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Molecular genetics of β-talassemia in Tripoli-Libya

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ABSTRACT

β-talassemia is an inherted blood disorder, in which the body does not make as much as β-globin as it should be. Hemoglobin is the part of a red blood cells (RBCs) that carries oxygen throughout the body. β-talassemia includes three main forms, major, intermadia, and minor. β-globin DNA mutations present in 14 subjects from 866 adult samples. β-talassemia, major and minor were analyzed by DNA sequencing of amplified DNA. Different β-talassemia mutations were identified. The three common three β-talassemia found in Libya were B°CD39 (C→T) N=6, B+IVS-I,6 (C→T) N=5, and B+IVS-1,110 (G→A) N=3.

Keywords: β-talassemia, DNA Sequencing, Libyan Adults, Tripoli.

1. Introduction

Hemoglobinopathies are the most common single gene disorders. They are inherited disease effecting the structure and synthesis of globin chains. They constitute a major public health problem in the world. They can be divided into two groups, such as hemoglobin variants and thalassemia [1]. Although in Libya these disorders are thought to be common,[2], [3,4], and 1999[5].

Thalassemia is a globin gene disorder that results in an effective rate of synthesis of one or more of the globin chains. About 1.5% of the global population (80 to 90 million) are carriers of β-thalassemia. More than 500 mutations are described as β-thalassemia. There are eight common mutations in Mediterranean countries [6] . β-thalassemia is common in Mediterranean countries Middle east, Central Asia, India, Southern China, the Far East as well as countries along the North coast of Africa and South America [7]. The highest carrier frequency is reported.

in Cyprus (14%) [8], and Sardinia (10.3%) [9].We concluded that there are three common β-thalassemia mutations in Tripoli area Bº CD39 (C→T), B^{+}IVS-1,6 (T→C), and B+IVS-1,110 (G→A).

2. Materials and Methods

2.1. Blood samples collection

Blood samples (5ml) were collected in vacutianers with EDTA as anticoagulant from 866 adult Libyan samples. The blood were stored at 4ºC.

2.2. Hematological determination

Hematological values were obtained on a Hematology Autoanalyser System 9000 .The RBC count ($x10^2$/L), Hb concentration (g/dl), MCV (in fl) and MCH (in pg). The MCV, MCH and HhA_2 values were especially useful parameters in diagnosis of β-thalassemia heterozygous. The MCV below 80 fl, MCH value below 27 pg and Hb A_2 above 3.5% were a sign of the presence of β-thalassemia carrier.

2.3. Hb A2 Quentitation

The Helena β-Thal Hb A_2 Quick column procedure (Microchromatographic method) was used for the quantitation of Hb A_2. The accurate Quantitation of Hemoglobin A_2 (HbA_2) is essential for diagnosis of several aneamias and evlevated Hb A_2 is widely regarded as efficient evidence for the diagnosis of β-thalassemia trait.

2.4. DNA isolation from whole blood (Micro Method)

This method had been described by Saiki et al 1986. The DNA was stored at -20ºC. The yield of a genomic DNA was equivalent to one microgram, in 25ul of lysate.

2.5. Agarose gel electrophoresis

The standard method used to Saprate, identify and purify DNA fragments was electrophoresis through agarose gel. The technique was simple, rapid to perform and capable of resolving mixture of fragments. The location of DNA within the gel could be determined directly. Bands of DNA with gel were stained with low Concentrations of the fluorescent intercalcting dye ethidiem bromide. As little as ug of DNA can then be detected by direct examination of gel in ultraviolet light.

2.6. PCR using tbr polymerase

The amplification of genomic DNA was performed on automeated thermal cycler (Perkin Elmer. Norwalh CT, USA [10] and [11] . The DNA was subsequently stored at -20ºC until further use.

