

Frequency and genotyping of alpha thalassemia in individuals undergoing premarital screening

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Abstract

Objective: To evaluate the frequency of alpha thalassemia and detect mutations in the alpha genes in individuals undergoing premarital screening.

Methods: The cross-sectional study was conducted at King Fahad Central Hospital, Jazan, Saudi Arabia, from January 2018 to May 2019, and comprised blood samples of individuals visiting the premarital screening clinic. The samples were analyzed for complete blood counts and haemoglobin electrophoresis. Molecular analysis of samples suspected for alpha thalassemia was done using alpha-globin StripAssay kit. Data was analysed using SPSS 20.

Results: Of the 3,970 samples analysed, 1,859(46.83%) were from males and 2,111(53.17%) from females. The overall frequency of suspected alpha thalassemia was 4.43% based upon haematological parameters including complete blood count and haemoglobin electrophoresis. Overall, 80 suspected samples were selected for genetic analyses, and, of them, 76 (95%) were positive for deletional and non-deletional mutations of alpha-globin genes, while 4 (5%) were negative for any of the 21 mutations tested.

Conclusion: Alpha thalassemia was found to be highly prevalent in the study area.

Keywords: Alpha thalassemia, $-\alpha^{3.7}$, $-\alpha^{IVS1}$, α^{PolyA1} , premarital screening. (JPMA 71: 101; 2021)

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Introduction

Alpha (α) thalassemia (Thal) is a clinical condition characterised by molecular aberrations in the α genes or their regulatory elements, resulting in reduced synthesis or absence of α chains. Contrary to beta (β) gene, which produces globin chains as one gene one chain synthesis, a globin chain synthesis occurs as a result of two coding genes $\alpha 1$ and $\alpha 2$ located on chromosome 16. Deletions and/or non-deletional mutations affecting one or both α genes are the major molecular genetic lesions in α -Thal. Almost 40 deletional and 70 non-deletional mutations has been reported to cause α -Thal.¹⁻³ Clinical features of carriers and patients with α -Thal show considerable variations ranging from asymptomatic to severe haemolytic anaemia or intrauterine death (IUD).⁴

Alpha-Thal (α^+ and α^0) cases have shown six most common deletions. Deletions found in α^+ Thal affect single gene and are of two types i.e. $-\alpha^{3.7}$ and $-\alpha^{4.2}$. Africa, Mediterranean region and Asia are more prevalent with $-\alpha^{3.7}$. While $-\alpha^{4.2}$ deletions are most common in Southeast Asian and the Pacific islands. Alpha⁰ Thal is characterised by double-gene deletions. Examples of α^0 genes deletion include α^{-SEA} , α^{-MED} , $-\alpha^{20.5}$ and α^{-FIL} . As their names suggest, these are

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more commonly found in Southeast Asia, the Mediterranean region and the Filipina respectively.^{5,6}

Saudi Arabia is one of the countries where haemoglobinopathies (HbPs) are the most prevalent. It was mandated by a royal decree in 2004 to do premarital screening for these genetic disorders. Since then, every Saudi individual getting married in the country has to obtain premarital screening certificate from an authorised premarital screening centre. During the premarital screening programme, all individuals are screened for HbPs, including sickle cell anaemia and Thal. A significant number of individuals are reported as "suspected for α -Thal"; as neither red cell indices nor biochemical parameters helps in establishing the definite diagnosis of α -Thal with one or two α gene deletions. Molecular studies are helpful in identifying the genetic lesions of α gene(s). The limitations of haemoglobin (Hb) electrophoresis in the diagnosis of α -Thal and significant number of suspected α -Thal cases have been reported earlier.⁷

A number of studies reported the prevalence of α and β Thal in the Eastern Province of Saudi Arabia,⁸⁻¹⁰ with no reported study of α -Thal in the Southwestern Region, especially the Jazan Region. The current study was planned to fill the gap by evaluating the frequency of α -Thal and to detect mutations in the α genes in individuals undergoing premarital screening in Jazan.

Subjects and Methods

The cross-sectional study was conducted from April 2018 to May 2019 at King Fahad Central Hospital, Jazan, Saudi Arabia. After approval from the institutional ethics review committee and the scientific research ethics committee of Jazan University, the sample size was calculated in the light of population and prevalence variance reported in literature⁹ at 95% level of significance and 5% margin of error. The sample was raised using simple random sampling technique from among those visiting the premarital screening clinic.

Those included were physically normal individuals of either gender aged 18-45 years with no history of recent illness, medication and blood transfusion. Those excluded were individuals with clinical history of blood disorders or other systemic disorders, and those with a history of blood transfusion.

