The Detection of the most Frequent β-thalassemia Mutation in Kirkuk Ethnic Population by β – Globin Strip Assay MED and Reverse Hybridization

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ABSTRACT

Background: There are about 941 beta-thalassemia patients in Kirkuk city, around 362 major thalassemia cases (186 females and 176 males), 257 minor cases (119 males and 138 female) , and 322intermedia cases (142 males and 180 females), and there is an increase in number. This study aims to detect the frequent mutations in this city, and there has been no other study done before. At the same time, our objective is to determine the possible number of thalassemic patients in the following years. Additionally, decrease the number of consanguinity marriages in the families at risk. Methods: A total of 50 patients with Beta-thalassemia were screened for 22 B- globin mutations: -101 [C > T] – 87 [C > G], -30 [T > A] , codon 5 [-CT] , codon 6 [G > A] HbC , codon 6 [A > T] HbS , codon 6 [-A] , codon 8 [-AA] , codon 8/9 [+ G] , codon 15 [TGG > TGA] , codon 27 [G > T] Knossos , IVS1.1[G > A], IVS 1.5 [G> C], IVS 1.6 [T > C], IVS 2.1 [G > A] , IVS 2.745 [C > G] , IVS 2.848 [C > A]. 97.5 % characterized and 2.5 % are uncharacterized cases were further evaluated Results: the most frequent mutation was: codon 8 [- AA] (21.25%), IVS2.1 [G > A] (20%), IVS1.110 [T > G] (16.25%), codon 8/9 [+ G] (12.5%), IVS 1.5 [G > C] (7.5%). Conclusion: β–globin strip assay MED (Vienna lab, Vienna, and Austria), covering > 90% of β–globin defects, and the results are accurate, fast, and easy to detect mutations.

Keywords: β-thalassemia mutation, Kirkuk, β-globin strip assay MED, reverse hybridization.

1. Introduction

The most frequent single-gene disorder is β-thalassemia. The majority of thalassemias are inherited as a mendelian recessive trait. The reason is that they are so widespread, and severe anemia is induced in both heterozygous compound and homozygous states, β-thalassemias are the most important types of thalassemia.

Thalassemias are structurally normal; however, imbalanced globin chain production is recorded as thalassemia. For example, it is a quantitative reduction in output of the gen altering the amino acid sequence of protein production [1]. β-thalassemia is a genetic condition in which the production of structurally normal β-globin chains is decreased while the synthesis of α-chains is unaffected. Because the underlying mutations are heterogeneous, the clinical severity of the anemia varies.

The basic defect in β-thalassemia is imbalanced globin chain synthesis. The excess α-chains produced in β-thalassemia are highly unstable, and they quickly precipitate and bind to the membranes of red cell precursors and red cells.

Producing inclusion bodies that cause oxidative membrane damage when linked to the membrane skeleton and Extensive premature destruction of red blood cell precursors in the bone marrow by apoptosis (ineffective erythropoiesis) [2].

β-thalassemia and disorders are caused by a variety of mutations. These mutations have the potential to impact every step of the globin gene expression pathway, including transcription, processing of the messenger ribonucleic acid (mRNA) precursor, translation of mature mRNA, and the chain's post-translational integrity. There have been almost 400 mutations identified [3].

2. Materials and Methods

Patients

The study randomly collected 50 patients in age categories ranging from (2.2 – 60). They are (32 females and 18 males), 35 of them were unrelated individuals, and 15 patients were related to studying the mutation's inheritance model in different families. The blood samples were collected from Azadi hospital thalassemia center between 21-11-2021 to 14-12-2021 with (minor, intermedia, and major) thalassemia patients from different ethnicities (Arab, Kurd, and Turk).

Major and intermedia cases are blood transfusion-dependent patients. Sampling occurs before the transfusion. To overestimate specific mutations frequency, one member of each family member was enrolled. The ethical agreement was taken from each patient During sampling. Informed consent was obtained from all subjects.

