DETECTION OF COMMON BETA THALASSEMIA MUTATIONS AMONG EGYPTIAN PATIENTS

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Thalassemia is a globin gene defect that results in a decreased rate of synthesis of one or more of the globin chains and a reduced rate of synthesis of the hemoglobin. Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis (Bain, 2006). Beta-thalassemia is caused by the reduced (ß+) or absent (ß0) synthesis of the beta globin chains of the hemoglobin (Hb) tetramer, which is made up of two alpha globin and two beta globin chains (alpha2 beta2). Beta-thalassemia homozygotes may develop either thalassemia major or thalassemia intermediate (Olivieri and Weatherall, 2001). The decreased amount or absence of beta globin chains result in a relative excess of unbound alpha globin chains which precipitate in erythroid precursors in the bone marrow leading to their premature death and ineffective erythropoiesis. The degree of globin chain reduction is determined by the nature of the mutation at the beta globin gene (Galanello and Origa, 2010). High prevalence of beta thalassemia is present in developing countries due to the health care problems and shortage of the healthcare delivery strategy. The highest incidences of beta thalassemia are reported in populations of Mediterranean, Middle-East, Transcaucasia and Cyprus (Weatherall *et al*., 2007). Therefore, a prevention program for beta thalassemia in these countries is highly needed (Al-Allawi *et al*., 2006). As a result of Egypt position in the Mediterranean area and in the center of the Middle East, β-thalassemia is considered as a major public health problem (El-Beshlawy *et al*., 1999). Because of the limited resources of Egypt, its healthcare system is unable to deal properly with beta thalassemia. Therefore, preventive approaches for the identification of carrier patients, genetic counseling and prenatal diagnosis are urgently needed (Ahmed *et al*., 2002). Many mutations had been detected which cause β-thalassemia, the information available concerning the underlying molecular defects in β-thalassemia has not yet been fully characterized (Omar *et al*., 2005). β-thalassemia mutations varies significantly among different geographical areas, therefore the success of carrier screening and

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prenatal diagnosis depends on the availability of information of prevalent mutations of such area (Edison *et al*., 2008).

MATERIALS AND METHODS

Subjects

The studied patients were a group of attendants to the hematology clinic of Abulrish hospital, Cairo University, Egypt, suffering from β-thalassemia disease. Diagnosis of beta thalassemia was based on clinical examination, history and hematological investigations. All examination and investigations were done in accordance with the Cairo University and human Ethical Clearance Committee guidelines for clinical researches. All personal, family and medical histories of the patients were obtained with informed consent.

Methods

Patients were clinically classified into thalassemia intermediate and thalassemia major with consideration to; the age of disease onset, the age of first transfusion, frequency of blood transfusion, hepatosplenomegaly, facial and growth affection. Venous blood was withdrawn under complete aseptic conditions for CBC, retics, DNA extraction, amplification and detection of mutations by reverse hybridization technique.

β-Globin stripassay

DNA was extracted from peripheral blood leukocytes obtained from blood samples according to standard protocols and commercial kits (β-Globin StripAssay MED™). Multiplex PCR amplification reaction using biotinylated primers for the extracted DNA was done according to standard protocols and commercial kits (β-Globin StripAssay MED™). The amplified beta globin products were then hybridized to a test strip containing wild type and mutant oligonucleotide probes immobilized as parallel lines. Detection of the affected alleles was done by the color of the Bound biotinylated sequences according to commercial kits (β-Globin StripAssay MED™).

Statistical analysis

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

RESULTS AND DISCUSSION

This study included thirty seven Egyptian patients (23 males and 14 females) who were confirmed to have β-thalassemia, 17 patients with thalassemia major manifestations and 20 patients with thalassemia intermediate. Twenty three male patients present in this study accounted for 62% of the studied patients 14 thalassemia major patients and nine thalassemia intermediate patients. Fourteen

female patients in this study accounted for 38% of the studied patients, 3 thalassemia major patients and 11 thalassemia intermediate patients. Regarding hematological data, when comparing thalassemia major and thalassemia intermediate groups, there were significantly higher reticulocytes, platelets and white blood cells (WBCs) and significantly lower hemoglobin (Hb), with no significant differences between patients with thalassemia major and thalassemia intermedia with regard to mean corpuscular volume and mean corpuscular hemoglobin (Table 1). El-Shanshory *et al*. (2014) reported that there were significantly lower red blood cells (RBCs), hemoglobin (Hb), and significantly higher reticulocytes, platelets and white blood cells (WBCs) in patients with thalassemia major compared with patients with thalassemia intermedia with no significant differences between patients with thalassemia major and thalassemia intermedia regarding mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

