e-ISSN: 0975-9506, p-ISSN:2961-6093

Available online on www.ijpqa.com doi: 10.25258/ijpqa.16.7.14

International Journal of Pharmaceutical Quality Assurance 2025; 16(7); 70-81

Original Research Article

Phenotypic Expression of Hemoglobinopathies: A Single Centre Study

Sonal Paul[1], Jina Bhattacharyya[2], Smita Das[3], Dhanjit Haloi[4], Damodar Das[5], Sewali D Talukdar[6]

[1]Consultant, Hematologist, Saroj Gupta Cancer Centre and Research Institute, Kolkata, W.B.
[2]Professor, Department of Clinical Hematology, Gauhati Medical College & Hospital, Assam
[3]Associate Professor, Department of Clinical Hematology, Gauhati Medical College & Hospital, Assam
[4]Register, Department of Clinical Hematology, Gauhati Medical College & Hospital, Assam
[5]Assistant Professor, Department of Clinical Hematology, Gauhati Medical College & Hospital, Assam
[6]Register Clinical, Department of Hematology, Gauhati Medical College & Hospital, Assam

Received: 01-04-2025 / Revised: 16-05-2025 / Accepted: 05-06-2025
Corresponding Author: Dr. Smita Das
Conflict of interest: Nil

Abstract

Background: Hemoglobinopathies are among the most prevalent genetic disorders worldwide, with high phenotypic heterogeneity. The northeastern region of India shows a predominance of hemoglobin E and (HbE)-related variants.

Methods: This prospective cross-sectional study was conducted at a tertiary care centre in N E India. Seventy patients aged over six months and diagnosed with hemoglobinopathy were included. Detailed demographic data, clinical features, hematological parameters, HPLC results, serum ferritin levels analysed. Genetic mutation profiles were analysed in twenty-five of these patients. Transfusion dependency and phenotypic outcomes were also studied.

Results: HbE-related disorders were the most common, seen in 65.7% of cases. HbE/β-thalassemia accounted for 44.2%, followed by beta-thalassemia major (17.1%) and sickle cell disease (5.7%). Nearly 48.5% of the patients were transfusion-dependent. Poorly transfused individuals had significantly higher serum ferritin, hepatosplenomegaly, short stature, delayed puberty, and endocrinopathies. Among genetically analyzed cases, the most frequent mutations were c.79G>A (HbE variant) and IVS1-5(G>C) (β-thalassemia), with compound heterozygosity often predicting a severe phenotype.

Conclusion: HbE/β-thalassemia is the predominant hemoglobinopathy in Assam with diverse clinical presentations. Transfusion status and chelation significantly influence growth and endocrine health. Mutation profiling enhances understanding of disease severity and can guide individualized care. Population-based studies are warranted to further characterize the regional mutation spectrum and phenotypic variability.

Keywords: Hemoglobinopathies-HbE/β-thalassemia-Transfusion dependence-Genetic mutations-Northeast India.

Introduction

Hemoglobinopathies are the most common genetically inherited disorders. The World Health Organization (WHO) estimate that 5% of world's population are carriers for the genetic hemoglobin (Hb) disorders.

In India, the prevalence of hemoglobinopathies is around 4.2%.[1] The incidence of hemoglobinopathies also differs in different parts of India. In Orissa [2] HbS is very common whereas in Northeast and East India, the commonest hemoglobinopathy seen is HbE. [3,4] Hb D-Punjab occurs with greatest prevalence (2%) in Sikhs in Punjab.[5] The ICMR study showed that HbE was mainly seen in Assam (23.9%) and Kolkata in West Bengal (3.92%).[6]

Abnormalities of Hb involve thalassemia and Hb variants which are due to abnormal globin chain synthesis. Thalassemia is characterized by reduced synthesis of normal Hb due to absence or decrease in the synthesis of one or more types of globin polypeptide chains. These disorders result from both the underproduction of Hb and imbalanced globin chain synthesis, leading to a shortened red cell survival rate.

