ORIGINAL ARTICLE

# Relation of Xmn-1 Polymorphism & Five Common Indian Mutations of Thalassaemia with Phenotypic Presentation in β-Thalassaemia

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**Abstract**

Xmn-1 polymorphism is a known factor, which increases foetal haemoglobin production. Among, b thalassaemics in India five mutations are common. Disease severity was assessed based on age of presentation, age when received first transfusion and blood transfusion in ml/kg/year. Data was divided into three Xmn-1 categories, (+/+), (-/+), (-/-) and intergroup correlation was made. Mutations were divided into 2 groups, (group I) IVS 1-1 as one of its variables and (group II) without IVS 1-1. They were correlated. In Xmn-1 +/+ category 66.66% were diagnosed after one year of age, in mutations (group I) 53.57% had age of diagnosis after 1 year. 77.77% in Xmn-1 +/+ received their first blood transfusion after 1 year of age, in mutations (group I) 64.28% received their first blood transfusion after 1 year of age. About 66.66% patients in Xmn-1 +/+ category received blood <200ml/kg/year as against 72.22% in Xmn-1 -/- category. In mutations group I 57.14% received blood transfusion <200 ml/kg/year as against 68.18% in group II. It is concluded that the presence of Xmn-1 polymorphism and IVS 1-1 mutation leads to a milder phenotypic presentation causing a delay in onset of blood transfusions but dose not effect the amount of blood received /kg/year.

**Key Words**

Thalassaemia, Mutations, Xmn-1 Polymorphism, Phenotype, IVS 1-1.

## Introduction

Among the inherited disorders of blood, thalassaemia constitutes a major bulk of genetic diseases in India. It causes a high degree of morbidity (moderate to severe haemolytic anaemia) among vulnerable segments of the society. It has been estimated that in India with a population of 100 million at the millenium (2000) and a birth rate of 25/1000, there would be about 4.5 million carriers and about 9000 infants born each year with haemoglobinopathies (1). In a more recent multicentric study on the causes of referral for genetic counselling, it has been reported that thalassaemia/haemophilia (8.8%) is one of the top four diseases of haematological disorders in India (2). In view of this heavy genetic load, frequent blood transfusions, high cost of treatment and management, physical trauma along with psychological and mental harassment to the patients and their families, the preventive genetic approach has been realized to be the most suitable for the Indian setting.

Thalassaemia has proved to be the problem of haemoglobin (Hb) production. All the Hb's are tetrameric proteins composed of four polypeptide chains of two types α and β. The a-type globin chains are coded in humans by a complex of genes on chromosome 16 and the β-type chains by a complex of genes on chromosome 11. Studies of families in which the variants segregate have confirmed that most thalassaemia mutations map to the β-globin gene complex on chromosome 11. Those that

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did not map to the $\beta^0$-globin complex mapped to the $\alpha$-globin gene complex on chromosome 16. Genetic studies have indicated that the severe cases are caused by homozygosity for a gene that in heterozygous combination with its normal allele produces mild anaemia.

The carriers of β-thalassaemia have levels of haemoglobins – $A_2$ and F, which can be greater than 3.5% and 2% of the total haemoglobin respectively (3). Among the genetic factors known to affect HbF production are DNA sequence variations within the β-globin gene cluster. In particular, the (C→T) variation at position – 158 upstream of the $G_\gamma$ globin gene, which is detectable by the restriction enzyme Xmn-1. The sequence variation has been shown to increase HbF levels in b-thalassaemia anaemia (4).

It is well established that each population has a few common mutations along with a large number of rarer ones. Several studies undertaken in Indians (5,6) have shown that five mutations are common among Indians and account for about 90% of the mutant alleles. These include IVS 1-5 (G→C); 619 bp deletion; codon 8/9 (+G); IVS 1-1 (G→T); and codon 41/42 (-TCTT). These are present in different parts of India in varying frequencies. This information is useful for diagnosis in Indians. Hence the present study was done to find the relation of Xmn-1 polymorphism and five common Indian mutation with phenotypic presentation in b-thalassaemia.

