Molecular Detection of Some Mutations Associated with Beta-Thalassaemia in Iraq

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Abstract

The study was carried out in period between "1 August 2005 to 30 of December 2006". Blood samples of 80 clinically thalassaemic patients were collected from three thalassaemia centers in Iraq, namely: Ibn Albalady central thalassaemia center in Baghdad, and also from Kerbalaa and Al-Qadissya governorates. Blood samples were also collected from 56 apparently healthy individuals to serve as a control group.

DNA was isolated from blood and used for molecular detection of seven types of β-thalassaemia mutations (IVS1 nt.1 G--A , IVS1nt.5 G--C, IVS1 nt.6 T--C, IVS1 nt.110 G --A, codon 39 C--T, IVS2 nt.1G--A, and IVS2 nt.745 C--G) using the PCR based technique called ARMS (Amplification Refractory Mutation System).

Five out of seven of these diagnosed mutations were reported for the first time in Iraq, and the most frequent β-thalassaemia mutations were codon 39 and IVS1 nt.110 with the proportions (26.76%) and (20.34%), respectively. No IVS2nt.745 was detected within the studied samples.

Genotypic distribution of the samples indicated that there is no significant difference ($p > 0.05$) between the frequency of homozygotes and heterozygotes within patient group, while there is a significant difference at ($P < 0.01$) in comparison with the control group.

The study of association between the number and the types of mutations revealed that 28(58%) of positive cases have single mutation in a homozygous state or heterozygous state which significantly associated at ($P < 0.05$) with β-thalassaemia mutations, whereas 20(42%) of these cases have compound mutations. The most frequent association appeared between IVS1 nt.110 and Codon 39 mutations.

Finally, mutations within families, pointed to a positive correlation between the types of mutations in sons or daughters and their fathers and/or mothers; this indicates the accuracy of the ARMS technique in detection of β-thalassaemia mutations. This conclusion should be taken with caution due to the limited number of families.

Key words: Beta-thalassaemia, mutation, ARMS-PCR, Iraq.

الخلاصة

أجريت الدراسة في الفترة ما بين شهري أب 2005 م و كانون الأول 2006 م. جمعت عينات الدم من 80 مصاب سريريا بالثلاسيميا – بيتا من ثلاثة مراكز ثلاسيميا في العراق هي (مستشفى ابن البلدي في بغداد, مستشفى الأطفال في كربلاء و مستشفى الأطفال في الديوانية), و كذلك من 56 شخص من الأصحاء ظاهريا. استخلص الحامض النووي (DNA) من جميع العينات, و اجري التشخيص الجزيئي لسبعة أنواع من الطفرات الوراثية المسببة للثلاسيميا– بيتا و هي:
IVS2 nt.745, IVS2 nt.1, codon 39, IVS1 nt.110, IVS1 nt.6, IVS1nt.5, IVS1 nt.1 بأستعمال تقنية الـ(ARMS).

بينت الدراسة أن خمسة من الطفرات المشخصة قد سجلت لأول مرة في العراق وأن أكثر نسبة للطفرات المسببة للثلاسيميا– بيتا كانت للطفرتين (codon 39) و(IVS1 nt.110) بنسبة (26.76 %) و (20.34%) على التوالي. و كشفت الدراسة أيضا ان الطفرة (IVS2 nt.745) لم يتم تشخيصها ضمن العينات المدروسة.

أظهر التوزيع الوراثي للعينات عدم وجود فرق إحصائي مهم ($P > 0.05$) بين الحالات المتجانسة الزيجة (Homozygous) و المتغايرة الزيجة (Heterozygous) ضمن مجموعة المرضى بينما كان هناك فرقا إحصائيا معنويا ($P< 0.01$) عند مقارنتها مع مجموعة السيطرة.

إن دراسة الترافق بين عدد ونوع الطفرات, أظهرت أن 28 (58%) من الحالات الموجبة كان لها طفرات مفردة بشكل متجانسة الزيجة (Homozygous) لكلا الأليلين أمتغايرة الزيجة (Heterozygous) لأليل واحد, في حين أن 20 (42%) كانت ذات طفرات مركبة (Compound), وأن أكثر ترافق بين أنواع الطفرات في الحالات المركبة لوحظ بين الطفرتين codon 39 و IVS1not. 110.

