Correlation of Cytogenetic, Molecular and Clinical Findings in Thalassemia Patients at a Tertiary Care Hospital

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ABSTRACT

BACKGROUND

Thalassemia syndromes are most common monogenic disorders which can be inherited by children from their parents. This study aimed to evaluate the correlation of cytogenetic (1p36 deletion), molecular (common mutations) and clinical findings such as haematologic parameters, age at presentation, nutritional status and transfusion requirements with β-thalassemia patients.

METHODS

In total, 140 β-thalassemia patients were clinically classified into β-thalassemia major (TM) or intermedia (TI). The cytogenetic analysis for 1p36 deletion was carried out in suspected patients with phenotypic dysmorphic, developmental delay and mental retardation by karyotyping. ARMS-PCR was performed to identify the common mutations in β-thalassemia patients. All data were analyzed by SPSS software.

RESULTS

Karyotyping was performed in 10 β-Thalassemia patients for 1p36 del. based on their physical appearance and intelligence; but, none of them were found to be positive for 1p36 del. Total 101 (72.14%) patients were of thalassemia major and 39 (27.86%) were of thalassemia intermedia. Among the thalassemia major patients, 71 (70.30%) have common mutation. In our study the IVS I-5 G→C mutation was most common 62 (87.32%) in thalassemia major patients. The clinical parameters such as Hb (gm/dl), HbA2 (%), HbF (%), MCV (fL), MCH (pg) and MCHC (gm/dl) were significantly associated with β-Thalassemia major.

CONCLUSIONS

In β-Thalassemia patients, 1p36 deletion was not observed. In our study, the IVS I-5 G→C mutation was the most common [62(87.32%)]. There were significant differences on the age of first transfusion, Hb (gm/dl), HbA2 (%), HbF (%), MCV (fL), MCH (pg) and MCHC (gm/dl) in between β-Thalassemia major and intermedia.

KEY WORDS

Cytogenetics, Thalassemia, Mental Retardation, Mutation, Genetic Disorder

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BACKGROUND

Thalassemia is one of the most common monogenic disorders in the world but it is a very heterogeneous disease at the clinical and molecular levels.[1-4] This is an inherited group of haemolytic anaemias, including beta-thalassemia and alpha-thalassemia, caused by absent or reduced production of the globin chains of haemoglobin (hb).[5-6]

These alterations depend on the degree of imbalances formed between α- and non-α-globin chains synthesis.[6] The incidence of this disease is high in many parts of the Indian subcontinent.[7] The β thalassaemias pose a significant health burden in India. The average prevalence of β thalassemia carriers is 3–4% which translates to 35 to 45 million carriers in our multi-ethnic and culturally and linguistically diverse population of 1.21 billion people which also includes around 8% of tribal groups according to the Census of India 2011. Several ethnic groups have a much higher prevalence (4-17%).[8,9]

The haematological and clinical spectrum of beta-thalassemia is very wide (Mild, intermedia and major) producing a diverse spectrum of clinical manifestations. So, the determination of factors causing such different clinical presentation of the disease has clinical importance. The underlying genetic mutation is one of the determinants of clinical presentation of beta thalassemia.[10]

β-Thalassemia major patients have severe anaemia, microcytic and hypochromic anaemia and hepato-splenomegaly. These thalassemia patients generally come to medical attention within the first two years of life.[11] Previously, there was no specific arrangement of congenital abnormalities associated with thalassemia and no constitutional chromosomal abnormalities have been identified in β-Thalassemia patients. Recently, a study reported that thalassemia patients with mental retardation was associated with microcephaly and congenital cataract, both having loss p36 position in chromosome 1 in Indian population.[12] The p36 short arm deletion on chromosome 1, associated in a syndrome with mental retardation and multiple congenital anomalies.[13] This monosomy 1p36 is the most common (Occurring in 1 in 5,000 births) terminal deletion syndrome in humans.[14,15]

Thalassemia major or thalassemia intermedia may develop by homozygous or compound heterozygous mutation. Thalassemia major are generally diagnosed within 2 years of age and regular blood transfusions is required for survival.[16] Whereas intermedia are diagnosed later and do not require regular blood transfusion for survival. The clinical indices of β-thalassaemias are greatly variable such as mild (Silent) mutations, mild hypochromic anaemia, moderate and severe lifetime transfusion-dependent anaemia and multi organ involvement.[17]

