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ORIGINAL RESEARCH

Mutation analysis of β-thalassemia in East-Western Indian population: a recent molecular approach

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Background: β-Thalassemia is the most prevalent genetic disorder in India. Its traits and coinheritance vary from mild to severe conditions, resulting in thalassemia minor, intermediate, and major, depending upon many factors.

Purpose: The objective of this study was to identify the incidence of β-thalassemia traits, their coinheritance, and mutations, as well as to support the patients already diagnosed with β-thalassemia in East-Western Indian population for better management.

Patients and methods: Seventy-five referral cases for β-thalassemia were analyzed for various β-thalassemia traits, heterozygosity, and homozygosity conditions. Blood phenotypic parameters using cell counter and capillary electrophoresis were investigated. Analyses of eight common mutations of thalassemia in India were carried out using polymerase chain reaction-amplification refractory mutation system, end point polymerase chain reaction, and DNA sequencing methods.

Results: Of these (75) referral cases from East-Western Indian region, 68 were positive for β-thalassemia (90.67%). The majority of case types were of β-thalassemia minor (49, 65.33%), followed by HbE traits (6, 8.0%) and β-thalassemia major, including heterozygous and homozygous (5, 6.66%; 4, 5.33%) types and then HbE homozygous (2, 2.66%), as well as one each of the HbE/β-thalassemia and HbD/β-thalassemia (1, 1.34%) combination. Mutation analysis also revealed that the highest frequency of mutation was c.92+5G>C (41, 60.29%) followed by deletion 619bp (9, 13.23%) and c.79G>A (8, 11.76%) in our study group. Five cases (nos. 24, 27, 33, 58, and 71) exhibited coinheritance between β^0/β^+ (2), β^0/β D (1), and c.124_127delTTCT/β^+ or β^0 (2) affecting the Rajasthani and Gujarati populations in our study of the Western region of India.

Conclusion: We strongly recommend these Western populations for genetic screening before adopting reproductive technologies and interracial marital relations.

Keywords: β-thalassemia traits, coinheritance, hematogram, capillary electrophoresis, PCR-ARMS, DNA sequencing, mutation analysis, East-Western India

Introduction

β-Thalassemia is one of the hemoglobinopathies belonging to a class of genetic disorders. It occurs due to mutation in β-gene of autosomal chromosome 11.[1] The incidence of β-thalassemia trait in India is 3.3% with 1%–7% of couples being affected annually.[2] Approximately 300 mutations would occur in this type, affecting β-chain globin synthesis. If the synthesis of two β-chains is absent (β^0/β^0), the person has β-thalassemia major (Cooley's anemia). This condition follows severe microcytic and hyochromic anemia. The person requires lifelong transfusion. β-Thalassemia minor is asymptomatic and results in microcytosis and mild anemia and HbA2 level increases, designated as β^+/β or β^0/β. Usually thalassemia intermedia is a condition between the major and minor forms depending on the severity of the anemic condition (β^+/β^+/or β^0/β^+) among other cases.[3,4]

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Others are HbE trait, HbE homozygous, HbD/β-thalassemia, and HbE/β-thalassemia hemoglobinopathies. The latter one, HbE/β-thalassemia, is maximum in Thailand.[5] The gene mutation takes place in another one of β-gene only in addition to β-thalassemia minor/allele (β^0/β or β^+/β), leading to coinheritance. In India, such coexisting HbE/β-thalassemia and HbD/β-thalassemia are less debated and occur in some parts of India, Pakistan, and Iran.[6,7] Recently, a report was published in Eastern Indian population about the status of thalassemia and hemoglobinopathies and suggested that more such studies are necessary in other regions of India.[8] Prevalence of common hemoglobinopathies in Gujarati population was documented by Patel et al[9] in screening programs, where β-thalassemia minor cases were maximum comparatively. Hence, we report β-thalassemia, HbE, HbD traits, and their coinheritance as well as the mutation analysis of β-thalassemia distribution in East-Western Indian population using electrophoresis, polymerase chain reaction-amplification refractory mutation system (PCR-ARMS), end point PCR, and gene sequencing technology in our study.

Patients and methods

Patient selection

Blood samples of 75 referral cases of both sexes varying in age from 6 months to 38 years were collected from Gujarat (17), Rajasthan (40), Maharashtra (7), Assam (3), and West Bengal (6) in India for β-thalassemia testing after duly filling the patient consent form in our Supratech Micropath Research Institute, Ahmedabad. These patients were referred at random. This project was approved by Human Ethical Committee (HEC) of Gujarat University, Ahmedabad (GU/HEC-001/15), in 2015 for investigation.