و أخيرا تمت دراسة الطفرات الوراثية ضمن العوائل المشمولة بالدراسة حيث أظهرت النتائج وجود تلازم ايجابي بين أنواع الطفرات الموجودة عند الأبناء أو البنات و بين ما هو موجود عند إبائهم و/ أو أمهاتهم و هذا يدل على دقة هذه التقنية (ARMS) في الكشف عن هذه الطفرات.

Introduction

Beta (β)-thalassaemia, one of the most widespread genetic disease in the word, is an autosomal recessive disorder caused by mutations in the β -globin gene which is located as a cluster on the short arm of chromosome 11[1]. It is characterized by microcytosis and hemolytic anaemia which result from a variety of molecular defects that intervene with the normal synthesis of the β -globin chains of haemoglobin.[2, 3]

Recently, more than 200 different mutations have been detected affecting the diverse levels of β -globin gene expression and cause β -thalassaemia.[1,4] These mutations are not uniformly distributed, but have a geographical specificity and racial origin, as each is characterized by the presence of few common mutations and variable numbers of rare ones.[5,6,7,8]

Arab people share common history and culture; originally, they are quite heterogeneous, a fact that should be reflected in their genetic background.[9] β -thalassaemia is endemic in all Arab world, this probably may due to the presence of malaria previously in that region. The frequency of carriers varies from 1% to 5%.[9, 10]

The molecular basis of β-thalassaemia was studied in various Arab countries.[11,12, 13,14,15,16,17,18,19]

In Iraq, at molecular level, there is an only one study that deal with the molecular identification of 2 types of β-thalassaemia mutations.[20]

There for the present study aims to:

1- Molecular detection of the most common types of mutation associated with β-thalassaemia among Iraqi population.

2- Build a data base for β-thalassaemia mutations types in Iraq and other neighboring countries.

3- Provide a molecular detection tool for β-thalassaemia mutations and a molecular base for prenatal and premarital genetic diagnosis

that have a great value for preventive programs which could be part of general scheme to increase awareness on the importance of counseling among many other preventive measures of genetic diseases.

Materials and Methods

Patients group:

Eighty random β-thalassaemic patients from different Iraqi populations were taken from three thalassaemic centers in Iraq are:

1 Ibn Al-Baladi pediatrics hospital (in Baghdad).
2 Karbalaa pediatrics hospital (in Karbalaa).
3 Al-Qadissya pediatrics hospital (in Al-Qadissya)

Control group:

Fifty six of apparently healthy individuals from different Iraqi (Arab,Kord, and Torkoman) populations were randomly included in the study.

Blood Sampling:

Two ml of blood was collected by vein puncture from all patients to cover the two main β-thalassaemia phenotypes (Major and Intermedia) according to the clinical and haematological physician diagnosis, and also collected from 11 patients' fathers and/or mothers for thalassaemia minor group. Blood was also collected from apparently healthy individuals as a control group.

The collected blood samples in EDTA anticoagulant tubes were divided into two aliquot: the first aliquot was transmitted directly for haematological examination, and the second aliquot was transmitted within 2-24 hours using cooling container for DNA extraction and molecular analysis.

Isolation of genomic DNA:

The genomic DNA isolated from the whole fresh blood collected in EDTA anticoagulant tubes for molecular studies was applied using Wizard genomic DNA purification kits using salting out method.[21]

ARMS Analysis:

ARMS (Amplification Refractory Mutation System) method was adopted to characterize mutations of the DNA samples in the medical genetic laboratory in National Institute of Genetic Engineering & Biotechnology (NIGEB) in Tehran, Islamic Republic of Iran.

Mutation Selection:

According to the common distribution of β-thalassaemia mutations in the neighboring countries[9,10,13,22,23], seven mutations were chosen for molecular diagnosis in Iraq. The types of mutations and the number of examined samples for each mutation according to the phenotype of patient and control groups are shown in table (1).

