db-Thalassemia Patients With Homozygous Xmn-1 Polymorphism That Are Characterized By A Milder Phenotype

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Citation

S Ashraf. *db-Thalassemia Patients With Homozygous Xmn-1 Polymorphism That Are Characterized By A Milder Phenotype*. The Internet Journal of Hematology. 2010 Volume 7 Number 2.

Abstract

Thalassemia intermedia is an inherited group of disorders with clinical presentations varying between the thalassemia carrier state and thalassemia major phenotype. This variability depends on the heterogeneous molecular mechanism of the disease. db-Thalassemia is a heterogeneous disorder characterized by elevated levels of Hb F in adult life. It is a rare disorder in Pakistan. The homozygous variety ^{G}g (^{A}g db)$^{\circ}$ are rare , present with a wide range of clinical phenotypes, and belong to the group of thalassemia intermedia. We determined clinical, haematological and genetic features of Inv/Del G_g ($A_g$db)$^{\circ}$db in 100 thalassemia intermedia patients, six of which had Inv/Del G_g ($A_g$db)$^{\circ}$ mutations, all were homozygous (+/+) for Xmn-1 polymorphism. Among pathological features, high levels of hemoglobin F, late clinical presentation, hepatosplenomegaly, variable transfusion dependence and an absence of alpha chain deletions were observed. This study explains strong link between the Xmn-1 Gg-polymorphism and the Asian-Indian Inv/Del. These findings explain the determined capacity of patients to produce fetal hemoglobin in delta beta-thalassemia. These patients lack alpha chain deletions and may potentiate the qualification of factors favouring HbF production as a prominent source of the thalassemia intermedia phenotype.

INTRODUCTION

Thalassemia is an inherited haematological disease characterised by beta chain mutations resulting in an early onset of anaemia due to reduced rate of synthesis of one or more globin chains [1]. Clinical manifestations are variable and can be associated with all the spectrum of the disease [2]. Clinical types of thalassemia are thalassemia minor which is the carries stat, thalassemia major which is the sever form and is transfusion dependent, and thalassemia intermedia [3].

Thalassemia intermedia presents with a variable phenotype ranging between the thalassemia minor and thalassemia major. Patients are characterized by multiple pathological features including high levels of HbF, late clinical presentation, hepatosplenomegaly and variable transfusion dependence [4].

[illegible]thalassemia are inherited conditions characterized by persistent expression of fetal (A and G) globin chains in adults, usually caused by deletions in the and globin genes, generating fusion genes [5, 2]. This may either leave both $G_$ and $A_$ genes intact or $G_$ alone intact while $A_$ affected. Hence, the disorders are called ()or ($A_$) thalassemia. The deletion results in a high production of active globin chain in adult life. The mechanism of the continued G globin chains productions is not yet elucidated. However, the loss of regulatory elements within the - genes as well as the rearrangement within beta gene complex bring the enhancer closer to the promoter of G globin gene. Moreover, the loss of competition between -, -, and the gene promoters for common locus control region (LCR) is suspected to be involved [4]. HbF synthesis in the post-fetal life seems to be favoured by non-linked factors such as Xmn-1 restriction site (CT change in G at position -158) [5].

-Thalassemia presents as thalassemia intermedia phenotype. Different ameliorating factors are associated to the thalassemia intermedia resulting in its mildness. XmnI polymorphism characterized by the C-T nucleotide change at position -158 is known to be associated with increased production of fetal hemoglobin which is typical trait of thalssemia [5]. In addition, reduced globin chains imbalance is also contributing to the milder phenotype [6].

In this study, we temped to understand the mechanism for

thalassemia. We examined clinical, haematological and genetic features of thalassemia patients and the roles of Xmn-1 polymorphism and alpha thalassemia were validated in 100 thalassemia intermedia patients.

DESIGN AND METHODS

This study was carried out on 100 known thalassemia intermedia cases. The criteria for the inclusion was the patient to be known thalassemic, age at commencement of transfusion was more than three years and the interval between the transfusion was at least one month. Their brief clinical history was taken. Blood counts absolute values and haemoglobin electrophoresis were carried out. Blood samples were first screened for common beta chain mutation found in Pakistani population and then screened for Inv/Del G (A) ° , Xmn-1 polymorphism and alpha chains deletions.