The two principal types of thalassemia are alpha (α) and beta-thalassemia. They arise due to the reduced rate of synthesis of the corresponding α-chain and β-chain. The prevalence of beta-thalassemia trait and sickle cell in various parts of India is around 3%–17% and 1% 44%, respectively, because of

consanguinity, caste, and area endogamy. HbE-β thalassemia and sickle-cell anaemia are also common Hb disorders, which are prevalent in many parts of India. [5-8]

The clinical spectrum of these disorders varies from asymptomatic conditions (beta-thalassemia minor) to serious disorders such as thalassemia major that require regularblood transfusions and extensive medical care. Patients may present with generalized weakness, skeletal deformities, abnormal facies, growth retardation.[9]

Transfusion dependent thalassemia patients are at risk for significant iron overload and deposition of iron in liver, myocardium and other organs leading to cirrhosis, liver fibrosis, myocarditis, cardiomyopathy, arrhythmias, growth retardation and other serious manifestations. Early initiation of iron chelation therapy is essential to prevent iron overload and toxicity. In some cases, splenectomy can reduce the symptoms and lead to a better overall survival. [9,10]

Aims and Objectives: The primary objective of this study was to study the demographic characteristics of the study population with phenotypic expression of all haemoglobinopathies and its corelation with genotype. The secondary objective was to study underlying nutritional deficiency and magnitude of transfusion dependency.

Materials and Methods

This Prospective Hospital based Cross sectional study was conducted in Department of Clinical Haematology, Gauhati Medical College, Assam in a period of one year.

Sample size: Total 70 patients were chosen based on the inclusion and the exclusion criteria.

Method of collection of data

Interviewing patients and the attendants and through physical examination with relevant investigations.

The institutional ethics committee cleared the study. Informed consent was taken from the patients/ parents of children before proceeding data collection. Pre-structured and pretested pro-forma was used to collect the data. The baseline data including the socio-demographic history including ethnic origin, age, sex was collected after interviewing the patients and the attendants.

A total of 70 patients attending the OPD of Clinical Haematology department were studied as per the inclusion and exclusion criteria. A detailed history was taken with emphasis on personal history, parent consanguinity, family history of hemoglobinopathies, family history of endocrine disorders, number of blood transfusions/month and duration of disease. Thorough physical examination was performed including anthropometric measurements, pallor, jaundice, presence of haemolytic facies, organomegaly, Tanners staging and splenectomy.

Laboratory investigations: 3ml of venous blood was collected in EDTA vials from each individual to perform complete blood count, peripheral smear, reticulocyte count, and High-Performance Liquid chromatography. 3ml blood in each of the two plane vacutainers was collected for serum ferritin level, thyroid stimulating hormone (TSH), free thyroxine 3 (FT3), free thyroxine 4(FT4), Liver function test, renal function test and viral markers. HbA2 level of >4% was used as a cut-off for diagnosis of beta thalassemia trait. Genetic Analysis including Beta globin gene mutation and Alpha globin gene mutation studies were performed were feasible.

Methods of tests

All patients were investigated for complete blood counts and red cell indices on automated analyser (Sysmex 500 5-part coulter counter). Giemsa-stained peripheral blood smears were examined for red cell morphology.

Quantitative analysis of Hbs A, F, E/A2, Hb S was done using HPLC (Bio Rad D 10 Hemoglobin Testing System). The Bio-Rad D 10 is a fully automated HPLC system to separate and determine area percentages for haemoglobin A2 and F and to provide quantitative determinations of abnormal haemoglobins. 1–2 ml of whole blood samples was collected in EDTA vials and were stored at 2–8 degree Celsius. EDTA anticoagulated 5 µl whole blood samples were mixed with 1.0 ml of haemolysis reagent to each sample vial and were analysed in batches. The prepared samples were injected sequentially into the analysis stream at 6.5-min intervals and separated by the cation exchange cartridge using a phosphate ion gradient generated by mixing two buffers of different ionic strengths to elute the different haemoglobins. HbA2/F calibrator and two-level controls were analysed at the beginning of each run.