2.7. Direct Sequencing of PCR Amplified DNA

DNA sequencing information has led to many major advances in molecular Biology. The accurate determination of the nucleotide sequence of DNA is often an essential part of the analysis of the structure and function of genes. The two main sequencing techniques in current are the chemical degradation method of [12] and the Enzymatic method of Sanger [13].

3. Results and Discussion

3.1. Molecular pathology of β-Thalassemia

Hematological data for all 14 cases with β-Thalhassemer, (4 patients and 10 anaemic heterozygotes) from screening are summarized in table 1. The ages between 22 and 28 years, RBC were between 3.45 and 6.0×10^2 /L, Hemoglobin value ranged from 6.9 and 11.4 g/dl. The MCV value were variable between 45.6 and 74.5 fl, while the MCH values were between 16.7, 24.1pg and Hb A_2 were between 3.2% and 6.2%.

Table .1. hematological and Hb compotation of 14 β-thalassemia from screening studies. (aljala Hospital Tripoli-Libya

Case	Gender-years	RBC10^{12} /L	Hb g/dl	MCV fl	HCH pg	Hb A_2 %	HB –A %	Hb F %	Genotypes
LA320*	F- 25	5.69	11.4	65.4	20.0	5.4	61.4	33.2	CD39T/B^A
LA431*	F- 25	3.9	7.8	62.1	20.0	4.5	63.3	32.2	CD39T/B^A
LA521*	F- 28	5.76	11.1	59.4	19.3	4.9	85.1	10.0	CD39T/B^A
LA661*	F- 30	5.50	9.2	54.6	16.7	6.2	84.7	9.1.	CD39T/B^A
LA783*	F- 32	4.88	9.2	61.6	19.2	5.2	82.3	11.1	CD39T/B^A
LA866*	F- 37	5.00	12.6	68.4	19.8	5.3	8.5	10.1	CD39T/B^A
LA630*	F- 22	3.5	7.1	67.3	21.4	6.1	74.9	14.8	IVS-1,6C/IVS-I,6C
LA211*	F- 26	3.62	7.7	68.5	21.2	3.5	74.2	19.7	IVS-1,6C/IVS-1,6C
LA115*	F- 27	3.45	7.5	24.1	3.5	3.5	71.0	14.9	IVS-1,6C/IVS-1,6C
LA189*	F- 28	3.46	7.3	72.6	21.1	5.5	80.0	4.9	IVS-1,6C/IVS-1,6C
LA190*	F- 34	6.07	12.1	66.0	19.5	4.9	68.0	26.5	IVS-1,6C/IVS-1,6C
LA75*	F- 26	4.88	9.6	60.2	19.7	4.0	68.1	27.0	IVS-1,110A/B^A
LA801*	F- 24	4.76	6.9	64.4	18.8	5.2	86.7	9.3	IVS-1,110A/B^A
LA585*	F-24	5.35	10.8	63.9	20.3	5.2	85.0	10.0	IVS-1,110A/B^A

* **LA Libyan Adult**

3.2. DNA Sequesing

The determination of the sequence of the amplified DNA samples (suing dideoxy method) contained the β-globin gene of 14 β-thalassemia. All samples were sequenced and the type of mutation was determined, Five sample which B^+IVS-1,6 (C), gave the sequence C at position number 6 of B-IVS-1 instead of T for both homozygotes and heterozygotes (fig 3.1).

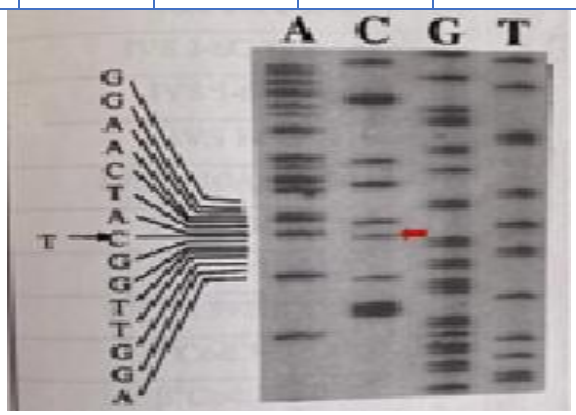

Fig. 3.1. Sequencing gel of the emplified of β-globin gene IVS,1,6, T→C homozygote

Three samples which were B^+IVS-I,110 (A) heterozygotes gave the sequence A at position number 110 of B^+IVS-I,110 Instead of G (fig3.2).