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The research council of college science at Kirkuk University (in Iraq) approved our study. 2 ml of blood was aspirated. Before sampling, the patients and their parents were asked to fill out a special questionnaire form during their periodical visit for clinical examination. A direct interview with patients and their parents has been adopted in filling the questionnaire. Later and before transfusion, about 2 ml of their blood samples were collected in a sterile EDTA tube. Clinically diagnosed beta-thalassemia (minor, intermedia, and major) patients, mutation analysis, was performed by the β–globin strip assay MED (Vienna lab, Vienna, and Austria).

3. METHODOLOGY

The procedure comprises these steps: 1) DNA isolation, 2) PCR amplification using biotinylated primers, and 3) Hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines (see Figure 1). Streptavidin-alkaline phosphatase and color substrates are used to detect the bound biotinylated sequences.

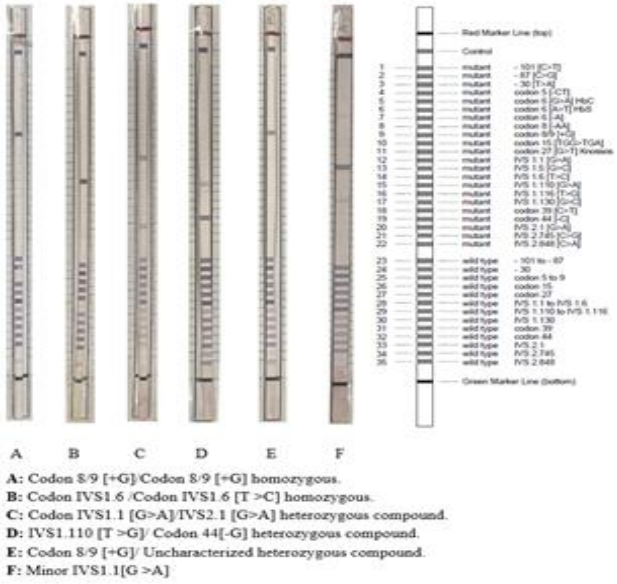

Fig. 1: Mutation detection on Strip assay for six of our patients.

4. RESULTS

The most noticeable mutant alleles diagnosed in studied patient were: Codon 8 [-AA] at 21.25%, codon 20 IVS2.1 [G >A] at 20%, IVS1.110 [T >G] at 16.25%, codon 8/9 [+ G] at 12.5 % , IVS1.5 [G > C] at 7.5 % , codon 44 [-C] at 2.5 %, codon 39 [C > T] at 2.5 % , codon 5 [-CT] at 2.5 % , codon 6 [A > T] at 2.5 %, IVS1.116 at 3.75 % , IVS1.1 [G > A] at 2.5 % , IVS1.6 [T > C] at 3.75 % and uncharacterized at 2.5% (see Table 1).

Table (1): Type of mutations, number of alleles, and their frequencies.

Mutation	Number of alleles	Mutation % ratio
Codon 8 [-AA]	17	21.25 %
Codon 20 IVS 2.1 [G>A]	16	20 %
IVS 1.110 [G>A]	13	16.25 %
Codon 8/9 [+G]	10	12.5 %
IVS 1.5 [G>C]	6	7.5 %
Codon 44 [-C]	2	2.5 %
Codon 39 [C>T]	2	2.5 %
Codon 5 [-CT]	2	2.5 %
Codon 6 [A>T]	2	2.5 %
IVS 1.116 [T>G]	3	3.75 %
IVS 1.1 [G>A]	2	2.5 %
IVS 1.6 [T>C]	3	3.75 %
Uncharacterized	2	2.5 %
	80	100 %

The most common genotype among tested individuals was as follows:
Codon8 [- A A] / IVS2.1 [G>A] in 9 cases (22.5%)
IVS1.110 [G > A] / IVS1.110 [G > A] in 5 cases (12.5 %).
Codon 8/9 [+ G] / codon 8/9 [+ G] in 4 cases (10%).
As shown below in Table 2.

Table (2): Genotype frequency in studied patients.

