DOI: 10.7860/JCDR/2018/27646.11463

Original Article

Genetics Section

Mutation Analysis of Beta-thalassaemia in 30 Families of India: A Report

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ABSTRACT

Introduction: The β-thalassaemia is one of the haemoglobinopathies related to genetic disorders. It occurs due to mutation in β-gene of autosome 11. In India, it affects 1-7% of couples annually. Reports are available in few states of India about β-thalassaemia affected families. But much data is not reported in families of various states of India. Further, the incidence of case index and mutations in parents and siblings of these families are limited.

Aim: To analyse patient case index and mutation analysis of parents and siblings {Children and Chorionic villus sampling/ Amniotic fluid (CVS/AF) cases} of 30 families of India and to develop preventive measures.

Materials and Methods: A total of 101 referral cases of 30 families filled consent forms and then blood was drawn in a sterilised tube from each case (71) for the study. The AF/CVS (30) were also included. The red cell markers like Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW) etc., biochemical, case types and molecular analysis were done using respective techniques for red cell indices, Haemoglobin (Hb) types and mutation analysis.

Results: We report a number of 30 referral families (101 cases) having parents (60), children (11) and CVS/AF samples (30) for their β-thalassaemia, as these traits also cause β-gene mutations. Out of these 101 cases, 88 (87%) cases were positive for this disease. Only 74 (73%) were detected carriers. The case analysis in present cohort indicated parents (56.5%), CVS/AF (19.8%) and proband (children) (10.9%) were found affected. All children moreover, were affected and did, not have CVS/AF samples. The mutations analysis, indicated c.92+G>C (50/94; 53.19%) was maximum and parents contributed 62% followed by siblings (38%) with CVS/AF (22%) and proband (16%) in present cohort. Thus, in the present study, mutation analysis further pointed out that parents transmit these to the offsprings in the subsequent generation who would be the targets of thalassaemia disease.

Conclusion: The present study, points out that mutations transfer from parents to offspring follows the laws of inheritance. Case index study showed parents constituted high percent of cases followed by CVS/AF and children/probands, comparable to mutation analysis in present cohort. Hence, carrier parents must undergo counseling and genetic testing to confirm their genetic disorder to limit the burden of the disease.

Keywords: Beta-thalassaemia traits, Red cell markers, Referral cases

INTRODUCTION

India has been suffering from various genetic disorders. One class of them is haemoglobinopathies. Beta-thalassaemia occurs as a result of β-gene mutation in β-chain globin synthesis presenting in autosome 11 [1]. The incidence of β-thalassaemia in India is 3.3% with 1-7% of couples being affected annually [2,3]. The β-globin synthesis is controlled by two alleles (β/β) of β-gene. Based on haematological indices, including Hb variants, these patients are classified as β-thalassaemia major (β0/β0), intermedia (β+/β+ or (β0/β+) and minor (β+/β or (β0/β). The β-thalassaemia major condition requires blood transfusion followed by β-thalassaemia intermedia with occasional transfusion. The third type is 'mild' [4]. Patel AP et al., documented common haemoglobinopathies like α- and β-thalassaemias in screening program of population [5].

In few occasions, coinheritance exists in certain patients between HbE, HbD with β-thalassaemia traits (β0/β+) leading to compound heterozygous condition in addition to homozygosity [6-8]. Recent studies in west-east population of India also detected coexistence of Hb variants with β-thalassaemia minor cases, including major and intermedia condition and such homozygotic and heterozygotic condition are sometimes fatal [9-11]. These conditions may be transferred to the next generation from the parents affecting offsprings. Few population studies are done, only restricted to Western and Northern India, in relation to this genetic condition [10,12]. Hence, the study was undertaken in 30 families from various regions of India referred to Supratech Micropath Laboratory and Research Institute, Ahmedabad, Gujarat, India (2015-2016), to analyse case index and mutations in them.