Hematological analysis and DNA extraction

Hematogram report was carried out on CELL DYN RUBY automated cell counter. Hemoglobin (Hb) levels were estimated by Sebia Capillary 2 Flex piercing electrophoresis. The DNA was extracted from 2 mL of EDTA blood using PerkinElmer Prepito DNA Blood 250 Kit automatic machine. The kit was used according to the manufacturer's instructions. The extracted genomic DNA was used as a template and was kept at 4°C until further use after routine DNA check.

Amplification, purification, and cycle sequencing

The primers were synthesized from the Eurofins, India. The amplification reaction was performed in Veriti Thermal Cycler. The PCR products were loaded on a 2.5% agarose gel, and the amplicons were visualized under ultraviolet transillumination after staining with ethidium bromide. PCR product cleanup using USB ExoSAP-IT kit (Affymetrix, Santa Clara, CA, USA) and cycle sequencing using BigDye TerminatorV3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) were used for further mutation identification.

Mutation analysis

The β-globin gene mutations were first characterized using two sets of allele-specific PCR-ARMS to detect eight common mutations in India including c.92+5G>C, deletion 619 bp, c.79G>A (p.E27K), c.47G>A (p.Trp16Ter), c.364G>C (p.E122Q), c.27_28insG, c.51delC, and c.124_127delTTCT. Unknown β-thalassemia genes were further characterized by direct DNA sequencing using 3500 Genetic Analyzer Applied Biosystems (ABI) for all coding regions and exon–intron boundaries to detect uncommon point mutations and small rearrangements in the β-globin gene. The c.92+5G>C mutation was detected by Sanger sequencing and PCR-ARMS, and deletion 619 bp was done by end point PCR (gel electrophoresis). Other mutations were analyzed only by Sanger sequencer. The data were analyzed using CodonCode Aligner v5.0.2 (CodonCode Corporation, Centerville, MA, USA) and Mutation Surveyor v5.0 (Softgenetics, State College, PA, USA). Mean and percentage were calculated wherever necessary.

Results

β-Thalassemia and other traits

Referral cases of 75 at our Supratech Micropath Research Institute, Ahmedabad, from 2015 to 2016 were analyzed for β-thalassemia and other traits based on Hb levels blood indices and mutation analysis from different parts of India. The affected (68) contributed 90.67% of the referral cases. High percentage (65.33%) had β-thalassemia followed by HbE trait (8%) and β-thalassemia major (heterozygous 6.66%; homozygous 5.33%). Others were HbE homozygous (2.66%), HbE/β-thalassemia, and HbD/β-thalassemia contributed only 1.34% each. Thus, 49 cases (65.33%) had β-thalassemia minor followed by HbE trait (8%) and β-thalassemia major (compound heterozygous 6.66% and homozygous 5.33%). Two were HbE homozygous (2.66%) and HbE/β-thalassemia and HbD/β-thalassemia contributed only 1.34% each. These hemoglobinopathies are well supported by increased levels of mean HbA2 (08.66%) with decreased mean corpuscular hemoglobin (MCH), mean corpuscular volume values, and altered mean HbD (1.25%), HbF (7.21%), and HbE (2.73%) levels, measured by capillary electrophoresis (Tables 1 and 2 and Figure 1).

Table 1 Classification of thalassemia traits in our study

Nos	Types of thalassemia traits	Characteristics	Cases	Percentage
1	β-thalassemia minor	Increased HbA2 with ↓ MCV and ↑ RDW	49	65.33
2	HbE trait	↑ HbA2/E, HbF, and ↓ HbA and ↑ RDW	6	8.00
3	β-thalassemia major (compound heterozygous)	↑ HbA2 with ↓ MCV, ↑ HbF, and ↑ RDW	5	6.66
4	β-thalassemia major (homozygous)	↑ HbA2 with ↓ MCV,↑ HbF, and ↑ RDW	4	5.33
5	HbE homozygous	↑ HbA2/E, HbF, and ↓ HbA and ↑ RDW	2	2.66
6	Combination of HbE/β-thalassemia	↑ HbA2/E, HbF, and ↓ HbA and ↑ RDW	1	1.34
7	Combination of Hb-D/β-thalassemia	↑ HbAD, HbA2, and HbF ↑ (sometimes) and ↑ RDW	1	1.34

Notes: Total cases 75; affected cases 68 (90.67%).
Abbreviations: ↑, increase; ↓, decrease; Hb, hemoglobin; HbF, fetal hemoglobin; MCV, mean corpuscular volume; RDW, red blood cell distribution width.

