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Spectrum of Mutations in Beta-Thalassemia in Maharashtrian Population

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KEYWORDS

Beta-thalassemia, IVS 1-5 G>C, Molecular study, Mutation analysis.

ABSTRACT:

Background: Beta thalassemia is an auto-recessive disorder that shows the most common gene mutation in hemoglobinopathies. The average prevalence of β-thalassemia carriers in India is 3-4%. Nationally, IVS 1-5(G>C) is the single most common mutant allele and represents 54.7% of all β-thalassemia mutations.

Aim and objectives: To identify the beta-globin gene mutations causing beta-thalassemia (β-thalassemia) in Maharashtrian population.

Material and methods: This prospective observational study was carried out for 18 months in a tertiary health centre. 3ml of blood samples were collected in EDTA vacutainers from patients who were requested for hemoglobinopathy screening. These patients were examined simultaneously for complete blood count (CBC) and peripheral blood smear (PBS). CBC was done with the help of a fully automated hematology analyzer (five-cell part differential cell counter) further screening for hemoglobinopathy was carried out with Bio-rad variant II Beta Thal Short. Deoxyribonucleic acid (DNA) was isolated from beta-thalassemia cases using Gentra Pure gene- KIT. Biotinylated primers were used to amplify the region of interest in the beta-globin gene. The amplicon was used for Reverse Dot Blot (RDB) to study the following common mutations Codon 8/9 (+G), Codon 15 (G– A), IVS 1:5 (G-C), Codon 30 (G-C), Codon 41/42 (-TCTT), IVS 1:1 (G-T), IVS 1:1 (G-A) and 619 base pair (bp) deletion.

Results: The most common mutation found is IVS 1:5 (G-C) (74%) along with Cod 16 and IVS-II which are uncommon in the study population.

Conclusion: The pathogenic beta thalassemia variants, identified during the present study can be employed for the diagnosis, carrier and prenatal screening, and planning therapy of thalassemia.

INTRODUCTION

Haemoglobinopathies are inherited, autosomal recessive, disorders of haemoglobin. Haemoglobin is a tetramer. It has two subunits each of β and α- globin chains. Haemoglobin synthesis is controlled by β-globin gene in chromosome 11 and α-globin gene in chromosome 16.[1] Beta globin gene, present on chromosome 11 (11p15.15), is a cluster composed of five genes in order of expression : ε (HBE), Gγ (HBG2), Aγ (HBG1), δ (HBD), and β (HBB).[2] Any genetic defects involving these genes leads to diminished, abnormal or no synthesis of adult haemoglobin, resulting in a condition called **"Haemoglobinopathy".** The term haemoglobinopathy comprises alpha thalassaemia, beta thalassemia and structural variants of the haemoglobin (Hb S, HbE, Hb O). Around 7% of individuals globally possess a variant gene responsible for abnormal haemoglobin production.[3] Beta thalassaemia is the most severe and prevalent form in India. Carrier rates fluctuate between 0.3% to 15%.[4] India showcases a diverse range of beta-thalassemia mutations, including the notably prevalent IVS1-5 (G>C).[5] In Maharashtra, the molecular spectrum of beta-thalassemia shows significant diversity due to the varied ethnic backgrounds and genetic makeup of the

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population. Key findings indicate that IVSI-5(G>C) mutation is notably prevalent, accounting for a substantial portion of beta-thalassemia alleles. Other mutations Cod 30(G>C), Cod 15(G>A) reflects on genetic heterogeneity in Maharashtra.[6,7] It is highly recommended to screen the population at large before marriage / pregnancy– the target population mainly being senior college students, premarital age group, newlywed couples (Mass Screening). However, if this is not done, it becomes important that the presence of haemoglobinopathies in the expectant parents is detected in a timely manner. During the antenatal period, the screening needs to be offered early in pregnancy – at the first antenatal visit / registration, to allow time for fathers to be screened. An early detection helps the couple to make timely informed choice.

MATERIAL AND METHODS

This prospective observational study was conducted to detect specific β-thalassemia mutations in diagnosed cases of beta-thalassemia by HPLC. It was carried out for a period of eighteen months from August 2022 – December 2023 in a tertiary care hospital in western India. The study received approval from the institutional ethics committee. A total of 80 cases that satisfied inclusion and exclusion criteria (infants less than 3 months), were included in the study. 3ml of blood samples were collected in EDTA vacutainers from confirmed cases of Beta thalassemia and processed for DNA extraction and mutation analysis following written consent for adults >18 years and assent for <18 years of age. Haemoglobin High-Performance Liquid Chromatography was performed on Bio-rad variant II Beta Thal Short. Gentra Pure gene- KIT- Method was used for DNA isolation. The probes were immobilized to the membrane. Biotinylated Primers were used to amplify the region of interest.

