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For further information about this article or if you need reprints, please contact:

Dr. Hala Mohamed Lotfy,
4 Petroleum Buildings,
Ahmed Orabi
St. Mohandessen,
Cairo, No. 63, Egypt

Tel: 0020233057435,
0020129257586

Study of Possible Genetic Factors Determining the Clinical Picture of Thalassemia Intermedia

¹N. Kaddah, ²S. Rizk, ³A.M. Kaddah, ³K. Salama and ³H. Lotfy

The aim of this study was to evaluate some of the genetic factors involved in ameliorating the severity of β thalassemia among a group of Egyptian children with thalassemia intermedia. The study included 22 patients who were diagnosed on clinical basis as β thalassemia intermedia. Their age ranged between 3 and 21 years. They were screened for the most common seven genetic mutations of β -thalassemia evaluated in Egyptian studies: IVS1-6, IVS1-110, IVS2-1, IVS2-745, IVS1-1, -87 and codon 39, also screened for -158 Xmn polymorphism and co-inheritance of α -gene deletions. Present results showed that, the frequency of IVS1-6 was found to be 22.7% and of IVS1-110 was 18.2%, while IVS2-1, IVS2-745, IVS1-1, -87 and codon 39 were undetected. The -158 Xmn polymorphism was detected in 2 out of 22 cases (9%) and co-inheritance of α -thalassemia was 5 out of 22 cases (22.7%) mm. This study showed that, the ameliorating factors in β -thalassemia intermedia may include the inheritance of mild β thalassemia allele as IVS1-6, the presence of -158 Xmn polymorphisms or co-inheritance of α -gene deletions. Identification of genetic pattern in thalassemia intermedia is essential for genetic counseling and prenatal diagnosis and also for the proper management of those patients.

Key words: Thalassemia intermedia, Egyptian children-mutations, α deletion, ameliorating factors

¹Department of the Hematology, Cairo University, Cairo, Egypt

²Department of Clinical Pathology, Cairo University, Cairo, Egypt

³Department of Paediatrics, Cairo University, Cairo, Egypt

INTRODUCTION

Thalassemia intermedia is a clinical form that is extremely heterogeneous and encompasses a wide spectrum of phenotypes ranging in severity from a severe anemia, with hepato-splenomegaly and marked thalassemia-like bone modifications, to a moderate microcytic hypo chromic anemia with barely enlarged spleen and almost absent facial bone alterations (Galanello and Cao, 1998).

Any inherited factor able to reduce the extent of the globin chain imbalance may therefore, result in milder forms of thalassemia (Cao and Mai, 2000). This may result from the presence of mild β thalassemia mutations which allow a residual synthesis of β globin chains, coinheritance of α thalassemia mutations and coinheritance of genetic determinant for the continuous synthesis of γ globin into adult life (Qatanani *et al.*, 2000). The study aimed at evaluating some of the genetic factors involved in ameliorating the severity of β thalassemia among a group of Egyptian children with thalassemia intermedia.

MATERIALS AND METHODS

The study was conducted on 22 clinically diagnosed β thalassemia intermedia patients, attending the hematology clinic of the children hospital, Cairo University, from April 2007 to March 2008. Their age ranged from 3 and 21 years with a mean of 12 years. Twenty two patients fulfilled the inclusion criteria of this work which were as follows: (1) having delayed age of presentation beyond the age of 3 years; (2) patients who were able to maintain Hb level of 6 g dL^{-1} without blood transfusion; (3) patients who had their overall frequency of blood transfusion less than those of thalassemia major. Patients who did not fulfill the inclusion criteria were excluded. All patients were subjected to full history taking, full clinical examination and also, the following investigations: (1) complete blood count; (2) red cell indices (MCV, MCH, MCHC) using automated cell counter (Advia 120); (3) Hb electrophoresis which was carried out on cellulose acetate membranes (Helena laboratories Beaumont, Texas) in tris-EDTA borate buffer, pH 8.4 (Lewis *et al.*, 2001); (4) Genetic studies were also performed including; DNA analysis and detection of point mutations of β thalassemia which was done using ARMS technique, in which genomic DNA was extracted from peripheral blood leukocyte of EDTA anticoagulated blood (Miller *et al.*, 1988), extraction was done from peripheral blood using QIA amp DNN blood Mini Kit: (catalog No. 51104).

Then DNA was amplified by a PCR method based on allele specific priming which is called the Amplification

Refractory Mutation System (ARMS) (Varawalla *et al.*, 1991). ARMS primers were designed to detect normal and mutant DNA, The nucleotide at the 3 end of the mutant primer was complementary to the change of DNA sequence caused by mutation that was being looked for and that of the normal primer was complementary to the normal DNA sequence .