Table 2 Sex wise distribution of Hb variants and mutations in our study

Nos	Age, years	Sex	HbA (%)	MCV (fL)	MCH (pg)	RDW (%)	HbA2 (%)	HbE (%)	HbF (%)	HbD (%)	Mutations	Genotype	Inference/ clinical report	Technique(s)
1	24	Female	94.30	75.00	24.00	14.60	5.00	0.0	0.70	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
2	27	Male	94.50	67.00	18.20	17.00	4.70	0.0	0.80	–	c.47G>A (p.Trp16Ter)	B^{0}/β	β-thalassemia minor	SS
3	25	Female	93.95	69.50	20.80	17.10	5.40	0.0	0.65	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
4	28	Male	93.75	75.50	18.40	41.30	5.40	0.0	0.85	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
5	25	Female	94.61	69.00	21.00	17.80	4.80	0.0	0.59	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
6	5	Female	65.65	69.90	18.50	15.10	33.60	0.0	0.75	–	c.92+5G>C	B^{+}/β^{+}	β-thalassemia minor	SS and PCR-ARMS
7	28	Male	94.35	68.00	21.00	18.00	4.70	–	0.95	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
8	28	Female	93.40	76.40	23.80	15.60	5.40	–	1.20	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
9	30	Male	92.50	75.00	22.40	14.70	6.60	–	0.90	–	Not detected*	β/β	Normal	SS and GE
10	25	Female	93.95	74.90	18.60	15.00	5.00	–	1.05	–	c.92+5G>C	B^{+}/β	β-thalassemia minor	SS and PCR-ARMS
11	30	Male	96.80	79.90	28.00	14.00	2.60	–	0.60	–	Not detected	β/β	Normal	SS and GE
12	33	Female	66.50	71.00	27.90	14.60	22.96	9.84	0.70	–	c.79G>A (p.Glu27Lys)	β^{E}/β	HbE trait	SS
13	33	Male	92.80	70.10	20.90	16.90	6.60	–	0.60	–	c.47G>A (p.Trp16Ter)	β^{0}/β	β-thalassemia minor	SS
14	20	Female	94.55	68.60	18.50	16.20	4.70	–	0.75	–	c.92+5G>C	β^{+}/β	β-thalassemia minor	SS and PCR-ARMS
15	28	Male	94.00	76.30	19.40	17.40	5.20	–	0.80	–	c.92+5G>C	β^{+}/β	β-thalassemia minor	SS and PCR-ARMS
16	6	Male	87.50	67.00	18.10	19.40	2.20	–	10.30	–	c.92+5G>C	β^{+}/β^{+}	β-thalassemia minor	SS and PCR-ARMS
17	26	Female	93.80	68.50	19.40	16.20	5.70	–	0.50	–	619 bp deletion	β^{0}/β	β-thalassemia minor	GE
18	28	Male	95.20	68.30	20.80	15.50	4.60	–	0.20	–	c.92+5G>C	β^{+}/β	β-thalassemia minor	SS and PCR-ARMS
19	1	Female	94.90	70.20	19.20	16.90	2.70	–	2.40	–	c.92+5G>C	β^{+}/β	β-thalassemia minor	SS and PCR-ARMS
20	29	Male	17.90	67.50	18.40	16.30	68.90	12.70	0.50	–	c.79G>A (p.E27K)	β^{E}/β	HbE trait	SS
21	24	Female	66.15	69.00	27.80	14.50	23.52	10.08	0.25	–	c.79G>A (p.Glu27Lys)	β^{E}/β	HbE trait	SS
22	31	Male	94.35	74.20	21.00	16.40	5.20	–	0.45	–	c.92+5G>C	β^{+}/β	β-thalassemia minor	SS and PCR-ARMS
23	27	Female	93.45	67.00	22.80	17.70	5.90	–	0.65	–	c.92+5G>C	β^{+}/β	β-thalassemia minor	SS and PCR-ARMS
24	1	Male	6.70	69.20	21.40	38.20	3.30	–	90.0	–	c.47G>A (p.Trp16Ter) and c.92+5G>C	β^{0}/β^{+}	β-thalassemia minor	SS and PCR-ARMS

(*Continued*)

Table 2 (*Continued*)