Reverse Dot Blot (RDB) was used to study the following mutations Cod 8/9 (+G), Cod 15 (G– A), Cod IVS 1:5 (G-C), Cod 30 (G-C), Cod 41/42 (-TCTT), IVS 1:1 (G-T), IVS 1:1 (G-A) and 619 bp deletion. For identification of mutations not included in our panel, the samples were outsourced to a molecular laboratory for Sanger sequencing.

The data was statistically analysed using Statistical Package for Social Sciences (SPSS version 24.0 IBM Corporation, USA) for MS Windows. Percentage and prevalence of various mutations associated with thalassaemia were calculated and presented in a tabular format.

RESULTS

Out of 80 cases of β-thalassemia studied, n=39 aged between 26 – 30 years (48.8%). 12 cases (15.0%) were aged below 18 years, 16 cases (20.0%) were between 18 – 25 years and 13 were more than 30 years of age. The mean ± SD of age of cases studied was 24.52 ± 9.76 (Table 1)

Females, n=45 were the predominant study population (56.2%) and n=35 (43.8%) were male. The male-to-female sex ratio is 1.0:0.78. (Table 2)

Overall, out of 80 cases of β -thalassemia studied, the most prevalent type of mutation was IVS 1:5 (G-C) type, present in 60 cases (75.0%), the second most pervasive mutation was COD 30 (G-A), present in 9 cases (11.3%), the third most prevalent type of mutation was COD 41/42 (-TCTT), present in 04 cases (5.0%), and 3 cases of COD 15 (G>A). Lesser prevalent mutations include: 2 cases each (2.5% each) had COD 8/9 (+G) and COD 1:1 (G>A), 1 case each (1.2%) had COD 16(-C) and IVS 2 type of mutations in the study group. 619bp deletion and IVS 1:1 (G>T) remained undetected in this study population. (Tables 3,4 & 5).

Table I: Age distribution (n=80).

Age group (years)	No. of cases	% of cases
<18	12	15.0
18 – 25	16	20.0
26 – 30	39	48.8
>30	13	16.3
Total	**80**	**100.0**

Table II: Sex distribution (n=80).

Sex	No. of cases	% of cases
Male	35	43.8
Female	45	56.2
Total	**80**	**100.0**

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Table III: Distribution of impression on Hb-HPLC (n=80).

Impression on Hb-HPLC	No. of cases	% of cases
β Thalassemia Homozygous (Major)	10	12.5
β Thalassemia minor (Heterozygous)	68	85.0
B-Compound Heterozygous	2	2.5
Total	**80**	**100.0**

Table IV: Distribution of prevalence of various β -thalassemia mutations(n=80).

al cases (n=80)	Present		Absent	
Mutation	**No. of cases**	**% of cases**	**No. of cases**	**% of cases**
COD 8/9 (+G)	2	2.5	78	97.5
COD 15 (G-A)	3	3.8	77	96.3
COD 16 (-C)	1	1.2	79	98.8
COD 30 (G-A)	**9**	**11.3**	71	88.8
COD 41/42 (-TCTT)	4	5.0	76	95.0
IVS 1:1 (G-T)	0	0.0	80	100.0
IVS 1:1 (G-A)	2	2.5	78	97.5
IVS 1:5 (G-C)	**60**	**75.0**	20	25.0
IVS 2	1	1.2	79	98.8
619 bp deletion	0	0.0	80	100.0

Abb: COD= Codon, bp= base pair

Table V: Distribution of prevalence of various β -thalassemia mutations (n=80).

		Homozygous (n=10)		Heterozygous (n=68)		Compound Heterozygous(n=2)		Total (n=80)	
Mutation		**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
COD 8/9 (+G)	Present	0	0.0	**2**	**2.9**	0	0.0	**2**	**2.5**
	Absent	10	100.0	66	97.1	2	100.0	78	97.5
COD 15 (G-A)	Present	0	0.0	**3**	**4.4**	0	0.0	**3**	**3.7**
	Absent	10	100.0	65	95.6	2	100.0	77	96.3
COD 16	Present	**1**	**10.0**	0	0.0	0	0.0	**1**	**1.2**
	Absent	9	90.0	68	100.0	2	100.0	79	98.8
COD 30 (G-A)	Present	0	0.0	**9**	**13.2**	0	0.0	**9**	**11.2**
	Absent	10	100.0	59	86.8	2	100.0	71	88.8
COD 41/42(-TCTT)	Present	**1**	**10.0**	**3**	**4.4**	0	0.0	**4**	**5.0**
	Absent	9	90.0	65	95.6	2	100.0	76	95.0
IVS 1:1 (G-T)	Present	0	0.0	0	0.0	0	0.0	0	0.0
	Absent	10	100.0	68	100.0	2	100.0	80	100.0
IVS 1:1 (G-A)	Present	**2**	**20.0**	0	0.0	0	0.0	**2**	**2.5**
	Absent	8	80.0	68	100.0	2	100.0	78	97.5
IVS 1:5 (G-C)	Present	**8**	**80.0**	**50**	**73.5**	**2**	**100.0**	**60**	**75.0**
	Absent	2	20.0	18	26.5	0	0.0	20	25.0
IVS 2	Present	0	0.0	**1**	**1.5**	0	0.0	**1**	**1.2**
	Absent	10	100.0	67	98.5	2	100.0	79	98.8