By this technique screening was done for the 5 most common β -thalassemia (mutations at IVS.1 position 110(G...A), IVS.1 position 6 (T...C), IVS.1 position 1(G...A), IVS.2 position 1(G...A) and codon 39(C...T)) (Kazazian *et al.*, 1984; El-Beshlawy *et al.*, 1993). Two other mutations(IVS.2 position 745 (C-G), -87 (C-G)) were also screened. Detection of Xmn.1 polymorphism 5 to the $\text{G}\gamma$ -Globin gene using RFLP technique (Winichagoon *et al.*, 2000) and detection of α -thalassemia co amplifying alpha globin genes in a single PCR (Tang *et al.*, 2001) was also done. Simple descriptive statistics, such as mean, median and range, were used to summarize the results. The t-test was used to compare numerical data. The Chi-square test was used to compare nominal data. Overall, p-values less than 0.05 were considered significant.

RESULTS

This study included 22 patients. They were diagnosed on clinical basis as β -thalassemia intermedia Egyptian children. Their age ranged from 3 to 21 years with a mean of 12 years. As regards the genetic study of thalassemia intermedia patients, ten (10/22) cases (45.5%) were +ve for β -thalassemia mutations, while the other twelve (12/22) cases (54.5%) were not detected by these seven common mutations studied. Table 1 shows the genetic results of the study group. IVS1-6 was present in seven cases, three of them (13.6%) were present in homozygous state, while the remaining 4 cases (18.4%) were present in compound(double) heterozygous state with IVS1-110 i.e., IVS1-6/IVS1-110.

IVS1-110 was present in seven cases, one of them (4.5%) was present in homozygous state, while 4 cases (18.4%) were present in double heterozygous state with IVS1-6, i.e., IVS1-6/IVS1-110, the remaining 2 cases (9%) were present in heterozygous state i.e., IVS1-110/--.

The incidences of IVS1-1,-87(C...G), IVS2-745(C...G), cd 39(CAG.....TAG) and IVS2-1 were undetected.

The incidence of co-inheritance of Xmn-1 polymorphism was detected in two cases (9%).The frequency of co-inheritance of α -thalassemia was 5/22 of cases (22.7%), a single (α 1) deletion was detected in 1/22 (4.5%) of cases, while a double (α 2) deletion was detected in 4/22 of cases (18.2%).

Table 1: Collective genetic study of thalassemia intermedia patients

	Count	(%)
β-thalassemia mutations	10	45.5
IVS1-6	3	13.6
(Homozygous)		
IVS1-110	1	4.5
(Homozygous)		
IVS1-110/--*	2	9.0
(Heterozygous)		
IVS1-6/IVS1-110	4	18.4
(Double Heterozygous)		
Undetected	12	54.5
Gamma mutations		
Xmn1-158 (C.....T)	2	9.0
Negative	20	91.0
α-mutations		
Yes:	5	22.7
α 1 deletion	1	
α 2 deletion	4	18.2
No	17	77.3

*Not detected by proposed method used

DISCUSSION

Homozygous β thalassemia usually result in thalassemia major, a clinically severe anemia, in which patients require regular blood transfusion and iron chelation therapy, however a milder form of this disorder exists, which has been termed thalassemia intermedia, in which patients might be transfusion independent and require only occasional transfusion (Qatanani *et al.*, 2000). This milder clinical presentation is usually due to a less marked imbalance of the α/non α globin chain ratio, which may be due to presence of mild β-thalassemia mutation with residual β globin chains, coinheritance of α thalassemia or the inheritance of genetic determinants for continuous synthesis of γ globin into adult life (Thein, 1993; Ratips, 1997).

Analysis of the genotype of Egyptian patients with thalassemia intermedia is important for early diagnosis of milder disease, thus avoiding regular blood transfusion and also help to establish genotype phenotype correlation. Twenty two Egyptian thalassemia intermedia patients had a genetic study which revealed that the frequency of IVS1-6 was the commonest (22.7%) (10 alleles of 44 total thalassemia alleles). This was in agreement with the results of Di Marzo *et al.* (1988), (26%), El Beshlawy *et al.* (1999), (22.4%). And more than the results of Zahed (2001) who reported a frequency of 13.6% in a randomly selected thalassemia patients. IVS1-6 was present in seven cases, three of them presented in homozygous state and the remaining four were in double heterozygous state with IVS1-110. The mild clinical picture in these cases were explained by Ho *et al.* (1998) and Qatanani *et al.* (2000), who stated that IVS1-6 is a mild β-thalassemia allele and it was associated with the thalassemia intermedia in either homozygous or double