Nos	Age, years	Sex	HbA (%)	MCV (fL)	MCH (pg)	RDW (%)	HbA2 (%)	HbE (%)	HbF (%)	HbD (%)	Mutations	Genotype	Inference/ clinical report	Technique(s)
25	30	Male	95.55	73.90	21.50	15.90	4.20	–	0.25	–	c.47G>A (p.Trp16Ter)	β^0/β	β-thalassemia minor	SS
26	35	Female	93.70	69.50	20.80	17.20	5.70	–	0.60	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
27	5	Male	4.00	66.90	18.50	15.90	58.80	25.80	11.40	–	c.79G>A (p.Glu27Lys) and c.92+5G>C	β^0/β^+	β-thalassemia minor	SS and PCR-ARMS
28	30	Female	93.20	68.00	22.30	16.60	6.20	–	0.60	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
29	32	Female	2.90	67.30	18.20	17.00	61.80	28.20	7.10	–	c.79G>A (p.Glu27Lys)	β^E/β	HbE trait	SS
30	4	Male	4.00	69.50	21.60	37.50	1.10	–	94.90	–	c.51delC	β^0/β	β-thalassemia minor	SS
31	26	Female	93.50	66.40	21.70	18.20	5.90	–	0.60	–	c.27_28insG	β^0/β	β-thalassemia minor	SS
32	2	Female	1.40	65.30	18.40	16.70	2.20	–	96.40	–	c.27_28insG	β^0/β^0	β-thalassemia minor	SS
33	27	Male	1.55	75.30	22.80	16.30	3.80	–	0.25	94.40	c.27_28insG and c.364G>C (p.E122Q)	β^0/β^D	HbD/β-thalassemia	SS
34	27	Female	96.10	70.30	21.90	17.20	3.60	–	0.30	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
35	30	Male	95.40	68.50	21.20	17.70	4.10	–	0.50	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
36	27	Female	94.75	62.20	20.40	16.80	4.80	–	0.45	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
37	30	Male	93.65	64.20	19.60	15.70	5.80	–	0.55	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
38	23	Female	93.45	69.80	22.50	14.70	6.20	–	0.35	–	c.47G>A	β^0/β	β-thalassemia minor	SS
39	26	Male	97.10	75.90	24.50	13.40	2.80	–	0.10	–	Not detected	β/β	Normal	SS and GE
40	29	Female	94.95	68.90	22.30	16.90	4.80	–	0.25	–	92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
41	31	Male	96.10	69.30	20.50	15.70	3.90	–	0.00	–	92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
42	5	Female	94.75	69.70	22.40	15.80	5.00	–	0.25	–	92+5G>C	β^+/β^+	β-thalassemia minor	SS and PCR-ARMS
43	8	Female	80.00	68.50	21.60	16.80	14.00	6.00	0.00	–	c.79G>A	β^E/β	HbE trait	SS
44	31	Female	94.27	64.20	19.50	15.80	5.28	–	0.45	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
45	33	Male	93.00	69.40	20.40	17.80	6.60	–	0.40	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
46	4	Female	3.70	67.00	19.20	15.50	1.60	–	94.70	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
47	33	Female	94.40	69.50	20.70	17.60	5.40	–	0.20	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
48	35	Male	94.50	64.10	19.70	16.90	5.20	–	0.30	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
49	30	Female	95.60	63.10	19.90	16.00	4.20	–	0.20	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
50	32	Male	94.80	70.00	20.10	15.80	4.80	–	0.40	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
51	26	Female	94.40	68.90	19.40	17.10	4.90	–	0.70	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
52	26	Male	94.65	69.90	20.30	17.60	4.80	–	0.55	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
53	$^1/_2$	Female	94.70	68.10	18.90	17.90	2.40	–	2.90	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
54	35	Female	91.10	69.50	20.80	17.00	8.20	–	0.70	–	Not detected*	–	–	SS and GE
55	38	Male	91.50	63.10	19.50	16.00	7.90	–	0.60	–	Not detected*	–	–	SS and GE

(*Continued*)

Table 2 (*Continued*)