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619 bp deletion	Present	0	0.0	0	0.0	0	0.0	0	0.0
	Absent	10	100.0	68	100.0	2	100.0	80	100.0

Abb: COD= Codon, bp= base pair

Table VI: Mutations identified in the study and their variant details[14,15,16,17,18,19,20]

S. No:	Mutations	ClinVar Identifiers	HGVS Nomenclature (MANE select)	VARIATION ID	TYPE AND LENGTH	LOCATION	MOLECULAR CONSEQUENCE	CLASSIFICATION AND IMPACT
1	IVS-1-5 G>C	NM_000518.5 (HBB):c.92+5 G>C	NM_000518.5:c.92+5 G>C MANE SELECT	15447	Single nucleotide variant, 1 bp	11p15.4	Intron variant	Germline-Pathogenic
2	CD 30 (G>A) Other names: IVS I (-1) AGG> AAG (Arg>Lys)	NM_000518.5 (HBB):c.92G >A (p.Arg31Lys)	NM_000518.5:c.92G>A MANE SELECT	36337	Single nucleotide variant, 1 bp	11p15.4	Missense	Germline-Pathogenic/ Likely pathogenic
3	CD 8/9 (+G) Other names: HBB:c.27_28insG cd8/9+G	NM_000518.5 (HBB):c.27dup (p.Ser10fs)	NM_000518.5:c.27dup MANE SELECT NM_000518.5:c.27dupG MANE SELECT	36308	Duplication, 1 bp	11p15.4	Frameshift	Germline-Conflicting classifications of pathogenicity Pathogenic Uncertain significance
4	CD 41/42 (-CTTT) Other Names: 41/42-TTCT	NM_000518.5 (HBB):c.126_129del (p.Phe42fs)	NM_000518.5:c.126_129del MANE SELECT NM_000518.5:c.126_129delCTTT MANE SELECT	15417	Deletion, 4 bp	11p15.4	Frameshift	Germline-Pathogenic/ Likely pathogenic
5	CD 15 TGG> TGA	NM_000518.5 (HBB):c.48G >A (p.Trp16Ter)	NM_000518.5:c.48G>A MANE SELECT	38646	Single nucleotide variant, 1 bp	11p15.4	Nonsense	Germline-Pathogenic

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6	IVS-II-837 (T>G)	NM_000518.5 (HBB):c.316-14T>G	NM_000518.5:c.316-14T>G MANE SELECT	36313	Single nucleotide variant, 1 bp	11p15.4	Intron variant	Germline-Pathogenic/ Likely pathogenic
7	CD 16 GGC>GG-^ (CD 16 -C)	NM_000518.5 (HBB):c.51del (p.Lys18fs)	NM_000518.5:c.51delC MANE SELECT	15414	Deletion, 1 bp	11p15.4	Frameshift	Germline-Pathogenic

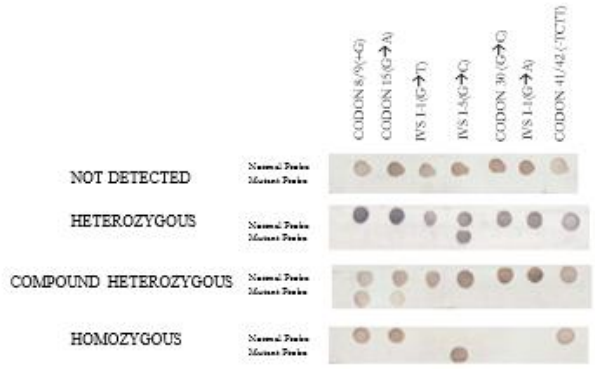

Figure I: Mutation detection by Reverse Dot Blot

DISCUSSION

β-Thalassaemia both in its heterozygous and homozygous forms is typically inherited following an autosomal recessive pattern as per Mendelian genetics. This is caused by a germline mutation in chromosome 11p15.4.[8]

The alpha and beta loci determine the structure of the two polypeptide chains in adult haemoglobin. Beta-globin cluster genes are arranged as: 5'-epsilon --gamma-G -- gamma-A -- delta -- beta-3'.[9]

In this study, overall, the majority of the study population comprises individuals in the reproductive age group with a slight inclination towards females than males. The study population for homozygous beta-thalassaemia predominantly includes infants and young adults.