MATERIALS AND METHODS

Case Selection

A total of 30 families reports of clinicians varying in age from 1-38 years were collected from Gujarat, Rajasthan, Maharashtra, Assam and West Bengal of India, for β-thalassaemia testing after duly filled patient consent forms were registered at the Research Institute, Ahmedabad during the period, (2015-2016). The project is approved by Human Ethical Committee (HEC) of Gujarat University, Ahmedabad (Gu/HEC-001/15) in 2015. The CVS/AF samples were sent from the respective approved collection center with details, where contaminated case samples were excluded by the doctors before delivery to research centre. Total of 101 cases depending upon sample index which included parents (60), children (11) and AF/CVS (30). Distal radius fractures with gross dorsal tilt, is geometry which cannot be acceptably corrected or if corrected not maintained by closed means such as casts or external fixators.

Haematological Analysis and DNA Extraction

Haematogram report including red cell indices was carried out on automated cell counter (CELL-DYN Ruby). The Hb levels were estimated by Sebia Capillary 2 Flex piercing electrophoresis. The DNA was extracted from EDTA blood, AF and CVS 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 DNA check routinely.

Amplification, Purification and Cycle Sequencing

Polymerase Chain Reaction (PCR) primers were designed using the oligocal (http://biotools.nubic.northwestern.edu/OligoCalc.html) to determine properties of designed primers and also use Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast) to check our region of interest to be covered the HBB gene exon 1,2,3 along with 619 bp deletion [13,14]. Primers were synthesised at 100 pmol/uL scale and cartridge purified (Eurofins India). PCR amplification was carried out using Takara TaqTM Hot Start Version PCR mix with each primer at 10 pM concentration under standard conditions. PCR amplifications were all carried out in duplicate alongside a known normal control sample. After initial denaturation at 95°C for five minutes, 45 cycles of PCR were performed in veriti thermal cycler (Apllied Biosystem, USA) with the PCR conditions as denaturation at 95°C for 20 seconds, annealing (for HBB exon 1,2,3 and 619 bp deletion) at 59°C, 55°C, 56°C and 64°C for 20 seconds and 72 for 30 seconds and following an extension at 72°C for seven minutes. Successful PCR product were purified prior to sequencing using ExoSAP-IT reagent (Affrymetrix-USA) according to the manufacturer's protocol. Purified PCR products were Sanger sequenced in both forward and reverse orientations using the same primer sequences used for PCR at 10 pM, final concentration using BigDye® v3.1 according to manufacturer's cycling conditions. BigDye® v3.1 sequencing reactions were then purified using EDTA and sodium acetate according to the manufacturer's protocol and analysed on 3500 genetic analyser (Applied Biosystems USA). Sanger sequencing data were analysed using CodonCode Aligner v5.0.2, (CodonCode Corporation Centerville, USA) and Mutation Surveyor v5.0, (Softgenetics Pennsylvania, USA).

Mutation Analysis by PCR and Gene Sequencing

The beta globin gene mutations were first characterised using two sets of allele specific PCR or Amplification Refractory Mutation System (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-thalassaemia genes were further characterised by direct DNA sequencing using 3500 Genetic Analyser Applied Biosystems (ABI) for all coding regions and exon-intron boundaries to detect uncommon point mutations and small rearrangements in the β-globin gene. Sanger sequencing data were analysed using CodonCode Aligner v5.0.2, (CodonCode Corporation Centerville, USA) and Mutation Surveyor v5.0, (Softgenetics Pennsylvania, USA).

RESULTS

The present study comprised of 30 families of India referred to the present medical research center. These families possessed 60 parents (father/mother), 11 children and 30 CVS/AF samples contributing to 101 cases.

Haematogram Analysis

All affected cases had altered Hb traits (HbA2, HbE, HbD and HbF) except 13 normal cases (Nos. 7, 9) of [Table/Fig-1] and 3 (2), 4 (2), 6, 10 (2), 12, 15, 17, 18 of [Table/Fig-2] respectively. These were followed by changed red cell indices MCV, MCH and RDW values of the particular thalassaemia disease in families with/without children (probands) [Table/Fig-1,2].