β-thalassemia mutations in 37 patients with 74 alleles were studied and revealed the presence of nine different β-globin mutations with IVS 1-110(G-A) accounted for about 34% of the studied alleles, IVS 1-6(T-C) represent about 23.5%, IVS 1-1(G-A) account for 19% and 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)) accounted for 4% and IVS 1.5 (G-C) represented by 1.5% (Fig. 1). These results were in partial agreement with the findings of El-Fadaly *et al*. (2015), Elmezayen *et al*. (2015) and El-Beshlawy *et al*. (2012).

Three mutations (IVS 1-110(G-A), IVS 1-6(T-C) and IVS 1-1(G-A)) were accounted for about 76% of mutations in our studied alleles. The other six less common mutations (IVS 2-848(C-G), Codon 27(G-T), IVS 2-745(C-G), IVS 2.1 (G-A), Codon 39(C-T) and IVS 1.5 (G-C)) represented about 24% (Table 2). El-Fadaly *et al*. (2015) found that the most frequent mutant alleles detected by reverse dot-blot PCR accounted for (72.5%) ((IVSI-110 G -A (31.25 %), IVSI-6 T-C (21.25 %), and IVS I-1 G -A (20%)). Also, Youssef *et al*. (2012) reported that the most common mutation encountered among carriers was IVSI-110, which was detected in 17/37 of carriers (46%) followed by IVSI-1 (6/37=16.2%) and then IVSI-6, which was detected in 5/37 of carriers (13.5%).

IVS 1-110(G-A) was found to be the most common homozygous mutations in six out of 14 homozygous cases (about 43%). In compound heterozygous cases, IVS 1-110(G-A) also was the most common heterozygous mutation which accounted for 13 out of 23 of the heterozygous cases representing about 56%. IVS 1-6(T-C) represented the second common homozygous mutations three out of 14 homozygous cases about 21%. In compound heterozygous cases, IVS 1-6(T-C) was the second common compound heterozygous mutation present in 11 out of 23

of the heterozygous cases accounted for 47% followed by IVS 1-1(G-A) which represented the second common homozygous mutations where three out of 14 homozygous cases about accounted for 21%. In compound heterozygous cases, IVS 1-1(G-A) occupied the third place in compound heterozygous mutations accounted for eight out of 23 of compound heterozygous cases representing about 34% (Fig. 2). These results were in agreement with those of El-Beshlawy *et al*. (2012) and Jiffri *et al*. (2010). On the other hand, El-Gawhary *et al*. (2007) and Elmezayen *et al*. (2015) reported that IVS 1-6(T-C) was more frequent than IVS 1-110(G-A).

Finally, Establishment of β-thalassemia mutations data base may be an essential step in the management of the diseases by carrier detection, prenatal diagnosis in high risk families, and thus can provide a useful tool in the prevention strategy for β-thalassemia.

SUMMARY

Beta-thalassemia is one of most common autosomal recessive disorders worldwide. Populations in the Middle-East, Mediterranean region, Central Asia, Indian and Far East countries show high prevalence for thalassemia. It is also relatively common in populations of African descent. The highest incidences are reported in Cyprus, Sardinia, and South East Asia. In Egypt, the genetic information concerning the molecular defects in β-thalassemia has not yet been fully investigated. The current study aims to detect the most common β-globin gene mutations in Egypt among β-thalassemic patients by using PCR and reverse hybridization method in an attempt to estimate the incidence of β-thalassemia mutations, a step in an assessment strategy of β-thalassemia management. Thirty seven confirmed β-thalassemia Egyptian patients were included in this study (twenty three males and fourteen females, seventeen thalassemia major and twenty thalassemia intermediate patients). Nine β-globin mutations were found in this study. IVS 1-110 was represented by 34% of the studied alleles while IVS 1-6 was represented by 23.5%, IVS 1-1 was represented by 19%, Codon 27 was represented by 6.5%, IVS 2-848 was represented by 6.5%, IVS 2-745 was represented by 2.1%, IVS 2.1 was represented by 2.5%, Codon 39 was represented by 4% and IVS 1.5 was represented by 1.5%. β-globin mutation (IVS 1-110[G>A]) was found to be the most common homozygous mutation while, β-globin mutation (IVS 1-110[G>A]/ IVS 1-6[T>C]) was found to be the most common heterozygous mutation. β-globin mutations (IVS 1-110[G>A], IVS 1-6[T>C] and IVS 1-1[G>A]) were found in 76% of allelic mutations. . In conclusion, establishment of β-thalassemia mutations data base may be a step in managing the diseases by carrier detection, prenatal diagnosis in high risk families, and thus can provide a tool in the prevention strategy of β-thalassemia.