## Material & Methods

Fifty patients representing 49 families and consisting of 33 males and 17 females, who were homozygous for β-thalassaemia and selected randomly at Preeti Tuli Thalassaemia Unit, Department of Paediatrics, Sir Ganga Ram Hospital, New Delhi were investigated. The patients were transfusion dependant and were categorized having transfusion dependant thalassaemia major. All of them were treated in the hospital with regular follow up. The patients were Indians with their families originating from different parts of Indian subcontinent. Their ages range from 3 months to 32 years. An informed consent was obtained.

Disease severity was assessed according to the following variables: age at the time of presentation, age of first transfusion and blood transfusion in ml/kg/yr.

The patients were screened for common thalassaemia mutations and for Xmn-1 polymorphism. The mutations of father and mother were evaluated only in case of doubtful results.

### DNA analysis

Five thalassaemia mutations, which are found commonly in Indian subjects, were investigated using genomic amplification of b-globin gene by polymerase chain reaction (PCR). Genotypes of the patients were analyzed at the Department of Genetics, Sir Ganga Ram Hospital, and New Delhi.

### DNA Mapping

Cellular DNA was prepared from the buffy coat cells of potassium EDTA anticoagulated blood samples by phenol chloroform extraction (7). DNA polymorphism analysis in the b globin gene cluster, $\alpha$ 5' $\beta$ globin haplotype was performed. A Hind II polymorphism with a e-globin gene probe, two Hind III polymorphisms with a g-globin gene probe and two Hind II polymorphisms with a $\psi$ β-globin gene probe (8). In addition each DNA sample was digested with Xmn-1 and hybridized to a $\gamma$ gene probe to determine the Xmn-1 polymorphic site at position -158 relative to the γ-globin gene (9).

### Detection of Mutations

Five b-thalassaemia mutations have been commonly found in Indians. One of them a 619bp deletion at the 3' end of the β-gene is detectable directly by restriction analysis. For the others, four sets of oligonucliotides were used as specific hybridization probes. Oligonucleotides of 19, 20, 21 and 23 base-pairs were synthesized on a DNA synthesizer (Applied Biosystems) and used as specific hybridization probes for detection of β-thalassaemia mutations at codons 8/9(+G), codon 15(G→A), codon 41/42 (-CTTT), IVS 1-1 (G→T) and IVS 1-5 (G→C).

## Results

The whole data was divided into three major groups according to the Xmn-1 +/+, -/+, -/-. The intergroup correlation with the various variables was made. Correlation was also carried out between mutation groups, which were divided into, first (group I) that had IVS 1-1 as at least one of its variables and second (group II) without IVS 1-1 variable. These groups were correlated with various phenotypic variables. P values were

calculated using student's t test and ANOVA analysis of variables. Pearson correlation between the various groups was also done. The results thus tabulated are as follows.

**Age at the Time of Diagnosis**

In Xmn-1 +/+ 3 (33.33%) were diagnosed less than 1year of age where as 6 (66.66%) were more than 1 year of age, the mean was 2.80 with a S.D. of 2.67. In Xmn-1 -/- 12 (66.66%) were diagnosed before one year where as 6 (33.33%) were diagnosed after 1 year of age, the mean was 2.03 with a S.D. of 3.15. In Xmn-1 -/+ 12 (52.17%) were diagnosed before 1 year of age and 11 (47.82%) after, the mean was 1.16 with a S.D. of 1.50. The P value thus calculated was 0.204, which was not significant.

A correlation was also calculated between Xmn-1 +/+ and -/- after dividing the whole cohort into two groups, one, who were diagnosed before 1 year of age and the second, who were diagnosed after 1 year of age. Though majority i.e. 66.66% in +/+ group were diagnosed after 1year of age, the P value calculated was 0.127 which was not statistically significant (Table I).

Among the group I i.e. with at least one IVS 1-1 variable, 13 (46.42%) had age at diagnosis below 1year. In the group II i.e. without IVS 1-1, 14 (63.63%) had age at diagnosis below 1 year. The P value calculated was 0.226, which was not significant. This shows that though majority of patients in the group with IVS 1-1 were diagnosed after 1 year of age, it was not statistically significant (Table II).

