

## The Prevalence and Heterogeneity of Beta Thalassemia Mutations in The Western Maharashtra Population: A Hospital Based Study

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**KEY WORDS** Beta thalassemia; molecular Heterogeneity;  $\beta^+$  thalassemia;  $\beta^0$  thalassemia; S $\beta^+$  and S $\beta^0$  thalassemia in India; Beta thalassemia mutations in India.

**ABSTRACT** The present study was undertaken with the objective to study molecular heterogeneity of  $\beta$  thalassemia, to correlate the phenotype with the genotype in  $\beta$  thalassemia cases, and to know the incidence of different  $\beta$  thalassemia mutations in western Maharashtra population, from the cases referred to the Genetic Clinic, B.J. Medical College, Pune. Total of 114 subjects were studied. These included 85 (74.6%) known  $\beta$  thalassemia carriers, 25 (21.9%) cases of  $\beta$  thalassemia major and 4 (3.5%) cases of sickle thalassemia. Total of 58 (50.9%) males and 56 (49%) females were studied. Amongst the total 114 cases studied 102 (89.5%) showed presence of IVS 1-5 mutation, while 12 (10.5%) cases showed presence of cd 41/42 mutation. Amongst the population studied the types of  $\beta$  thalassemia prevalent are  $\beta^+$  thalassemia,  $\beta^0$  thalassemia,  $\beta^+\beta^0$  thalassemia, S $\beta^+$  thalassemia S $\beta^0$  thalassemia. Amongst these  $\beta^+$  thalassemia is the most prevalent type. Frequency of blood transfusion (B.T.) was different in thalassemia patients with different beta globin genotypes. Hematological findings showed that presence of cd 41/42 mutation in homozygous state produces more severe phenotype than that of IVS 1-5 mutation. IVS 1-5 mutation in combination with sickle mutation produces less severe phenotype than that of cd 41/42 mutation with sickle mutation. The comparative incidence of IVS 1-5 mutation in W.M. population is higher than that reported for Maharashtra and T.N. Incidence of cd 41/42 mutation is similar to that found in Tamil Nadu and North-West Pakistan and higher than that reported for Maharashtra. The communities, which showed higher incidence of cd 41/42 mutation, are Muslim and Navbudha. An exclusive occurrence of IVS 1 5 (G-C) and cd 41/42 mutations in Western Maharashtra population will help us to prevent beta thalassemia by prenatal diagnosis.

### INTRODUCTION

$\beta$  Thalassemias are characterized by reduced or absent  $\beta$  globin gene expression. The incidence of  $\beta$  thalassemia trait in India ranges from 3.5 to 15%. Every year more than 10,000 children with thalassemia major are born in India (Varawalla

et al. 1991). The treatment available for thalassemia major is not satisfactory, so the prevention of disease by carrier detection and prenatal diagnosis is very important.  $\beta$  thalassemia can be broadly classified into two types,  $\beta^+$  thalassemia and  $\beta^0$  thalassemia. Due to molecular heterogeneity these two forms also show variable clinical and hematological pattern. In  $\beta^0$  thalassemia there is a total absence of  $\beta$  chain synthesis. In homozygous state it leads to severe transfusion dependent disease. In  $\beta^+$  thalassemia there is a reduced synthesis of  $\beta$  globin chains. The clinical and hematological picture is extremely variable. It is shown that these two forms of thalassemia in heterozygous state do not show significant hematological difference, but when these combine with HbS gene the result is different phenotype (Millard et al. 1977).

$\beta^+\beta^0$  Thalassemia – This is a compound heterozygous state of  $\beta$  thalassemia. If  $\beta$  thalassemia gene is of severe variety its interaction with  $\beta^0$  thalassemia produce typical  $\beta$  thalassemia major with high level of HbF. (Weatherall et al. 1981). Sickle cell  $\beta^+$  and sickle cell  $\beta^0$  thalassemias are the compound heterozygous states known as sickle thalassemias. Sickle cell  $\beta^+$  thalassemia is found to have milder course than sickle cell  $\beta^0$  thalassemia. Sickle cell  $\beta^+$  thalassemia produces HbS, HbF and HbA<sub>2</sub> with variable amount of HbA. The severe forms have low HbA levels (3 - 15%). Mild forms have higher HbA levels (18-30%) (Donaldson et al. 2000).

