

MOLECULAR SPECTRUM OF ALPHA-THALASSEMIA IN JORDAN

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ABSTRACT

Objective: The aim of this study was to define the spectrum of α -thalassemia determinants existing in Jordan.

Methods: A total 286 suspected α -thalassemia subjects including 29 hemoglobin Hb (Hb H) patients were examined by polymerase chain reaction and restriction enzyme digestion. Polymerase chain reaction product was examined by agarose gel electrophoresis.

Results: Five different α -thalassemia determinants were characterized in 336 chromosomes. The most prevalent α -thalassemia determinant was the single gene deletion $-\alpha^{3.7}$ (45%). The non-gene deletion α^{5nt} accounted for 27% of thalassemic chromosomes, followed by the non-gene deletional determinant $\alpha^{T-Saudi}$ (23%). The two-gene deletional determinant $--^{MED}$ was characterized only in 4% of thalassemic chromosomes. Triplicated α -gene determinant was observed in two heterozygous individuals ($\alpha\alpha\alpha/\alpha\alpha$). Four different genotypes were found to be responsible for Hb H disease. Homozygosity for the non-deletional determinant $\alpha^{T-Saudi}$ ($\alpha^{T-Saudi}\alpha/\alpha^{T-Saudi}\alpha$) was observed in the majority of those patients (76%) and was found to be associated with high Hb H levels. Less commonly, Hb H disease occurred as a result of compound heterozygosity between $--^{MED}$ determinant with other determinants; ($--^{MED}/\alpha^{T-Saudi}\alpha$), ($--^{MED}/\alpha^{5nt}\alpha$), ($--^{MED}/\alpha^{3.7}\alpha$).

Conclusion: The outcome of this pilot study provides valuable and basic information about the spectrum of α -thalassemia mutations in Jordan that might be useful in setting a strategy for molecular diagnosis of α -thalassemia carrier status and Hb H disease in this country.

Key words: Alpha thalassemia, Hemoglobin H, Molecular

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Introduction

Alpha thalassemia is one of the most common single gene disorders in humans.⁽¹⁾ Most frequently alpha thalassemia results from the loss of one (α^+) or both of the duplicated α genes (α^0) from chromosome 16.⁽²⁾ Carriers of the deletional forms of α thalassemia ($-a/\alpha$), ($-a/-a$) or ($--/\alpha\alpha$) are

clinically normal but have a mild hypochromic, microcytic anemia. Loss of three α genes ($--/-\alpha$) results in Hb H disease, a hemolytic anemia with variable clinical course. Infants who inherit no α genes develop severe anemia, which results in death at or shortly after the time of birth (Hb Bart's hydrops fetalis syndrome). The most widely occurring single gene deletions (α^+) are the $-\alpha^{3.7}$ and

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the $-\alpha^{4.2}$. The double α -gene deletions in *cis*, such as the $--^{SEA}$, $--^{FIL}$, and $--^{THAI}$ are most common in Southeast Asia, while the $--^{MED}$ and the $--^{20.5}$ double gene deletions (α^0) occur most frequently in the Mediterranean area.⁽³⁾ Less commonly, α -thalassemia results from point mutations and small deletions within the α -globin genes or in the regulatory sequences.^(4,5) The most common non-gene deletional mutations (α^T/α^T), existing in Mediterranean populations are two-point mutations in the polyadenylation site (Poly A) of the α_2 globin gene; $\alpha^{T-Saudi}$ also known as $\alpha^{PA-Saudi}$ (AATAAA>AATAAG), and α^{PA} (AATAAA>AAGAAA).⁽⁶⁻⁸⁾ The other important mutation existing in the region is the pentanucleotide (α^{5nt}) deletion of the first splice donor site of the α_2 -globin gene.⁽⁹⁾

In Jordan, like in other Mediterranean countries various hemoglobinopathies are common. Although several studies have been carried out to define the frequency and the spectrum of β -thalassemia,⁽¹⁰⁻¹²⁾ the incidence and spectrum of α -thalassemia are still unknown in this country. This study was carried out in order to acquire the spectrum of α -thalassemia variants in Jordan.