Nos	Age, years	Sex	HbA (%)	MCV (fL)	MCH (pg)	RDW (%)	HbA2 (%)	HbE (%)	HbF (%)	HbD (%)	Mutations	Genotype	Inference/ clinical report	Technique(s)
56	4	Female	94.50	75.00	23.10	14.00	5.00	–	0.50	–	Not detected*	–	–	SS and GE
57	5	Female	93.30	75.60	25.00	14.10	6.10	–	0.60	–	Not detected*	–	–	SS and GE
58	22	Female	93.80	69.00	21.40	38.20	5.80	–	0.40	–	c.124_127delTTCT** and c.92+5 G>C	**/β^+	β-thalassemia minor	SS and PCR-ARMS
59	26	Male	94.30	63.20	19.50	16.10	5.20	–	0.50	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
60	29	Female	93.55	67.40	19.10	17.80	6.10	–	0.35	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
61	30	Female	93.95	69.10	21.80	17.80	5.50	–	0.55	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
62	28	Female	94.00	69.00	21.40	38.20	5.40	–	0.60	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
63	30	Male	93.60	69.40	20.70	17.00	5.90	–	0.50	–	c.27_28insG	β^0/β	β-thalassemia minor	SS
64	27	Female	94.40	69.00	21.80	17.40	5.20	–	0.40	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
65	30	Male	94.30	67.10	18.20	19.10	5.00	–	0.70	–	c.27_28insG	β^0/β	β-thalassemia minor	SS
66	24	Male	93.20	69.00	21.70	17.80	6.10	–	0.70	–	619 bp deletion	β^0/β	β-thalassemia minor	GE
67	26	Male	91.75	62.40	19.90	18.20	7.90	–	0.35	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
68	26	Female	94.35	68.90	20.90	17.60	4.90	–	0.75	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
69	23	Male	93.80	73.90	20.10	14.60	5.50	–	0.70	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
70	27	Female	94.40	74.30	19.30	15.90	5.00	–	0.60	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
71	24	Male	0.0	72.90	21.00	14.50	3.40	–	96.60	–	c.51_51delC and c.124_127delTTCT**	$\beta^{0/**}$	β-thalassemia minor	SS
72	26	Male	94.20	68.40	21.00	14.00	5.20	–	0.60	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS
73	27	Female	69.80	74.20	20.30	14.40	20.73	8.97	0.50	–	c.79G>A (p.E27K)	β^E/β	HbE trait	SS
74	28	Male	68.40	70.20	21.80	16.90	21.70	9.30	0.60	–	c.79G>A (p.E27K)	β^E/β	HbE trait	SS
75	27	Male	95.40	64.20	20.10	16.40	3.90	–	0.70	–	c.92+5G>C	β^+/β	β-thalassemia minor	SS and PCR-ARMS

Notes: Total cases: 75; age range (1/2 to 38 years). Case nos: 24, 27, 33, 58, and 71 had double mutations (compound heterozygous). Mean HbA = 81.37 (96.8%–97.8%), mean MCV = 69.53 (83–100 fL), mean MCH = 20.92 (27–32 pg), mean RDW = 17.85 (11.5%–14.5%), mean HbA2 = 8.66 (2%–3.5%), mean HbE = 2.73% (absent), HbD = 1.25% (absent), and HbF = 7.21 (0.0%–1.0%). Figures in parentheses indicate normal range/values.
*Large deletion and duplication are not identified in our study. **Genotype novel.
Abbreviations: GE, gel electrophoresis; Hb, hemoglobin; MCV, mean corpuscular volume; PCR-ARMS, polymerase chain reaction-amplification refractory mutation system; RDW, red blood cell distribution width; SS, Sanger sequencing.

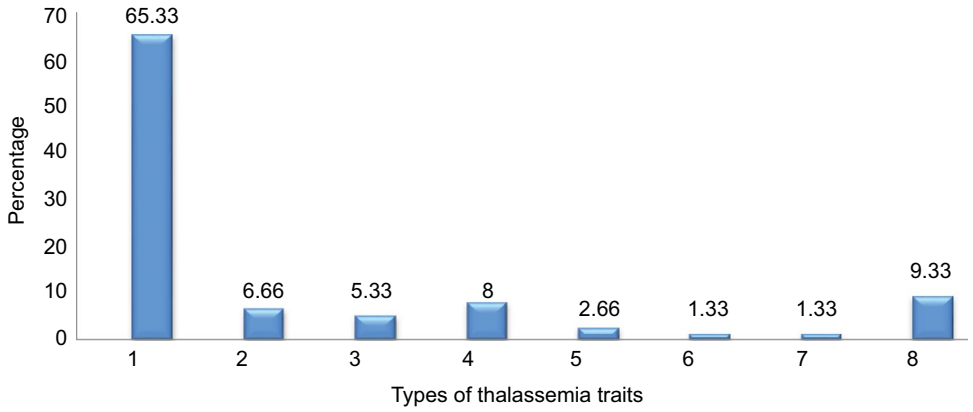

Figure 1 Percentage (%) distribution of β-thalassemia traits.