Seven different  $\beta$  thalassemia mutations are commonly known to occur in Asian Indians, which are IVS 1 5 (G-C), cd 41/42, cd 8-9, IVS 1 1 (G-T), 619 bp deletion, nonsense codon 15, frame shift at codon 16 (Varawalla et al. 1991; Kazazian et al. 1984).

The distribution of  $\beta$  thalassemia mutations on Indian subcontinent has been studied. IVS 1-5 mutation is the most common along with that cd 41/42 mutation is also present. The highest incidence of IVS 1-5 mutation is recorded in Tamil Nadu (81%) while that of cd 41/42 in Bengal (20%). (Varawalla et al. 1991; Thein et al. 1988).

The present study was undertaken with the objective to study molecular heterogeneity of  $\beta$  thalassemia, to correlate the phenotype with the genotype in  $\beta$  thalassemia cases, and to know the incidence of different  $\beta$  thalassemia mutations in western Maharashtra population, from the cases referred to the genetic clinic B.J. Medical College, Pune. The patient's population studied is from the western Maharashtra region.

### MATERIALS

Subjects studied were the known cases of  $\beta$  thalassemia and sickle thalassemia, which were referred initially to the genetic clinic for the diagnosis of hemoglobinopathies. Total of 114 subjects were studied. These included 85 (74.6%) known  $\beta$  thalassemia carriers, 25 (21.9%) cases of  $\beta$  thalassemia major and 4 (3.5%) cases of sickle thalassemia. Total of 58 (50.9%) males and 56 (49%) females were studied, from Western Maharashtra region.

### METHODS

Initially family history of each subject was studied with the help of following proforma. Name, Age, Sex, Address, Community, Consanguinity, pedigree, blood transfusion given, age at the onset of disease. Diagnosis of  $\beta$  thalassemia was done by the standard hematological methods. Cellulose acetate electrophoresis was done at alkaline pH, (Dacies and Lewis 1995) HbA<sub>2</sub> quantitation, HbF quantitation was done on Bio-Rad Variant (Wilson et al. 1983; Fuscharoen et al. 1988). Sickling test was done for confirmation of sickle cell anemia. Then 5 cc. blood of the diagnosed cases was collected in the wintrobles bulb and DNA isolation was performed by phenol chloroform extraction method (John 1991).

ABMS PCR was carried out for detection of  $\beta$  thalassemia mutations (Thein et al. 1988; Newton et al. 1989). DNA samples were screened for

IVS 1.5 (G-C), frame shift at cd 8/9, IVS 1 1 (G-T), 619 bp deletion, nonsense codon 15 mutations. A 25  $\mu$ l reaction mixture contained 0.5  $\mu$ g genomic DNA, 5 p mol of each oligonucleotide primer, 200  $\mu$ M of each dNTP in 10 mM tris-Cl, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>. The thermal cycling was done as follows. Initial cycle of 95°C for 5 min followed by 25 cycles of 94°C 1 min, 65°C 1 min 15 sec. Last cycle of extension at 72°C for 5 min. The amplified product was electrophoresed using 0.5 X TBE buffer using 2% agarose gel. The bands were visualized on U.V. transilluminator after EtBr staining.