Methods

A total of 286 subjects (572 chromosomes) were studied among which 29 were Hb H disease patients. Patients were selected upon having red cell indices suggestive of thalassemia carrier status (MCV<80 fl and MCH<25 pg) after β -thalassemia and iron deficiency were excluded.

Blood samples were vacuum collected at Princess Iman Center for Research and Laboratory Sciences using Na-EDTA as anticoagulant. Hematological parameters were obtained from an automated counter, Sysmex XE-2100 (Sysmex-Toa Medical Electronics Co. Kobe, Japan). Red cell lysates were examined on cellulose acetate electrophoresis at pH 8.6. Hb A₂, Hb F and Hb H fractions were measured by high performance liquid chromatography (HPLC) using BioRad Variant II. Hb H disease was confirmed by electrophoresis and the demonstration of Hb H inclusion bodies by supravital staining.

Genomic DNA was isolated by DNA isolation kit (Instagene genomic isolation kit. BioRad. USA). Detection of α -thalassemia mutations $-\alpha^{3.7}$, triplicated α -gene, $-\alpha^{4.2}$, $--^{MED}$, $--^{20.5}$ was achieved by

gap polymerase chain reaction (PCR) using published primer sequences with some modifications to the original technique.⁽¹³⁾ Positivity for deletional α -thalassemia was confirmed by hybridization technique using (mDx α_1 gene & mDx α_2 gene, BioRad) gene amplification product. Restriction enzymes used were, *Stu* I for the ($\alpha^{T-Saudi}$) mutation,⁽¹⁴⁾ *Nla* III for the (α^{PA}) mutation⁽¹⁵⁾ and *Hph* I for the (α^{5nt}) mutation.⁽⁹⁾ Restriction enzymes were obtained from (NE Biolabs, Beverly; MA, USA) 10 units of restriction enzyme were added to 25 μ l of the PCR product and incubated at 37 C° overnight. For Gap PCR detection of deletional α -thalassemia mutations the following conditions were used; each 50 μ l reaction contained 200 μ M of each dNTP, 1.5 mM MgCl₂, 2.5 μ g BSA, 10% DMSO, 250- 500ng of genomic DNA and two unit of Taq DNA polymerase (Ampli Taq Gold polymerase, Perkin Elmer) in the supplied reaction buffer. Reactions were performed in icycler BioRad. USA. The program was initiated with denaturation at 96°C for five minutes followed by 32 cycles of 95°C denaturation for one minute, 62°C for 75 sec, and 72°C extension for 135 sec. The reaction was completed with final extension at 72°C for 10 minutes. After amplification, 10 μ l of product were electrophoresed through 1% agarose gel in 1x Tris-EDTA-Borate-buffer at 10 volts/cm for one hour. The ethidium bromide-stained gel was visualized and photographed under a UV transilluminator. α -globin gene sequence analysis was performed for 16 samples and was carried out at the Weatherall Institute of Molecular Medicine (John Radcliffe Hospital, Oxford, UK).

Results

Five different α -thalassemia determinants were observed including deletional, non- deletional and triplicated alpha gene. The gene-deletional determinants were the α^+ thalassemia deletion ($-\alpha^{3.7}$) and α^0 thalassemia deletion ($--^{MED}$). The non-gene deletional determinants were the ($\alpha^{T-Saudi}$), sometimes referred to as α^{PA1} , and the (α^{5nt}) mutation. The $-\alpha^{3.7}$ mutation was detected in 45% Jordanian thalassemic chromosome. The α^{5nt} determinant accounts for 27% of α -thalassemic chromosomes and the $\alpha^{T-Saudi}$ was characterized in 23% of α -thalassemic chromosomes. This mutation affects the poly A addition site of the

Table I. α -thalassemia chromosome frequency observed in 286 Jordanian individuals (572 chromosomes) carrying α -thalassemia

Type of chromosomes	No. of chromosomes	Frequency
$-\alpha^{3.7}$	151	45%
α^{5nt}	91	27%
$\alpha^{T-Saudi}$	79	23%
$--_{MED}$	15	4%
$\alpha\alpha\alpha$	2	1%
Total No. of (thalassemic chromosomes)	338	
$\alpha\alpha$ (normal chromosomes)	234	