Table 1: Type of mutations and the number of examined samples for each mutation according to the phenotypes of β–thalassaemia

	Phenotype				
Mutation (substitution)	Major	Intermedia	Minor	Control	Total
IVS1nt.1 (G—A)	31	12	11	37	91
IVS1nt.5 (G—C)	18	6	8	16	48
IVS1nt.6 (T—C)	16	7	6	11	40
IVS1nt.110 (G—A)	35	13	11	45	104
Codon 39 (C—T)	46	14	11	29	100
IVS2nt.1 (G—A)	23	10	10	21	64
IVS2nt.745 C—G)	11	6	5	10	32
Total	180	68	62	169	479

Primers Selection and ARMS-PCR Programmes:

Primer sets and PCR programmes selected for ARMS analysis were previously

described by [4, 22, 24].

ARMS-PCR Protocol:

For each mutation, out of the 7 types of β-thalassaemia samples were tested according to phenotypes of the disease for both patient and control groups using a specific ARMS primer set for each mutation. Two ARMS-PCR reactions (two tubes) were performed for each sample: one for identifying the presence of the normal allele (using the normal primer) and the other for the presence of mutant allele (using the mutant primer) [4, 24]. The molecular analyses of the samples were repeated to cover the seven types of β-thalassaemia mutations.

ARMS-PCR analysis:

ARMS-PCR technique was used for molecular screening the seven studied types of β-thalassaemia mutations using a specific set of primers for each mutation and a set of internal control primers. The ARMS-PCR products and the ladder marker were resolved by electrophoresis. 3 µl of loading blue dye (15% ficoll, 0.05% bromophenol blue) plus 10 µl of the product were mixed and loaded on 2 % agarose gel (2g agarose/100 ml 0.5X TBE buffer) and run at 100 volt for approximately one hour. The gel was stained with ethidium bromide solution (0.5 µg/ml) for 15-30 minutes; In addition, bands were visualized on UV transiluminator.[4,24]

Biostatistical Analysis:

Percentages, frequencies, means, standard deviation were calculated. The Chi-square was adopted to test for difference and association significance. The Ratio of the odds was taken to represent the Relative Risk (RR) of the different mutations. All statistical analysis was carried according to Bland 2003[25].

ARMS-PCR analysis and screening:

In all successful ARMS-PCR reactions, the internal control product of 861 bp molecular weight was observed, this is considered as a mandatory sign of successful reaction on gel electrophoresis. The band was located between 800 bp and 900 bp on 100 bp ladder marker. Also, the ARMS-PCR products of suspected size of normal, heterozygous and /or homozygous cases for each type of the studied mutations were observed. For normal cases, the ARMS-PCR products were found within the normal primer reactions. In positively diagnosed heterozygous patients, the ARMS-PCR products were found within both normal and mutant reactions but only within mutant primer reactions in homozygous cases. For example the ARMS-PCR products (419 bp) of IVS1nt.110 are observed as shown in figure (1), ARMS-PCR products of IVS1 nt.110 mutation turn on a 2% agarose gel. Lane 1: A DNA marker (100 bp). All samples contains an internal control band (861 bp). Sample 1: contains an amplified product in the normal (N) but lacks it in the mutant (M) primer; hence, implying a healthy individual. Sample 2: contains an amplified product in both normal (N) and mutant (M) primers; assigning the individual to heterozygous genotype. Sample 3: contain an amplified product in the mutant (M) primers indicating to the homozygous genotype.