A dual-wavelength filter photometer analysed the haemoglobin elution from the cartridge by detecting the absorbance changes at 415 nm and the secondary filter at 690 nm corrected the baseline for effects caused by mixing buffers with different ionic strengths. Total acceptable area of each analysis ranged from 1 to 3 million volts. 3ml of blood was collected by vein puncture and allowed to clot. The separated serum was used for the estimation of ferritin levels using ELISA based serum ferritin assay kit (Acculite ferritin kit) by chemiluminescence immunoassay method. Thyroid function was assessed by measuring serum level of

T3, T4 and TSH using commercial quantitative ELISA kits.

Liver function test and renal function test were performed by using automated biochemistry analysers.

After analysis of the samples, parents and siblings of the patients were screened and counselled.

Inclusion Criteria: Patients of all age groups (>6 months) diagnosed with haemoglobinopathy.

Exclusion Criteria: Patients less than 6 months of age,patients suffering concurrently with any other major illness and Patients who did not give consent.

Statistical Method: Data was entered in Microsoft Excel. Statistical analysis was done by SPSS (Statistical Package for Social Science) software (by IBM company). Data was analysed using the following statistical tests. Histogram and Pie diagrams were used for descriptive statistics. Frequency, percentage, Mean and standard deviation were used for all quantitative data. Chi Square test was used to evaluate association between categorical variables. Analytical data were analysed using appropriate tests of significance (Independent Student t test and ANNOVA) .A "p" value less than 0.05 is considered as statistically significant at 5% level of significance.

Results and Observation

Age distribution: In the present study majority of the patients belonged to age group of 12 years &less (60%) paediatric, while 40% of the patients were adults.

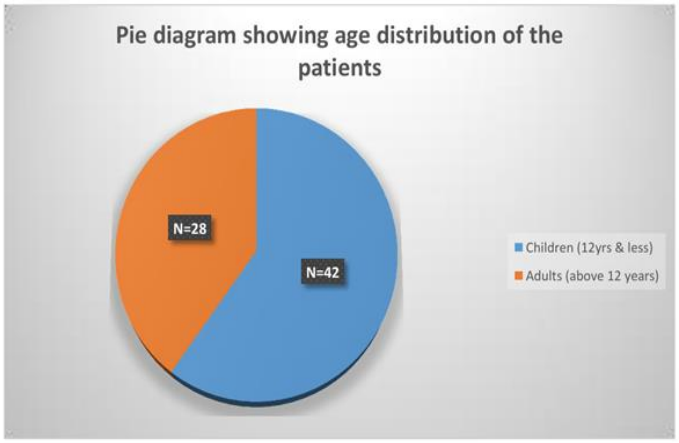

Figure 1: Pie diagram showing age distribution of the patient

Sex distribution: 53% of the patients were male and 47% were female. Demographic distribution: 51% of the patients were from rural areas and 49% were from urban. Distribution of Hemoglobinopathies: 46(65.7%) patients had a variant of HbE disorder. 31(44.2%) patients had HbE Beta thalassemia, 10(14.2%) had HbE disease and 5(7.1%) had Hb E trait. 12 patients (17.1%) had beta thalassemia major while 5 (7.1%) had beta thalassemia trait. 4 patients (5.7%) had sickle cell anaemia while 2 patients (2.8%) had sickle cell trait. There was one case of alpha thalassemia diagnosed by genetic studies.