Case Analysis

Out of 101 cases, 88 (87%) were positive for the disease. Of these, carriers were c 74 cases (73%). Fourteen cases were of homozygotes eight (6/8 children., Case Nos. 1, 2, 6, 7, 8 and 10 of [Table/Fig-1,2] CVS/AF., (Case Nos. 8 and 14 of [Table/Fig-2] and others six were compound heterozygotes (4/6 children case Nos 3,4,5 and 11 and one each CVS and father (Case Nos. 6 (2) in [Table/Fig-1]. Further, all children of 11 families (6 homozygous, 4 compound heterozygous with one carrier (case number 9) were affected. No children had CVS/AF samples as their age ranged one to five years [Table/Fig-1]. The carriers cases were parents 57 (56.7), CVS/AF 20 (19.8%) and 11 children/probands (10.9%) cases were affected followed by 13 (12.8%) cases of normal [Tables/Fig-1,2].

Mutation Analysis

Mutation analysis from common eight mutations in India using PCR and Sanger gene sequencing indicated maximum percent mutation was c.92+5G>C (50/94) following with 619 bp deletion (14/94), c.79G>A p.E27K. (11/94), c.27_28insG (9/74), c.47G>A (p.Trp16Ter (6/94), c.364G>C p.E122Q (2/94) and c.124_127delTTCT (2/94) respectively of 94 mutations. Thus, parents contributed 58/94 (62%) children (Proband) 15/94 (16%) and CVS/AF 21/94 (22%) making siblings 38% [Table/Fig-3].

DISCUSSION

In the present study, 30 families included 60 parents and 41 (11+30) siblings (Children+CVS/AF), who were analysed for RBC markers, case types and mutation analysis for β-thalassaemia traits. The Hb traits and red cell markers indicated majority of cases possessed β-thalassaemia minor which were asymptomatic, with nearly normal cases. The HbA2 levels were higher including microcytosis and mild anaemia. Few cases of HbA2, HbE, HbD and HbF were also altered in contrast to Hb levels. This condition was supported by red cell indices, where MCV, RDW and MCH values were different.

In the present study, the case index consisted of 60 parents, 11 child/probands and 30 CVS/AF samples. The probands of 11 families affected with six homozygous and four with compound heterozygous with one carrier. Out of 30 CVS/AF carriers, 17 cases followed by two homozygous and one case with heterozygous condition. In parents (60), 56 (51+5) cases were carriers followed by one with heterozygotic condition (case 6). In present cohort, the total carriers detected were parents (56), proband (1) and CVS/AF (17) leading to total 74. However, total affected cases were 88. Probands/children had no CVS/AF samples as all these were related to parents (30). These data implicated that CVS/AF samples (19.8%) were more affected than children (10.9%) after parents (56.5%) in the present study. However, it was noticed that proband/children affected were more with homozygous (6) and compound heterozygous states (4) with one carrier than CVS/AF cases. These cases were well supported by clinical data including altered Hb types like HbA2, HbE and HbD followed by changed red cell indices for each case types, in addition to molecular analysis, using ARMS-PCR and Sanger sequencing. The present study is supported by others who documented population affected by thalassaemia in Gujarat and Maharashtra [12,15,16].

Total mutations in the present study detected were 94 of which c.92+5G>C (50) was the major type in all 30 families following with deletion 619 bp 14 including adults and siblings (probnad/children and CVS/AF). Same data were also obtained in earlier studies in population of Gujarat and Maharashtra [3,12,15,16]. Recent study, reported by Shah PS et al., also support present