Mutation analysis

We have analyzed conventional mutations of eight in Indian population using PCR-ARMS and end point PCR allayed with using Codon code Aligner V6.0.2 and mutation survey 5.0 software for exact specific mutation nomenclature from 68 affected cases. The data showed that c.92+5 G>C was higher (41, 60.29%), followed by nine cases of deletion 619 bp (13.23%), eight cases of c.79G>A (p.E27K) (11.76%), and five cases each of c.27_28insG (7.35%) and c.47G>A (p.Trp16Ter) (7.35%) with two cases each of c.124_127delTTCT (2.94%) and c.51delC (2.94%) and one case each of c.364G>C (p.E122Q) and c.47G>A (p.Trp16Ter) (1.47%), respectively, with no sex difference as female and male ratio was (1:1.13) (Table 3). Deletion 619 bp was only detected by gel electrophoresis, and c.92+5G>C was identified using PCR-ARMS and also gene sequencing as that of others (Figure 2A–G). In Rajasthan and Gujarat, where more are accumulated (40 and 17) respectively, in both cases, the most frequent mutation is c.92+G>C (26 and 10) followed by 619 bp (3 and 4) and 619 bp deletions (Table 3 and Figure 3).

Discussion

β-Thalassemia is one of the heterozygous inheritable disorders in India. It causes reduced or absence of β-chain synthesis of Hb. Its variants in addition to carrier identification and prenatal analysis are necessary for its management and to avoid marriages between carrier of mutated genes including consanguineous types.[4,10–12] Hence, from 75 referral cases of Western and Eastern India, the blood was collected to identify various traits and mutations accurately using electrophoretic and molecular diagnostic techniques in our laboratory including coinheritance with β-thalassemia. Of the total referral patients, 68 cases were affected having 90.67% in this study. Of these, 65.33% of β-thalassemia traits (49) were detected followed by HbE trait and β-thalassemia major with HbE homozygous and their HbE/β-thalassemia and HbD/β-thalassemia coinherited cases depending on altered Hb, MCH, red blood cell distribution width, and MCV values. It indicated that β-thalassemia cases (carriers) are maximum followed by others and support the data of previous workers in India.[13–16] Similarly, Hb patterns were measured and presented in the study of Mondal and Mandal,[8] who obtained few cases of HbE, HbD traits, and β-thalassemia major comparatively. This could be due to changing lifestyles, environmental and genetic factors, and coinheritance of HbE, HbD, and/or α-thalassemia with β-thalassemia carriers.[17,18] Further, these factors may also be the cause of β-thalassemia major with heterozygosity/homozygosity who are less in number requiring blood transfusion. Similarly, coinheritance of HbE/β-thalassemia and HbD/β-thalassemia and HbE homozygous cases were also reduced in number in our report. HbE trait had six cases having less severity of clinical condition. However, Olivieri et al[17] mentioned that these conditions may vary from severe to mild depending upon genetic and environmental factors, and such patients are also less frequent to support our data. We detected one each of HbD/β-thalassemia and HbE/β-thalassemia cases in addition to HbE patients with variable phenotypic indices expressing mild heterozygous state.[19–22] These patients may require transfusion in severe condition only, due to coinheritance of the disease.[23]

Further, we extended our investigation on molecular analysis of mutations of β-thalassemia and systematically using latest molecular biology tools such as PCR-ARMS, end point

Table 3 Percentage of mutation types in our thalassemic cases (68) of different regions

Nos	Mutation types	Mutation nos (73)*	Male (34)	Female (39)	Mutation percentage	Region (state) wise mutation
1	c.92+5G>C	41	18	23	60.29	Rajasthan 26, Gujarat 10, West Bengal 3, Maharashtra 2
2	619 bp deletion	9	3	6	13.23	Gujarat 4, Rajasthan 3, Maharashtra 2
3	c.79G>A (p.E27K)	8	4	4	11.76	Assam 3, Rajasthan 2, Maharashtra 1, West Bengal 2
4	c.47G>A (p.Trp16Ter)	5	4	1	7.35	Gujarat 2, Rajasthan 2, Maharashtra 1
5	c.364G>C (p.E122Q)	1	1	0	1.47	Rajasthan 1
6	c.27_28insG	5	2	3	7.35	Rajasthan 2, Gujarat 1, Maharashtra 1, West Bengal 1
7	c.51delC	2	2	0	1.47	Rajasthan 2
8	c.124_127delTTCT	2	0	2	1.47	Rajasthan 2

Notes: State/region wise mutations: Rajasthan: 40, Gujarat: 17, Maharashtra: 7 (Western India: 40 + 17 + 07 = 64), West Bengal: 6, Assam: 3 (Eastern India: 06 + 03 = 09). *Five with compound heterozygous (double mutations); M:F = 1:1.13 (32/36 = 68).
Abbreviations: F, female; M, male.