Presently, the molecular basis (Mutation) of β-thalassemia has been studied worldwide.[10] Maximum thalassemia mutations are contributed by small deletions, insertions or point mutations within the coding regions (CDS: coding sequence) and the exon-intron junctions.[10] The prevalence of β-thalassemia trait was 2.78 % and range from 1.48 to 3.64 % in different states. HbE trait was mainly seen in Dibrugarh in Assam (23.9 %) and Kolkata in West Bengal (3.92 %).[18] Now, more than 200 mutations have been known in β-thalassemia patients in chromosome 11, β-globin gene, resulting in a total or partial deficit of the synthesis β-globin chain and reduce of the production of adult haemoglobin (HbA).[6,11] The most common mutations for β-thalassemia in India are IVS I-5 (G-C), IVS I-1 (G-T), Co 41/42 (-CTTT), Co 8/9 (+G) and 619 bp del.

Our aim was to find out further information regarding anaemia since there are previously reported cases of anaemias associated with this 1p36 region with genetic disorder which is characterized by haematological abnormalities, other shows the 'putative tumour suppressor gene' in chronic myelocytic leukemia and the other is one inherited erythroblastopenia, commonly known as 'Diamond-Blackfan anaemia' (DBA), caused by mutation in the gene encoding ribosomal protein L11 (RPL11). In this study we also explore the correlation of cytogenetic, molecular and clinical findings such as haematologic parameters, age at presentation, nutritional status and transfusion requirements with β-thalassemia patients. Our data show the genotype–phenotype correlation of each mutation.

METHODS

Patient Selection

One hundred and forty beta-thalassemia patients who attended the Thalassemia Clinic, Department of Paediatrics, King George's Medical University, Lucknow were registered in this study after the haematological confirmation of thalassemia. Informed written consent was obtained from patients, as per the institute guidelines. The sample size was taken based on the convenience of the study. The study protocol confirms to the ethical guidelines of the 1975 Declaration of Helsinki. This study was approved by Institutional ethical committee (No. 69/Ethics/R.Cell-17) at K. G. Medical University, Lucknow. All beta thalassemia patients, who were being transfused and managed for the clinical symptoms and manifestation of the disease were included in the study. Any unconfirmed blood transfusion dependent children were excluded from the study. Beta-thalassemia patients were clinically classified into major and intermedia on the based criteria such as age at presentation, average Hb level at the steady state and transfusion frequency history, HbE and HbS. A whole clinical history along with blood transfusion events were recorded. Patients were also examined for growth parameters (Height, Weight, and Nutrition). The cytogenetics analysis was carried out in suspected patients with phenotypic dysmorphic, developmental delay and mental retardation.

Karyotyping for Chromosomal Abnormalities

Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2005). The blood sample was collected in heparin vials. 500 ul of blood sample was culture in 5 ml of media from (Gibco PB Max) up to 70 hours. 5 ml of exponentially growing cells were treated with 30 µl of Colcemid (10 µg/ml Gibco KaryoMax) and incubated for 1 hour at 37^0C and centrifuge at 1000 rpm for 10 minutes. Discard the supernatant suspend the cells in 5 ml of hypotonic solution and incubate at 37^0C for 30 minutes. Cells were fixed by the addition of 5 ml of fixative which consist of 3:1 cold methanol/acetic acid mixture. The fixation was repeated 3 times, and centrifuged after each addition. Metaphase cells were stored at -20°C in 1 ml fixative. This cold suspension of

metaphase cells was dropped from 30 cm height onto a horizontal microscopic slide and the fixative was allowed to evaporate at 56^{0}C temperature on a hot plate. Trypsin powder (0.15 gm) was added in 50 ml of phosphate buffer saline (PBS) solution. Depending on the ageing of the slide, dip the slides in trypsin solution for 3 to 5 seconds. Dip the slides in cold normal saline to stop trypsin activity and the wash under tap water. Keep the slides in Giemsa solution for 5 to 7 minutes and wash in tap water. Slide was covered with a 24x60 mm cover glass and sealed with DPX solution. Metaphase imaging was done on a Nikon eclipse 90i inverted microscope (Nikon Microsystems, Japan). Per slide a representative number of pictures was taken for analysis (At least 25 spreads per sample). Evaluation of chromosome counts per cell in each picture was done using the Genikon software.

Hematological Analysis

Haemoglobin, red blood cell counts, and red cell indices, were assessed. Automated high-performance liquid chromatography (HPLC) system was used for analysing the haemoglobin variants. This system utilized double-wavelength detection (416 and 690 nM). This cationic exchange column chromatography enables qualitative determinations of Hb A2, Hb F and abnormal haemoglobins in 6.5 minutes on a haemolysate prepared from 5 ml of venous blood.