Family No	Age	Sex	HbA (%)	MCV fL	MCH pg	RDW (%)	HbA2 (%)	HbE (%)	HbF (%)	HbD (%)	Mutations	Inference/clinical report	Genotype
01	25	M	93.95	74.90	18.60	15.00	4.70	-	1.05	-	c.92+5G>C	Thalassaemia minor	β+/β
	28	F	93.40	76.40	23.80	15.60	5.40	-	1.20	-	c.92+5G>C	Thalassaemia minor	β+/β
	05	D/P	65.65	69.90	18.50	15.10	33.60	0.0	0.75	-	c.92+5G>C(Homo)	Thalassaemia major	β+/β+
	-	AF	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
02	20	M	94.55	68.60	18.50	16.20	4.70	-	0.75	-	c.92+5G>C	Thalassaemia minor	β+/β
	28	F	94.00	76.30	19.40	17.40	5.20	-	0.80	-	c.92+5G>C	Thalassaemia minor	β+/β
	06	S/P	87.50	67.00	18.10	19.40	2.20	-	10.30		c.92+5G>C(Homo)	Thalassaemia major	β+/β+
	-	AF	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
03	26	M	93.80	68.50	19.40	16.20	5.70	-	0.50	-	619bp deletion	Thalassaemia minor	β0/β
	28	F	95.20	68.30	20.80	15.50	4.60	-	0.20	-	c.92+5G>C	Thalassaemia minor	β+/β
	04	S/P	58.0	73.90	21.50	15.90	4.90	-	16.70	-	619bp deletion and c.92+5G>C	Thalassaemia major	β0/β+
	-	AF	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
04	23	M	93.50	66.40	21.70	18.20	5.90	-	0.60	-	619bp deletion	Thalassaemia minor	β0/β
	28	F	5.80	66.20	17.80	16.90	61.80	28.2	0.20	-	c.27_28insG	Thalassaemia minor	β0/β
	11/2	S/P	1.4	65.42	20.65	17.45	2.20	-	96.4	-	619bp deletion and c.27_28insG	Thalassaemia major	β0/β0
	-	CVS	-	-	-	-	-	-	-	-	619bp deletion	Thalassaemia minor	β0/β
05	35	M	93.45	69.80	22.50	14.70	6.20	-	0.35	-	c.92+5G>C	Thalassaemia minor	β+/β
	37	F	2.90	67.30	18.20	17.00	61.80	28.20	7.10	-	c.79G>A p.Glu27Lys	HbE trait	β E/β
	01	D/P	4.00	66.90	18.50	15.90	64.50	31.00	0.50	-	c.79G>A p.Glu27Lys and 92+5G>C	HbE/β-Thal	βE/β+
	-	CVS	-	-	-	-	-	-	-	-	c.79G>A p.Glu27Lys	HbE tarit	βE/β
06	25	M	93.50	66.40	21.70	18.20	5.90	-	0.60	-	c.27_28insG	Thal-trait	β0/β
	27	F	1.55	75.30	22.80	16.30	3.80	-	0.25	94.40	c.27_28insG and c.364G>C p.E122Q	HbD/β-Thal	β0/βD
	1/1/2	D/P	1.40	65.30	18.40	16.70	2.20	-	96.40	-	c.27_28insG (Homo)	Thalassaemia major	β0/β0
	-	CVS	-	-	-	-	-	-	-	-	c.27_28insG and c.364G>C p.E122Q	HbD/β-Thal	β0/βD
07	29	M	94.95	68.90	22.30	16.90	4.80	-	0.25	-	92+5G>C	Thalassaemia minor	β+/β
	31	F	96.10	69.30	20.50	15.70	3.90	-	0.00	-	92+5G>C	Thalassaemia minor	β+/β
	05	D/P	94.75	69.70	22.40	15.80	5.00	-	0.25	-	92+5G>C (Homo)	Thalassaemia major	β+/β+
	-	AF	-	-	-	-	-	-	-	-	Not detected	Normal	-
08	31	M	94.27	64.20	19.50	15.80	5.28	-	0.45		c.92+5G>C	Thalassaemia minor	β+/β
	33	F	93.00	69.40	20.40	17.80	6.60	-	0.40	-	c.92+5G>C	Thalassaemia minor	β+/β
	04	D/P	3.70	67.00	19.20	15.50	1.60	-	94.70	-	c.92+5G>C (Homo)	Thalassaemia major	β+/β+
	-	AF	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
09	35	M	91.10	69.50	20.80	17.00	8.20	-	0.70	-	c.92+5G>C	Thalassaemia minor	β+/β
	38	F	91.50	63.10	19.50	16.00	7.90	-	0.60	-	c.92+5G>C	Thalassaemia minor	β+/β
	04	D/P	94.50	75.00	23.10	14.00	5.00	-	0.50	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	AF	-	-	-	-	-	-	-	-	Not Detected	Normal	-
10	28	M	93.60	69.40	20.70	17.00	5.90	-	0.50	-	c.92+5G>C	Thalassaemia minor	β+/β
	31	F	93.80	68.50	19.40	16.20	5.70	-	0.50	-	c.92+5G>C	Thalassaemia minor	β+/β
	04	S/P	86.20	68.50	21.60	16.80	3.5	-	10.30	-	c.92+5G>C (Homo)	Thalassaemia major	β+/β+
	-	AF	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
11	27	M	93.50	66.40	21.70	18.20	5.90	-	0.60	-	c.92+5G>C	Thalassaemia minor	β+/β
	30	F	94.50	67.00	18.20	17.00	4.70	0.0	0.80	-	c.47G>A (p.Trp16Ter)	Thalassaemia minor	β0/β
	01	S/P	6.70	66.90	18.50	15.90	3.3	-	90	-	c.47G>A (p.Trp16Ter and c.92+5G>C	Thalassaemia major	β0/β+
	-	CVS	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β

