Molecular Characterization of beta-Thalassemia Mutations in Baghdad

Waleed Abdelaziz Omer
MBChB, MSc, PhD

Background: There are currently more than 200 known mutations in the beta globin gene that cause thalassemia syndrome in the world and each ethnic population has its own unique and frequency of beta globin mutations. Delineation of the beta-thalassemia mutations in specific community is a prerequisite for implementation of preventive program in that community.

Aim of the study is to characterize the spectrum of beta globin gene mutations in Baghdad patients.

Patients and methods: thirty one thalassemic patients were included; they were transfusion dependent and they were diagnosed and registered in thalassemia centers in Baghdad. After DNA extraction from venous blood and PCR based DNA amplification, the allele's characterization was achieved by reverse hybridization to specific oligonucleotide probe designed to detect 22 beta-thalassemic mutations.

Results: Eleven alleles causing beta-thalassemia in Baghdad were identified, and these alleles with their frequencies were:
IVS 2.1 (G→A)29.03% , IVS 1.110 (G→A)17.74% , cod 44 (-C)14.51%, IVS 2.745 (C→G)11.29% , cod 5 (-CT)9.67% , IVS 1.6 (T→C)4.83% , IVS 1.5 (G→C)3.22% , IVS 1-25 (25 bpDEL)3.22%, cod 8 (-AA)3.22%, cod 39 (C→T)1.61% and cod 8/9 (+G)1.61%.

Twenty three (74.19%) patients presented as thalassemic patient during the first year of life. Twenty five (80.64%) patients have homozygous alleles.

Twenty two (75.86%) families have consanguineous relation between the parents and eleven (37.93%) families have more than one affected individual,

Conclusions:

1- Eleven β-globin alleles causing β-thalassemia syndrome in Baghdad were characterized; most of these mutations are of Mediterranean type and few are of Kurdish and Asian Indian types.

2- Most of the patients are homozygous, and of severe (β^o) Thalassemia, and showed consanguineous relation between their parents.

3- The characterization of the most common beta-thalassemia mutations provides a foundation for prenatal genetic counseling that will be part of a thalassemia prevention program in Baghdad.

Key words: thalassemia, beta globin genes mutations, Baghdad, oligonucleotide, reverse hybridization

Introduction

The thalassemias are a heterogeneous group of genetic disorders of hemoglobin synthesis, all of which result from a reduced rate of production of one or more of the globin chains of hemoglobin.

The β-thalassemias are the most important types of thalassaemia because they usually produce severe anemia in their homozygous and compound heterozygous states[1].

Genetic counseling, population screening, prenatal diagnosis and the option of termination of affected pregnancies remain the mainstay strategy in the control of beta thalassemia major. This was demonstrated in Cyprus, Greece and in many countries worldwide. Many studies have confirmed and shown the benefits of a thalassemia prevention program [2].

There are currently more than 200 known mutations in the beta globin gene .Prenatal diagnosis requires identification of the mutation spectrum in each population, then it is possible to do direct identification for these mutations in the majority of the population using monoplex and multiplex amplification refractory mutation system(ARMS) or dot blotting [3,4].

Characterization of beta thalassemia mutations have been carried out in countries around Iraq ; in Saudi Arabia [5] , in Syria[6], in Jordan[7], in Kuwait[8], in Turkey(9) , and in Iran(10) .

In Iraq a limited study has been carried out in Dohuk governorate region [11]

Aim of the study:- is to characterize the spectrum of beta globin gene mutations in Baghdad in patients with beta- thalassemia major who are registered in thalassemia centers, using PCR - based DNA diagnostic techniques.

Patients Materials and Methods

This study was conducted during the period: from Faberuary 2007 to December 2007.

Thirty one thalassemic patients were included in this study; the patients were diagnosed and registered as thalassemia major in thalassemia center in **Al-Karama hospital** (30 patients), and thalssemia center in **Ibn-Al-Baladi hospital** (1patients) in Baghdad governorate.

A case sheet for each patient, including: name, sex, date of birth, age of presentation, consanguinity of parents had been prepared.

For each patient two ml of venous blood was withdrawn from the antecubital fossa and was put in a 2.5 ml EDTA tube (AFMA-Dispo-Jordan) and subjected to the following investigations:

1- DNA extraction: carried out in the Laboratory of Bone Marrow Transplant center in Children Welfare Teaching Hospital, of Medical City,

Baghdad-Iraq, using Wizard Genomic Purification Kit (Promega corporation-USA), according to the method described by the manufature .