Genotype in 40 unrelated patients	Number ratio %	
(8+20) Codon 8 [-AA] / IVS 2.1 [G>A]	9 / 22.5 %	Characterized by Strip assay MED
(15+29) IVS 1.110 [G>A] / IVS 1.110 [G>A]	5 / 12.5 %	
(9+25) Codon 8/9 [+G] / Codon 8/9 [+G]	4 / 10 %	
(8+13) Codon 8 [-AA] / IVS 1.5 [G>C]	4 / 10 %	
(15+19) IVS 1.110 [G>A] / Codon 44 [-C]	2 / 5 %	
(8+25) Codon 8 [-AA] / Codon 8 [-AA]	1 / 2.5 %	
(4+6) Codon 5 [-CT] / Codon 6 [A >T] Hbs	2 / 5 %	
(12+20) Codon IVS 1.1 [G>A] / IVS 2.1 [G>A]	1 / 2.5 %	
(9+20) Codon 8/9 [+G] / IVS 2.1 [G>A]	1 / 2.5 %	
(13+20) IVS 1.5 [G>C] / IVS 2.1 [G>A]	1 / 2.5 %	
(14+28) IVS 1.6 [T>C] / IVS 1.6 [T>C]	1 / 2.5 %	
(16+29) IVS 1.116 [T>G] / IVS 1.116 [T>G]	1 / 2.5 %	
(8+12) Codon 8 [-AA] / IVS 1.1 [G>A]	1 / 2.5 %	
(8+14) Codon 8 [-AA] / IVS 1.6 [T>C]	1 / 2.5 %	
(18+33) Codon 39 [C>T] / Codon 39 [C>T]	1 / 2.5 %	
(20+33) IVS 2.1 [G>A] / IVS 2.1 [G>A]	2 / 2.5 %	
(15+Uncharacterized) IVS 1.110 [G>A]	1 / 2.5 %	
(13+16) IVS 1.5 [G>C] / IVS 1.116 [T>G]	1 / 2.5 %	
(9+Uncharacterized) Codon 8/9 [+G]	1 / 2.5 %	
	40 / 100 %	

5. Discussion

The most frequent β-globin mutation in this study was codon 8 [- AA] responsible for (21.2%) 0 of detected alleles followed by IVS2.1 [G > A] (20%) and IVS1-110 (16. 25%), codon 8/9 [+G] (12.5%), IVS1.5 [G> C] (7.5%), codon 44 [-C], codon 39 [C > T], codon 5 [- CT], codon 6 [A > T], was (2.5%), IVS1.116 [T > G], IVS1.6 [T> G] was (3.75 %) and uncharacterized was 2.5% (see Table 2).

The population of Kirkuk city consists of three ethnicities (Turkish, Arabic, and Kurdish). When we compare other studies done in different Iraqi cities, we notice diverse results despite a common mutation between all studies.

Because of the distribution of ethnic Arabs in the south, the ethnic Kurd in the north, and ethnic Turk in the middle (especially in Kirkuk city) leads to different results in every Iraqi city according to the differences in ethnic populations in every city.

As an example, when we examine the results of [4], the most frequent mutation is IVS2.1 [G > A], followed by IVS1.6 and IVS1.110 which were performed in Duhok city using Strip assay. In our study, IVS2.1 is the second common mutation, and IVS1-110 is the third as in al-Allawi [4].

According to [5], in a study done in Baghdad, the most frequent mutation is IVS1.110, followed by codon 39, and all the patients were ethnic Arabs using a specific PCR reaction [5].

The study (Hassan and al-attar, 2017) was conducted in Erbil city using a multiplex PCR technique. The most frequent mutation is codon 8/9 and codon 8 in ethnic Kurds.

In Karbala, the most frequent mutation is IVS1.110 and codon 39 using ARMS – PCR Jeddoa et al. [6].

In the study [7], the IVS1.5 [G > C] and codon 8/9 [+ G] were most common in ethnic Arabs of Al- Basrah city using ARMS – PCR [7].

Similarly, the same results are obtained in Al- Muthanna city in ethnic Arabs conducted by Al-Fartosi et al. [8]. In another study carried out in Al-Muthanna, the most frequent mutations were IVS2.1 [G > C], IVS1.5 [G >C] [9].

According to all studies, the mutation IVS1.110 is the most common mutation in the south of Iraq as the results of Ninawa, Babylon, Karbala, Baghdad, Wasit, and in our current study (the third most frequent mutation) because of the presence of ethnic Arabs in Kirkuk city too [6, 10, 11].