Amongst the homozygous group, intron variant mutations, IVS 1-5 (G>C), (NM_000518.5(HBB):c.92+5G>C) was most prevalent. Other mutations detected were IVS 1-1 (G>A). This was specific to the homozygous population. Two cases showed co-expression of IVS 1-5 (G>C) with COD 41/42(-TCTT) and COD 16 respectively.

In the heterozygous beta-thalassaemia mutated population, IVS 1-5 (G>C) predominate. Followed by missense mutation in COD 30 (G>A) (NM_000518.5(HBB):c.92G>A (p.Arg31Lys), frameshift mutation in COD 41/42 (-TCTT) (NM_000518.5(HBB):c.126_129del (p.Phe42fs) and non-sense mutation COD 15 (TGG>TGA) (NM_000518.5(HBB):c.48G>A (p.Trp16Ter) and few showed frameshift mutation in COD 8/9 (NM_000518.5(HBB):c.27dup (p.Ser10fs). Two cases showed compound heterozygous condition i.e. IVS 1-5 (G>C) with HBD, Sickle cell disease respectively.

This study population did not show mutation for IVS 1-1(G>T) or 619bp del.

Similar studies conducted over various regions in India by Sinha S[7], Chakrabarti P et al.[10] Satapathy N et al[11] Mishra AS and team[12] aligns with our findings.

The differences, though, lie in prevalence of other mutations which shows regional differences. In our study, COD 30 (G>A) followed by COD 41/42 (-TCTT) and COD 15 (TGG>TGA) were more prevalent than Cod 8/9, IVS1-1 (G>T) and 619 bp deletions remained undetected.

Chakrabarti P et al.[10] Prevalence of beta-thalassemia mutations was studied in two northern parts of India. Mutations were analysed in 62 patients. The study revealed four major mutations: IVS1 nt5 (G→C), F.S 8/9 (+G), F.S 41/42 (-TCTT) and del 619bp, which accounted for 94.1% of all alleles studied. In particular, the IVS1 nt5 (G → C) mutation was most frequent, followed by F.S 8/9 (+G). The results concluded that IVS1 nt5 (G → C) and F.S 8/9 (+G) are dominant mutations in these Indian states.

Satapathy N et al.[11] conducted a study analysing β gene mutations in populations from northern Odisha, India,

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involving 98 patients. Most notable were IVS I-5(G->C), Cd 41/42(-CTTT)." The research also identified other mutations IVS I-1(G->T), cd 15(G->A), Cd 8/9(+G),) Cd 30(G->C). This research contributes significantly to understanding the genetic landscape of β-thalassemia in this specific geographic area of India.

Amongst the Gharwal population, Mishra AS and team[12] showed that the spectrum of other mutations included IVS 1-1 G-T, Codon 41/42 (-TCTT), Codon 8/9, and the 619bp deletion.

Selvaraj B et al.[13] determined beta-thalassemia mutations among 387 heterozygous carriers in Chennai. IVS 1-5 (G-C) mutation was particularly common.

As discussed earlier, although these studies confirm the existing knowledge of IVS 1-5 (G>C) being most prevalent mutation, it also points the spectrum of mutation involving IVS II. As opposed to the rare variants: IVS II-1 (G>T) and IVS II 781 (C-G), identified amongst the heterozygous beta thalassaemia population in Chennai, our study identified the variant IVS II 837 (T>G).

This analysis also helps in categorizing and assessing the severity of pathogenicity (classified according to ClinVAR) and helps for better proper counselling of patients, especially of those newly diagnosed during pre-natal checkups.

The various mutations identified in this study and their respective variant details along with its pathogenicity is summarised in the table below. (Table 6) [14,15,16,17,18,19,20]

CONCLUSION

As is seen in our study, most of the patients are those in the reproductive age group and were referred for hemoglobinopathy screening as a part of ante-natal screening. It is alarming that the awareness regarding the hereditary hemoglobinopathies is still insignificant.

The data collected in this study is representative of the common mutations seen in the state of Maharashtra as it is conducted in a tertiary centre.

In light of the persistently distinct inherited divisions in Indian society resulting from marriages within castes and communities, community-specific mutation testing paves a path for an effective implementation of genetic education, early screening, and primary prevention initiatives.

Increasing incidence of β-thalassemia worldwide, calls for efficient measures for reduction in β-thalassemia births at national levels. The heterogeneity of β-thalassemia alleles and early screening and identification minimizes the burden of genetic testing of foetuses.

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Conflicts of Interest- Nil

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List of Abbreviations:

Abbreviation	Definition
a) Hb – HPLC	Hemoglobin High-Performance Liquid Chromatography
b) CBC	Complete Blood Count
c) PBS	Peripheral Blood Smear
d) PCR	Polymerase Chain Reaction
e) RDB	Reverse Dot Blot
f) Cod/ CD	Codon
g) bp	Base Pair