2-DNA amplification and mutation identification: carried out in Italy by Dott. Marcello Morgutti (Servizio di Genetica, IRCCS "Burlo Garofolo", via dell 'Istria 65/1, 34100 TRIESTE, Tel.:0403785424, Fax: 0403785540, e-mail: morgutti@burlo.trieste.it) using β-Globin StripAssay™ kit (ViennaLab Labordiagnostika GmbH- Austria) which intended for the identification of β-globin gene mutations based on polymerase chain reaction(PCR) followed by reverse hybridization to specific wild and mutant oligoprobes designed to detect 22 β–thalassemia mutations (ViennaLab Labordiagnostika GmbH).

The assay cover 22 beta globin mutations as following: -87(C→G), -30(T→A), Codon 5(- CT), hemoglobin C (HbC), Hemoglobin S (HbS), Codon 5(-A), Codon 8(-AA), Codon 8/9(+G), Codon 22(7bp del), Codon 30(G→C),IVS 1.1(G→A), IVS 1.2(T→A), IVS 1.5(G→C), IVS1.6 (T→C), IVS 1.110(G→A), IVS 1.116(T→G),IVS 1-25(25bp del), Codon 36/37(-T), Codon 39(C→T), Codon 44(-C), IVS 2.1(G→A), IVS 2.745(C→G) (ViennaLab Labordiagnostika GmbH)

Results

Thirty one patients with 62 alleles from 29 families were studied; all were of Arab origin for father and mother (table 1). The following criteria were studied in addition to the characterization of the alleles:

1-Sex: 18/31 (58.06 %) patients were males and 13/31 (41.93 %) were females with ratio of male: female was 1.3:1.

2-Age: the mean of age was 9.93 years (SE+/- 1.05) with range of 2-27 years.

3-Age of presentation: 23/31 (74.19%) patients presented during the first year of life; 8/31 (25.8%) presented during the second year of life.

4-Consangunity of parents: 22/29 (75.86%) families have consanguineous relation between the parents, while 7/29 (24.13%) families have no consanguineous relation between the parents.

5-Genotype: 25/31 (80.64%) patients have homozygous alleles and in 23/25 (92%) of these patients there was consanguinity between their parents, while 2/25 (8%) have no consanguinity between their parents.

Six out of 31(19.35%) patients have compound heterozygous (c. heterozygous) alleles and in only one of them (16.77%) there was consanguinity between the parents, while 5/6 (83.33%) patients have no consanguinity between their parents.

6-Other affected siblings: 11/29 (37.93%) families have more than one affected individual, while 18/29 (62.06%) families have only one affected individual.

7-Characterization of the alleles: 31/31 (100%) of the alleles were characterized.

8-Types of the mutations: 11 types of mutations were identified and these are : IVS 2.1 (G→A), IVS 1.110 (G→A) , cod 44 (-C) , IVS 2.745 (C→G) , cod 5 (-CT) , IVS 1.6 (T→C) , IVS 1.5 (G→C) , IVS 1-25 (25 bpDEL) , cod 8 (-AA) , cod 39 (C→T) and cod 8/9 (+G).

9-Frequency of the mutations (table 2):

-IVS 2.1 (G>A) is the most frequent mutation and it constitutes 29.03 %(18/62) of all alleles.

-The mutation IVS 1.110 (G>A) is the second most common mutation and it constitutes 17.74% of all alleles.

-The least frequent mutation was cod 8/9 (+G) which constitutes 1.61 %(1/62) of all alleles.

Discussion

In this study **eleven β-globin gene mutations** have been characterized in the thalassemic patients in Baghdad. Up to our knowlwdge this is the first study that delineates the β-globin mutations in the thalassemic patients in Baghdad.

The eleven mutations which have been characterized in the studied group determined that the main β-thalassemia mutations in Baghdad are of **Mediterranean origin**; with some mutations were of **Kurdish** and **Asian Indian** origins; The mutations IVS 2.1 (G>A), IVS 1.110 (G>A), IVS 1.6 (T>C), cod 5 (-CT), cod 39 (C>T), cod 8 (-AA) and IVS 2.745 (C>G) are included in the Spectrum peoples [12,13] and these mutations constitutes of β-thalassemia mutations in Mediterranean

77.39% of all types of mutations in the studied group. The mutation cod 44 (-C), a Kurdish mutation [12] constitutes only 14.51% while the Asian Indian mutations IVS 1.5 (G>C), IVS 1-25 (25 bpDEL) and cod 8/9 (+G)[12] constitute 8.05% of all mutations. These findings (the high frequency of Mediterranean mutations and the low frequency of Asian Indian mutations) are similar to the findings obtained in the surrounding countries especially Syria, Jordan and Saiudi Arabia – table 3.

