

## MOLECULAR BASIS OF BETA THALASSEMIA MUTATIONS IN EGYPTIAN PATIENTS

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### ABSTRACT

Thalassemia is a wide range hereditary disease with high incidence in Egypt along with the high frequency of consanguineous marriages. Investigation the heterogeneity, molecular basis, and natural history are the most effective methods to deal with the thalassemia to develop effective method for management and prevention including the prenatal diagnosis. The current study aims to detect the most common  $\beta$ -globin gene mutations in Egypt among  $\beta$ -thalassemic patients by using PCR based reverse hybridization method (StripAssay) for the most prevalent 22  $\beta$ -globin gene mutations in the mediterranean population in an attempt to estimate the incidence of each mutation, and an attempt to improve our control strategy of  $\beta$ -thalassemia. This study included a total of 37 confirmed  $\beta$ -thalassemia ethnic Egyptian patients (23 males and 14 females) out of them 17 patients were a thalassemia major and 20 were a thalassemia intermediate. Evaluation of  $\beta$ -thalassemia mutations revealed that, the presence of 9 different  $\beta$ -globin mutations. The most frequent mutation were IVS 1-110[34%], IVS 1-6(23.5%), IVS 1-1(19%), Codon 27[6.5%], IVS 2-848[6.5%], IVS 2-745[2.1%] and IVS 2.1 [2.5%], Codon 39[4%]), and IVS 1.5 [1.5%]. IVS 1-110[G>A] is the commonest homozygous mutation found in 6 out of 14 homozygous cases accounting for about 43% of homozygous mutations while, IVS 1-110[G>A]/ IVS 1-6[T>C] is the commonest heterozygous mutation found in 6 out of 23 heterozygous cases accounting for about 26% of heterozygous mutations. In conclusion,  $\beta$ -Globin Strip Assay is useful rapid screening method for  $\beta$ -globin gene mutations which can provide an insight into the detection of individual patients, to take the proper measurement, and thus could be used as a basis for genetic counseling and prenatal diagnosis.

**Keywords:**  $\beta$ -Thalassemia, mutations,  $\beta$ -Globin Strip Assay (reverse dot-blot PCR), Egyptian

### INTRODUCTION

Beta( $\beta$ )-thalassemias are a group of genetic (autosomal recessive) blood disorders characterized by decreased or absent  $\beta$ -globin chain synthesis (1). High prevalence is present in developing countries whereas the health problem is prominent and shortage of the healthcare

delivery strategy. The highest incidences are reported in populations of

African descent, Mediterranean, Middle-East, Transcaucasia, Central Asia, Indian and Cyprus (2). Therefore, a thalassemia prevention program in these countries is highly needed (3). The position of Egypt in the center of the Middle East and as in many Mediterranean countries,  $\beta$ -thalassemia in Egypt considered as a major public health problem (4). In Egypt, it was reported that out of 1.5 million births, there are around one thousand child suffering

from  $\beta$ -thalassemia major (5). Due to the limited resources of Egypt, its healthcare system is unable to deal properly with such a large number of sick babies. Therefore, community based prevention system must be that includes identification of carrier patients, genetic counseling and prenatal diagnosis (6-8). More than 200 mutations are detected to cause  $\beta$ -thalassemia, the information available concerning the underlying molecular defects in  $\beta$ -thalassemia has not yet been completed. (9).  $\beta$ -thalassemia mutations varies significantly among different geographical areas, there for The success of carrier screening and prenatal diagnosis depends on the information of prevalent mutations of such area (10-14).

Previously, it was reported that the human  $\beta$ -globin gene has been genotyped using several methods such as a denaturing gradient gel electrophoresis (15), and PCR, followed by restriction digestion (16). These methods take long time for diagnosis (17).

In present study, a reverse-hybridization assay was designed for the rapid and simultaneous screening of 22  $\beta$ -globin gene mutations in a single procedure, which give the advantage of accuracy, sensitivity, and applicability of assay useful for prenatal diagnosis.

## **SUBJECTS AND METHODS**

### **Subjects**

The study participants were among a group of attendants to the hematology clinic of Abulrish hospital, Cairo University, Egypt. They comprised males and females suffering from  $\beta$ -thalassemia disease. Diagnosis of  $\beta$ -thalassemia disease was based on history, clinical examination and hematological investigations. All investigations were done in accordance with the Cairo University, health and human Ethical Clearance Committee guidelines for clinical researches. After obtaining informed consent, all participants

were questioned in regard to their personal and family medical histories.

