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Genetic Basis of Saudi *beta thalassemia* Identification of Mediterranean and Asian Mutations

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Thalassemias are of frequent occurrence in different parts of the World and different regions differ in the molecular basis of the *thalassemia*, where in every population 5-6 mutations occur frequently while others are rare. We compared the frequency of mutations identified in 66 β-thalassemia patients with those reported in some of the Mediterranean, Gulf and Asian countries. Our results showed that IVS-I-110 (G→A) and IVS-I-5 (G → C) were the most frequently encountered mutations in our study group. The former is a mutation which is frequent in the Mediterranean countries, while the latter was mainly encountered in the Gulf region and Asian countries. The next most frequent mutation in Saudis was IVS-II-1 (G → A). This mutation is wide spread and was reported to occur in the Mediterranean, Gulf and Asian countries. CD-39 (C → T) was the next most frequent mutation in Saudis. It was reported in most of the Mediterranean and the Gulf countries, but was either rare or non-existent in Pakistan, Iran and India. IVS-1-3' end (-25) was mainly reported in the Gulf countries and at a low frequency in the Mediterranean and Asian countries. The next most frequent mutation in Saudis was CD 6 (-A). This was not reported in any of the Gulf and Asian countries, but was reported in most of the Mediterranean countries. CD 5 (CCT→ -CT) which occurred at a frequency of 1.8% in Saudis was also reported in most of the Mediterranean, Gulf and Asian countries. Four mutations were encountered at a frequency of less than 1% in the Saudi patients. Comparison showed that some were more common in the Mediterranean countries, while others were more frequent in the Gulf and Asian countries. Our study showed that Saudi Arabia occupies a central position in regards to the nature of mutations which cause β-thalassemia in this population. We suggest detailed β- globin gene haplotype analysis to identify the origin of these mutations. In addition, studies are also needed on a larger number of samples from different regions of Saudi Arabia.

Key words: β-*thalassemia*, β-*globin gene*, Saudi Arabia, Mediterranean countries, Gulf, Asian countries.

Hereditary blood disorders (HBD) are a heterogenous faction of genetic defects that affect the red cells membrane, the red cell enzymes, or the structure or amount of hemoglobin synthesized in the red cells[1].

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The β-thalassemias are among the more frequently encountered HBD's that are characterized by anomalies in the synthesis of the β-globin chains of hemoglobin A (HbA), the normal adult hemoglobin, resulting in deficient and variable levels of β-globin chains, thus elevating the α/β ratio. These variations result in phenotypes ranging from severe life threatening, transfusion dependent anemic state to clinically asymptomatic conditions, with hypochromic microcytic red cells, depending

on the amount of hemoglobin synthesized. There are three main forms of the β-thalassemia i.e. β-thalassemia major, β-thalassemia intermedia and β-thalassemia minor, depending on the amount of normal HbA available in the red cells, which depends largely on the type of mutation producing the thalassemic state [2]. The β-thalassemias result mainly from point mutations and rarely from gene deletions, where part of the gene or the entire β-globin gene may be deleted [3-7]. A significant variability is encountered in the molecular basis of the b-thalassemia, where over a few hundred mutations have been reported in and around the β-globin gene, located on the short arm of chromosome 11 at 11p15.5. Some mutations result in deceased amount of β-globin chains producing β^+ phenotype, while others result in complete absence of the β-globin chains resulting in β^0 phenotype. Majority of the mutations are transmitted in an autosomal recessive manner, though a few dominant mutations have also been reported [8-10]. The overall distribution of mutations shows significant variability in the different World populations and interestingly, in each region[6-8] mutations are more specific and are commonly encountered while others are rare. Hence, there are mutations specific to the populations in the Mediterranean regions, the Chinese, the Japanese, the Africans, the Arabs in the different Middle Eastern countries, etc. Even within the same country, different areas frequently show differences in the nature of the mutations reported in each area. This variability has resulted in numerous studies exploring the molecular basis of mutations in different populations.

In Saudi Arabia, our studies showed the presence of β-thalassemia, in the different parts of the country at a variable prevalence [11-13]. Studies exploring the molecular basis of the β-thalassemia showed specific mutations in the different parts of the country [14-18]. In this study we compared the mutations types reported in Saudis, with those

Table 1. Results of hematological parameters in the adult and children β-thalassemia patients.