 - Thalassemia can be diagnosed by the identification of characteristic abnormal DNA fragments which span the breakpoint of the deletion. The reaction mixture consisted of 1 l of primer, 20 l of buffer mixture and 0.1 l of taq polymerase. 2 l of DNA was added to each mixture tube. The PCR reaction included denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute and a final extension was carried out at 72°C for 3 minutes. Number of cycles carried out were 30. The amplified product was run on 6% acrylamide gel and results were recorded. Normal DNA as negative control, heterozygous and homozygous DNAs for delta beta mutations were run as controls with each batch.

Xmn-1 can recognize the C-T polymorphism at position – 158 from the Cap site of the - globin gene [7]. In order to demonstrate this polymorphism, a 641bp fragment of DNA flanking the polymorphism was amplified using the following primers.

5’ – GAA CTT AAG AGA TAA TGG CCT AA 5’ – ATG ACC CAT GGC GTC TGG ACT AG

The reaction mixture was prepared by adding 20 l of buffer, 1 l of primer, 0.1 l of Taq and 2 l of DNA. The PCR conditions were 1 minute denaturation time at 94°C, annealing at 60°C for 1 minute, extension at 72°C for 1 minute and 3 minutes of final extension at 72°C. Number of cycles carried out were 30. Tubes were removed from the PCR machine and the amplified fragment was digested with 10 units of enzyme Pdm-I (Fermentus) at 37°C overnight and the results were recorded after electrophoresis on 6% Acrylamide gel. Samples from a normal individual and known homozygous and heterozygous for Xmn-1 polymorphism were used as controls.

The common -thalassemia -2 determinants were detected by PCR conditions as explained for the $^{3.7}$ and $^{4.2}$ deletion by Baysal and Huisman 1994 [8].

RESULTS

Inv/Del G (A) ° , mutation was found in 6 of the patients. Four of the patients were Punjabi, one Baloch and one was Mahajir. Parents of all these patients were unrelated to each others. Their ages at present were 5- 32 years, three of the patients presented at 3 years of age, one at 4, one at 10 and the other at 32 years of age. Two of the patients presented with mild facial changes while the rest did not have any facial change. One of the patients have had marked splenomegaly and had undergone splenectomy, mild spleneomegaly was observed in three patients while the spleen of rest of the two was normal. Two of the patients had mild degree of jaundice and one had moderate jaundice. Two of the patients were getting transfusion every month, one getting transfusion after an interval of two months while the rest of three patients needed to be transfused after three months. Their haemoglobin level before transfusion was found to be 8 – 12.5 g/dl. HbF was found to be 97 – 100% and haemoglobin A_2 was absent in these patients.

All these patients were homozygous for Xmn-1 polymorphism (+/ +) and had / genotype. Results are summarized in table 1.

Figure 1

Table I: Genetic and clinical features of ° in the study

Features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (yrs)	3	17	14	25	32	5
Age at 1st transfusion	3	3	4	10	32	3
Transfusion Interval (months)	90	1	1	2	3	3
Total number of transfusion per year	1	12	12	6	4	4
Consanguinity	Unrelated	Unrelated	Unrelated	Unrelated	Unrelated	Unrelated
Ethnic group	Punjabi	Mahajir	Baloch	Punjabi	Punjabi	Punjabi
Facial changes	None	Mild	none	None	Mild	none
Hepatosplenomegaly	Mild	Mild	Mild	Absent	-	Absent
Splenectomy	-	-	-	-	+	-
Jaundice	+	+	-	-	++	-
Xmn-1 genotype	+/+	+/+	+/+	+/+	+/+	+/+
Alpha chain deletions	αα /αα	αα /αα	αα /αα	αα /αα	αα /αα	αα /αα
Pre transfusion HGB g/dl	12.5	8	9	10	9	11.9
MCV pg	72	69	71	72	71	70
HbF %	100	98	100	100	97	100
A_2 %	-	-	-	-	-	-
A %	-	2	-	-	3	-

DISCUSSION

Thalassemia intermedia is a heterogeneous group of disorders, their clinical severity varies from thalassemia carrier state to the transfusion-dependent thalassemia major phenotype. These patients do not depend on the regular transfusion for their survival. Variable clinical severity of thalassemia intermedia is a result of their genetic heterogeneity. The common hallmark of the defined molecular mechanism for thalassemia intermedia is a reduction in the imbalance of and non- globins chains synthesis. One of the mechanisms which reduces the imbalance of and non- globins chains is increase in the production of -globin chains which results from the coinheritance of genetic determinants such as thalassemia or hereditary persistence fetal hemoglobin (HPFH) [6]. Co-inheritance of thalassemia has an ameliorating effect on the severity of the disease and thus usually results in the thalassemia intermedia phenotype. Homozygotes and compound heterozygotes for these high HbF determinants are relatively rare and their clinical features have not been well documented [9].