Blood Collection and DNA Extraction

The blood sample of thalassemia patients were collected in EDTA vials. Genomic DNA was isolated from blood by standard commercial kits (QIAamp DNA Mini Kit) according to the manufacturer's instructions.

Molecular Analysis

Multiplex ARMS PCR was used to amplify different fragments corresponding to the coding regions of the concerned genes for β thalassemia mutation. PCR analysis was performed first for five common mutations. These mutations are IVS I-5 (G-C), IVS I-1 (G-T), Co 41/42 (-CTTT), Co 8/9 (+G) and -619bp deletion. We used ARMS-PCR for each mutation in the following manner: the DNA was amplified using a set of three primers fitted to amplify either the wild type or mutated allele as described by Newton et al. (1989) [19].

Mutation	Nucleotide Sequence	Tm (^{0}C)	Product Size
IVS1-1 M	5'-TTAAACCTGTCTTGTAACCTTGATACGAAA	56	281 bp
IVS1-5 M	5' -CTCCTTAAACCTGTCTTGTAACCTTGTTAG	59	285 bp
Cd 8/9 M	5'-CCTTGCCCCACAGGGCAGTAACGGCACACC	70	225 bp
Cd 41/42 M	5'-GAGTGGACAGATCCCCAAAGGACTCAACCT	64	439 bp
619 bp del	5'-GAG TCA AGG CTG AGA GAT GCA GGA-3	61	242 bp
Internal Control C1	5'-CAATGTATCATGCCTCTTTGCACC	56	861 bp
Internal Control C2	5'-GAGTCAAGGCTGAGAGATGCAGGA	59	
Reverse Primer B1	5'-ACCTCACCCTGTGGAGCCA	58	
Reverse Primer B2	5' -CCCCTTCCTATGACATGAACTTAA	54	

Table 1. List of the Primers

Statistical Analysis

Continuous data were summarized as Mean ± SD while discrete (categorical) data in percentage. Categorical variables in two groups were compared using the Chi-square test. Continuous variables in two groups were compared by t-test. The p-value<0.05 was considered significant. All the analysis was carried out using SPSS 16.0 version (Chicago, Inc., USA).

RESULTS

One hundred forty beta-thalassemia patients who attended the Thalassemia Clinic, Department of Paediatrics, King George's Medical University, Lucknow were registered in this study. Basic characteristics of patients are shown in Table 2. Range of age among thalassemia patients was between 4 and 408 months Mean age of patients was 40.19±53.00 months. Out of 140 beta thalassemia patients, 101 patients had beta thalassemia major and 39 patients had beta thalassemia intermedia.

	n	Mean	Median	SD	Min	Max
Age (Months)						
Age (months	140	40.19	18.00	53.00	4.00	408.00
Age 1-12 months	56	8.91	9.00	2.87	4.00	12.00
Age 13-24 months	33	20.73	24.00	3.64	12	24.00
Age 24-60 months	25	45.36	48.00	11.50	30	60.00
Age > 60 months	26	127.31	108.00	69.61	66	408.00
Gender	**n**	**%**	-	-	-	-
Male	101	72.14	-	-	-	-
Female	39	27.86	-	-	-	-
Religion						
Hindu	91	65.0	-	-	-	-
Muslim	49	35.0	-	-	-	-
Clinical Investigation	**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
Hb	135	5.11	5.10	1.37	1.70	9.30
HbA2	140	6.23	3.1	12.94	0.00	81.10
HbF	140	54.55	52.75	30.87	0.30	97.80
MCV	140	69.36	68.95	11.85	6.50	99.50
MCH	140	24.29	23.75	4.95	14.40	46.40
MCHC	140	35.11	34.70	5.19	17.60	49.10
RDW %	140	32.27	33.20	7.41	0.90	46.50
Ferritin Blood	132	1764.52	1705.0	688.98	193.80	4495.0

Table 2. Baseline Characteristics of Beta-Thalassemia Patients

The mean age of first visit of β-Thalassemia patients were 34.40±41.37 and 55.21±73.83 months in major and intermedia, respectively in our Thalassemia clinics. Most of β-Thalassemia patients (67.32%) were visit within 24 months. Age of first transfusion was 9.59±10.44 and 39.00±36.61 in β-Thalassemia Major and β-Thalassemia Intermedia, respectively. The mean age of first visit, age of first transfusion, height (cm), weight (kg) and clinical parameters (Hb, HbA2, HbF, MCV, MCH and MCHC) were significant different in between β-Thalassemia Major and β-Thalassemia Intermedia (Table 3). The β-Thalassemia was more common in male, 77.23% in major and 58.97% in intermedia. Out of 101, total 66 (65.35%) patients were belong to Hindu religion whereas 40 (34.65%) patients were belong to Muslim religion in β-Thalassemia Major. Whereas 64.10% Hindu and 35.90% Muslim religion in β-Thalassemia intermedia.