This approach proved to be useful for the mutation screening of β-thalassaemia alleles in the present study for the advantages of high detection rate and sensitivity and the use of a simple methodology based on a non-radioactive detection method.[4]

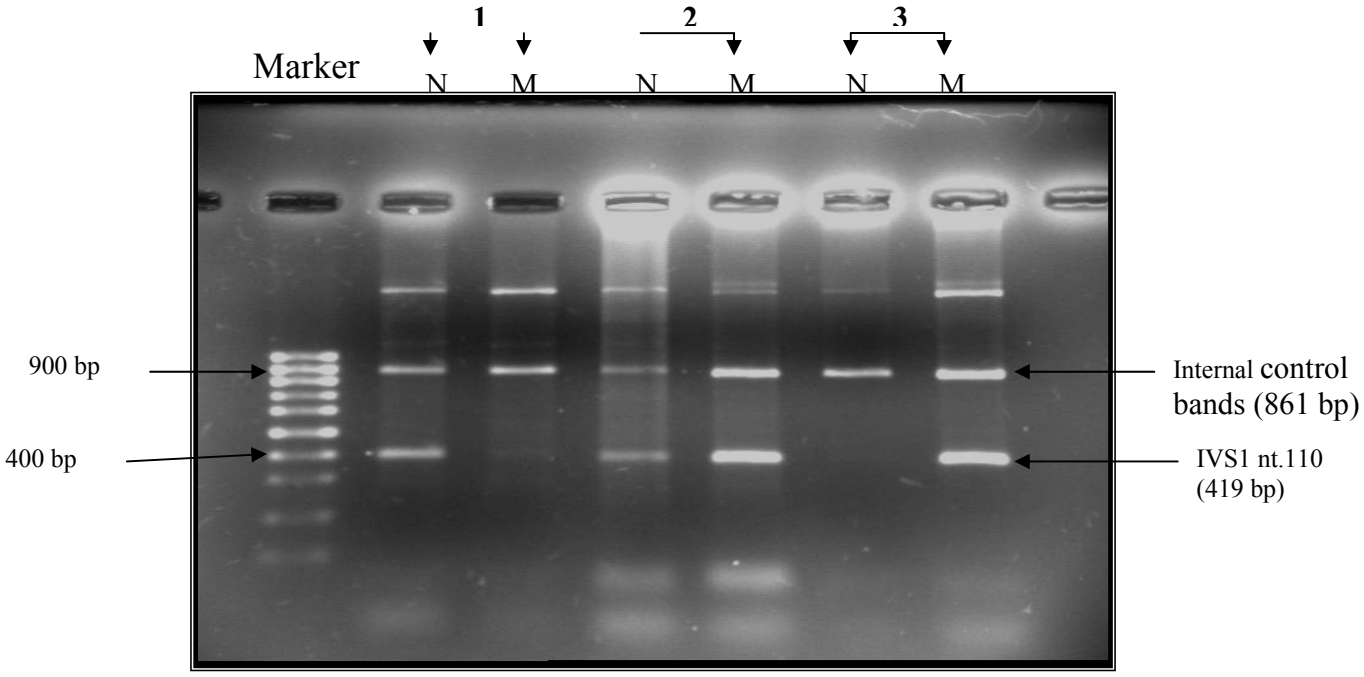

Figure 1: ARMS-PCR products of IVS1nt.110 β-thalassaemia mutation on 2% agarose gel at 100 voltages for one hour.

Molecular diagnosis of β-thalassaemia mutations:

The results of molecular diagnosis of the seven studied types of β-thalassaemia mutations are shown in table (2). The number and percentages of the studied types of mutations in patients group were 19(26.76%) for Codon 39 and 12 (20.34%) for IVS1 nt.110, followed by 6 (18.75%) of IVS1 nt.5; 4 (13.79) IVS1 nt.6; 5 (9.26%) IVS1 nt.1 and 2 (4.65%) IVS2 nt.1; no IVS2 nt.745 was detected in any of the examined samples (Figure 2).

The results of the control group, showed that 2(6.9%) of studied apparently healthy cases were positive for codon 39 and 1(2.7%) for IVS1 nt.1.

The Chi-square revealed that there is a significant difference at ($P<0.01$) in frequencies of β-thalassaemia mutations between patients and control groups.

The Chi-square indicated a highly significant difference in the frequency of different mutations within patient group and the frequency of the same mutations between patients and control groups (chi-square with 18 degrees of freedom =31.65; $P<0.001$). IVS1nt1 was less than expected within patient group. The major contribution to the Chi-square value came from Codon 39, where the observed value was 19 while the expected is only 10 within the patient group and the control group to a lesser extent. IVS2nt 745 observed was also less than the expected within the patient group.