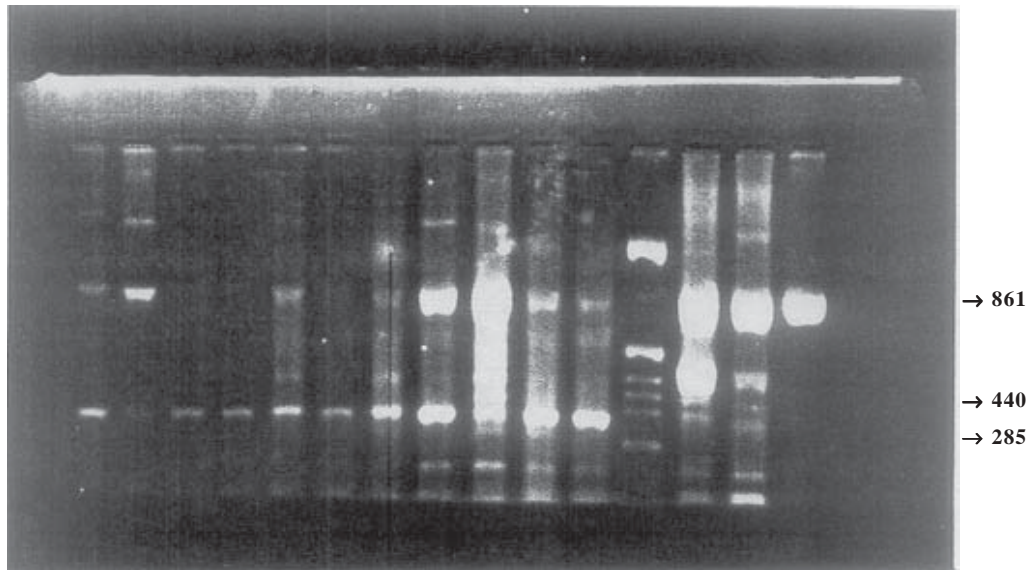
### RESULTS

Amongst the total 114 cases studied 102 (89.5%) showed presence of IVS 1-5 mutation (Fig. 1), while 12 (10.5%) cases showed presence of cd (41/42) mutation (Fig. 2).

Amongst the total 85 cases of thalassemia trait, IVS 1-5 mutation was present in 77 (91.7%) cases while cd 41/42 mutation was present in 7 (8.3%) cases. Amongst total 25 cases of thalassemia Major IVS 1-5 mutation was present in 22 (88.0%) cases while cd 41/42 mutations were presents in 3 (12.0%) cases. Amongst the 4 cases of sickle thalassemia, IVS 1-5 mutation was present in 2 (50%) cases while cd (41/42) mutation was present in 2 (50%) cases (Table 1).

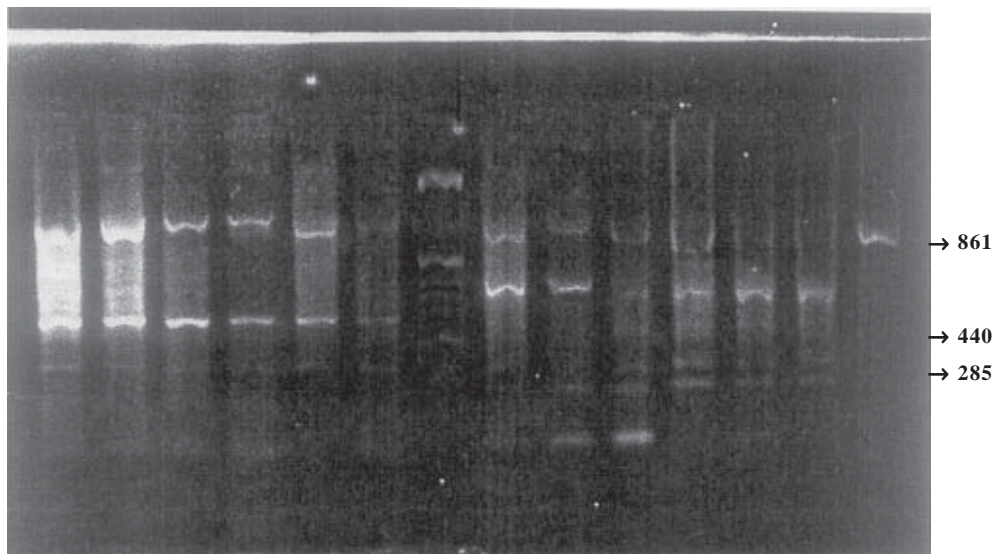
### Genotype Phenotype Correlation

In case of  $\beta$  thalassemia carriers with cd 41/42 mutation ( $\beta^0$  thalassemia) the average HbA<sub>2</sub> was 5.57% and HbF 1.4% (Table 2). The couples with this mutation had history of death of siblings. In cases of  $\beta^0$  thalassemia carriers with IVS1-5 mutation the average Hb was 12.4g%, HbA<sub>2</sub> 5.4% and HbF 2.2% (Table 2). Amongst 78 carriers of IVS1-5 mutation 6 couples showed history of death of siblings at 5 months to 5 yr. of age. Amongst the total 7 carriers of cd 41/42 mutation, 4 couples showed history of death of their siblings at 9 months to 3 yr. of age (Table 3). Amongst the two cases of homozygous  $\beta^0$  thalassemia the average Hb was 6.0 g%. HbA<sub>2</sub> 4.0% and HbF 79.0% one child received blood transfusion at 1-2 months interval up to 3 years of of age and then died, another child received blood transfusion at 1-month interval and died at 11 months of age



**Fig. 1.** A photograph showing results obtained after electrophoresis of amplified product using mutation specific Primers IVS 1-5 G-C and cd 41/42.