The distribution of beta-thalassemia in major 101 (72.14%) and intermedia 39 (27.86%) patients (Table 4). In beta-thalassemia in major 71 (70.30%), the common mutations are further classified in to IVS 1-1 G→T, IVS I-5 G→C, Cd 8/9 +G, Cd 41/42 (-TCTT) and 619 bp deletion. Out of 71, maximum number of patients 62 (87.32%) have IVS1-5 G-C common mutation, whereas 7.04% in Cd 8/9+G, 2.82% and 1.41% in IVS 1-1 G→T and 619 del each. Whereas, in β-Thalassemia intermedia 39 (29.70%), out of 25, 2 (5.12%) have IVS1-5 G-C common mutation, Whereas 5 (12.82%) in HbS, 12 (30.77 %) in HbE, 1 (2.65%) in HbS+ IVS I-5 G→C, 3 (7.69%) in HbE+ IVS I-5 G→C and 2 (5.13%) in HbE+Cd 41/42 (-TCTT).

	β-Thalassemia Major (n=101)		β-Thalassemia Intermedia (n=39)		p
Age Months)	**Mean**	**SD**	**Mean**	**SD**	
Age (months	34.40	41.37	55.21	73.83	0.037*
	n	**%**	**n**	**%**	
Age 1-12 months	42	41.58%	14	35.90%	0.045*
Age 13-24 months	26	25.74%	7	17.95%	
Age 24-60 months	20	19.80%	5	12.82%	
Age > 60 months	13	12.87%	13	33.33%	
Age of First Transfusion (month)	**Mean (n=101)**	**SD**	**Mean (n=27)**	**SD**	
	9.59	10.44	39.00	36.61	<0.001*
Weight (kg)	8.10	5.41	16.74	7.31	<0.001*
Height (cm)	74.49	17.16	106.59	22.56	<0.001*
Gender	n	%	n	%	
Male	78	77.23%	23	58.97%	0.051
Female	23	22.77%	16	41.03%	
Religion					
Hindu	66	65.35%	25	64.10%	0.890
Muslim	35	34.65%	14	35.90%	
Clinical Investigation	**Mean**	**SD**	**Mean**	**SD**	
Hb (gm/dl)	4.86	1.34	5.77	1.21	<0.001*
HbA2 (%)	3.55	4.51	13.19	22.13	<0.001*
HbF (%)	63.14	29.73	32.32	21.38	<0.001*
MCV (fL)	71.20	9.74	64.60	15.23	0.003*
MCH (pg)	25.34	4.77	21.57	4.37	<0.001*
MCHC (gm/dl)	36.045	4.881	32.69	5.24	<0.001*
Ferritin Blood (ng/ml)	1810.8	682.06	1613.8	701.04	0.165

Table 3. Comparison of Baseline Characteristics in Beta-Thalassemia Major and Intermedia Patients

Mutation	n	%
β-Thalassemia major (n=101)		
Common Mutation	71	70.30
IVS 1-1 G→T	1	1.41
IVS I-5 G→C	62	87.32
Cd 8/9 +G	2	2.82
Cd 41/42 (-TCTT)	5	7.04
619 del	1	1.41
Unidentified Mutation	30	29.70
β-Thalassemia intermedia (n=39)		
Common Mutation	25	64.10
IVS I-5 G→C	2	5.12
Combined Mutation		
HbS	5	12.82
HbE	12	30.77
HbS+ IVS I-5 G→C	1	2.65
HbE+ IVS I-5 G→C	3	7.69
HbE+Cd 41/42 (-TCTT)	2	5.13
Unidentified Mutation	14	35.90

Table 4. Genotypic Distribution of β-Thalassemia Major Patients (n=101)