The overall picture indicated that the association of Codon 39 is the most prominent feature of association followed by IVS1nt.5 and 110. These results reflect the point which indicates that the most common three types of β-thalassaemia mutations in different Iraqi population were codon 39, IVS1 nt.110 and IVS1 nt.5. In previous results (20), at the molecular level, of 40 Iraqi patients showed that the frequency of IVS1 nt.110 was (25%), IVS1 nt.1 was (5%); no mutation was detected in IVS1 nt.6 locus.[20]

Using the same information the Relative Risk (RR) of the mutations was calculated and presented in table (3) for the patient and control and for positive and negative within the patient group only; When RR was calculated for patient and control, IVS1nt.110 was the highest (36) followed by IVS1nt.5 (RR=18) and IVS1nt.6 (RR=12). The relative risk of IVS1nt.1 and Codon 39 was very low (0.31 and 0.39 respectively).
This means that the presence of 110, 5 and 6 was very decisive in the existence of thalassaemia.

When the RR was calculated for +/- thalassaemia within patient group, the highest was found for Codon 39 (1.99), 110 (1.39) and 5 (1.27). The difference in the size of RR calculated both ways might justify considering the RR of +/- within patients especially the results of which goes in line with the previously obtained results that the most effective mutations are those of 110, 5 and codon 39. Other mutations though frequent, the RR of their presence is small.

Comparison with the frequencies of several mutations of those in some neighboring countries, showed that codon 39 and IVS1 nt.110 emerged as the most common mutations in Syria, Jordan, Lebanon, Bahrain, Kuwait, Saudi Arabia and some African countries like Tunisia and Algeria.[9,19,22]

The most widespread and common mutations are presumably the oldest one. This is true for Codon 39 and IVSI-110.The nonsense Codon 39(C--T) mutation, which is believed to be Roman in origin, is most frequent in the Western Mediterranean Arab countries and decreases in its frequency towards the East. However, this mutation also reaches a high frequency in some countries of the Arabian Peninsula, a fact that may be explained by gene flow and founder effect. Moreover, IVSI-110 (G--A), which is believed to have arisen in Turkey, reaches its highest frequencies in the Eastern Mediterranean Arab countries, and may probably have been introduced to other countries by many of settlers from the East, including Turkish, Greeks, or Phoenicians. This mutation is known to be a typical eastern defect, and the relative frequency of IVSInt.110 decreases rapidly along the northeast to southwest axis and reaching its maximum among Turkish Cypriots (72.19%) and Greek Cypriots (79.86%).[4,9,24,26]

Altay, 2002[27] reported that, in Mediterranean and Middle Eastern countries, four mutations {Codon 39(C--T), IVSI-110(G--A),IVS1nt.6 (T--C), and IVS1nt.1 (G--A)} which make up 75% of the total β-thalassaemia determinant mutations, and the regional differences in the frequency of various mutations may give some clues regarding the migration patterns and the ethnic background about the studied population.

Table 2: Molecular diagnosis of β- thalassaemia mutations in Iraq

Mutation	Patient				Control			
	Thalassemic (+)		Normal (-)		Thalassemic (+)		Normal (-)	
	No.	%	No.	%	No.	%	No.	%
IVS1nt.1	5	9.26	49	90.74	1	2.7	36	97.3
IVS1nt.5	6	18.75	26	81.25	0	0	16	100
IVS1nt.6	4	13.79	25	86.21	0	0	11	100
IVS1nt.110	12	20.34	47	79.66	0	0	45	100
Codon 39	19	26.76	52	73.24	2	6.9	27	93.1
IVS2nt.1	2	4.65	41	95.35	0	0	21	100
IVS2nt.745	0	0	22	100	0	0	10	100
Total	48	15.48	262	84.52	3	1.78	166	98.2