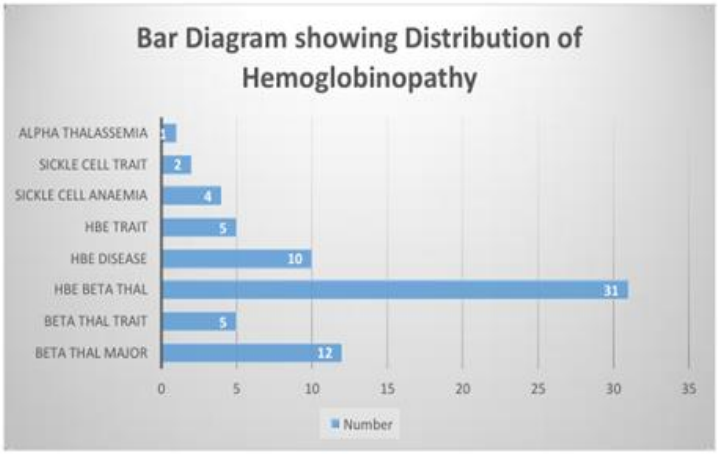

Figure 2: Bar Diagram showing Distribution of Hemoglobinopathy

Distribution of Consanguinity: In the present study, 8.57% of the patients had a history of parental consanguinity while 91.43% patients had parents with non-consanguineous marriage. Parental history of consanguineous marriage was found in 17.6% of patients. 16.6% (2/12) of the beta thalassemia major patients and 18% (4/22) of the HbE beta thalassemia patients had parents with a consanguineous marriage. In the beta thalassemia major subset, mean serum ferritin concentration in study subjects was 1513.3µg/l (range 1220-1900 µg/l).

Ethnic distribution: In our study, the tribal population of Ahom, Boro, Tea Tribe, and Sonowal each represented 6.67%, 6.67%, 3.33%, and 3.33% respectively.

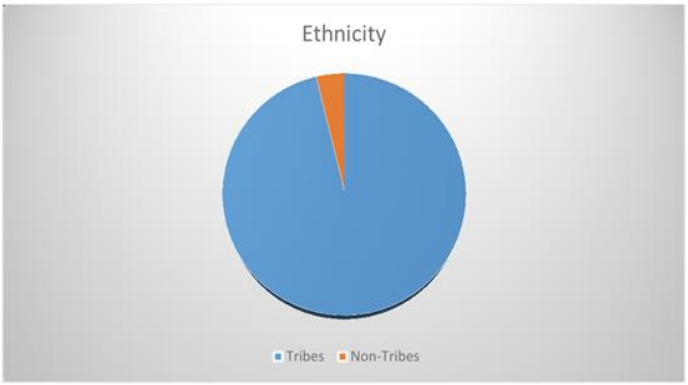

Figure 3: Ethnicity

Characteristic of transfusion dependent Thalassemia patients: In the present study, out of 70 cases, 34 patients were transfusion dependent (48.5%). Out of these, 12 patients (17.1%) had beta thalassemia major while 22(31.4%) patients had transfusion dependent HbE beta thalassemia. Mean hemoglobin concentration was 7.83g/dl (6.8-9.6). Splenomegaly was present in 83.3% patients, with a mean spleen size of 4.4cm (0-10 cm). Hepatomegaly was present in 55.9% patients with a mean liver size of 1.4cm (0-6cm). In the HbE Beta thalassemia patients, mean hemoglobin concentration was 7.8g/dl (6.3- 9.6), with a mean spleen size of 4.35cm (1- 10cm) and mean liver size of 1.2cm (0-3cm). 2 patients underwent splenectomy. Mean serum ferritin concentration in study subjects was 1568.1µg/l (range 1010-2100 µg/l).

Frequency of Blood transfusion as per age: We divided the transfusion dependent patients, according to total number of blood transfusions per year into three groups 6-12, 13-24, >24 transfusions per year. We divided the study subjects into three age groups (0-5, 6-10, ≥ 11years). The requirement of total number of blood transfusions per year according to these age groups is shown in a graphical form in the above figure.

Fig 4 shows that as the age of the patient increases, there is increasing requirement of blood transfusion. However, there was no patient ≥ 11years old requiring more than 24 transfusions per year.