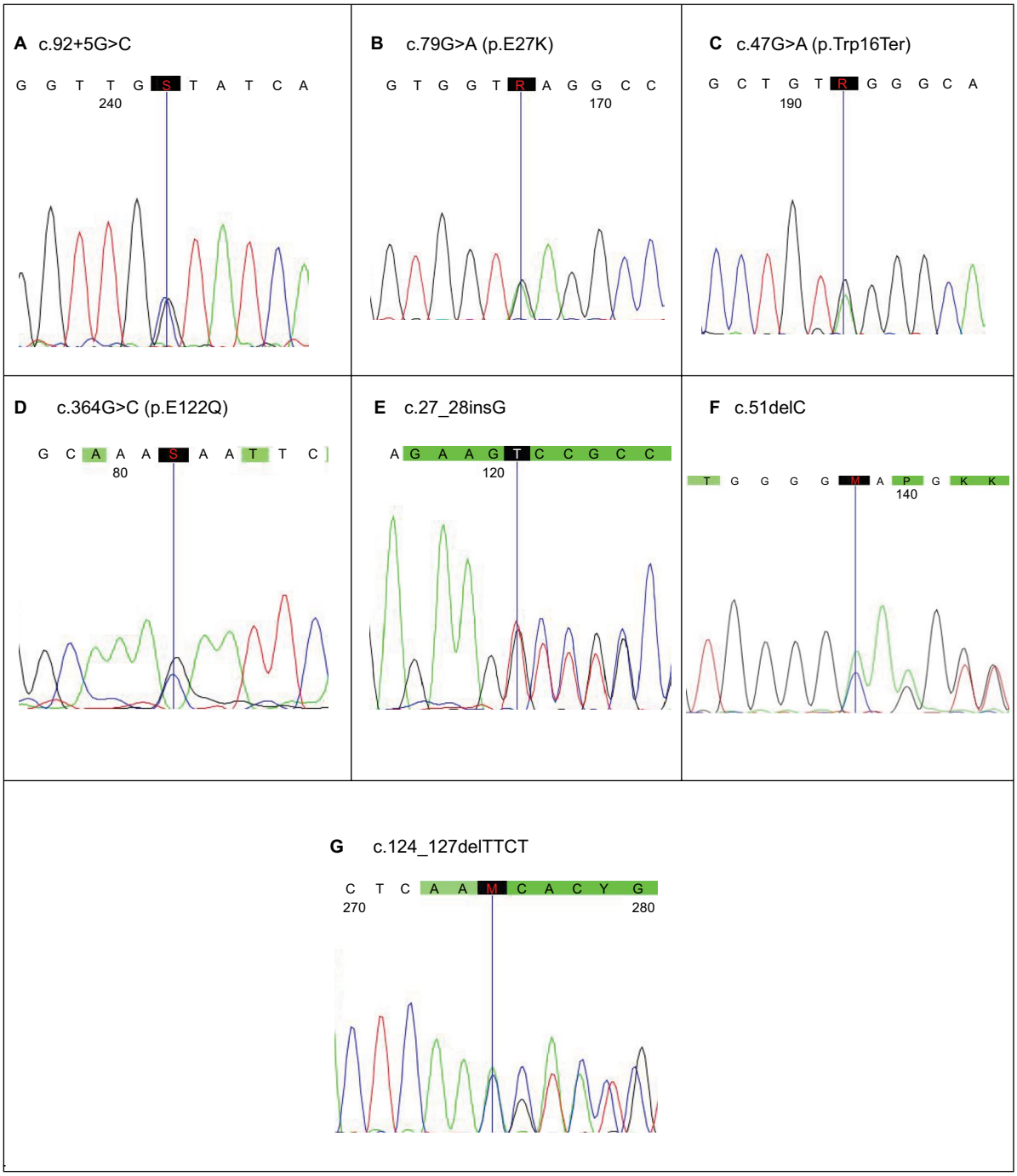

Figure 2 Seven common mutations identified by direct DNA sequencing.
Notes: (**A**) c.92+5G>C, (**B**) c.79G>A (p.E27K), (**C**) c.47G>A (p.Trp16 Ter), (**D**) c.364G>C (p.E122Q), (**E**) c.27_28insG, (**F**) c.51delC, and (**G**) c.124_127delTTCT. Nucleotide colors: A = green, T = red, G = black, and C = blue.
Abbreviations: S, G/C; R, G/A; K, G/T; M, C/A; Y, C/T.