-Thalassemias are heterogeneous disorders characterized by elevated levels of HbF in adult life [4]. It is a rare disorder in Pakistan [10]. Homozygous forms of (^{A} ^{G}) ° thalassemia which are rare were identified in this group of thalassemia intermedia patients and presented with a variable phenotype.

Many of the thalassemia are produced by the deletions of and globin genes. These are associated with persistent synthesis of chain at a much higher level than is observed in thalassemia. Production of high levels of chain leads to relatively a mild degree of globin chain imbalance and hence these conditions are much milder than the thalassemia. G thalassemia is characterized by the deletion of A genes and synthesis of G chains only. If the production of A is preserved in the mutation than both type of chains are produced and the condition is called ^{G} ^{A} thalassemia. At the molecular level these disorders are very heterogeneous and require the determination of underlying defects [11].

In this study 100 thalassemia intermedia patients were tested and six of them were found to have ^{G} (^{A}) ° Thalassemia deletions, all were homozygous for the deletion each having +/+ Xmn-1 and /. 100% haemoglobin F was found in four patients, but two of the patients had haemoglobin F of 97% and 98%. How ever almost complete absences of haemoglobin A_2 was observed in these patients. Four of the patients were Punjabi, one Baloch and one was Mahajir which describes the distribution of the gene among different ethnic groups of Pakistan.

Clinical presentation of these patients was mild as only two had mild facial changes and mild jaundice. These patients had mild hepatosplenomegaly, and variable transfusion dependence. One of them who presented at 32 years of age underwent splenectomy and requires transfusion after every 3 months having mean hemoglobin of 9g/dl. One of the patients was on transfusion at an interval of 3 months. Rest of the patients need transfusion after every 30 days. This reflects the variation in the clinical presentation of these patients. However these patients managed to maintain their hemoglobin level at 8g/dl or above [Table: I].

Although certain genotypes are often associated with a mild course, there is still considerable clinical variation with in each particular interaction. Xmn-1 polymorphism has been associated with increased the production of haemoglobin F in gene mutations that leave its promoter region intact [12, 13]. All of the patients in the present study with ^{A} ^{G} / ^{A} ^{G} had +/+ sites describing a strong linkage between the Xmn-1 Ggamma-polymorphism and the Asian-Indian Inv/Del. These findings may explain the ability of thalassemia patients being able to produce high levels of haemoglobin F.

Reduction in the imbalance of and non- globins chains

synthesis is a common hallmark to define the molecular mechanism for thalassemia intermedia [6]. None of the patients in this study demonstrated alpha chain deletions. Absence of alpha chain deletions in these patients further support the dependence of HbF favouring mechanisms being sufficient to produce a milder disease and strengthens the involvement of Xmn-1 polymorphism in the typical presentation of thalassemia.

Thus homozygous state of (^{A} ^{G}) ° is associated with thalassemia intermedia picture having the assistance of Xmn-1 polymorphism which may be one of the factors responsible for having high HbF levels. Further described by this study is the independence of alpha chain deletions which may have contributed by reducing the globin chain imbalance. These factors compliment thalassemia to present as a milder disorder.

Thus DNA based diagnosis is possible in the prenatal as well as in the postnatal period for the prediction of milder phenotype. A detailed molecular analysis is suggested for the thalassemic patients before the commencement of treatment. This will help to define their treatment approach and to avoid the consequences and exaggeration of disease.

ACKNOWLEDGMENT

I would like to extend my deepest gratitude to Gen. Suhaib Ahmed of Armed forces institute of pathology, Rawalpindi for his marvellous supervision in the study. Thanks are also due for the technical staff of the institute for their tremendous support.

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