the peripheral erythrocytes or H band on high performance liquid chromatography (HPLC). Quite often, but not always, there was a record of the clinical presentation and the complete blood count (CBC). This study is a retrospective study of all such samples analyzed from 1994 to 2005.

Blood was obtained by venipuncture into EDTA, complete blood count was done using ABX Pentra 120 cell counter (ABX France, Montpellier). The hemoglobin quantitation was determined using cation-exchange high performance liquid chromatography (HPLC) - Shimadzu LC-20AT, Shimadzu Corporation, Kyoto, Japan. DNA was isolated from peripheral leucocytes using phenol extraction. All the samples were screened for the α -thal-2 (-3.7 kb) deletion [17] and the α_2 -globin gene polyadenylation site (AATAAA→AATAAG) mutation using a PCR method and allele-specific oligonucleotide hybridization respectively as previously reported [17, 18]. All other samples that were not solved with these methods, were subjected to reverse-dot blot hybridization using the Vienna Lab StripAssay technique (Vienna Lab Diagnostics, Vienna, Austria).

The ethnic origins of the patients were documented by their nationalities. The different α -globin genotypes were identified and the allele frequencies calculated. The mean Hb level and RBC indices compared among patients with different genotypes. Clinical data were collected from the HbH disease patients, especially whether they were being followed in a hematology clinic and if they were on any medications or how often they were transfused with blood.

Data are presented as means \pm SD or as percentages as appropriate. Statistical differences between mean values among the major groups were tested using Student t test or ANOVA. Continuous variables were subjected to Chi-square test to determine statistically significant differences.

Results

Over the period of the study from 1994 to 2015, there were 400 samples received as suspected moderate to severe alpha thalassemia or HbH disease. Of these, 183 were females and 217 males, with ages ranging from 1 to 80 years and median of 6 years, although more than half (60%) of the patients were below 20 years of age. Most (90.8%) patients were Kuwaitis with a few other nationalities as shown in Table 1.

Table 2 shows the distribution of α -globin genotypes in the study. The most common (33.3%) was homozygosity for the $\alpha^{\text{PA-1}}$ (c.*94A > G), nondeletional α -thal allele, which gives a HbH disease phenotype, followed by heterozygosity for the same allele (32.3%). The compound heterozygosity of $-\alpha^{-3.7}/\alpha^{\text{PA-1}}$ was the next common at 20.5%. None of the other frequencies reached double digits.

Table 3 shows the frequencies of the individual alleles. The PA-1 allele was the most frequent, 0.733, followed by the $-\alpha 3.7$ kb deletion at 0.195. Interestingly, the double gene deletion $-\text{MED}$ was found at a frequency of 0.019. The other rare alleles are shown in the table. They include the $\alpha 0$ ($-\text{FIL}$) in a Filipino child, HbCS (c.427T > Cp.X143Gln), Hb Adana (c.179G > Ap.Gly60Asp), $\alpha^{\text{PA-2}}$ (c.*92A > G), Hb Icaria (c.427T > Ap.X143Lys)

None of the patients was on a chronic transfusion therapy, but many had received sporadic transfusions. The patients with the most severe phenotypes in the study were 2 siblings with $-\text{MED}/\alpha^{\text{CD19}}$. They both had poor growth parameters and baseline Hb of < 6 g/dl and are now regularly transfused every 6 to 8 weeks. Interestingly, they have 2 other affected siblings who do not require transfusions.

Discussion

This report is limited to patients who were suspected to have moderate to severe alpha thalassemia based on microcytic, hypochromic anemia, no laboratory evidence of iron deficiency and normal hemoglobin electrophoresis or HPLC. In addition, they had H inclusion bodies and/or H band on HPLC. The study has revealed the extent of the diversity among individuals with alpha thalassemia in Kuwait. As expected, the vast majority of the patients had the $\alpha^{\text{PA-1}}$ nondeletional allele, which has been reported as the commonest cause of HbH disease in Kuwait. The frequency of the allele in this study was 0.733. It

was found as homozygote in 33.3% of the patients, while heterozygotes accounted for 32.3%. It was present as a compound heterozygote with $-\alpha^{-3.7\text{ kb}}$ in 20.5% and in combination with other alleles like $-\text{MED}$, $-\alpha^{-5\text{nt}}$, $\alpha^{\text{cd}59}$ and $\alpha^{\text{PA}-2}$.

The third most frequent allele in this study turned out to be the α^0 , $-\text{MED}$, which was found in 13 patients, in whom it presented a HbH disease phenotype in 10. Of the latter, it was in compound heterozygosity with the $\alpha^{\text{cd}19-\text{G}}$ in 6 patients from 2 related families, with the $-\alpha^{-3.7\text{kb}}$, in 2 patients, the $\alpha^{\text{IVS}1-5\text{nt}}$ and the $\alpha^{\text{PA}-1}$ in 1 patient each.

The $\alpha^{\text{PA}-1}$ allele was first described from Eastern Saudi Arabia[10] and previous studies from Kuwait[2, 16] had highlighted its role in the etiology of HbH disease among our patients. It has also been reported as the commonest cause of HbH disease in Saudi Arabia[19], Jordan, [20], Bahrain[21] and UAE[22]. The allele is therefore widespread in the Arabian Gulf, especially in the countries adjoining Saudi Arabia. It is uniformly associated with a moderate thalassemia intermedia phenotype with only occasional blood transfusion requirement, usually with intercurrent infection [16]. HbH disease is mainly a disease of childhood in Kuwait. Most patients are referred to the clinic for investigation of persistent microcytic, hypochromic anemia not responding to iron therapy. Indeed, the anemia tends to improve as the child stops rapid growth and enters puberty.