Table 1: The results of beta-globin mutations and other clinico-pathological criteria of 31 thalassemic patients.

Code No.	Allele No. 1	Allele no. 2	Sex	Age year	Pres year	Con	Oth. Affe.
1	IVS 2.745 (C>G)	IVS 2.745 (C>G)	M	4	<1	+	1
2	IVS 1-25 (25 bpDEL)	IVS 1-25 (25 bpDEL)	F	9	<1	+	1
3#	IVS 1.110 (G>A)	IVS 1.110 (G>A)	F	11	3	+	1
4	IVS 2.1 (G>A)	IVS 2.1 (G>A)	M	3	<1	-	-
5	IVS 2.745 (C>G)	IVS 2.745 (C>G)	F	27	1.5	+	2
6	IVS 1.110 (G>A)	IVS 1.110 (G>A)	M	1	<1	+	1
7	IVS 1.110 (G>A)	IVS 1.110 (G>A)	M	17	3	+	-
8	IVS 1.110 (G>A)	IVS 1.110 (G>A)	M	7	<1	+	-
9#	IVS 1.110 (G>A)	IVS 1.110 (G>A)	F	12	3	+	1
10	IVS 2.1 (G>A)	IVS 2.1 (G>A)	M	12	4.5	+	-
11	IVS 2.1 (G>A)	IVS 2.1 (G>A)	M	4	<1	+	-
12	cod 5 (-CT)	cod 5 (-CT)	F	3	<1	+	-
13	cod 44 (-C)	cod 44 (-C)	M	2	<1	+	-
14	cod 44 (-C)	cod 8/9 (+G)	M	6	<1	-	-
15	IVS 1.110 (G>A)	IVS 2.1 (G>A)	F	10	<1	-	-
16	IVS 2.1 (G>A)	IVS 2.1 (G>A)	M	9	6	+	-
17*	cod 44 (-C)	cod 44 (-C)	M	5	<1	+	1
18*	cod 44 (-C)	cod 44 (-C)	M	6	<1	+	1
19	cod 44 (-C)	cod 44 (-C)	F	9	<1	+	1
20	IVS 2.1 (G>A)	IVS 2.1 (G>A)	F	9	<1	-	-
21	IVS 2.745 (C>G)	IVS 1.6 (T>C)	M	8	<1	+	-
22	cod 5 (-CT)	cod 5 (-CT)	F	10	<1	+	-
23	cod 39 (C>T)	IVS 2.1 (G>A)	F	17	<1	-	-
24	IVS 1.6 (T>C)	IVS 2.1 (G>A)	F	12	2.5	-	1
25	IVS 2.1 (G>A)	IVS 2.1 (G>A)	M	15	<1	+	1
26	IVS 2.745 (C>G)	IVS 2.745 (C>G)	F	20	<1	+	-
27	IVS 1.6 (T>C)	IVS 2.1 (G>A)	M	12	<1	-	-
28	IVS 2.1 (G>A)	IVS 2.1 (G>A)	F	12	<1	+	2
29	cod 8 (-AA)	cod 8 (-AA)	M	4	2	+	-
30	IVS 1.5 (G>C)	IVS 1.5 (G>C)	M	20	<1	+	2
31	cod 5 (-CT)	cod 5 (-CT)	M	12	<1	+	-

#: Sisters, *: Brothers, **: Brothers, F: Female, M: Male, Pres: Age of presentation, Con: Consanguinity, Oth. aff: other affected siblings.

Table 2: The mutations detected in the patients studied in Baghdad and the number of the alleles and frequency for each mutation.

The mutation	No. of alleles	% of alleles in Baghdad
IVS 2.1 (G>A)	18	**29.03**
IVS 1.110 (G>A)	11	**17.74**
cod 44 (-C)	9	**14.51**
IVS 2.745 (C>G)	7	**11.29**
cod 5 (-CT)	6	**9.67**
IVS 1.6 (T>C)	3	**4.83**
IVS 1.5 (G>C)	2	**3.22**
cod 8 (-AA)	2	**3.22**
IVS 1-25 (25bpDEL)	2	**3.22**
cod 39 (C>T)	1	**1.61**
cod 8/9 (+G)	1	**1.61**
Total	62	**99.95**

Table 3: The types, severity & frequency (%) of various β-thalassemia mutations identified in Baghdad & surrounding countries.