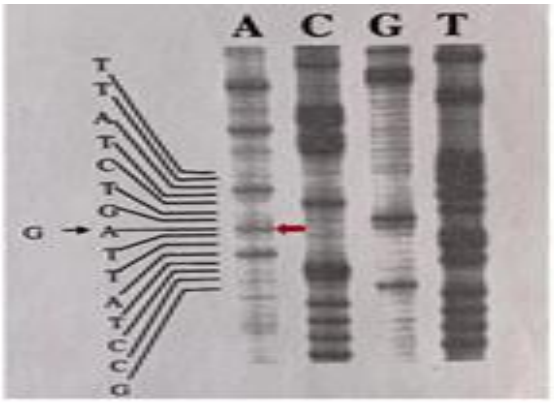

Fig.3.2. Sequencing gel of the amplified β-globin gene IVS, 1,110 C→A heterozygoe

Six which were BºCD39T heterozygotes gave C-T mutation in codon 39 (CAG for Gln to TAG for Stop) as indicated in appropriate section of the sequencing film (Fig 3.3).

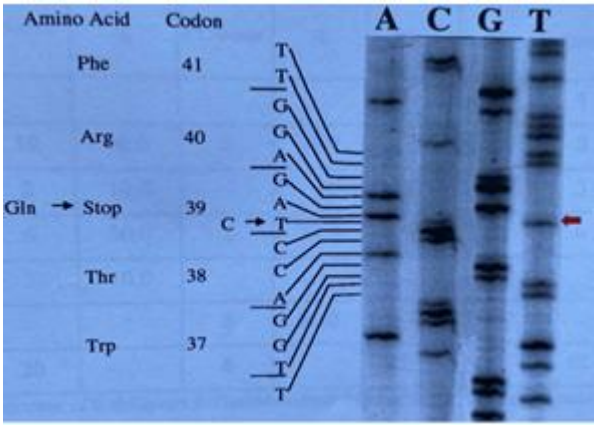

Fig.3.3. Sequencing of amplified codon 39 C-T heterozygote

4. Discussion

Molecular characterization of the β- thalassemia mutations:

The characerization of the β-thalassemia defection of 19 chromosomes has shown great molecular heterogencity with 3 different mutations being observed. These three mutations are Bº CD39 (C→T), B⁺IVS-1,6 (T→C), and B⁺IVS-1,110 (G→A). These data support the general observation that in most populations. A few mutation (8 or less) account for most of β-thalassemia mutations [14].

The most common mutations in libya is B⁺IVS-1,6 (T→C), is famous in about 35,7% (N= 5) of these three mutations. It is prevalent in many Mediterranean countries, being the most common mutation in Malta and account for 71,4% [15], and is the second most common mutation in Libya. The nonsense mutation Bº CD39 (C→T), is found in 42,8% (N=6), and is the most prevalent in many West and Central Mediterranean countries such Algeria [16] , Italy [17], and Algeria [18] ,this mutations reaches its heighst frequencey in Sardinia 95,7% [19].

The third most common mutations in Libya is B⁺IVS-1,110 (G→A), this mutation effects RNA processing and is found in about 21,5% (N=3). This mutation is the most prevalent in many East Mediterreanan countries ,such as Egypt [16] (Khan et al. 2021) ,Greece (Voskarida et al. 2019) [20], Italy[9], Lebanon 62% [18], and Cyprus where reached the highest at 77% (Bozkurt, 2007)[8].

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