		Adults			Children		
	Sex	Mean	SEM*	Sig**	Mean	SEM*	Sig**
RBC ($X10^{12}$)	M	5.14	0.23	NS	3.92	0.23	NS
	F	4.38	0.32		4.00	0.24	
WBC ($X\ 10^9$)	M	7.87	0.68	NS	10.09	0.92	NS
	F	8.00	0.48		8.58	0.80	
Hb (gm/dl)	M	12.93	0.44	0.047	10.18	0.46	NS
	F	11.06	0.75		10.44	0.38	
PCV (%)	M	36.58	1.69	.NS	28.94	1.45	NS
	F	32.67	2.17		29.40	1.33	
MCV (fl)	M	69.11	1.79	NS	70.40	2.68	NS
	F	75.22	3.79		75.33	2.68	
MCH (pg)	M	25.13	1.01	NS	26.75	1.05	NS
	F	25.88	1.46		27.01	1.39	
MCHC ($X10^{12}$)	M	35.66	0.92	NS	35.66	0.71	NS
	F	33.88	0.48		35.94	0.74	
Retic (%)	M	3.00	0.40.	NS	3.49	0.76	NS
	F	1.75	0.46		3.51	0.69	
HbA_2 (%)	M	5.48	0.63	0.016	4.24	0.33	NS
	F	3.64	0.32		3.72	0.37	
HbF (%)	M	2.56	1.63	NS	4.98	1.439	NS
	F	4.02	2.71		5.23	1.607	

*SEM: Standard error of the mean; **Sig: Significance of the results in the male and female patients. NS: Not significant. M; Males; F: Females.

reported in literature, in an attempt to highlight ethno-geographic variations in Saudi Arabs and the neighboring populations. This paper compares our findings in Saudi patients with those reported from the Mediterranean region, Gulf and Asian countries and shows overlap in the molecular basis of β-thalassemias in this part of the World.

METHODS AND MATERIALS

The study group included 66 patients, suffering from β-thalassemia, attending clinics of AA and AKM. The children were enrolled in the study after consent from the patents, while consent was taken from the adult patients. Blood was drawn by venepuncture in tubes with anticoagulants. Whole blood was used to determine the hematological parameters and red cell indices using Coulter Counter with hemoglobinometer attachment. HbA2 and HbF levels were measured according to published methods [19, 20]. Blood cells were separated by centrifugation at 1000rpm for 10 minutes and the red cells, buffy coat and plasma were carefully separated. The red cells were hemolysed by adding cold distilled water and the hemolysate was used for electrophoresis in agarose gel to determine the presence of abnormal hemoglobins[19]. DNA was extracted from the buffy coat and used for the genetic studies. The β-globin gene mutations were detected by Amplification Refractory Mutation System (ARMS) using primers specific for the mutated and the wild type allele, applying the standardized procedures[14, 15].

The mutations identified were manually assessed and allele frequencies were calculated. The results of the hematological and biochemical parameters were analyzed using SPSS version 18. Mean and standard error of the mean (SEM) were obtained separately for the male and female adults and children. The results of the different groups were compared using student's t test. P value <0.05 was considered statistically significant.

RESULTS

There were 66 thalassemia patients included in this investigation. Among these there were 18 adults (males: 9 and females: 9) and 48 children (<18 years) (m ales: 26 and females: 22). The hematological parameters in this group were as presented in the Table 1. Except for the level of hemoglobin and HbA_2 none of the other parameters showed any difference between the male and female patients. When the adults and children were compared, there were significantly lower values of RBC count, hemoglobin and PCV in the children, while WBC count, reticulocyte count and HbF levels were higher.

In the study group, two patients were identified as suffering from HbS/β+ thalassemia and one was suffering from HbS/β^0 thalassemia. The β-thalassemia mutations identified in the patients are presented in Table 2. Of the total 132 chromosomes investigated, mutation was identified on 112 chromosomes (84.85%) and no mutation was identified on 20 chromosomes

Table 2. Mutations identified in Saudi β-thalassemia patients

Mutation	Position/Type	Type of Thal	No. of alleles	Frequency
CD-39 (C → T)	Nonsense mutant	βo	14	12.8
IVS-1-3'end (– 25)	Splice junction	βo	12	11.0
IVS-II-1 (G → A)	Splice junction	βo	16	14.7
CD 6 (-A)	Frame shift	βo	4	3.7
CD 8/9 (+G)	Frame shift	βo	1	0.92
IVS-I-1 (G → A)	Splice junction	βo	1	0.92
CD 44 (-C)	Frameshift	βo	1	0.92
CD 5 CCT → (C	Frameshift	βo	2	1.84
CD 26 (+T)	Frameshift	βo	1	0.92
IVS-I-110 (G → A)	Internal IVS change	β+	27	24.8
IVS-I-5 (G → C)	Consensus change	β+	27	24.8
IVS-I-6 (T → C)	Consensus change	β+	1	0.92

Table 3. Comparison of mutations identified in Saudi b-thalassemia patients with those reported in literature in the Mediterranean, Gulf and Asian countries*