After taking written informed consent from the subjects enrolled, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) anti-coagulated tubes. Complete blood count (CBC) was done using Sysmex Kx2100 and Hb electrophoresis was carried out using Variant II Haemoglobin Testing System. Separation of S/D (haemoglobin S/ haemoglobin D), and A₂/C/E/O (haemoglobin A₂, haemoglobin C, haemoglobin E and haemoglobin O) or inconclusive results were confirmed by Sebia capillary electrophoresis system. After CBC and Hb electrophoresis, molecular analyses for α-Thal genes were carried out when the red cell indices were borderline or frankly low; red cell count was high; normal red cell distribution width (RDW); normal Hb A₂ levels; or normal Hb F levels. Genetic analyses of the samples suspected for α-Thal were carried using α-globin StripAssay kit (Vienna Lab Diagnostics GmbH, Vienna, Austria) according to the manufacturer's recommendations. This kit detects 21 different mutations, including deletional mutations -α^{3.7}, -α^{4.2}, -MED, -SEA, -THAI, -FIL, -(α)^{20.5}, gene triplication αα^{anti 3.7} and non-deletional point mutations i.e. codon 14, codon 59 (Hb Adana), initiation codon [ATG>ACG (α2)], codon 19, IVS-I [-5 nt (α2)], codon 59 (Hb Adana), codon 125 (Hb Quong Sze), codon 142 [Hb Constant Spring (Hb CS)], codon 142 (Hb Icaria), codon 142 (Hb Paksé), codon 142 (Hb Koya Dora), polyadenylation signal site (polyA1) [Saudi type], and polyA2 (Turkish type).⁸

Data was analyzed using SPSS 20. Kolmogorov-Smirnov test was used for checking the normality of data. It was expressed as mean±standard deviation (SD) and significance was checked using independent t-test. P<0.05 was considered statistically significant.

Results

Of the 3,970 samples analysed, 1,859 (46.83%) were from males and 2,111 (53.17%) from females. The overall frequency of suspected alpha thalassemia was 4.43% based upon haematological parameters including complete blood count and haemoglobin electrophoresis. Overall, 80 suspected samples were selected for genetic analyses, and, of them, 76(95%) were positive for deletional and non-deletional mutations of α globin genes, while 4(5%) were negative for any of the 21 mutations tested. Various HbPs, including sickle cell trait, sickle cell disease, β-Thal with sickle cell, β-Thal trait, β-Thal major, α-Thal (suspected) or Thal variants, Hb H disease and Hb E were found in the screened population (data not shown). CBC results were noted (Table 1). Frequency of α genotypes of the positive subjects showed 6 different alleles, including -α^{3.7}/-α^{3.7}, -α^{3.7}α/αα, -α^{IVS1} α/-α^{IVS1}, -α^{PolyA1} α/αα, -α^{3.7}α/α^{PolyA1}α and -α^{3.7}α/α^{IVS1}α (Table 2).

Table-1: Red blood cell (RBC) parameters and haemoglobin (Hb) electrophoresis of normal and alpha (α) thalassemia subjects.

Rubrics	Groups	Normal Mean ±SD	α thalassemia Mean ±SD	p-value
RBC (x10 ¹² /L)	Male	5.33±0.38	6.35±0.48	0.001
	Female	4.83±0.43	5.97±0.49	0.001
Hb (g/dl)	Male	14.81±1.71	14.19±1.09	0.001
	Female	12.92±1.00	12.55±1.00	0.009
MCV (fl)	Male	84.60±5.26	72.19±6.76	0.001
	Female	82.55±5.69	64.56±5.02	0.001
MCH (pg)	Male	27.76±3.29	22.4±1.40	0.001
	Female	26.51±1.89	21.09±1.93	0.001
RDW (%)	Male	13.78±1.00	17.15±2.27	0.001
	Female	14.36±1.23	18.85±2.66	0.001
Hb. A (%)	Male	97.04±0.19	97.7±0.50	0.001
	Female	97.13±0.36	97.75±0.42	0.001
Hb. A ₂ (%)	Male	2.91±0.23	2.25±0.44	0.001
	Female	2.03±0.36	2.21±0.40	0.001
Hb. F (%)	Male	0.6±0.1	0.5±0.1	0.961
	Female	0.5±0.2	0.8±0.01	0.012

MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, RDW: Red cell distribution width.

Table-2: Frequency of alpha (α) genotypes in the studied population (n=80).

Genotype	Phenotype	Mutation type	n (%)
αα/αα	Normal	Nil	04 (5)
-α ^{3.7} /-α ^{3.7} (Homozygous)	α thalassemia trait	Deletional	46 (57.5)
-α ^{3.7} α/αα (Heterozygous)	Silent carrier	Deletional	14 (17.5)
-α ^{IVS1} α/-α ^{IVS1} α (Homozygous) (α2 ^{IVS1})	α thalassemia trait	Non-deletional	02 (2.5)
-α ^{PolyA1} α/αα (Heterozygous) (α2 ^{PolyA1})	α thalassemia trait	Non-deletional	04 (5)
-α ^{3.7} α/αα ^{PolyA1} Compound heterozygous	α thalassemia trait	Deletional / Non-deletional	04 (5)
-α ^{3.7} α/αα ^{IVS1} Compound heterozygous	α thalassemia trait	Deletional / Non-deletional	06 (7.5)