### **Methods**

All affected patients were clinically classified into thalassemia major and thalassemia intermediate by collecting their peripheral venous blood for complete hematological examination with consideration to; the age of disease onset, the age of first transfusion, frequency of blood transfusion, hepatosplenomegaly, facial and growth affection.

### **$\beta$ -Globin stripassay**

Reverse dot-blot PCR was done using  $\beta$ -Globin StripAssay MED<sup>TM</sup>, (ViennaLab Diagnostics GmbH, Gaudenzdorfer Gurtel, Vienna, Austria). First, DNA was extracted from peripheral blood leukocytes obtained from EDTA anti coagulated blood samples according to standard protocols and commercial kits. The isolated DNA was subjected to multiplex PCR amplification reaction using biotinylated primers. The resulted amplified  $\beta$ -globin products are then selectively hybridized to a test strip containing wild type and mutant oligonucleotide probes immobilized as parallel lines. The color of the Bound biotinylated sequences is then developed. The assay covers 22 mutations, characteristics for the Mediterranean area.

### **Statistical analysis**

Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis as follows: quantitative variables Results are expressed as mean  $\pm$  standard deviation (SD) or number (%). While the qualitative variables as number and percentage. P value  $\leq 0.05$  was considered significant and  $< 0.001$  was considered highly significant.

## **RESULTS AND DISCUSSION**

This study included a total of 37 ethnic Egyptian patients (23 males and 14 females) who were confirmed to have  $\beta$ -thalassemia. Out of the  $\beta$ -thalassemia, 17 patients were a thalassemia major and 20

were a thalassemia intermediate. The range of the ages were from 3 to 10 years with a mean and SD of  $5.6 \pm 2.1$  years. Male patients account for 62% of the studied patients 14 male patients give a thalassemia major picture and 9 male patients had the thalassemia intermediate picture. Female

Hematological data of study subjects revealed that, there were significantly lower hemoglobin (Hb), and significantly higher reticulocytes, platelets and white blood cells (WBCs) in patients with thalassemia major compared with patients with thalassemia intermedia. Moreover there were no significant differences between patients with thalassemia major and thalassemia intermedia as regard mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) as shown in **Table 2**.

Evaluation of  $\beta$ -thalassemia mutations in 37 patients with 74 alleles revealed the presence of 9 different  $\beta$ -globin mutations. The most frequent mutation is IVS 1-110[G>A] which account for about 34% of the studied alleles followed by IVS 1-6(T-C) which represent about 23.5%. While IVS 1-1(G-A) account for 19%. Other less

frequent mutations as Codon 27[G>T] and IVS 2-848[C>G] presented by 6.5% for each mutation, IVS 2-745[C>G] and IVS 2.1 [G>A] presented by 2.5% for each mutation, Codon 39[C>T]) account for

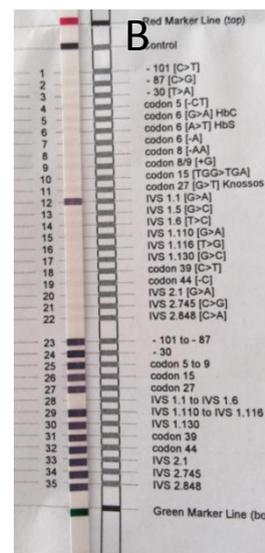
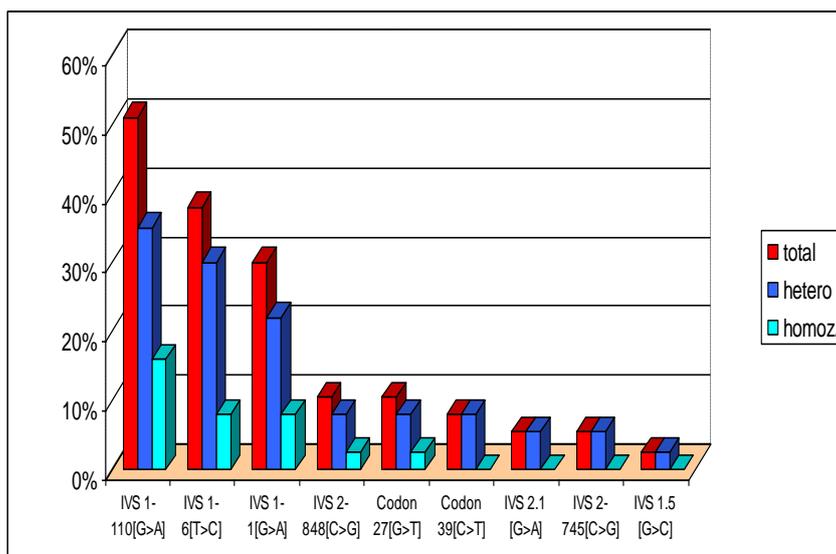
patients account for 38% of the studied patients 3 female patients give a thalassemia major picture and 11 female patients had the thalassemia intermediate picture, moreover the multiple physical and clinical examinations are shown in **Table 1**.