On the basis of mean fetal haemoglobin (Haemoglobin F or HbF), the distribution of types of mutation, MCV and MCH in β-Thalassemia major patients are shown in Table 5. Total 45.54% β-thalassemia major patients have less than 63.14 fetal haemoglobin (Haemoglobin F or HbF) with 63.04% common mutation and 36.96% unidentified mutation, in which 86.96% with MCV≤80 and 73.91% have MCH≤27. Whereas 54.46% β-thalassemia major patients have equal or greater than 63.14 fetal haemoglobin (Haemoglobin F or HbF) with 76.36% common mutation and 23.64% unidentified mutation, in which 72.73% with MCV≤80 and 70.91% have MCH≤27. On the basis of HbF level, the distribution of patients was comparable in common and unidentified mutation, moreover, slightly higher number of β-Thalassemia major patients have ≥ 63.14 HbF level with a smaller number of patients with MCV≤80 and MCH≤27. In this study, common mutation in beta thalassemia patients have greater HbF value compare to unidentified mutation.

Hb F	No. of Patients n (%)	β-Thalassemia Major		MCV ≤80	MCH ≤27
		Common Mutation	Unidentified		
<63.14	46 (45.54%)	29 (63.04%)	17 (36.96%)	40 (86.96%)	34 (73.91%)
≥ 63.14	55 (54.46%)	42 (76.36%)	13 (23.64%)	40 (72.73%)	39 (70.91%)
p-Value		0.147		0.081	0.074

Table 5. Distribution Types of Mutation, MCV and MCH on the Basis of Mean HbF in β-Thalassemia Major

On the basis of mean fetal haemoglobin (Haemoglobin F or HbF), the distribution types of mutation, MCV and MCH in β-Thalassemia intermedia patients are shown in Table 6. Total 53.85% β-thalassemia intermedia patients have less than 32.32 fetal haemoglobin (Haemoglobin F or HbF) with 71.43% common mutation and 28.57% unidentified mutation, in which 85.71% with MCV≤80 and 90.48% have MCH≤27. Whereas 46.15% β-thalassemia intermedia patients have equal or greater than 32.32 fetal haemoglobin (Haemoglobin F or HbF) with 55.56% common mutation and 44.44% unidentified mutation, in which 94.94% with MCV≤80 and 83.33% have MCH≤27. On the basis of HbF level, the distribution of patients was comparable in common and unidentified mutation, moreover, slightly higher number of β-Thalassemia intermedia patients have <32.32 HbF level.

Hb F	No. of Patient's n (%)	β-Thalassemia Intermedia (n=39)		MCV ≤80	MCH ≤27
		Common Mutation	Unidentified		
< 32.32	21 (53.85%)	15 (71.43%)	6 (28.57%)	18 (85.71%)	19 (90.48%)
≥ 32.32	18 (46.15%)	10 (55.56%)	8 (44.44%)	17 (94.94%)	15 (83.33%)

Table 6. Distribution Types of Mutation, MCV and MCH on the Basis of Mean HbF in β-Thalassemia Intermedia

Test	Sensitivity	Specificity	PPV	NPV
HbA2 (%)	87.3%	42.0%	70.4%	67.7%
HbF (%)	93.3%	43.8%	55.4%	89.7%
MCV (fL)	72.9%	28.8%	43.0%	59.0%
MCH (pg)	85.4%	35.6%	41.4%	81.1%

Table 7. Sensitivity, Specificity, NPV and PPV of HbA2, HbF, MCV and MCH to Predict β-Thalassemia Major

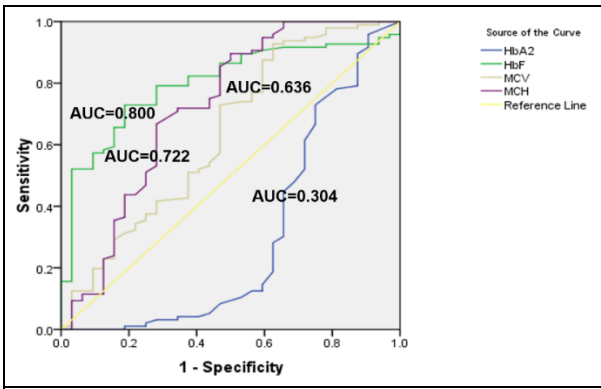

Figure 1. Receiver Operating Characteristic (ROC) Curve Analysis of HbA2, HbF, MCV and MCH in β-Thalassemia Major Patients. Each Receiver Characteristic Curve is Expressed as a Solid Line. AUC: Area Under the Curve

The sensitivity, specificity, positive and negative predictive values (PPV and NPV) were used to analyse the HbA2, HbF, MCV and MCH (Table 7). The cut-off value for HbA2, HbF, MCV and MCH were 3.2, 63.14, 71.20 and 25.34, respectively to make a diagnosis of β-Thalassemia major. With these cut-off

values, HbF and HbA2 had greater sensitivity of 93.3%, 87.3% and specificity of 43.8% and 42.0%, respectively in the diagnosis of β-Thalassemia major. The HbA2, HbF, MCV and MCH were showed significantly large area under the curve (AUC=0.487) on the ROC curve (Fig. 1).