[Table/Fig-1]: Haematological and molecular comparison of parents, proband/children and CVS/AF samples in 11 families.
M: Mother; F: Father; P: Proband; D: Daughter; S: Son; AF: Amniotic fluid; CVS: Chorionic villus sampling; MCV: Mean corpuscular volume; RDW: Red blood cell distribution width. Homozygous families with probands/children Nos. 1, 2, 6, 7, 8, 10 and had double mutations Nos. 3, 4, 5, 11 with probands/children (compound heterozygous). Case No. 9 with carrier. Two cases were normal. Case no 5 HbE carrier

Family No	Age	Sex	HbA (%)	MCV fL	MCH pg	RDW (%)	HbA2 (%)	HbE (%)	HbF (%)	HbD (%)	Mutations	Inference/clinical report	Genotype
01	24	M	94.30	75.00	24.00	14.60	5.00	0.0	0.70	-	c.92+5G>C	Thalassaemia minor	β+/β
	27	F	94.50	67.00	18.20	17.00	4.70	4.70	0.0	0.80	c.47G>A p.Trp16Ter	Thalassaemia minor	β0/β
	-	CVS	-	-	-	-	-	-	-	-	c.47G>A p.Trp16Ter	Thalassaemia minor	β0/β
02	25	M	93.95	69.50	20.80	17.10	5.40	0.0	0.65	-	c.92+5G>C	Thalassaemia minor	β+/β
	28	F	93.75	75.50	18.40	41.30	5.40	0.0	0.85	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	CVS	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
03	28	M	93.40	76.40	23.80	15.60	5.40	-	1.20	-	c.92+5G>C	Thalassaemia minor	β+/β
	30	F	96.80	79.90	28.00	14.00	2.60	-	0.60	-	Not detected	Normal	-
	-	AF					6.60				Not detected	Normal	-
04	25	M	93.95	74.90	18.60	15.00	5.00	-	1.05		c.92+5G>C	Thalassaemia minor	β+/β
	30	F	96.80	79.90	28.00	14.00	2.60	-	0.60	-	Not detected	Normal	-
	-	AF									Not detected	Normal	-
05	33	M	66.50	71.00	27.90	14.60	22.96	9.84	0.70	-	c.79G>A p.Glu27Lys	HbE Trait	βE/β
	33	F	92.80	70.10	20.90	16.90	6.60	-	0.60	-	c.47G>A (p.Trp16Ter)	Thalassaemia minor	β0/β
	-	CVS	-	-	-	-	-	-	-	-	c.79G>A p.Glu27Lys	HbE Trait	βE/β
06	24	M	94.95	68.90	22.30	16.90	4.80	-	0.25	-	c.92+5G>C	Thalassaemia minor	β+/β
	29	F	96.10	69.30	20.50	15.70	3.90	-	0.00		c.92+5G>C	Thalassaemia minor	β+/β
	-	AF	-	-	-	-	-	-	-	-	Not detected	Normal	-
07	24	M	17.90	67.50	18.40	16.30	68.90	12.70	0.50	-	c.79G>A p.Glu27Lys	HbE trait	βE/β
	26	F	95.20	68.30	20.80	15.50	4.60	-	0.20	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	AF	-	-	-	-	-	-	-	-	c.79G>A p.Glu27Lys	HbE trait	βE/β