Table 3: Relative Risk of mutations for Patient/control and within patients

Mutation	Patient/control	+/- within patients
IVS1nt.1	0.31	0.56
IVS1nt.5	18	1.27
IVS1nt.6	12	0.90
IVS1nt.110	36	1.39
Codon 39	0.59	1.99
IVS2nt.1	6	0.27
IVS2nt.745	0.0	0.0

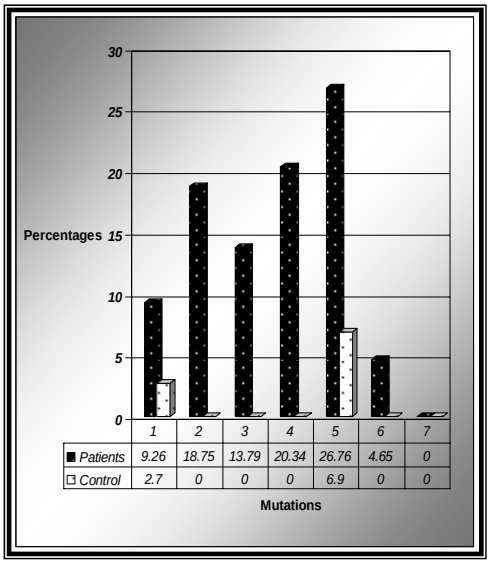

Figure 2: Frequencies of β-thalassaemia mutations in Iraq.
(1=IVS1 nt.1, 2= IVS1 nt. 5, 3= IVS1 nt.6, 4= IVS1 nt.110, 5= Codon 39, 6= IVS2 nt.1 and 7= IVS2 nt. 745).

Genotypic distribution of the samples:

For all studied mutations, the distribution of the samples according to the genotypes was given in table (4). The results has revealed that out of the total number of patients genotypes, 23(7.4%) homozygous and 25 (8.1%) were heterozygous for a single or compound different mutations. While only 3 (1.78%) out of 16 control were heterozygous or carrier cases. The Chi-square reflected no significant differences at ($P> 0.05$) between homozygosity and heterozygosity within patient group. In contrast, there was a significant difference at ($P< 0.01$) in genotypic frequencies of patients and control group. This might indicate that the homozygosity and heterozygosity of β-thalassaemia mutations under study can be equally distributed within thalassaemic patients.

When the presence of thalassaemia (Homozygous and heterozygous combined) and compared to the normal genotype for all mutations studied, a highly significant difference was noticed with a chi-square value of 16.8 for 6 degrees of freedom ($P<0.01$). The Observed and expected values were very close for IVS1 nt.1, IVS1 nt.5, IVS1 nt.6 and IVS1 nt.110; the observed genotype for codon 39 was much higher than the expected while it was much lower for IVS2 nt.1 and IVS2 nt.745.

The Chi square was also used to test for any significant difference/association between any mutation and genotype of patients.

There was double than expected homozygote and much lower heterozygotes. For IVS1 nt.6 and IVS1 nt.110 both heterozygotes and homozygotes are less than expected. The heterozygotes IVS1 nt.110 was higher than expected. For codon 39, there was less than expected normal and higher than expected homzygotes and heterozygotes. For IVS2 nt.1, both homo and heterozygotes are less than expected.

The total chi square value for the above comparisons was 20.25 for 12 degrees of freedom with a P value of just over 5%. Many cells within the chi-square are less than 1; results should be taken with caution.

In Tunisian population, homozygosity was observed for most frequent β-thalassaemia mutations as well as for rare ones; the finding suggests that the high rate of consanguinity.[19]

Table 4: Distribution of the samples according to the mutations and genotype

		Genotype of Patients						Genotype of Control					
		Normal		Homozygous		Heterozygous		Normal		Homozygous		Heterozygous	
Mutation	*N	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
IVS1nt.1	182	49	90.7	2	3.7	3	5.6	36	97.3	0	0	1	2.7
IVS1nt.5	96	26	81.2	3	9.4	3	9.4	16	100	0	0	0	0
IVS1nt.6	80	25	86.2	3	10.4	1	3.45	11	100	0	0	0	0
IVS1nt.110	208	47	79.7	5	8.47	7	11.9	45	100	0	0	0	0
Codon39	200	52	73.2	8	11.3	11	15.5	27	93.1	0	0	2	6.9
IVS2nt.1	128	41	95.4	2	4.65	0	0	21	100	0	0	0	0
IVS2nt.745	64	22	100	0	0	0	0	10	100	0	0	0	0
Total	958	262	84.5	23	7.4	25	8.1	166	98.2	0	0	3	1.78

* Number of examined chromosomes.