Out of 140 beta thalassemia patients, karyotyping was performed only in 10 patients for 1p36 del. based on their physical appearance and intelligent. Quotient only 10 patients were taken into consideration for 1p36 del, but none of them was found to be positive for 1p36 deletion. They all were normal (Fig. 2).

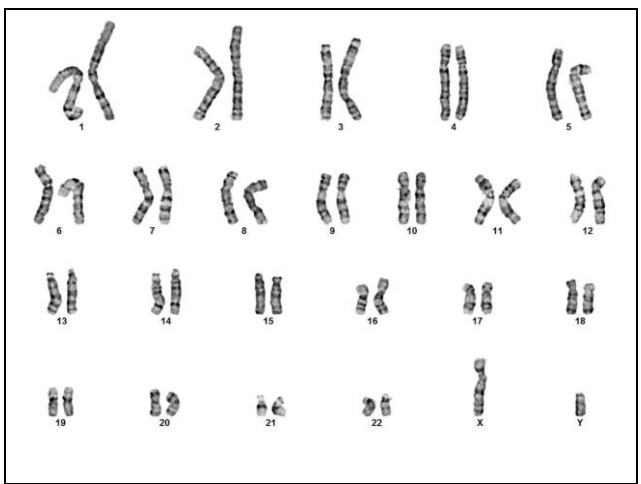

Figure 2. Karyotype Showing Normal Chromosome

DISCUSSION

Beta-Thalassemia is one of the most common monogenic disorders which can be transmitted from parents to their children. The type of β-globin gene mutation mainly affects the severity of beta-thalassemia disease. In this study we revealed the distribution of the β-globin gene mutations affecting the beta-thalassemia along with phenotype-genotype correlation in North Indian paediatrics population. Beta-thalassemia is the most important thalassemia problem in India. It can be linked with numerous clinical phenotypes ranging from thalassemia intermedia to thalassemia major. In our study, all thalassaemic patients are from Uttar Pradesh state. The treatment facilities for thalassemia patients including transfusion, chelation and specialist should be made available at medical college/district hospitals in various part of the state due regions where there is high incidence of thalassemia so that the families don't have to migrate to bigger cities for treatment.

The cytogenetic analysis of a distal short arm deletion of chromosome with dysmorphic features, develop mental and growth delays with microcytic anaemia. By cytogenetic analysis, the deletion appeared to involve 1p36.21 and 1p36.3. Maximum deletions in chromosome 1p36 are novel mutations and occur during the formation of gametes (eggs or sperm), before fertilization. The patients with 1p36 deletion their parents have a balanced/ symmetrical translocation. It means a 36' portion of chromosome one is relocated to another chromosome, when it happens, gametes are missing a portion of 36 in chromosome one during cell division before fertilization.[12]

In our study, we did not find 1p36 del. In beta thalassemia patients with phenotypic dysmorphic, developmental delay and mental retardation. Previously, De et al. (2014) revealed 46, XY, del (1) (p36.21) in the male who was also diagnosed as a 'beta thalassemia trait' and the other case was 46, XX, del (1) (p36.3) in the female who was diagnosed as a case of 'HbE-beta thalassemia'. Previously, Shapira et al. (1997) reported that the p36 deletion in chromosome one in a syndrome have multiple congenital abnormalities and mental delay.[13] This monosomy terminal deletion is most common (1 in 5,000 births) in humans.[14,15]

In our study, the maximum number (67.32%) of beta thalassemia patients was 1-24 months of age with severe haemolytic anaemia. Similarly, the mean age was 17.2±19.9 months, with 50% being diagnosed within the first year of life in an Indian cohort study.[20] Moreover, in 2016, a lower mean age of 3.7 years was reported in a similar study carried out in India.[21] Kattamis et al. (2005) reported that the age at which thalassemia presentation was noted to be 13.1 (2–36) months.[22] Our study was supported by Cao and Galanello, (2000),[23] who reported that the mean age of children with thalassemia to be 8.4±9.1 months. Moreover, Modell and Berdoukas.[24] also reported that the 60% of thalassemia patients with the mean age of 6 months had presented clinically in the first year of life. Thalassemia major is generally suspected in an infant younger than 24 months of age with severe microcytic anaemia and hepatosplenomegaly. Whereas Thalassemia intermedia shows at the later age with common but milder clinical findings.