Well No. 1 to 11 showing internal control band of 861bp and mutation specific band of 285 bp with mutation specific Primer IVS 1-5 G-C. Well no. 8 shows positive control for IVS 1 5. Well no.12 shows molecular weight marker (PBR 322 Hinf 1 digest), well no.13, 14 shows 440 bp band with cd 42/42 mutation specific primers, and the internal control bands of 861 bp, well no. 15 shows 861 bp band in negative control.



**Fig. 2.** A photograph showing results obtained after electrophoresis of amplified product using mutation specific Primers IVS 1-5 G-C and cd 41/42.

Well no. 1 to 6 shows an internal control band of 861 bp and a mutant band of 285 bp with mutation specific primers IVS 1 5. Well no. 7 shows molecular weight marker (PBR 322 hinf 1 digest). Well no. 8 shows positive control for cd 41/42 mutation. Well no.9-13 show internal control band of 861 bp and mutant band of 440 bp with mutant specific primers of cd 41/42. Well no. 14 shows negative control.

**Table 1: Distribution of beta thalassemia mutations according to the type of disease**

Disease Mutation	Thalassemia trait		Thalassemia major		Sickle thalassemia		Total No. of Cases	Frequency %
	No. of chro- mosomes	Per cent	No. of chro- mosomes	Per cent	No. of chro- mosomes	Percent		
IVS 1-5 (G-C)	78	91.7	22	88.0	2	50.0	102	89.5
Cd 41/42	7	8.3	3	12.0	2	50.0	12	10.5

**Table 2: Hematological findings in beta thalassemia cases from western Maharashtra region**

Type of thalassemia	State of Disease	Hb g%	HbA <sub>2</sub> %	HbF %	HbS %	HbA %	Mutation	No. of Cases	Per cent
$\beta^0$	Heterozygous	11.7	5.5	1.4	-	84.0	Cd 41/42	9	7.9
$\beta^0$	Homozygous	6.0	4.0	79.0	-	18	Cd 41/42	2	1.8
$\beta^0/\beta^+$	Compound Heterozygous	7.8	4.7	84.3	-	13.0	Cd 41/42 IVS 1 5	3	2.6
$S\beta^0$	Compound Heterozygous	8.0	5.0	25.0	68.0	5.0	Cd 41/42 Sickle	2	1.8
$S\beta^+$	Compound Heterozygous	13.0	3.5	28.0	61.7	19.8	IVS 15 Sickle	2	1.8
$\beta^+$	Heterozygous	12.4	5.4	2.2	-	83.0	IVS 1-5	76	66.6
$\beta^+$	Homozygous	7.05	3.9	48.6	-	54.0	IVS 1-5	20	17.5
Total	-	-	-	-	-	-	-	114	100.0

**Table 3: Average frequency of blood transfusion and age at the onset of disease in homozygotes and compound heterozygotes according to their genotype**

Genotype	Age at onset of disease	Freq. of B.T.	Age at death
Cd 41/42	6 months – 1.5 yrs	4-8 weeks	9m – 3yrs
Cd 41/42/ IVS 1 5	1 – 5 yrs	No/ once in life	1.5 yrs/ no death
IVS 1 5	7- 18 months	4-6 weeks	No death/5m-5yrs

(Table 3). In case of compound heterozygotes with IVS1-5 and cd 41/42 mutation i.e.  $\beta^+/\beta^0$  thalassemia the average Hb was 7.8% HbA<sub>2</sub> 4.7% and HbF 98% in one case and 75.0% in two cases studied. One child had received 1 blood transfusion in 11 years of life span while another has not received blood transfusion until now and both are still surviving. In 3<sup>rd</sup> case child had received only one B.T. and died at 1½ years of age. In case of homozygotes with IVS1-5 mutation average Hb was 7.05g% HbA<sub>2</sub> 3.9% and HbF 48.6%. The average frequency of B.T. was 2-4 weeks.