Table II. α -thalassemia genotypes observed in Hb H disease patients

Genotype	No. of patients	Hb(g/dl)	MCV(fl)	MCH(pg)	Hb A2(%)	Hb H(%)
$\alpha^{T-Saudi} / \alpha^{T-Saudi}$	22	9.2±1.0	59.6±7.6	18.7±1.4	1.2±0.4	16.3±5.2
$--_{MED} / \alpha^{T-Saudi}$	3	9.5±0.9	61.1±2.8	18.6±0.3	1.1±0.2	18.7±6.9
$--_{MED} / -\alpha^{3.7}$	2	9.6, 9.4	62.4, 60.8	18.1, 18.9	1.2, 1.3	8.5, 5.5
$--_{MED} / \alpha^{5nt}$	2	8.1, 8.4	60.0, 60.4	18.6, 17.9	0.9, 0.8	13.8, Hb Barts 4.5%
						18.7

α_2 gene and was first described in patients from Saudi Arabia.⁽¹⁶⁾ The α^0 ($--_{MED}$) determinant was detected in 4% and only two individuals were heterozygotes for triplicated α -gene ($\alpha\alpha\alpha$) determinant (1%). Homozygosity for $\alpha^{T-Saudi}$ ($\alpha^{T-Saudi} / \alpha^{T-Saudi}$) was observed in 76% (22 out of 29). Compound heterozygosity for α^0 $--_{MED}$ deletion with other determinants ($--_{MED} / -\alpha^{3.7}$), ($--_{MED} / \alpha^{T-Saudi}$) or ($--_{MED} / \alpha^{5nt}$) were responsible for the remaining 24% of the total number of Hb H disease genotypes. In one patient, a 28-years old pregnant woman with the genotype ($--_{MED} / \alpha^{5nt}$). Hb Barts was detected in measurable amounts (4%).

Discussion

It is believed that α -thalassemia in Jordan has been underestimated because Hb H disease is relatively uncommon. Initially, the evaluation of the possible occurrence of α -thalassemia determinants was based only on screening methods such as hematological indices and supravital staining of Hb H bodies. None of these methods is reliable or sensitive to detect α -thalassemia trait carriers. Other electrophoretic and immunological methods detecting the small amounts of Hb Bart's in newborn babies underestimate the frequency of α -thalassemia trait carriers.⁽¹⁶⁾ This problem was overcome by the application of PCR approach for the various α -thalassemia determinants.⁽¹⁷⁻¹⁹⁾

In this study we defined the spectrum of α -thalassemia mutations in Jordan. We reported only

the data in which molecular characterization was achieved and the α -thalassemia determinants were clearly defined. Since only the most common Mediterranean determinants have been investigated, we cannot entirely exclude the occurrence of other rare determinants in few suspected α -thalassemia cases. These cases were not included in the study. However, the outcome of DNA sequence analysis for these samples is not expected to affect significantly the data obtained in this study.

As expected, the $-\alpha^{3.7}$ determinant was the most common (45%), which is in consistence with the high incidence of $-\alpha^{3.7}$ genotype and its remarkable frequency in the Mediterranean region.⁽¹⁾ The second most frequent determinant was the α^{5nt} (27%). Fortunately, even in the homozygous state, both determinants are not associated with severe clinical phenotype. The frequency of the severe non-deletional determinant $\alpha^{T-Saudi}$ was strikingly higher than expected (23%), while the two-gene deletion $--_{MED}$ was not as common as reported in other countries in the region.⁽²⁰⁾ It was observed only in 4% of thallemic chromosomes. Heterozygosity for triplicated α gene ($\alpha\alpha\alpha$) was observed in two sporadic cases. Despite the rare occurrence of this determinant, the coinheritance of triplicated alpha gene with heterozygous β thallemia might result in severe clinical phenotype which has important implications for genetic counseling and prenatal diagnosis.⁽²¹⁾ Surprisingly, the α -thalassemia determinants $--^{20.5}$, and $\alpha PA2$

(AATAAA>AAGAAA), believed to be quite common in the Mediterranean, were not detected in our patients.^(20,22) More importantly is the lack of $-\alpha =^{4,2}$ in Jordan although it was found to be quite common in other Arab countries in the Gulf region,⁽²³⁾ probably because of the high numbers of immigrants of non-Arab ethnicities to these countries.