08	27	M	96.10	70.30	21.90	17.20	3.60	-	0.30	-	619 bp deletion	Thalassaemia minor	β0/β
	30	F	95.40	68.50	21.20	17.70	4.10	-	0.50	-	619 bp deletion	Thalassaemia minor	β0/β
	-	AF	-	-	-	-	-	-	-	-	619 b deletion (Homo)	Thalassaemia major	β0/β0
09	27	M	94.75	62.20	20.40	16.80	4.80	-	0.45	-	c.92+5G>C	Thalassaemia minor	β+/β
	30	F	93.65	64.20	19.60	15.70	5.80	-	0.55	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	CVS	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
10	23	M	93.45	69.80	22.50	14.70	6.20	-	0.35	-	c.47G>A (p.Trp16Ter)	Thalassaemia minor	β0/β
	26	F	97.10	75.90	24.50	13.40	2.80	-	0.10	-	Not detected	Normal	-
	-	CVS	-	-	-	-	-	-	-	-	Not detected	Normal	-
11	33	M	94.40	69.50	20.70	17.60	5.40	-	0.20	-	619 bp deletion	Thalassaemia minor	β0/β
	35	F	94.50	64.10	19.70	16.90	5.20	-	0.30	-	619 bp deletion	Thalassaemia minor	β0/β
	-	CVS	-	-	-	-	-	-	-	-	619 bp deletion	Thalassaemia minor	β0/β
12	30	M	95.60	63.10	19.90	16.00	4.20	-	0.20	-	c.92+5G>C	Thalassaemia minor	β+/β
	32	F	94.80	70.00	20.10	15.80	4.80	-	0.40	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	CVS	-	-	-	-	-	-	-	-	Not detected	Normal	-
13	26	M	94.40	68.90	19.40	17.10	4.90	-	0.70	-	c.92+5G>C	Thalassaemia minor	β+/β
	26	F	94.65	69.90	20.30	17.60	4.80	-	0.55	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	AF	-	-	-	-	-	-	-	-	c.92+5G>C	Thalassaemia minor	β+/β
14	22	M	93.80	69.00	21.40	38.20	5.80	-	0.40	-	c.124_127delTTCT	Thalassaemia minor	β*
	26	F	94.30	63.20	19.50	16.10	5.20	-	0.50	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	AF	-	-	-	-	-	-	-	-	c.124_127delTTCT (Homo)	Thalassaemia major	β*/β*
15	29	M	93.55	67.40	19.10	17.80	6.10	-	0.35	-	619bp deletion	Thalassaemia minor	β0/β
	30	F	93.95	69.10	21.80	17.80	5.50	-	0.55	-	619bp deletion	Thalassaemia minor	β0/β
	-	CVS	-	-	-	-	-	-	-	-	Not detected	Normal	-
16	28	M	94.00	69.00	21.40	38.20	5.40	-	0.60	-	619bp deletion	Thalassaemia minor	β0/β
	30	F	93.60	69.40	20.70	17.00	5.90	-	0.50		c.27_28insG	Thalassaemia minor	β0/β
	-	AF	-	-	-	-	-	-	-	-	c.27_28insG	Thalassaemia minor	β0/β
17	27	M	94.40	69.00	21.80	17.40	5.20	-	0.40	-	c.92+5G>C	Thalassaemia minor	β+/β