**Age at the Time of First Transfusion**

In Xmn-1 +/+ 2 (22.22%) received first transfusion before one year of age and 7 (77.77%) after one year of age, the mean was 3.46 with a S.D. of 2.50. In Xmn-1 -/- 12 (66.66%) received first transfusion before one year of age and 6 (33.33%) after, the mean being 2.45 with a S.D. of 4.36. In Xmn-1 -/+ 11 (47.82%) received their first transfusion before one year of age and 12 (52.17%) after, the mean being 1.54 and the S.D. was 1.56. P value calculated was 0.259, which was not statistically significant. As there was a wide variation between Xmn-1 +/+ and -/-, a correlation was calculated to see that, does presence of Xmn-1 polymorphism have a bearing on the age of first transfusion. The P value calculated was 0.037, which was significant. This show's that the presence of Xmn-1 polymorphism leads to a delayed need of blood transfusions (Table III).

It was seen that (64.28%) of the patients with at least one IVS 1-1 variable had received their first transfusion after 1 year of age. In the group without IVS 1-1 (68.18%) had received their first transfusion before 1 year of age. The P value calculated was 0.023, which was significant. This suggested that the presence of IVS 1-1 mutation lead to a delay in need of blood transfusion (Table IV).

**Blood Transfusion in ml/kg/yr**

The amount of blood received by the child in ml/kg/yr was calculated by standard formula = 300 x no.of transfusions per year / weight in kilograms. 300 being the approximate ml of packed cells at a PCV of 60%. They were divided into two groups. Group A, was those receiving blood < 200ml/kg/year and group B was of those who received blood > 200 ml/kg/year. It was found that in Xmn-1 +/+ category 6 (66.66%) were in group A as compared to Xmn-1 -/- category, where 13 (72%) were in group A. There was not much of variation in the two categories. The P value calculated was 0.402,which was not significant (Table V).

Correlation was also done with the five common Indian mutations. They were divided into two groups, first with at least one IVS 1-1 variable and second without IVS 1-1. In the first group there were 57% patients who received blood <200ml/kg/year. In the second group 68% received blood transfusion <200 ml/kg/year. There was not much variation in the two groups; the P value calculated was 0.559, which was not significant. This show's that there is no bearing of presence or absence of IVS 1-1 on the amount of blood a patient receives (Table VI).

**Table I**

**Age at diagnosis group analysis done using ANOVA and Student's Test**

| Age in year | 0—0.3 | 0.4—0.6 | 0.7—0.9 | 0.10—0.12 | 1.1—2 | 2.1—3 | 3.1—4 | 4.1—5 | > 5.1 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Xmn-1+/+ | 0 | 0 | 1 | 2 | 2 | 1 | 0 | 2 | 1 | 9 |
| Xmn-1 -/+ | 2 | 5 | 1 | 4 | 7 | 2 | 1 | 0 | 1 | 23 |
| Xmn-1 -/- | 0 | 4 | 2 | 6 | 2 | 0 | 1 | 1 | 2 | 18 |

**Table II**

**Mutation and age at the time of diagnosis cross-tabulation done using Pearson Correlations.**

| | Group I | Group II |
|---|---|---|
| Age<1yr | 13 | 14 |
| Age>1yr | 15 | 8 |
| **Total** | **28** | **22** |

**Table III**

**Age at first transfusion group analysis done using ANOVA and student's t test.**

| Age in year | 0—0.3 | 0.3—0.6 | 0.7—0.9 | 0.10—0.12 | 1.1—2 | 2.1—3 | 3.1—4 | 4.1—5 | > 5.1 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Xmn-1 +/+ | 0 | 0 | 0 | 2 | 1 | 2 | 0 | 3 | 1 | 9 |
| Xmn-1 -/+ | 0 | 6 | 0 | 5 | 6 | 3 | 2 | 0 | 1 | 23 |
| Xmn-1 -/- | 0 | 2 | 4 | 6 | 2 | 0 | 0 | 1 | 3 | 18 |

**Table IV**

**Mutation and age at first transfusion cross tabulation done using Pearson Correlations.**

| | Group I | Group II |
|---|---|---|
| Age< 1yr | 10 | 15 |
| Age> 1yr | 18 | 7 |
| Total | 28 | 22 |