In our study the Beta thalassemia was more prominent in male (72.14%) with a male to female ratio of 2.6:1. Similarly, various previous Indian studies have reported a higher male preponderance of up to 68% and 69.5%.[25,26] Shah et al. (2010) found that in western India, 62% (88 cases) of their studied cases with thalassemia were males, whereas 38% (54 cases) were females.[27] In 2015, a higher male to female ratio (2.5:1) was reported among Indian children with thalassemia, with 71.4% being males.[20] In addition, in 2016, a higher male predominance was reported among Saudi children with thalassemia as 70% were males, with a male to female ratio of 2.3:1.[28] Various other studies also reported that the male was slightly male predominance was detected in their study population.[21,29-31] Al-Kherbash et al. (2017) reported that the slight predominance was detected among as males represented 53.2% of the total studied cases, with a male to female ratio of 1.14:1.[32] In 2017, another study also reported that the male to female ratio 1.26 in thalassemia patients in Bangladesh.[29] A similar result was reported in an Indian study, in 2016, which found that 53.3% of their cases were males and 46.6% were females.[30] Zamani et al. (20015) reported that among their studied thalassaemic patients in Hamdan province in Iran, 54.9% were males.[30] In addition, Bejaoui and Guirat, (2013) found that in Tunisia, 55.4% of male patients were thalassaemic, whereas 44.5% were female patients.[31]

In our study, the incident of beta thalassemia was greater in Muslim population. The Muslim population in Uttar Pradesh is 19.3% whereas 27.86% thalassemia patients are Muslim. This is mainly due the consanguinity among them. A comparably lower consanguinity rate (53%) was detected in western India in 2010 as Shah et al. (2010 reported that the Muslim patients with thalassemia in their study were a result

of a consanguineous marriage.[27] In Shiraz city, Iran, Asadi-Pooya and Doroudchi [33] reported that 49.5% of thalassaemic patients were a result of cousin marriages. In 2016, consanguineous marriage was detected in 26.6% of the parents of thalassaemic Indian children.[21]

In this study the total 101 (72.14%) patients are thalassemia major and 39 (27.86%) are thalassemia intermedia. Out of 101 patients are thalassemia major, total 71 (70.30%) have common mutation. In our study the IVS I-5 G→C mutation was most common 62 (87.32%), whereas 2 (2.82%) Cd 8/9, 5 (7.04%) Cd41/42 and 1 (1.41%) IVS 1-1 and 619bp del in thalassemia major patients. In this study, out of 39 beta thalassemia intermedia patients, total 25 (64.10%) have common, HbS and HbE mutation. The HbE mutation was commonly found in intermedia 17 (68.0%), whereas 6 (24.0%) and 1 (4.0%) IVS 1-5. Similarly, Shah et al. (2017) reported that the 92+5 G>C (IVS-1–5) mutation is the maximum in cases (60.29%), of Rajasthan and Gujarat followed by deletion 619 bp.[34] Various previous studies reported the similar findings with others documented earlier in Gujarat, Maharashtra, and Rajasthan.[35-41] Whereas, Hassan et al. (2013), reported that the cd26 (A-G) HbE and cd41/42 (−TTCT) were higher in their studies in Thailand population.[42] Thong et al. (2005) demonstrated that the cd41/42 (−TTCT) and IVS-2 654 (C-T) were greater in Chinese population.[43] The incidence of these mutations 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.[44,45] The phenotype of compound heterozygous β-thalassemia can be intermedia or major. Individuals with β-thalassemia intermedia require RBC transfusion within few years after their birth, whereas β-thalassemia major would require medical treatment within two years.[36] The compound heterozygosity has led to $\beta^+ \beta^0$ type causing β-thalassemia major. Usually in $\beta^+ \beta^0$ type β-thalassemia, the Hb pattern shows HbA between 10 and 30%, HbF of 70–90% and HbA2 of 2–5%.[46] Murali et al. (2004) have checked the prevalence of different mutations in different districts of Andhra Pradesh and Karnataka.[47] They can observe that IVS1+5G>T mutation is the most popular around Andhra Pradesh and Karnataka. Krishna district of Andhra Pradesh has100% IVS1+5G>T mutation followed by Adilabad 87.5% and Hyderabad 77%. Kirti et al. (2004) conducted a survey on different mutations present in different states in South India.[48] They found that the IVS I-5 (G→C) mutation prevalence is more, Andhra Pradesh IVS I-5 (G→C) 66.18% Karnataka 45.76% Kerala 62.7% and Tamilnadu 56.6%. Sinha et al. (2009), reported that the IVSI-5(G>C) is more 67.7% in beta thalassemia patients.[49]