Phenotype - mutations association:

The association between phenotypes of β-thalassaemia and examined types of mutations in patient and control groups were studied, the results (table 5) showed that 26(51%) of positive diagnosed cases having major phenotypes, 15(29.4%) were intermedia and 7(13.7%) were minor phenotypes, while 3(5.9%) cases represented to control group.

The association between mutations and the phenotypes of thalassaemia: major, minor and intermediate was examined using the chi-square and comparison between observed and expected frequencies on the null-hypothesis revealed no significance ($P>0.05$). Testing between major, intermediate and minor for all total patient groups showed a highly significant association ($P < 0.01$). It is well established, and commonly observed, that patients with identical β-globin genotypes may have radically different clinical phenotypes, ranging from severe transfusion-dependant β-thalassaemia major to mild forms of thalassaemia intermedia. This heterogeneity has been the subject of intense study over the past twenty years although still not fully understood.[1, 28]

Table 5. Association between phenotypes and examined mutations in patient and control groups

phenotype			Mutations														Total	
			IVS1 nt.1		IVS1 nt.5		IVS1 nt.6		IVS1 Nt.110		Codon 39		IVS2 nt.1		IVS2 nt.745			
			+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Patients	Major	No	3	28	3	15	1	15	6	29	12	34	1	22	0	11	26	154
		%	50	32.9	50	35.7	25	41.7	50	32	57	43	50	35.5	0	34.4	51	36
	Intermedia	No	2	10	1	5	2	5	5	8	4	10	1	9	0	6	15	53
		%	33.3	11.8	16.7	11.9	50	13.9	41.7	9	19	13	50	14.5	0	18.8	29.4	12.4
	Minor	No	0	11	2	6	1	5	1	10	3	8	0	10	0	5	7	55
		%	0	12.9	33.3	14.3	25	13.9	8.3	11	14	10	0	16	0	15.6	13.7	12.9
Control		No	1	36	0	16	0	11	0	45	2	27	0	21	0	10	3	166
		%	16.7	42.4	0	38.1	0	30.5	0	49	10	34	0	33.9	0	31.2	5.9	38.8
Total		No	6	85	6	42	4	36	12	92	21	79	2	62	0	32	51	428
		%	6.6	93.4	12.5	87.5	10	90	11.5	88.5	21	79	3.1	96.9	0	32	10.6	89.4

Association between the mutation numbers and mutation types:

Table (6) shows the number and the types of mutations for positively diagnosed cases in patient group. There are 28(58%) cases of single mutation and 20(42%) cases have compound mutations. The difference was not significant (P>0.05). This indicates that there is no association between the mutation number and type.

The presence of single mutation as a homozygous condition (two alleles) or two different (compound) mutations as a heterozygous condition is sufficient for the induction of β-thalassaemia; the signs and severity of the disease depend on the phenotypic characteristics of the causative mutations and the clinical picture resulting from homozygosity of β^+ and β^o - thalassaemia which may be ameliorated by coinheritance of mutations in the gene encoding the α-globin chain associated with α-thalassemia , on the other hand, in the homozygous or double heterozygous state, β-thalassaemia can be lethal, especially in areas where health conditions are poor.[18,29]

Table 6: Association between mutation number and mutation type in positively diagnosed patients

Mutation No.	Mutation types														Total	
	IVS1 nt.1		IVS1 nt.5		IVS1 nt.6		IVS1 nt.110		Codon 39		IVS2 nt.1		IVS2 nt.745			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Single	4	80	4	66.7	2	50	6	50	10	52.6	2	100	0	0	28	58
Compound	1	20	2	33.3	2	50	6	50	9	47.4	0	0	0	0	20	42
Total	5		6		4		12		19		2		0		48	

Table (7) shows the genotypes of β-thalassaemia mutations in compound samples. The results revealed that 9 cases have compound mutations, 7 (77.85%) of these cases have two mutations while 2(22.15%) have three mutations. The more frequent genotypic association appeared in 6 cases (66.7%) between codon 39 and IVS1 nt.110; both being the most frequent mutations in Iraq.