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Table (1): Hematological data of the studied patients.

Genotypes	No.	Hb (g/dl)		RBCs (millions/cmm)		WBC (Thousands/cmm)	
		mean	SD	mean	SD	mean	SD
IVS 1-110[G/A]	6	9.200	1.0807	4.033	0.2251	12.17	6.047
IVS 1-6[T/C]	3	9.667	1.2741	4.067	0.0577	10.00	4.770
IVS 1-1[G/A]	3	8.533	1.1060	3.833	0.0577	10.17	1.528
IVS 1-110[G/A]/ IVS 1.6 [T/C]	6	9.550	1.4181	4.067	0.3559	7.83	1.780
IVS 1-110[G/A]/ IVS 1.1 [G/A]	2	8.400	0.4243	4.050	0.2121	6.75	2.475
IVS 1.6 [T/C] / IVS 1.1 [G/A]	3	10.133	0.5686	4.033	0.2887	7.50	.500
IVS 1-110[G/A]/ Co cdon 39[C/T]	2	8.000	1.5556	4.300	0.9899	8.50	2.121
IVS 1-110[G/A]/ Codon 27 [G/T]	1	9.600	.	3.800	.	8.50	.
IVS 1-110[G/A]/ IVS 2-745[C/G]	1	7.300	.	3.700	.	11.00	.
IVS 1-110[G/A]/ IVS 2.1 [G/A]	1	10.300	.	3.600	.	6.50	.
IVS 1.6 [T/C] / IVS 2.848 [C/A]	1	10.300	.	4.200	.	5.50	.
IVS 1.6 [T/C] / IVS 1.5 [G/C]	1	10.400	.	4.300	.	10.50	.
IVS 1.1 [G/A] / IVS 2.848 [C/A]	2	6.100	1.8385	3.650	0.3536	13.25	1.061
IVS 1.1 [G/A] / Codon 27 [G/T]	1	10.600	.	4.100	.	7.00	.
IVS 2-848[C/G]	1	7.900	.	4.100	.	16.00	.
Codon 27[G/T]	1	10.400	.	3.800	.	6.00	.
IVS 2.1 [G/A] / IVS 2-745[C/G]	1	9.500	.	3.600	.	6.50	.
Codon 27 [G/T] / Codon 39[C/T]	1	10.900	.	4.200	.	9.00	.
Total	37	9.181	1.4203	3.995	0.3100	9.36	3.645

Table (2): Genotype and phenotype features.

Genotypes	Phenotypes		Total
	Major	Intermediate	
IVS 1-110[G/A]	3	3	6
IVS 1-6[T/C]	1	2	3
IVS 1-1[G/A]	3	0	3
IVS 1-110[G/A]/ IVS 1.6 [T/C]	2	4	6
IVS 1-110[G/A]/ IVS 1.1 [G/A]	2	0	2
IVS 1.6 [T/C] / IVS 1.1 [G/A]	0	3	3
IVS 1-110[G/A]/ Codon 39[C/T]	2	0	2
IVS 1-110[G/A]/ Codon 27 [G/T]	0	1	1
IVS 1-110[G/A]/ IVS 2-745[C/G]	1	0	1
IVS 1-110[G/A]/ IVS 2.1 [G/A]	0	1	1
IVS 1.6 [T/C] / IVS 2.848 [C/A]	0	1	1
IVS 1.6 [T/C] / IVS 1.5 [G/C]	0	1	1
IVS 1.1 [G/A] / IVS 2.848 [C/A]	2	0	2
IVS 1.1 [G/A] / Codon 27 [G/T]	0	1	1
IVS 2-848[C/G]	1	0	1
Codon 27[G/T]	0	1	1
IVS 2.1 [G/A] / IVS 2-745[C/G]	0	1	1
Codon 27 [G/T] / Codon 39[C/T]	0	1	1
Total	17	20	37