This study is the first to report the presence of the α^0 , $-\text{MED}$ allele among Kuwaiti patients. In 3 cases, it was as simple heterozygotes while in 10 cases, it was as compound heterozygotes with the pentanucleotide (5nt) deletion in the $\alpha^{\text{IVS}1-5\text{nt}}$ in 1, the $\alpha^{\text{PA}-1}$ mutation in 1, the $-\alpha^{-3.7}$ in 2, and the $\alpha^{\text{cd}19(\text{G/A})}$ in 6 (from 2 related families). In all these instances, the phenotype was mild with moderate anemia. However, in 2 patients with the $-\text{MED}/\alpha^{\text{cd}19}$ genotype, the patients were stunted in growth and were on regular transfusion. The other rare alleles found in the study included the α^0 ($-\text{FIL}$) in a Filipino child, HbCS ($\alpha^{\text{Cd}142\text{ TAA} \rightarrow \text{CAA}}$), Hb Adana ($\alpha^{\text{cd}59\text{G/A}}$), $\alpha^{\text{PA}-2}$ ($\text{AATAAA} \rightarrow \text{AATGAA}$), Hb Icaria ($\alpha^{\text{Cd}142\text{ TAA} \rightarrow \text{AAA}}$).

Conclusions

This study has demonstrated the diversity of the alpha thalassemia alleles among Kuwaitis. While the deletional allele is quite widespread, the nondeletional $\alpha^{\text{PA}-1}$ is responsible for most of the moderate to severe HbH disease. Interestingly, the α^0 ($-\text{MED}$) allele is also encountered, with implications for pre-marital genetic counselling to prevent the birth of babies with alpha thalassemia major (Bart's hydrops fetalis).

List Of Abbreviations

PCR Polymerase chain reaction

PA Polyadenylation

MED Mediterranean

FIL Filipino

SEA Southeast Asia

Kb Kilobases

Hb Hemoglobin

CS Constant Spring

CBC Complete blood count

EDTA Ethylenediaminetetraacetic acid

SD Standard deviation

ANOVA Analysis of variance

UAE United Arab Emirates

Declarations

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Declarations

Authors' Contributions

1. AA was the lead physician who planned the study and carried out the analysis and wrote the first draft
2. MH was the head of the laboratory and carried out the molecular characterization. He contributed to the writing
3. JS, DT and MS all contributed to sample and data input/analysis
4. All authors have read and approved the manuscript

Ethics Approval and Consent to Participate

The study was approved by the Human Research Ethics Committees of the Faculty of Medicine, Kuwait University and the Ministry of Health, Kuwait.

Consent for Publication

No identifying images or other personal or clinical details of participants are presented. Hence, consent for publication is not applicable to our manuscript.

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Competing Interests

There are no other competing financial or non-financial interests.

Availability of Data and Materials

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1

Nationalities of the Patients of the Patients in the study

Nationality	n	%
Kuwaiti	317	90.8
Iraqi	10	2.9
Egyptian	6	1.7
Lebanese	4	1.1
Non Kuwaiti Bedouin	3	0.9
Syrian	3	0.9
Others	6	1.7
Total	349	100.0

Table 2

Distribution of Alpha Globin Genotypes of the Patients in the Study

Genotype	Number	%
$\alpha^{PA-1}\alpha/\alpha^{PA-1}\alpha$	133	33.3
$\alpha^{PA-1}\alpha/aa$	129	32.3
$-\alpha^{3.7}/\alpha^{PA-1}\alpha$	81	20.5
$-\alpha^{3.7}/-\alpha^{3.7}$	20	5.0
$_{-MED}/\alpha^{CD19}\alpha$	6	1.5
$\alpha^{CD59}\alpha/aa$	4	1.0
$-\alpha^{3.7}/aa$	3	0.8
$_{-MED}/aa$	3	0.8
$\alpha^{5nt}\alpha/\alpha^{5nt}\alpha$	3	0.8
$-\alpha^{3.7}/\alpha^{5nt}\alpha$	2	0.5
$-\alpha^{3.7}/_{-MED}$	2	0.5
$aa/\alpha^{CD19}\alpha$	2	0.5
$\alpha^{5nt}\alpha/\alpha^{PA-1}\alpha$	1	0.3
$_{-MED}/\alpha^{5nt}\alpha$	1	0.3
$_{-MED}/\alpha^{PA-1}\alpha$	1	0.3
$\alpha^{CS}\alpha/\alpha^{CD142}\alpha$	1	0.3
$\alpha^{PA-2}\alpha/aa$	1	0.3
$\alpha^{5nt}\alpha/aa$	1	0.3
$-\alpha^{4.2}/-\alpha^{4.2}$	1	0.3
$\alpha^{CD19}\alpha/\alpha^{CD19}\alpha$	1	0.3
$\alpha^{PA1}\alpha/\alpha^{PA2}\alpha$	1	0.3
$\alpha^{CD59}\alpha/aa$	1	0.3
$\alpha^{CD59}\alpha/\alpha^{PA-1}\alpha$	1	0.3
$\alpha^{CD59}\alpha/\alpha^{5nt}\alpha$	1	0.3

-SEA/- $\alpha^{3.7}$	1	0.3
Total	400	100

Table 3

Frequency of Alpha Thalassemia Alleles in the Study

Allele	n	Frequency
AATAAAàAATAAG (PA-1)	480	0.733
- α -3.7 single gene deletion	128	0.195
-MED double gene deletion	13	0.019
α 2 cd19 (-G)	10	0.015
α 2 IVS-1 (-5nt)	11	0.017
α 2 cd59 (GàA)	7	0.011
- α -4.2 single gene deletion	2	0.003
AATAAAàAATGAA	2	0.003
α 2 cd 142 (TàC) Hb Constant Spring	1	0.002
-FIL double gene deletion	1	0.002