Hb H disease patients of the sample analyzed, 29 individuals were Hb H disease patients. The most prevalent genotype was homozygosity for $\alpha^{T \text{ Saudi}}$ ($\alpha^{T \text{ Saudi}} \alpha^{T \text{ Saudi}} / \alpha^{T \text{ Saudi}} \alpha$) as many Jordanians are descendants from Saudi origin and because of the high rate of consanguinity among Jordanians.⁽²⁴⁾ This genotype accounted for 76% (22/29) of Hb H genotypes. The average Hb H fraction for this group was 16.3% (Table II).

Homozygotes for $\alpha^{T \text{ Saudi}}$ mostly have severe Hb H disease phenotype and detectable amounts of Hb H. It has been reported that homozygosity for $\alpha^{T \text{ Saudi}}$ might be very severe and in one instant, it caused fetal loss.^(8,25)

This is the contrary of homozygotes for the other non deletional mutation α^{5nt} detected in Jordan, those patients have thalassemia trait carrier phenotype with only a mild hypochromic microcytic anemia and no detectable Hb H fraction as shown in Table III.

Table III. α -thalassemia genotypes observed in asymptomatic carriers

α thalgenotypes	No. of patients	Frequency	Hb H %
$-\alpha^{3,7} \alpha / \alpha \alpha$	117	41%	ND*
$\alpha^{5nt} \alpha / \alpha \alpha$	75	26%	ND
$\alpha^{T \text{ Saudi}} \alpha / \alpha \alpha$	31	11%	ND
$--^{MED} / \alpha \alpha$	8	3%	ND
$-\alpha^{3,7} \alpha / -\alpha^{3,7} \alpha$	16	6%	ND
$\alpha \alpha \alpha / \alpha \alpha$	2	<1%	ND
$\alpha^{5nt} \alpha / \alpha^{5nt} \alpha$	7	2%	ND
$\alpha^{5nt} \alpha / \alpha^{T \text{ Saudi}}$	1	<1%	ND

ND*: Not detected by cellulose acetate electrophoresis and HPLC

The other Hb H disease patients showed compound heterozygous genotypes; ($--^{MED} / -\alpha^{3,7} \alpha$), ($--^{MED} / \alpha^{T \text{ Saudi}} \alpha$) and ($--^{MED} / \alpha^{5nt} \alpha$), together they accounted for 24% of Hb H genotypes. Compound heterozygosity for deletional α -thalassemia with non deletional type α -thalassemia ($-- / \alpha^T \alpha$) have Hb H disease, which is more severe than deletional type

Hb H disease ($-- / -\alpha$). Those patients might require blood transfusion, which is unusual in patients with the deletional type Hb H disease.⁽²⁶⁾ Table II shows that higher levels of Hb H are associated with these genotypes. However, the small number of patients with these genotypes might not be sufficient to draw conclusions.

Despite the high frequency of $-\alpha^{3,7}$ and α^{5nt} we believe that the absence of compound heterozygous cases with the genotype ($-\alpha^{3,7} / \alpha^{5nt} \alpha$) might be due to the fact that positive samples for $-\alpha^{3,7}$ with thalassemia trait phenotype were not further examined for other mutations.

The low incidence of α^0 thalassemia in Mediterranean populations accounts for the rare occurrence of Hb Bart's hydrops fetalis in the region.⁽²⁷⁾ However, the occurrence of Hb H disease associated with $\alpha^{T \text{ Saudi}}$ in Jordan might impose the requirement of specific screening and prevention also molecular characterization is also useful for the purpose of definitive diagnosis and counseling.

Based on our results, a suggested strategy for molecular diagnosis of α -thalassemia trait carriers among Jordanians should begin with the detection of $-\alpha^{3,7}$, α^{5nt} , $\alpha^{T \text{ Saudi}}$, and $--^{MED}$ determinants, respectively, while Hb H disease patients should first be examined for $\alpha^{T \text{ Saudi}}$ and $--^{MED}$ determinants, rather than for other determinants.

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