Mautation	Syria (21)	Lebanon (22-24)	Jordan (25)	Gaza (26)	Egypt (27-29)	Tunisia (30-31)	Algeria (32-34)	Saudi Arabia (This study)	E. Saudi Arabia (17)	Kuwait (35)	Bahrain (36)	UAE (37-40)	Oman (41)	Pakistan (42)	Iran (43)	India (44)
CD-39 (C → T)	6.4	0.5	2	11.5	1.5	27	27.6	12.8	20.3	7.3	24.2	5	1	0.3	-	-
IVS-1-3' end (– 25)	0.7	1	-	-	-	-	-	11	4.4	7.3	36	8	5.5	-	-	-
IVS-II-1 (G → A)	4	10	19.8	1	3	-	-	14.7	27.5	29	6.1	3.3	3.5	0.8	32.97	-
CD 6 (-A)	-	-	1	5	1	10.5	17	3.7	-	-	-	-	-	-	-	-
CD 8/9 (+G)	1.4	0.5	-	-	0.3	-	-	0.92	-	1.3	1.5	7	-	37.3	13.51	6.05
IVS-I-1 (G → A)	17	15	6.6	20	11.3	0.9	11.7	0.92	5.8	7.3	3	-	1	1.3	4.59	2.42
CD 44 (-C)	-	1	-	-	-	4.4	-	0.92	1.5	1	4.5	1.3	9.6	-	-	-
CD 5CCT→ -CT	8.5	4	3.3	10	2.4	1.8	-	1.84	1.5	-	-	4	0.5	1.3	-	6.05
CD 26 (+T)	-	-	-	-	-	0.9	-	0.92	-	-	-	-	-	-	-	-
IVS-I-110 (G→A)	24	33	22	37.5	*33*	12.3	24.7	24.8	-	-	1.5	1.7	0.5	-	8.38	-
IVS-I-5 (G → C)	-	5	5.5	-	-	-	0.4	24.8	23.2	18.8	16.7	55	61.6	38.9	-	41.94
IVS-I-6 (T → C)	4	15	6.6	7.5	13.6	6	3.3	0.92	4.4	-	-	-	-	-	-	-

*Only mutations identified in Saudis are compared. ( ) Reference number.

(15.15%). Of the mutations identified 3 were HbS mutation and 109 chromosomes carried β-thalassemia mutations (97.3%). A total of 12 different mutations were identified. Of these three were β+ mutations and the rest 9 were β^0 mutations. The most frequent mutations were IVS-I-110 (G → A) and IVS-I-5 (G → C). A table was prepared listing the mutations reported in some of the Mediterranean, Gulf and Asian countries, in comparison to mutations in Saudi Arabs and the comparison is presented in Table 3.

DISCUSSION

In this group of 66 patients, no mutation was detected on 20 of the chromosomes. This was either because these chromosomes did not carry any mutation and the patients having them were heterozygotes or they had mutations which were not detected during this investigation. Among the β-thalassemia mutations identified, the most frequent mutations in this group of Saudi β-thalassemia patients were IVS-I-110 (G→A) and IVS-I-5 (G → C). Both mutations produce a β+ phenotype. IVS-I-110(GA) is of frequent occurrence in most of the Mediterranean countries but occurs at a lower prevalence in the Asian countries, though its prevalence is 8.38% in Iran. In the Gulf countries it is either nonexistent or occurs at a low frequency as shown in the Table 3.

The other common Saudi mutation IVS-I-5 (G → C), and is the most frequently encountered mutation in the Asian and the Gulf countries; where in Oman[41] and UAE [37-40], frequencies as high as 61.5% and 55%, respectively have been reported. Both in India [44] and Pakistan[42], it is the most frequently encountered mutation. In contrast, in the Mediterranean countries, no cases with this mutation have been reported. It has been suggested that the occurrence of this mutation in the Gulf countries, could be easily attributed to the Spice trading routes, established a few thousand years ago between the Arabs and South Asian populations, and the most affected countries are Oman and United Arab Emirates, both at the southeastern side of the Gulf much close to South Asia than other countries [45]. Saudi Arabia is halfway between in the frequency of this mutation between the Mediterranean and Asian countries.

The third most frequent mutation in our study was IVS-II-1 (G→A) occurring at a frequency of 14.7%. This is a splice junction mutation, producing β^0-thalassemia phenotype. In another study [17], on the population in the Eastern region of Saudi Arabia, IVS-II-1 (G→A) was reported as the most frequent mutation (27.5%). Kuwait [35] and Iran[43] have this as the most frequent mutation causing β-thalassemia. However, in the other Gulf countries, and Pakistan, it occurs at a low frequency, and is either non-existent in India or occurs at a low frequency. Similar to IVS-I-5, IVS-II-1 seems to be restricted to some of the Arabic countries both in the Mediterranean region and in the Gulf [46]. Its frequency is high in Iran, but is either nonexistent or occurs at a low frequency in Pakistan and India. Several studies linking IVS II-1 to different β-globin gene haplotypes have suggested the association of this mutation with multiple backgrounds in world populations and multiple origins of the mutations. It was also speculated that the mutation occurred in the southwestern part of the Arabian peninsula, and subsequently was transported to neighboring areas by trading routes established some 2000 years ago[47].

