Research Article

Assessment of Molecular Changes of Transfusion Dependent Beta Thalassemia Children in El Minia Governorate and Their Correlations with Patients Clinical Outcomes

Gehan L. Abdel Hakeem*, Aliaa M. M. Hegazi, Mohamed H. M. Mahgoub* and Lina Ahmed Mahmoud***

* Department of Pediatrics, Minia University Hospital

** Department of clinical pathology, Minia University Hospital

Abstract

Beta Thalassemia represents a major public health problem in Egypt. The carrier rate varies between 5.5% to > 9%. It is estimated that there are 1000/1.5 million per year live births born with beta thalassemia.(1) β thalassemia occurs when there is a quantitative reduction of β globin chains that are usually structurally normal.(2) They are caused by mutations that nearly all affect the β globin locus and are extremely heterogeneous. Almost every possible defect affecting gene expression at transcription or post-transcriptional level, including translation, have been identified in β thalassemia.(3) These genetic defects lead to a variable reduction in β globin output ranging from a minimal deficit (mild β+ thalassemia alleles) to complete absence (β° thalassemia). **Aim of the work:** We aimed in this study to assess the molecular changes in transfusion dependent Beta thalassemia patients and the correlation of these molecular changes with their clinical outcomes. **Patients & methods:** This study will include 40 transfusion dependent β thalassemia patients with age range of 2 -18 years, recruiting the Pediatric Hematology unit in Minia University children hospital. **Study procedure:** β-Thalassemia mutation identification of samples will be performed by the reverse dot blot hybridization technique (RDB). For RDB, a panel of primers and probes using the beta globin strip assay well be used (β-Globin Strip Assay MED kit, VIENNA lab

Keywords: Beta Thalassemia, Patients Clinical Outcomes

All enrolled Patients were subjected to:

A-Clinical assessment

1- Full medical History taking including age, sex, age of starting transfusion, family history of consanguinity and similar conditions in family.
2- Clinical examination including general examination stressing on anthropometric measures plotted on growth chars. Systematic examination including chest, heart, abdominal, musculoskeletal, joints and neurological examination

B- laboratory work including

1-Routine lab investigations:

a- Complete blood picture
b- Hb electrophoresis
c- Serum ferritin
d-Liver functions
e-Renal functions
f- Amount of blood transfusions per year.

Conclusion

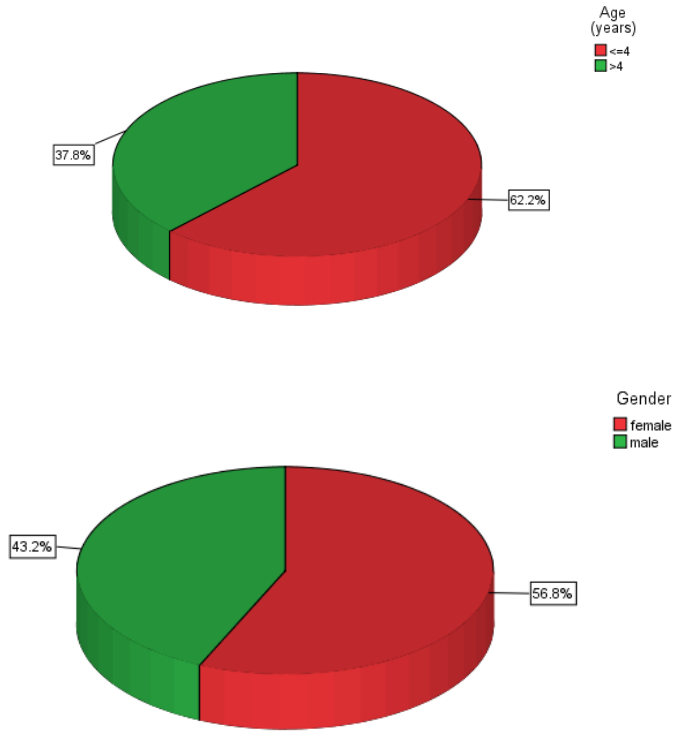
IVS 2-848 is the most common mutation in El-Minya Governorate followed by IVS 2-745

Results

Clinical and laboratory data of the studied groups were tabulated and statistically analyzed. Results of the present study are shown in tables and figures as follows:

Table (1): mutation type of studied group

Mutation type		Frequency	Percent
101 (C>T)	Wild	37	100.0
87 (C>G)	Wild	37	100.0
30 (T>A)	Wild	37	100.0
codon 5 (-CT)	Wild	37	100.0
codon 6 (G>A) HbC	Wild	37	100.0
codon 6 (A>T) HbS	Wild	37	100.0
codon 6 (-A)	Wild	37	100.0
codon 8 (-AA)	Wild	37	100.0
codon 8/9 (+G)	Wild	37	100.0
codon 15 (TGG>TGA)	Homozygous	1	2.7
	Wild	36	97.3
codon 27 (G>T) Knossos	Wild	37	100.0
IVS 1.1 (G>A)	Heterozygous	6	16.2
	Homozygous	7	18.9
	Wild	24	64.9
IVS 1.1 (G>A)	Mutant	13	35.1
	Wild	24	64.9
IVS 1.5 (G>C)	Wild	37	100.0
IVS 1.6 (T>C)	heterozygous	1	2.7
	homozygous	7	18.9
	wild	29	78.4
IVS 1.6 (T>C)	mutant	8	21.6
	wild	29	78.4
IVS 1.110 (G>A)	heterozygous	3	8.1
	homozygous	1	2.7
	wild	33	89.2
IVS 1.110 (G>A)	mutant	4	10.8
	wild	33	89.2
IVS 1.116 (T>G)	heterozygous	2	5.4
	wild	35	94.6
IVS 1.130 (G>C)	wild	37	100.0
codon 39 (C>T)	wild	37	100.0
codon 44 (-C)	heterozygous	2	5.4
	wild	35	94.6
IVS 2.1 (G>A)	homozygous	1	2.7
	wild	36	97.3
IVS 2.745 (C>G)	heterozygous	14	37.8
	homozygous	1	2.7
	wild	22	59.5
IVS 2.745 (C>G)	mutant	15	40.5
	wild	22	59.5
IVS 2.848 (C>A)	heterozygous	10	27.0
	homozygous	12	32.4
	wild	15	40.5
IVS 2.848 (C>A)	mutant	22	59.5
	wild	15	40.5
	Total	37	100.0



Discussion

The thalassemias have a high incidence in a broad area extending from the Mediterranean basin and parts of Africa, throughout the Middle East, the Indian subcontinent, Southeast Asia, and Melanesia in to the Pacific Islands.[1]

In the present study, a comprehensive analysis of the β-globin gene mutations was performed through simultaneous screening of 22 deletions and point mutations covered by the β-Globin Strip Assay.

Our results revealed that There were 13 mutations found to be absent in our study group.

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 27 (G>T) Knossos, IVS 1.5 (G>C), IVS 1.130 (G>C) & codon 39 (C>T).

In agreement with this finding detected some of the rare β-globin mutations to be -87 (C>G), codon 5 [-CT], codon 39 [C>T], codon 27 [G>T], and codon 8 [-AA] in a significant proportion of patients.[2]

In our study, the 9 other mutations were present as heterozygous (19%), homozygous (81%) or both as follow: IVS 2.848 (C>A) is the most common mutation (59.5%), IVS 2-745 (40%), IVS 1.1(G>A) (35.1%) & IVS 1.6(T>C) (21.6%) , Less common, IVS 1.110 (G>A) (10.8%), IVS 1.116 (T>G) (5.4%), codon 44 (-C) (5.4%), IVS 2.1 (G>A) (2.7%) & codon 15 (TGG >TGA) (2.7%).

In our study the most common mutation was IVS 2.848 at all and also was the most common homozygous mutation & the most common heterozygous mutation was IVS 2.745 (C>G).

In contrary to our results, on a study was done on attendants to the hematology clinic of Abulrish hospital, Cairo University, Egypt, found that the most common mutation is IVS 1-110 (34%) & most common heterozygous mutations are IVS 1-110(G>A) & IVS 1-6(T>C)[3]

In study conducted on patients attend pediatric hematology unit of Zagazig University Hospital revealed that IVS1-1, IVS1-110 and IVS1-6 were the commonest mutations (26.7%, 22.6% and 18.5% respectively)[4]

In study was conducted on Egyptian patients with β-thalassemia who were being treated in the Departments of Human Genetics and Hematology, Medical Research Institute, University of Alexandria, Egypt , stated that ten

different mutations were detected, the most frequent of which were IVS 1.6 [T>C] and IVS I.110 (G>A). These 2 common mutations accounted for 50% of the total tested chromosomes.[2]

These genetic variation between β thalassemia patients in our study , Alexandria, Cairo and Zagazig may be attributed to geographical distrbution as: IVS-1-110 (G>A) mutation is the most common mutation in Greece & Italy[5] as there has been a large community of Greeks in Egypt till 1952 mainly in Cairo & Alexandria.
Mutations detected in our study can be attributed to many factors, the most important factor is immigration

The most frequent thalassemia allele in the Turkish population is IVS-I-110 (G>A) mutation (40%), being the most common thalassemia mutation in the majority of the high risk regions of the Mediterranean The other common mutations in Turkey are: IVS-1-6(T>C), FSC-8(-AA), IVS-1-1(G>A), IVS-2-745(G>A), Cd39 (C>T), -30 (T>A) and FSC-5 (-CT) [6]
In our study IVS 2-745(G>A) was the 2nd most common mutation followed by IVS 1-6 (T>C)
In our study, most patients with IVS 2-745 didn't require chelation therapy (73.3%), they were the largest mutation group didn't require chelation (27%), while patients with IVS 1.1(G>A) were the largest group who required chelation (41%)
Most patients were on Deferasirox as chelation therapy (66.7%) followed by DFP (16.7%), DFO (8.3%) & combination Deferasirox & DFO (8.3%).

Patients with thalassemia major (TM) requiring regular blood transfusions accumulate iron at a rate of approximately 0.5 mg kg−1 day−1. This iron is toxic to the heart, liver, and endocrine system.[7] In our study, 71% of patients with IVS 1-1(G>A) & IVS 2-745(C>G) require blood transfusion ≥120 ml/Kg/year.

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