PCR, and Sanger Gene Sequencing. Data revealed 92+5 G>C (IVS-1–5) is the maximum in cases (60.29%), of Rajasthan and Gujarat followed by deletion 619 bp and is conformed with others documented earlier in Gujarat, Maharashtra, and Rajasthan.[2,9,12,15,24–26] But Hassan et al,[27] from Thailand, found cd26 (A-G) HbE and cd41/42 (–TTCT) were higher in their studies. Thong et al[28] presented cd41/42 (–TTCT) and IVS-2 654 (C-T) were maximum in Chinese population. Similarly, second highest mutation was 619 bp in (9, 13.23%) this study similar to that of other studies in Western India conducted by Sheth et al,[10] Grow et al,[13] Colah et al,[29] and Nigam et al.[16] The third largest mutation in our study was c.79G>A (p.E27K) followed by c.47G>A (p.Trp16Ter) and c.27_28insG different from other investigators,[2] followed by other mutations, ie, c.51delC, c.124_127delTTCT(novel), and c.364G>C (p.E122Q). The incidence of these mutations

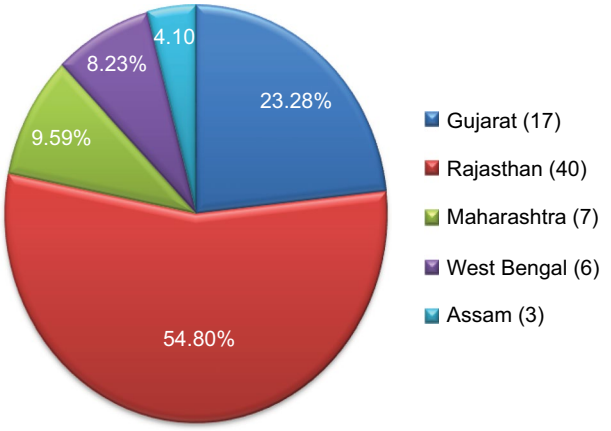

Figure 3 Region wise percentage distribution of mutations in β-thalassemia and numbers in parentheses indicate cases.

does not seem to be related to sex, as our sex ratio was 1:1.13 (M:F). The variation in occurrence of these mutations is related to regional, ethical, migration, interracial marriages, study plan, and other factors as mentioned by others.[12,29,30]

Conclusion

Our study showed that 68 cases were affected by β-thalassemia in our referral cases (75), from East-Western Indian region. β-Thalassemia carriers were 49 (65.33%) followed by HbE trait and β-thalassemia major with heterozygous and homozygous condition using hematological profiles. Detection of molecular analysis of mutations using PCR-ARMS, end point PCR, and gene sequencing methods revealed c.92+5G>C mutation exhibited higher incidence (26+10+2) followed by deletion 619 bp (3+4+2) in Rajasthan, Gujarat, and Maharashtra (Western India) as compared to West Bengal and Assam (3:0; 0:0) of Eastern India, respectively. This requires further elucidation. The variation in incidence of these mutations is dependent on ethnic diversity, migration, genetic factors, and other lifestyles. We, hence, recommend the mass screening, Prenatal Diagnostic Techniques, genetic counseling, transfusion programs and clinical management made available to these populations before adopting assisted reproductive and preimplantation technologies in India.

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Author contributions

Parth S Shah and Nidhi D Shah have contributed to writing results and discussion in the manuscript preparation when they visit India. Hari P Ray, Nikunj B Khatri, Ketan K Vaghasia, and Rutvik J Raval are involved in collection of blood from the patients after duly filled consent forms, blood analysis, DNA extraction, DNA sequencing, PCR-ARMS, end point PCR, and data analysis of 75 patients. Dr Sandip C Shah and Dr Mandava V Rao have contributed to preparation of reports after finalization of the results and preparation of the manuscript finally for submission to the journal. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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