According to our study, mutations 8 [- AA] and IVS2.1 are the common mutations in the middle of Iraq. It is important to mentioning that our work is the first one performed in Kirkuk city.

The frequency of the mutation IVS2.1 is increasing towards the north, as it is the commonest in the Duhok, Sulaymaniyah. According to the [12], it is the most common in the north of Iraq [12, 13] (see Table 3).

Table 3: Comparison between present study (Kirkuk) and other studies in Iraqi cites.

The studies in Iraqi cites	The most frequent mutation	Techniques used in the characterization	References
Erbil 2017 1	Cd 8/9 (8, 20 %), Cd 8 (6, 15 %), Cd 41 / 42 (4, 10 %), IVS 1.5 (3, 7.5 %)	Multiplex-PCR 40 patients	[10]
Baghdad 2009 2	IVS 1.110 (36%); CD 39 (25.3 %); CD 8/9 (9.3 %), CD 8 (4 %); IVS 1.110 & CD 8 (1.3 %); unknown (24 %)	Specific PCR Reaction 75 patients	[9]
Ninawa 2009 3	IVS 1.110 (27 %); IVS 1.6 (14.5 %); Cod 8 (12.5 %); Cod 39 (12.5 %); IVS 2.1 (12.5 %)	B. Globin Strip Assay Kit (24 patients)	[10]
Babylon 2015 4	IVS 1.110 (21 %); IVS 1.5 (10 %); IVS 1.110 and IVS -5 (1 %); undetermined (69%)	(ARMS-PCR) 100 patients	[11]
Al-Muthanna 2014 (100) 5	IVS 1.5 (53.8 %); Codon 8/9 (27.6%); Codon 15 (18.4%)	(ARMS-PCR) 100 patients	[8]
Kerbala 2018 6	(IVS 1.110) & Codon 39 (26 -76 %), (20 -34 %) respectively; IVS 2.744 undetermined.	(ARMS-PCR) 80 patients	[6]
Wasit 2016 7	IVS 1.116 [T>G] (39.6%); IVS 1.110 (10.4%); IVS 1.5 (6.6%); Codon 8/9 (2.8%)	Multiplex ARMS-PCR 106 patients	[5]
Basrah 2016 8	Codon 15 [G-A]; IVS 1.5 [G-C] (37.3%); and 18(21.7%) respectively; Codon 8/9 (16.9%); Codon 30 (9.6%)	(ARMS-PCR) 100 patients	[2]
Baghdad2013 9	Codon 8/9 (39.5%); IVS 1.5 (26.3%)	(ARMS-PCR) 100 patients	[7]
Duhok 10	IVS 2.1 (26.5%); Codon 44 (16.2%); IVS 1.6 (11%); Codon 39 (13.2%); Codon 8 (5.1%)	(ARMS-PCR) 68 patients	[12]
Alawi northern Iraq 11	(88.2%) of most seven mutations are IVS 11.1 [G → A], IVS 1.1 [G → A], Codon 8 [-AA]; Codon 39 [G-T]; Codon 8/9 [+G]; Codon 44 [-C]; Codon 5 [-CT]	Strip Assay MED 127 patients	[12]
Kirkuk 12	Codon 8 [-AA] (21.25%); Codon 20 IVS 2.1 [G>A] (20%); IVS 1.110[T>G] (16.25%); and Codon 8/9 [+G] (12.5%)	Strip Assay MED 50 patients	Present study
Sulaymaniyah 2020	IVS 2.1 [G>A] (41.2%); IVS 1.6 [T>C] (23.3%); IVS 1.110[G>A] (5%)	Strip Assay Kit MED 159 patients	[13]
13 Study on 102 Iraqi Arab Patients	IVS 11.1 [G>A], IVS 1.6 [T>C] and IVS 1.110 (G>A) in (31.4 %) (17.6 %) and (6.9 %)	Strip Assay MED	[4]
Al-Muthanna	IVS 11.1 [G>A], IVS 1.6 [G>C] have been previously reported	Gap-PCR 97 patients	[5]

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