In case of compound heterozygotes of sickle thalassemia with IVS 1-5 mutation, the average HbA<sub>2</sub> was 3.5 % HbF was 28.0%, average HbS was 61.7%, and HbA 19.8%. There was no history B.T. There was not any history of death of siblings in a couple with  $S\beta^+$  thalassemia. In case of compound heterozygotes of sickle thalassemia with cd 41/42 mutation, the average Hb was 8.0 g% HbA<sub>2</sub> 5.0%, HbF 25.0%. HbS 68.0 and HbA 5%. There was no history of B.T. Couples in

which one member was carrier for cd 41/ 42 mutation along with sickle mutation, showed history of death of siblings.

#### Comparative Incidence of $\beta$ Thalassemia Mutation in Western Maharashtra Population with Other Parts of India

In Maharashtra incidence of IVS 1-5 mutation reported is 54%, that in Sindh is 12%, Punjab 38%, Bengal 60%, Tamil Nadu 81%, Gujarat 41%. (Varawalla et al. 1991). Incidence of IVS 1-5 mutation reported by us for W.M. region is around (90.4%) which is quite higher than that reported for Maharashtra.

Incidence of cd 41/42 mutation reported for Maharashtra is 7.6% that for N.W. Pakistan is 10%, Bengal 20%, Tamil Nadu 10%. Incidence reported by us for W.M. Region is 9.6% which is higher than that reported for Maharashtra.

The communities that showed presence of cd 41/42 mutation are Muslim (4), Navbudha (3), Lingayat (2), Matang (1), Brahmin (1).



## DISCUSSION

Amongst the population studied the types of  $\beta$  thalassemias prevalent are  $\beta^+$  thalassemia,  $\beta^0$  thalassemia,  $\beta^+/\beta^0$  thalassemia,  $S\beta^+$  thalassemia  $S\beta^0$  thalassemia. Amongst these,  $\beta^+$  thalassemia is the most prevalent type.

The incidence of IVS 1-5 mutation is 89.5% and cd 41/42 mutation is 10.5% in Western Maharashtra population.

$\beta^0$  thalassemia in Asian Indians is found to be caused by either cd 41/42 mutation, 0.6 kb deletion or 619 bp deletion (Spritz and Orkin 1982; Kazazian et al. 1984).  $\beta^0$  thalassemia in Western Maharashtra population is found to be caused by cd 41/42 mutation only.

An exclusive occurrence of IVS 1 5 mutation is reported in Orissa state of India. (Kulozik et al. 1991). In Western Maharashtra population also around 90% cases showed presence of this mutation.

Hematological findings showed that presence of cd 41/42 mutation in homozygous state produces more severe phenotype than that of IVS 1-5 mutation. Heterozygotes of these two mutations do not show significant hematological difference. IVS 1-5 mutation in combination with sickle mutation produces less severe phenotype than that of cd 41/42 mutation with sickle mutation.

Frequency of B.T. was different in thalassemia patients with different genotypes. Frequency of blood transfusion was 2-4 Wks in homozygotes of IVS 1-5 mutation, it was similar in homozygotes of cd 41/42 mutation but the shorter lifespan was seen in these. In compound heterozygotes of IVS 1-5 and cd 41/42 mutations the frequency of blood transfusion was very less (once or twice in a lifespan) or there was no history of blood transfusion, but early death was seen in some cases (Table 3).

The comparative incidence of IVS 1-5 mutation in Western Maharashtra population is higher than that reported for Maharashtra and Tamil Nadu. Incidence of cd 41/42 mutation is similar to that found in Tamil Nadu and N.W. Pakistan and higher than that reported for Maharashtra. The communities, which showed higher incidence of cd 41/42 mutation are Muslim and Navbudha.

An exclusive presence of two mutations IVS 1-5 and cd 41/42 in Western Maharashtra population can be correlated to the independent origin of these mutations in this population group. This will definitely help us to prevent beta

thalassemia and sickle thalassemia by prenatal diagnosis.

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