**Table V**

**Correlation between amount of blood transfused in ml/kg/yr. and Xmn-1 polymorphism done using Pearson Correlations.**

| Blood transfused | < 200 | > 200 | Total |
|---|---|---|---|
| Xmn-1 +/+ | 6 | 3 | 9 |
| Xmn-1 -/+ | 12 | 11 | 23 |
| Xmn-1 -/- | 13 | 5 | 18 |

**Table VI**

**Correlation between amount of blood transfused in ml/kg/year with the mutation groups (group I=with at least one IVS 1-1 variable, group II=without IVS 1-1) done using Pearson Correlations.**

| Blood transfused | Group I | Group II |
|---|---|---|
| < 200 ml | 16 | 15 |
| > 200 ml | 12 | 7 |
| Total | 28 | 22 |

## Discussion

The effect of -158 C > T mutation on expression of Gg globin gene has been the subject of considerable interest. The association of some b-globin mutations with Xmn-1 site with elevated HbF expression has been previously published. The role of increased HbF response as an ameliorating factor has become evident in patients who were mildly affected despite being homozygotes or compound heterozygotes for $\beta^0$ or $\beta^+$ thalassaemia (10).

The patients were also assessed based on the age at diagnosis. In Xmn-1 + / + 33.33% were diagnosed at less than 1 year of age where as 66.66% were diagnosed after 1year of age. In Xmn-1 - / - category 66.66 % were diagnosed before 1 year of age and 33.33% after. In Xmn-1 - / + category 52.17% were diagnosed before 1 year of age and 47.82% after. However the P value calculated was not statistically significant. In the mutational analysis it was noted that 53.57% of patients were diagnosed after 1 year of age and in the group without IVS 1-1 63% were diagnosed before 1 year of age. The P value was not significant. This has also been noted in other studies, which state that the age at diagnosis may vary according to the awareness of the family and economic status (11).

In Xmn-1 + / + category 22.22% received their first transfusion below 1 year of age and 77.77% after 1 year, the mean being 3.46 with a S.D. of 2.50. In Xmn-1 - / - category 66.66% received first transfusion before 1 year and 33.33% received after 1 year of age, the mean being 2.45 with a S.D. of 4.36. In Xmn-1 - / + category 47.82% received blood transfusion before 1 year and 52.17% after 1 year of age the mean being 1.54 with a S.D. of 1.56. Though the P value was not statistically significant, its suggests that in Xmn-1 + / + category majority of the patients received their first blood transfusion after 1 year of age. A correlation was then calculated between Xmn-1+/+ and -/- to determine the effect of presence of Xmn-1 polymorphism on the age of transfusion. The P value calculated was significant. This show's that presence of Xmn-1 polymorphism delays the need of blood transfusions. The analysis of mutation groups revealed that the presence of IVS 1-1 mutation delays the onset of blood transfusion. This has also

been suggested in some studies which recognized that Xmn-1 + / + along with IVS 1-1 mutation has an effect which increases the HbF level which result's in slightly milder than expected disease in homozygotes and transfusion requirements are generally after 2 years of age (12,13).

It was also noted that in Xmn-1 + / + category 66.66% were transfused <200ml/kg/yr. In Xmn-1 - / - category 72.22% patients received blood <200ml/kg/yr. Thus the presence or absence of Xmn-1 polymorphism does not have an effect on the amount of blood transfused in ml/kg/yr. Correlation was also seen between the two mutational groups and it was found that this also had no bearing on the amount of blood transfused. It has been known that blood transfusion requirement increase under certain conditions such as sever anaemia, congestive cardiac failure, hyperspleenism, infections. Hypertransfusion is also carried out in thalassaemia major in certain patients. These factors influence the frequency of transfusion and thus the amount of blood transfused in ml/kg/year is not a reliable indicator for depicting the phenotipic presentation (11,14).

## Conclusion

A positive correlation in presence of Xmn-1 polymorphism and IVS 1-1 mutation was noted in age of receiving first blood transfusion. However it was noticed that age at diagnosis and blood transfusion in ml/kg/yr had no correlation with genotypic presentation. Thus it is concluded that the presence of Xmn-1 polymorphism and IVS 1-1 mutation lead to a milder phenotypic presentation and a delay in onset of blood transfusion.

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**ERRATUM**

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