Discussion

The prevalence of α -Thal in different Arab countries has been studied extensively.^{8,10-15} The current study was the first done in the Jazan area determining the prevalence of α -Thal gene polymorphisms in individuals visiting premarital screening centre. The overall frequency α -Thal was found to be 4.43% based upon haematological and molecular analysis. This finding is not in agreement with a study conducted two decades ago reported 0.55% percent individuals had α -Thal in this region.¹⁵ It also showed significantly higher difference in the prevalence of α -Thal in different regions of Saudi Arabia.¹⁵ In general practice at the premarital screening centres, these individuals are reported as "suspected for α -Thal" and no definite diagnosis is made. It is highly recommended that the screening of α -Thal shall be included for effective screening programme, especially in the highly prevalent areas of the Saudi Arabia. The base of inclusion criteria was low red cell indices (microcytosis) and normal Hb electrophoresis. Prevalence of α -Thal in microcytic and hypochromic anaemia has been reported in several studies.¹⁶⁻¹⁸ Overall frequency of α -Thal in microcytic and hypochromic anaemia was 12.7%, 46% and 78% in Indian, Turkish and Iranian population respectively.¹⁶⁻¹⁸ The current study showed the highest percentage of α -Thal in subjects with low red cell indices and normal Hb electrophoresis.

Molecular analysis showed six different mutations in α genes in the studied population. The most common deletional mutation detected was $-\alpha^{3.7}$ (75%). Out of 75% $-\alpha^{3.7}$ positive cases, 57.5% individuals were homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$) and $-\alpha^{3.7}\alpha/\alpha$ heterozygosity was found in 17.5% cases. High prevalence of $-\alpha^{3.7}$ has been reported by other researchers as well.^{8,10,11,19} Heterozygous poly A mutation ($-\alpha^{\text{PolyA1}}\alpha/\alpha$) was found in 5% of the studied population. Poly A mutation has already been reported in Saudi population of Eastern province.^{8,19,20} One study in the Eastern province found 1.78% of $-\alpha^{\text{PolyA1}}$ mutation in transfusion-dependent α -Thal patients and healthy individuals.¹¹

About 5% of the studied population also showed a compound deletional and non-deletional mutation i.e. $-\alpha^{3.7}\alpha/\alpha^{\text{PolyA1}}\alpha$. This mutation has also been reported previously among the Saudis.^{8,19} A compound deletional mutation and firstly detected non-deletional mutation i.e. $-\alpha^{3.7}\alpha/\alpha^{\text{IVS1}}\alpha$ was found in 7.5% population. To the best of the our knowledge, this mutation has not been reported previously in Saudis or nationals of any other country in the Gulf region. The presence of this mutation in Jazan area may be due to the studied population belonging to a

Table-3: Comparison of alpha (α) thalassemia gene mutations in Saudi Arabia and some Arab countries.

Country	Mutations (%)					Reference No.
	$-\alpha^{3.7}$	$-\alpha^{\text{IVS1}}$	$-\alpha^{\text{PolyA1}}$	$-\alpha^{3.7}\alpha/\alpha^{\text{PolyA1}}\alpha$	$-\alpha^{3.7}\alpha/\alpha^{\text{IVS1}}\alpha$	
Algeria	10.78	–	–	–	–	21
Bahrain	32	12	53%	–	–	22
Iraq	9.8	–	–	–	–	23
Jordan	32	18	25	–	–	24
Kuwait	10	3.3	86.7	–	–	25
Saudi Arabia	64	–	41	–	–	20
	21.8	–	5.2	–	–	8
	43.5	–	1.78	–	–	11
United Arab Emirates	44.5	1.4	0.7	–	–	26

specific area and consanguineous marriages. A number of common mutations reported in Saudis including $-\alpha^{4.2}$, $--\text{MED}$, $--\text{SEA}$, $--\text{THAI}$, $--\text{FIL}$, $-(\alpha)^{20.5}$, gene triplication $\alpha\alpha\alpha^{\text{anti } 3.7}$ were not observed in this study. Reasons may be low frequency of these mutations, different ethnic groups and geographic location. Homozygous $-\alpha^{\text{IVS1}}$ gene deletion was found in 2.5% cases. Again, to the best of our knowledge and extensive literature search, this mutation has not been reported in any study conducted in the country, but this mutation has been reported in other Arab countries (Table 3).

The current study has its limitations. Gene sequencing for α -Thal-positive individuals was not done due to limited funds and limited time. Similar studies shall also be conducted in other parts of the region, like Sabia, Al Fifa, Farasan Island, to establish guidelines and adding the screening of α -Thal in the premarital screening programme.

Conclusion

A significant number of individuals had α -Thal in the Jazan area. It is recommended that screening of α -Thal shall be included in the premarital screening programme.

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Conflict of interest: None.

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