Mutations of This study	Origin [14]	Bagh	Syri (11)	Jord (7)	SA (14)	Kuw(14)	Iran (11)	Turk (11)	Severity (13)
IVS 2.1 (G>A)	Med	29.03	4	15	15	29	33.9	4.9	β^o
IVS 1.110 (G>A)	East Med	17.74	24	25	22	Reco[14]	4.8	41.4	β^+
cod 44 (-C)	Kurd	14.51	0	0	7.5	1	2.6	1.3	β^o
IVS 2.745 (C>G)	Med	11.29	1.4	14.2	0	0	Reco(16)	Reco (17)	β^+
cod 5 (-CT)	Med	9.67	8.5	3.8	0	0	0.7	2.3	β^o
IVS 1.6 (T>C)	West Med	4.83	4	8.3	7	7.3	1.1	10.6	β^+
IVS 1.5 (G>C)	Asia-India	3.22	0	5.5[14]	10	18.8	7.6	1.2	β^+
IVS 1-25 (25 bpDEL)	Asia-India	3.22	0.7	0	14	7.3	-	0	β^o
cod 8 (-AA)	Med Turk	3.22	0.7	0	10	3	4.5	5.7	β^o
cod 39 (C>T)	West Med	1.61	6.4	4.6	20	7.3	1.7	4	β^o
cod 8/9 (+G)	Asia-India	1.61	1.4	0	2.5	1.3	4.8	1.3	β^o
Undetermined alleles		5.38	12.8	1.6			19.1	9.5	
IVS 1.1 (G>A)	Med	0.0	17.0	6.6[14]	7.0	7.3	2.9	5.3	β^o

Bagh: Baghdad, Med: Mediterranean, Jord: Jordan, S.A.: Saudi Arabia, Kuw: Kuwait, Kurd: Kurdish, Turk: Turkish
Reco: the mutation is recorded irrespective to (%)

Beside that , all the mutations characterized in this study have been previously described in other populations in the surrounding countries and the most common mutation determined in this study are also the most common mutations in the surrounding countries:

The mutation IVS 2.1 (G>A) (splice junction mutation), the most common mutation in this study was, also, the most common mutation in Kuwait, northern and southern of Iran and the 2nd most common mutation in Jordan [7,8,10]

It has been recorded in the most of Arab countries [14].

The mutation IVS 1.110 (G >A) (create new splice site), which was the 2nd most common mutation in this study was, also, the 2nd most common mutation in southern Iran [16] and the predominant mutation in Syria, Jordan, Turkey and in Saudi Arabia [5,6,7,9].

It is the most common cause of beta-thalassemia in Mediterranean countries, especially eastern Mediterranean region, but reaches lower frequencies in countries around the Arabian Gulf. [14].

The cod 44(-C) (frameshift mutation) is a mutation of Kurdish origin, and it was the 3rd most common mutation in this study; it was of higher frequency in Iraq than in countries around Iraq; a finding similar to that obtained by in the Duhok governorate and although this mutation was considered as a Kurdish mutation with some authors having hypothesized that this mutation arose in the Duhok region ,the mutation is relatively of high frequency in Saudi Arabia; this being explained by the assumption that this mutation arose independently in these two regions[11,12,14]..

The IVS 2.745 (C > G) (mutant creating new splice site) is the fourth most common mutation in this study; it is a Mediterranean mutation, and it was detected in Mediterranean Arab Countries and found at its highest frequency in north Jordan (12%) [14].The cod 5(-CT) (frameshift mutation) is a Mediterranean mutation, and it was found in all Arab Mediterranean Countries except Algeria [14].

The IVS 1.6 (T > C) (consensus change mutation) present with a relatively high frequency in most Mediterranean Arab Countries. Typically the clinical course of patients with this mutation is mild in nature [14]. The IVS 1.5 (G > C) (consensus change mutation) occur in the Mediterranean region. It is the most common mutation in United Arab Emirate (55%) and Oman (62%) and is quite frequent in neighboring Kuwait and Saudi Arabia [14]. The IVS 1-25 (25 bpDEL) (splice junction mutation) was found in Iran and in Arab counties around Iraq, in low frequencies, but reaches its highest frequency in countries of the Arab Peninsula, especially in Bahrain where it reaches 36% [14]. The mutation cod 8 (-AA) (frameshift mutation) is of low frequency in countries around Iraq and in Arab countries except Saudi Arabia, where it reaches 10 %(14).

The mutation cod 39 (C >T) (nonsense mutation) has the highest frequencies in Western Arab Countries such as Tunisia, Algeria and Morocco [14, 18, 19].

The cod 8/9 (+G) (frameshift mutation) was found in all counties around Iraq (except Jordan), in low frequencies, but reaches its highest frequency in countries of the Arab Peninsula [14].