heterozygous states without the presence of other known ameliorating factors. The second most frequent allele in this study was IVS1-110 (18.2%) (8 alleles of 44 total thalassemia alleles). This is in agreement with Zahed (2001), who stated that IVS1-110 is the most common mutation in the eastern Mediterranean especially in Arab countries (12%-88%) and that the frequency of IVS1-110 in Egypt was 3. The results of this work were also close to those of Noveletto *et al.* (1990) (16) (27.1%), El-Beshlawy (1999) (40%) . IVS1-110 was present in 7 cases out of 22(31.8%). In one case, IVS1-110 was present in homozygous state, in two cases, IVS1-110 was present in heterozygous state and in four cases and IVS1-110 was present in double heterozygous state with IVS1-6. The presence of IVS1-6 with IVS1-110 explained the mild clinical picture of the four cases, this is supported by Qatanani *et al.* (2000), who stated that double heterozygous with IVS1-6 is a mild β⁺. While, the presence of IVS1-110 in heterozygous state in two case, may be present with unknown mutation IVS1-110/ not detected by our method used which may be one of silent or mild genes, or it might be a genetic defect outside the gene remote, This is in agreement with, Cao *et al.* (1994) and Cao and Moi (2000), who stated that the principal factors which are able to reduce the globin chain imbalance in β-thalassemia thereby determining an attenuated phenotype are either homozygosity for mild or silent β-thalassemia or less consistently compound heterozygosity for mild/silent mutation and a severe one. In the present study, the -158 Xmn-1 polymorphism was detected in 2/22 cases (9%), (case no 4, 10). This is in agreement with studies in Brazil by Fonseca *et al.* (1998) (18) which showed that it was observed in 3 out of 31 cases (9.67%). The genotype of one patient was IVS1-110 in a homozygous state, which is expected to be severe thalassemia major, however the presence of gamma mutation -158 (Xmn-1 polymorphism) was the only ameliorating factor in this case. This shows the impact of inheritance of such polymorphism. This is in agreement with Bollekens and Forget (1991) and Cao and Moi (2000) who stated that the genetic determinants for maintaining a high γ chain production rate in adulthood may result in a reduction of the α/non α chain imbalance, thereby determining a mild phenotype when co-inherited with homozygous severe β thalassemia. Also, Cao and Moi (2000) stated that in a limited number of otherwise typical β thalassemia heterozygotes, the β thalassemia mutations remain uncharacterized as they show a completely normal DNA sequence within the β-globin gene transcription unit. In these, the casual mutation may lie in the upstream LCR or in the 3' enhancer. Other mutations e.g., cd39(C...T), was not detected in this study, this is in

agreement with Elgawhary (2008), who reported that cd39 was not detected in any of β thalassemia alleles studied. Also, IVS1-1 was not detected, this in contrast to Noveletto *et al.* (1990), who reported a frequency of 10.4%. The frequency of -87 was not detected, while Zahed (2001) reported a frequency of 1.2%. The frequency of IVSII-745, IVSII-I in this study was also not detected; this is in contrast to Zahed (2001) who reported that the frequency of IVSII-745 is 6%. The frequency of co-inheritance of alpha thalassemia in the present study was 5/22 (22.7%) of the beta thalassemia cases. A single $\alpha 1$ deletion represents 1/22 of cases (4, 5%); while a double $\alpha 2$ deletion represents 4/22 of cases (18.2%). In a study done by Ho *et al.* (1998) the co-inheritance of alpha thalassemia (single alpha gene deletion and a non deletion alpha thalassemia variant was present in 8/65 (12.3%) of various beta genotypes. As there is only one patient with non deletional alpha thalassemia variant, so the incidence of co-inheritance of single alpha gene deletion was 7/65 (10.8%). In agreement with the present study, Viprakasit *et al.* (2004) reported that the amounts of free α -globin chains appear to be a major factor contributing to differences in hematologic and clinical severity in patients with β -thalassemia. From this study we can conclude that ameliorating factors in β -thalassemia intermedia may include the inheritance of mild β -thalassemia allele as IVS1-6, the presence of -158 Xmn polymorphisms or co-inheritance of α -gene deletions. Identification of genetic pattern in thalassemia intermedia is essential for genetic counseling and prenatal diagnosis and also for the proper management of those patients since, they have a different risk factor in relation to thalassemia major.

CONCLUSION

From the present study we can conclude that ameliorating factors in β thalassemia intermedia may include the inheritance of mild β -thalassemia allele as IVS1-6, the presence of -158 Xmn polymorphisms or co-inheritance of α -gene deletions. Identification of genetic pattern in thalassemia intermedia is essential for genetic counseling and prenatal diagnosis and also for the proper management of those patients since they have a different risk factor in relation to thalassemia major.

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