4% and IVS 1.5 [G>C] represented by 1.5% as shown in **Fig. 1**.

IVS 1-110[G>A] represents the most common homozygous mutations 6 out of 14 homozygous cases about 43%. In compound heterozygous cases, IVS 1-110[G>A] also is the most common heterozygous mutation account for 13 out of 23 of our heterozygous cases representing about 56%. Next IVS1-6 [T>C] represents the second common homozygous mutations 3 out of 14 homozygous cases about 21%. In compound heterozygous cases, IVS 1-

6[T>C] is the second common compound heterozygous mutation present in 11 out of 23 of our heterozygous cases account for 47%. Followed by IVS 1-1[G>A] which represents the second common homozygous mutations 3 out of 14 homozygous cases about 21%. In compound heterozygous cases, IVS 1-1[G>A] occupy the third place in compound heterozygous mutations account for 8 out of 23 of compound heterozygous cases representing about 34%

A



**Fig 1.β-Globin gene mutations.** (A) Diagram describes the distribution and frequency of β-thalassemia mutations among carrier patients. (B) Representative of the test strip used in the study

Beta Thalassemia is a group of hemoglobin diseases caused by a reduction (b+) or abolish (b0) in the synthesis of β-globin chains. Carrier individuals can be either compound heterozygous, or homozygous for β-thalassemia [18].

β-Thalassemia is the most common genetically inherited β-globin disorder in Egypt.(19). Combined effects of high carrier rates and high frequency of consanguineous marriages make prevalence of β-thalassemia particularly high in Egypt (20).Up to date in Egypt, carrier detection and genetic counseling and carrier identification is essentially presented to families with an affected individual. In these families, the phenotypic and genotypic evaluation is usually performed in these families to determine the prognosis and to offer the comprehensive genetic counseling. For further reduce the incidence of new births of children with β-thalassemia (21.)

Despite efforts to develop a therapy or bone marrow transplantation for β-thalassemia, still the prenatal diagnosis followed by termination of the affected fetus remains the best form of prevention.

Until now, more than 200 different mutations have been described in patients with β-thalassemia. So the aim of this study is to find rapid, sensitive accurate method to detect the affected individuals, carrying the β-globin gene mutations and applicability of this method to use in prenatal diagnosis and further prevention of the disease.

In our studied, the commonest symptoms in the subjected patients were Pallor and jaundice while the most common signs werehepatomegaly and splenomegaly. This is in accordance with (22, 23).

In 37 patients with 74 alleles revealed the presence of 9 different β-globin mutations. The most frequent mutation is IVS 1-110[G>A] which accountfor about 34% of the studded alleles followed by IVS 1-6(T-C) which represent about 23.5%. These results are in agreement with **El Fadaly et al, 2015, El-Shanshory et al 2014, Elmezayen et al., 2014 and El-Beshlawy et al, 2012,** (20, 22, 24- 25).

IVS 1-110[G>A] is the commonest homozygous mutation found in 6 out of 14 homozygous cases accounting for about 43% of homozygous mutations. IVS 1-

110[G>A]/ IVS 1-6[T>C] is the commonest heterozygous mutation found in 6 out of 23 heterozygous cases accounting for about 26% of heterozygous mutations. Our results are in agreement with the results of **El-Beshlawy et al, 2012, and Jifri et al, 2010** (25- 26). On the other hand **El -Gawhary et al., 2007** and **Elmezayen et al., 2014** (27, 24) reported that IVSI-6 is more frequent than IVSI-110.

#### CONCLUSION

Knowledge of  $\beta$ -thalassemia mutations and their incidence may be a step in the heterogeneity detection of thalassaemic carrier patients; Consequently, prenatal diagnosis in families at-risk can reduce the incidence and the severity of the disease and thus can provide an insight into the prevention strategy for this disease.

#### Declaration of Interest:

The authors report no conflicts of interest

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