The next most frequent mutation in Saudi's was CD-39 (C → T). This is a non-sense mutation producing a β^0-thalassemia phenotype. This splice junction mutation reported to occur in most of the Mediterranean and Gulf countries though at significantly variable frequencies ranging from 0.5 in Lebanon to 27.6 in Algeria (Table 3). This substitution at CD-39 (C→T), is classified primarily as a Western Mediterranean mutation. However, in a study from the Eastern Saudi Arabia, the frequency was over 20% [17]. Among the Gulf countries Bahrain[36] has the highest frequency (24.2%), while among the Asian countries the frequency at which CD-39 (C→T) exists is low, where in Pakistan the frequency was 0.3% [42], while from Iran and India [43-44], no case was reported.

A 25 base pair deletion in IVS 1 [IVS-1-3'end (– 25)], is a splice junction mutation and is also a frequent mutation in Saudi β-thalassemic, occurring at a frequency of 11%. It is encountered in almost all the Gulf countries with variable frequencies, where in Bahrain the frequency is as high as 36% [36]. However in the Asian countries it

is almost nonexistent. Also in the Mediterranean countries the frequency is either very low or it is non-existent.

CD 6 (-A), a frame shift mutation producing a β^0-thalassemia phenotype, was not reported in any of the Gulf countries, nor in the Asian countries, while in most of the Mediterranean countries it was reported though at significantly variable frequencies (Table 3). In our study this occurred at a frequency of 3.7%, but was not reported in a study from the Eastern Province.

CD 5 (CCT → -CT), a frameshift mutation producing a β^0-thalassemia phenotype, occurs in most of the Mediterranean, Gulf and Asian countries, though at variable frequencies (Table 3). In the Saudis the frequency in our study was 1.84%, and was almost the same in a study from the Eastern province (1.5%).

IVS-I-6 (T → C), IVS-I-1 (G →A), CD 8/ 9 (+G) and CD 44 (-C), were all present in our patients but each at a frequency less than 1%. IVS-I-6 (T-C) occurs at high frequencies in European countries, where in Portugal, Italy and Yugoslavia high occurrence has been noted. Also in the Eastern Mediterranean Arab countries it shows frequent occurrence. However, in the Gulf countries and in India, Pakistan and Iran, it is either nonexistent or occurs at a low frequency (Table 3). Saudi Arabia has a low frequency for this mutation. IVS-I-1 (G-A) also occurs at a low frequency in our patients, though in a study from the Eastern province the frequency was reported as 5.8% (17). It occurs at a higher frequency in the Mediterranean countries, compared to countries in the Gulf areas and in Asia (Table 3). CD8/9 +G, a frame shift mutation producing β^0-thalassemia, is more common in the Asian countries though it is reported at low frequencies in some of the Mediterranean. In Pakistan, frequencies as high as 37.3% have been reported [42]. Finally, CD 44 a deletion producing frameshift, resulting in β^0-thalassemia is present in most of the Gulf countries, and in Asia and the Mediterranean countries low prevalence is reported in a few countries and in many it does not exist.

In a recent study reported from the Western Saudi Arabia[16], 23 mutations were identified to cause β-thalassemia in the western region of Saudi Arabia. Of these there were 7 frequent mutations which were IVS-II-1 (G>A), IVS-I-110 (G>A), IVS-I-5 (G>C), codon 39 (C>T), codon 26 (G>A) [Hb E or β26(B8)Glu→Lys, GAG>AAG], frameshift codons (FSC) 8/9 (+G), and IVS-I-1 (G>A). Except for codon 26 (G>A) [Hb E or β26(B8)Glu→Lys, GAG>AAG] mutation, which we did not identify in our patient group, all others were the same as those observed during our study (Table 2). Genetic heterogeneity was reported in this study and it was shown that 10 mutations were of Asian or Asian/Indian origin, two were Kurdish, one Chinese, one Turkish, one Saudi, and the remainder were of Mediterranean origin.

In conclusion, this comparison places Saudi Arabia in a fairly central position, where mutations seen in the Mediterranean countries, and the Gulf and Asian countries are encountered. An overlap can be seen clearly, where some mutations frequent in either the Mediterranean countries or Asian countries occur at an intermediate frequency in Saudis. Some mutations more specific to the Gulf are present in the Saudis.

Further studies are required on a larger sample size, and on Saudis from different regions of the country to conduct a microdistribution study of the mutations. On the other hand, it is also required to study the -globin gene haplotypes associated with each mutation, in an attempt to shed more light on the origin and spread of the β-thalassemia mutations.

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