The clinical expression of compound heterozygosity for β-thalassaemia gene varies and ranges between a severe transfusion dependent thalassaemia major and a mild anaemia resembling thalassaemia minor.

In contrast, compound heterozygosity for mild/silent β^+ and sever mutations produce a variable phenotypes, ranging from thalassaemia intermedia to thalassaemia major; therefore, the presence of this genotype does not predict a mild phenotype. This finding reflects the phenotypic complexity of compound mutations associated with β-thalassaemia.[29,30]

Table 7: Genotypes of β -thalassaemia mutations in compound samples

No.	First mutation	Second mutation	Third mutation
1	N/IVS1nt.110	N/Codon 39	-
2	N/IVS1nt.5	N/Codon 39	-
3	N/IVS1nt.1	N/Codon 39	-
4	N/IVS1nt.6	N/IVS1nt.110	N/Codon 39
5	N/IVS1nt.110	N/Codon 39	-
6	N/IVS1nt.6	N/IVS1nt.110	N/Codon 39
7	N/IVS1nt.110	N/Codon 39	-
8	N/IVS1nt.5	N/Codon 39	-
9	IVS1nt.110/IVS1nt.110	N/Codon 39	-

Mutations within families:

Blood samples were taken from some patients and their fathers and /or mothers for molecular identification of β-thalassaemia mutations in order to study the flow of mutations within families. The genotypes of β-thalassaemia mutations are shown in table (8). The results revealed that there was a positive correlation between the type of mutation in son or daughter and/or his /her parents in families number 2 to 6; this might be due to the autosomal recessive inheritance patterns of β-thalassaemia mutations where the parents of an affected child are obligate heterozygotes, therefore, carry a single copy of a disease – causing β-globin gene mutation.

These results also reflect the accuracy of ARMS technique in molecular detection of β-thalassaemia mutations.

As for the families number 7,8 and 9, the unknown (un diagnosed) mutations appeared, this may indicate that the other types of β-thalassaemia mutations are found, However, further studies for a new mutations are needed to get a complete picture of molecular basis of β-thalassaemia mutations in Iraq. These findings should be taken with caution due to the limited number of families.

Table 8. Genotype of β-thalassaemia mutations within families

No. of Family	Father	Mother	Son or daughter
1	-	N/cd39	IVS1nt.110
2	N/cd39	N/cd39	cd39/cd39
3	-	N/IVS1nt.5	cd39/IVS1nt.5
4	-	N/IVS1nt.5	IVS1nt.5/IVS1nt.5
5	N/IVS1nt.110	-	IVS1nt.110/IVS1nt.110
6	N/IVS1nt.6	-	IVS1nt.6/IVS1nt.6
7	unknown	-	unknown
8	unknown	-	unknown
9	unknown	Unknown	unknown

Conclusions

The following conclusions are drawn:

1- Beta – thalassaemia is a major public health problem in Iraq. Geographically it is extremely heterogeneous among the population.
2- Seven different β-thalassaemia mutations have been identified in the Iraqi population. Codon 39 (C--T) and IVS 1nt.110 (G--A) are the most frequent β-thalassaemia mutations with proportions (26.76%) and (20.34%), respectively.
3- Five out of seven of the observed β-thalassaemia mutations are recorded for the first time in Iraq.
4- All of the existing mutations are the result of a sever β^{+} or β^{o} - thalassaemia phenotypes. These findings explain the genetic biodiversity (phenotype – genotype correlation) of β-thalassaemia.
5- ARMS provide a valuable molecular tool for β- thalassaemia diagnosis in Iraq.

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