In our study, the β-thalassemia major is characterized by reduced Hb level (<4.86 g/dl), mean corpuscular volume (MCV) 71.2 fl and mean corpuscular Hb (MCH) 25.34 pg. Whereas intermedia is also characterized by reduced Hb level (<5.77 g/dl), mean corpuscular volume (MCV) 64.60 fl and mean corpuscular Hb (MCH) 21.57 pg.

Similarly, Galanello et al. (1979), reported that the β-Thalassemia major was characterized by reduced Hb level (<7 g/dl), mean corpuscular volume (MCV) > 50 < 70 fl and mean corpuscular Hb (MCH) > 12< 20 pg.[50] Whereas, β-Thalassemia intermedia was characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor was characterized by reduced MCV and MCH, with increased Hb A2 level.[50]

In this study β-thalassemia major patients 46 (45.54%) have less than 63.14 fetal haemoglobin, in which 29 (63.4%) common mutation and 17 (36.96%) unidentified mutation with 40 (86.96%) patients have MCV≤80 and 34 (73.91%) patients have MCH≤27. Whereas 55 (54.56%) patients have ≥ 63.14 fetal haemoglobin, in which 42 (76.36%) common mutation and 13 (23.64%) unidentified mutation with 40 (72.73%) patients have MCV≤80 and 39 (70.91%) patients have MCH≤27. This study shows that the beta thalassemia patients with common mutation have greater HbF value. Whereas the greater β-thalassemia major patients have less common mutation, MCV≤80 and MCH≤27 are slightly reduced in ≥63.14 HbF group as compared to < 63.14 HbF. MCV of less than 80 fL was reported in 96.5% cases of the β-thalassemia minor cases, 54.2% cases of sickle/β-thalassemia, 100% (5/5) cases of HbE disease, 100% (2/2) cases of HbE/β-thalassemia, 40% (2/5) cases of HbE traits, 100% (6/6) cases of HbD Punjab (100%), and 76.9% cases in α-thalassemia trait. An MCH value less than 27 pg was observed in 100% of β-thalassemia minor cases, 92% of α-thalassemia trait, 100% with sickle/β-thalassemia cases, 100% of HbE disease, 100% of HbE/β-thalassemia, 60% of HbE trait, and 83.3% of HbD Punjab. Similar results were reported earlier.[51,52] The cases of β-thalassemia minor showed reduction in MCV and MCH values, and, thus, these parameters found to be important in diagnosis of β-thalassemia carriers. The degree of microcytosis and type of thalassemia mutation has shown wide variations in ranges of MCV.[52]

In our study, the cut-off values of HbA2 3.2%, HbF 63.14%, MCV 71.20 fl and MCH 25.34 pg were suggested to be associated with a high probability of β-thalassemia major. Our study was supported by a Indian study conducted by Bhukhanvala et al. (2013), who reported that the cut-off values of MCV 78.0 fl or less, MCH 28 pg or less, and HbA_2 more than 3.8% for β-Thalassemia trait diagnosis.[53] Previously, Soliman et al. (2014) found that the cut-off values of MCV 63.14 and MCH fl or less were suggested to be associated with a high probability of β-Thalassemia trait.[54] Though, Parthasarathy, (2012) observed that the cut-off values of MCV below 76 fl was suggested to be associated with a high probability of β-Thalassemia trait in Indian population.[55]

CONCLUSIONS

The 1p36 deletion in β-Thalassemia patients was not observed. In our study, the IVS I-5 G→C mutation was most common [62 (87.32%)]. There were significant differences on the age of first transfusion, Hb (gm/dl), HbA2 (%), HbF (%), MCV (fL), MCH (pg) and MCHC (gm/dl) between β-Thalassemia major and intermedia.

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