These similatities can be explained by: *Firstly*: The patients included in this study were of Arab origin and the majority of people in Syria, Jordan, Saudi Arabia, and Kuwait are of Arab origin.
Secondly: In the past and before Christ (B.C.) many tribes migrated from Arab peninsulua to various regions of the Midlle East including Iraq.
Thirdly: The introduction of Islam and development of the Umawia and Abbaside National States made Iraq and its surrounding countries as a one state. All

these events have played a role to continous intermarriages and admixtures of the Middle Eastern people which led to a degree of similarity in the mutations between Iraq and its neigbouring countries. While the differences in β-thalassemia mutations between patients in this study and patients in the surrounding countries are:

Firstly: the mutant IVS 1.1(G→A) was recorded in most countries around Iraq with various frequencies [7,11,14] but it was not detected in patients in this study, although the mutation was included in the panel of the mutations that should be detected by the kit used. However, the small sample may have played a role in such a result.

Secondly, the mutant cod 44(-C) was of higher frequency in Baghdad (according to this study) than in countries around Iraq; a finding similar to that obtained by (Al-Allawi et al) in the Duhok governorate. In comparing the results of this study with that of (Al-Allawi et al) which was carried out on thalassemic patients in Dohuk region, there were significant difference ($p< 0.05$) in the distribution of mutation between Baghdad and Duhok; these differences are expected as the people of Duhok are of Kurd origin and their contact is more with Turkish Kurd than with Arab people in Baghdad-table-4.

Table 4: shows the comparison of mutation distribution between Baghdad and Duhok governorates.

The mutation	No. & % of alleles in Baghdad	No. & % of alleles in Dohuk
IVS 2.1 (G>A)	**18**(29%)	**19**(18.3%)
IVS 1.110 (G>A)	**11**(17.7%)	**2**(1.9%)
code 44 (-C)	**9**(14.5%)	**13**(12.5%)
IVS 2.745 (C>G)	**7**(11.3%)	-
code 5 (-CT)	**6**(9.7%)	**11**(10.6%)
IVS 1.6 (T>C)	**3**(4.8%)	**9**(8.7%)
IVS 1.5 (G>C)	**2**(3.2%)	**7**(6.7%)
code 8 (-AA)	**2**(3.2%)	**3**(2.9%)
IVS 1-25 (25bpDEL)	**2**(3.2%)	-
code 39 (C>T)	**1**(1.6%)	**9**(8.7%)
code 8/9 (+G)	**1**(1.6%)	**8**(7.7%)
Undetermined	-	**12**(11.5%)
IVS 1.1 (G>A)	-	**9**(8.7%)
cod 22 (-7bp)	-	**1**(1%)
Cod 30 (G>C)	-	**1**(1%)
Total	**62**	**104**

The most common mutations encountered in this study are of severe type (β^o); the mutations IVS 2.1 (G>A), cod 44 (-C), cod 5 (-CT), cod 39 (C>T), cod 8 (-AA), IVS 1-25 (25 bpDEL) and cod 8/9 (+G) are associated with β^o-thalassemia (13) and these constitute 62.87% of allele characterized– table 3; this explain why most of the studied patients present as thalassemic patients in the first year of life.

Consanguineous marriages: The results of this study showed that in most of the families (75.86%) the parents have consanguineous relation, and this explain the high incidence (80.64%) of homozygosis in the studied group; these results are similar to many studies carried out in the surrounding countries(6, 8, 9, 20).

This study demonstrated another problem, which is the continuous birth of affected children in the same family; the results of this study showed that 38.7% of families in the studied group have more than one affected sibling and 11.3% have 3 affected siblings, so initiation of a preventive program for beta-thalassemia is a corner stone in the management of this disease [2, 12, 21, 22]

So characterization of the common mutations carried out in this study will provide a sound foundation on which to base a preventive program for thalassemia, and, also, these findings will facilitate the improvement of medical services such as carrier screening, genetic counseling and prenatal diagnosis.

Conclusions

1- Eleven β-globin alleles causing β-thalassemia syndrome in Baghdad were characterized; most of these mutations are of Mediterranean type and few are of Kurdish and Asian Indian types.
The frequency of these mutations was as follow: IVS 2.1 (G→A) , IVS 1.110 (G→A) , cod 44 (-C), IVS 2.745 (C→G) , cod 5 (-CT) , IVS 1.6 (T→C), IVS 1.5 (G→C) , IVS 1-25 (25 bpDEL) , cod 8 (-AA), cod 39 (C→T) and cod 8/9 (+G).

2- Most of the patients are homozygous, and of severe (β^o) Thalassemia, and showed consanguineous relation between their parents

3- More than 1/3 of the families have more than one affected sibling.

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