	30	F	94.30	67.10	18.20	19.10	5.00	-	0.70	-	c.27_28insG	Thalassaemia minor	β0/β
	-	AF	-	-	-	-	-	-	-	-	Not detected	Normal	-
18	27	M	69.80	74.20	20.30	14.40	20.73	8.97	0.50	-	c.79G>A p.Glu27Lys	HbE trait	βE/β
	28	F	68.40	70.20	21.80	16.90	21.70	9.30	0.60	-	c.79G>A p.Glu27Lys	HbE trait	βE/β
	-	AF	-	-	-	-	-	-	-	-	Not detected	Normal	-
19	24	M	69.80	74.20	20.30	14.40	21.73	7.97	0.50	-	c.79G>A p.Glu27Lys	HbE trait	βE/β
	31	F	93.80	73.90	20.10	14.60	5.50	-	0.70	-	c.92+5G>C	Thalassaemia minor	β+/β
	-	AF	-	-	-	-	-	-	-	-	c.79G>A p.Glu27Lys	HbE	βE/β

[Table/Fig-2]: Haematological and molecular comparison in parents and CVS/AF samples in 19 families.
M: Mother; F: Father; P: Proband; D: Daughter; S: Son; Af: Amniotic fluid; CVS: Chorionic villus sampling; MCV: Mean corpuscular volume; RDW: Red blood cell distribution width. Homozygous families with CVS/AF Nos. 8, 14 and case number 6 had double mutations with father (1) and CVS (1) (compound heterozygous). Total normal cases 07, 09 form table-1 13/101; only 11 cases were normal. Case Nos. 5, 7, 18, 19 where each had two HbE carries of parents and CVS/AF

Nos.	Mutation types	No. of Mutations	Mother (30)	Father (28)	Proband (15)	CVS/AF (21)	Percentage (%)
1	c.92+5G>C	50	17	15	9	9	53.19
2	619 bp deletion	14	6	3	2	3	14.89
3	c.79G>A (p.E27K)	11	4	2	1	4	11.40
4	c.27_28insG	9	1	4	2	2	9.57
5	c.47G>A (p. Trp16Ter)	6	1	3	1	1	6.38
6	c.364G>C p.E122Q	2	0	1	0	1	2.12
7	c.124_127delTTCT	2	1	0	0	1	2.12

[Table/Fig-3]: Percent mutation types in families, probands/children and CVS/AF cases.
Total mutations: 94; Mutations in parents (Adult) 58/94 (62%); Probands (Children) 15/94 (16%); CVS/AF 21/94 (22%)

data who reported West-East population of India where mutation, c.92+5G>C and deletion 619 bp were dominant [4]. It means that offspring inherited the mutations from parents of the respective families as present studies follow hereditary laws. The 22% and 16% of mutations inherited from parents (62%) to CVS/AF and probands/children respectively. These mutations may cause thalassaemia disease, if such members in a family marry. In theory, 31% of families get affected in 5% of general population with this haemoglobinopathy [17]. However, the study by Mishra AK and Tiwari A obtained 76% siblings, as carrier of beta-thalassaemia mutations (18) (6/74), c.364G>C p.E122Q (2/74) and c.124_127delTTCT (2/74) respectively of 94 mutations [18]. Thus, parents contributed 58/94 (62%) children (Proband) 15/94 (16%) and CVS/AF 21/94 (22%) making siblings 38% thalassaemia disease [Table/Fig-3]. However, present data indicated about 36/94 (38%) siblings were detected of β-thalassaemia disorder. The discrepancy is due to sampling size, region, family types and other factors including RBC indices [19,20].

Homozygous and compound heterozygous (double mutations) states also appeared in present investigation where proband/children were more affected in comparison to CVS/AF cases in referrals sited earlier requiring blood transfusion. Compound heterozygous state included coinheritance of HbD/β0, HbE/β+ and β0/β+ which are to be noted for thalassaemia major types. Incidence of such heterozygotic victims are also fatal similar to homozygosity in present report as detected by Olivieri NF et al., [7]. These data thus confirmed that parents and siblings of 30 families had common mutations following the inheritance patterns of genetics, where c.92+5G>C and deletion 619 bp were dominated amongst eight common mutations analysed in present referral patients.

In the present study, 30 referral families with 101 cases, 88 (87%) cases detected positive for β-thalassaemia disease with 74 carriers (73.2%) and 11 families (11/30) had probands (children), who were affected and had no CVS/AF samples. In all parents (56.5%), followed by CVS/AF (19.8%) and children (10.9%) cases registered positive. Similarly, percent mutation of c.92+5 G>C was maximum followed by deletion 619 bp and others in parents (62%) following with CVS/AF (22%) and proband (16%). Such parent carriers in families would be screened and counseled to avoid the risk and burden of this disease in the society. Multi screening centers are a need in India for screening of this genetic disease to control.

CONCLUSION

Finally we would like to conclude that internal distraction patting is a simple, easy, reliable and more suitable method of treatment. This cohort emphasised screening of the population to control this genetic disorder. Hence, multi-centric testing opportunities need to be made available for this purpose to limit the burden of this disease in India.

ACKNOWLEDGEMENTS

Authors acknowledge the support extended by medical and non-medical staff of the Supratech Micropath laboratory and Research Institute, Ahmedabad, Gujarat, India.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 16, 2017**
Date of Peer Review: **Apr 06, 2017**
Date of Acceptance: **Nov 25, 2017**
Date of Publishing: **May 01, 2018**