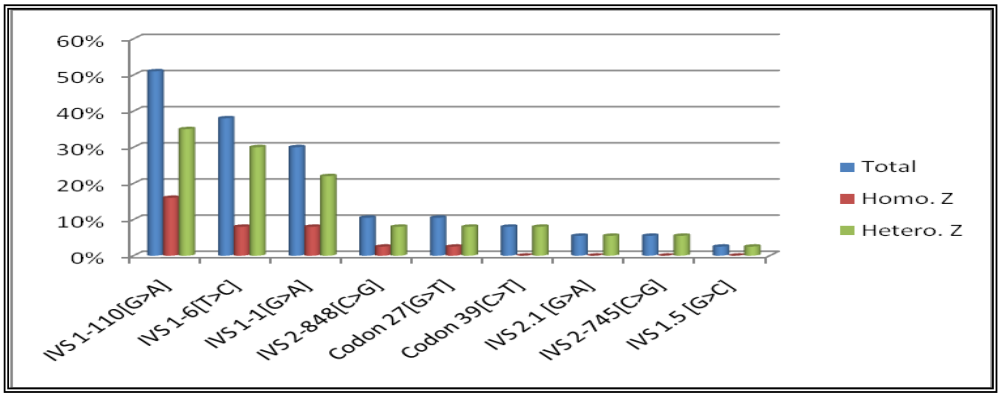

Fig. (1): Diagram of the frequency of β-thalassemia mutations among patients.

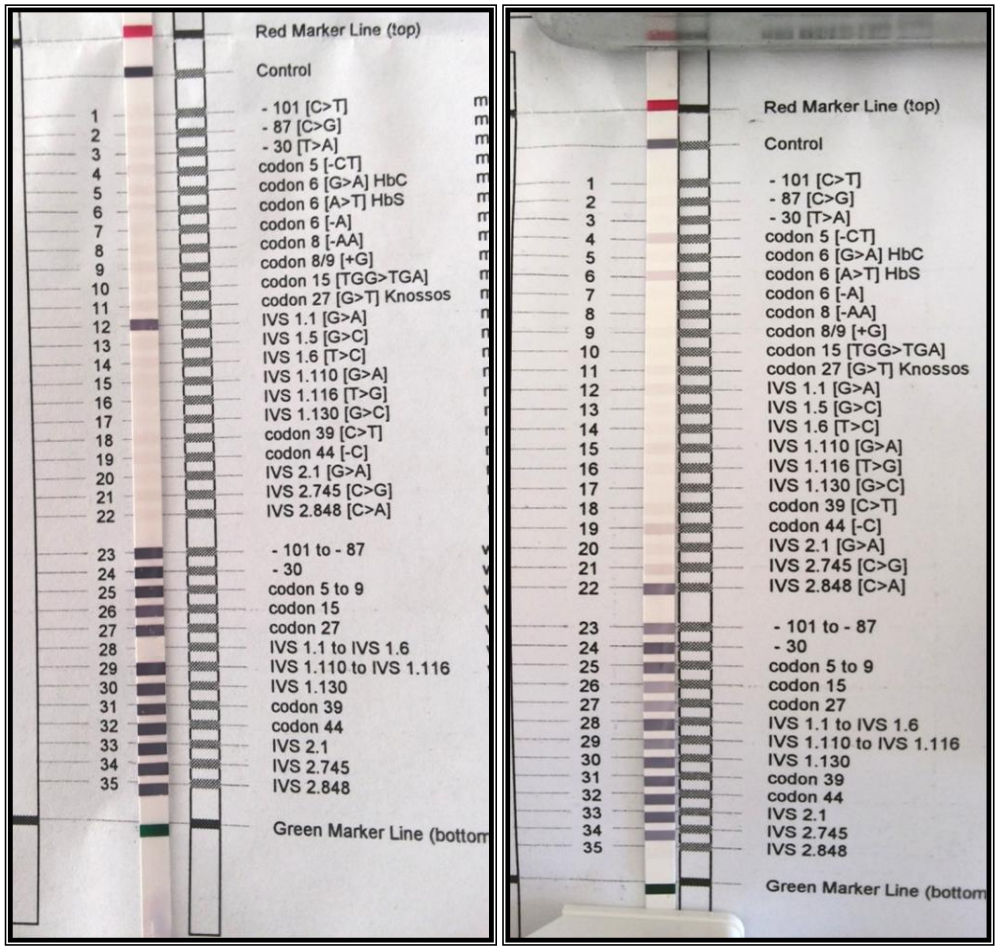

Fig. (2): Results of the test strips used in the study (homozygous IVS 1-1(G-A) and homozygous IVS 2-848(C-G)).