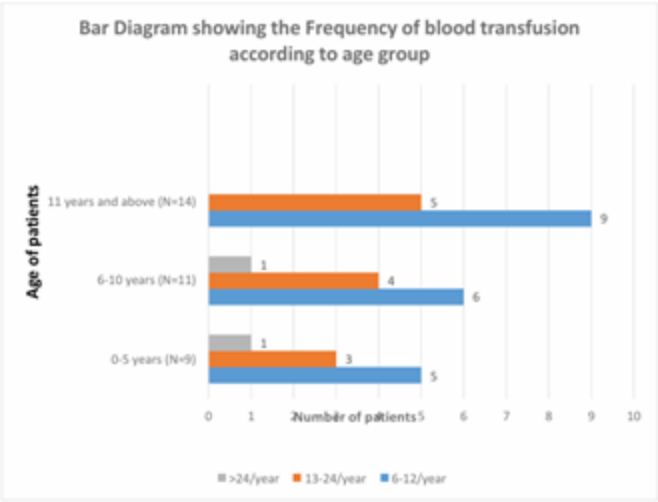

Figure 4: Bar Diagram showing the frequency of blood transfusion according to age group

Serum ferritin level: It ranged from 1500-1700 mcg/l irrespective of the age group. None of the transfusion dependent thalassemia patients in our study had a normal serum ferritin.

Comparision of Phenotypic profile between well and poorly transfused: Well Transfused (Maintaining a pre transfusion Hb ≥9 g/dl) and poorly transfused (Maintaining a pre transfusion Hb≤ 9 g/dl) (As per ICMR guidelines) [82] were compared. The study had 10 and 24 number of patients respectively. From the table it is clearly evident, that inadequately transfused patients have poor growth and development, delayed puberty, presence of haemolytic facies and endocrinopathies which is statistically significant. They also have increased liver size (1.58cm vs 0.6cm) and higher serum ferritin levels, which are statistically significant. Short stature was seen in 12.5% of the poorly transfused individuals, delayed puberty and hypothyroidism was seen in 20.8% and 8.3% of the inadequately transfused patients. There was only one well transfused (pre transfusional Hb>9), well chelated (S.ferritin 950µg/l) transfusion dependent Hb E beta thalassemia patient. This patient did not have any hemolytic facies, endocrinopathy, no hepatomegaly, however had mild splenomegaly of 1cm.

Characteristic of non-transfusion dependent Thalassemia patients: In the present study,51.4% patients had a non-transfusion dependent phenotype. The mean Hb concentration in beta thalassemia trait patients is 8.98g/dl. This is because in our study there were 2 beta thalassemia trait patients who had concomitant iron deficiency anaemia and G6PD deficiency, probably causing a decrease in the mean hemoglobin. Among, Hb E/beta thalassemia patients, mean Hb is 10.1 g/dl. In sickle cell disease patients, mean Hb is 8.9 g/dl while in sickle cell trait patients, it is 10 g/dl. Hepatomegaly was not seen in any of the patients while splenomegaly was seen in 41.7% of the patients. Among HbE beta thalassemia patients, 55.5 % patients did not have splenomegaly, 44.5% patients had mild enlargement in spleen size. (1-2cm) 2 patients with non-transfusion dependent HbE beta thalassemia had a concurrent G6PD deficiency and iron deficiency anaemia respectively. Patients with HbE Beta Thalassemia had the highest average ferritin level of 671.56 ng/mL, followed by beta thalassemia trait with an average ferritin level of 430.4 ng/mL. 28 patients (77.7%) of the patients never required a blood transfusion, however the mean ferritin among various subgroups was slightly higher.

Spectrum of Hb E disease: This study revels, 46(65.7%) patients had variant of HbE disorder. 31(44.2%) patients had HbE Beta thalassemia, 10(14.2%) had HbE disease and 5(7.1%) had Hb E trait. Majority of the patients who were transfusion dependent were children with an average age of 10.5 years. Mean pre transfusion haemoglobin was 7.8 in transfusion dependent HbE- beta thalassemia patients as opposed to 10.1 in the NTDT subset. Mean Hb was 12.1 and 13.9 in Hb E disease patients and HB E trait patients respectively. Average liver size was 1.2 cm, ranging from 0 to 3 cm in transfusion dependent HbE beta thalassemia, while the mean spleen size was 4.15 cm ranging from 1cm to 10cm. Splenectomy was done in 2 patients. Mean serum ferritin was 1568, ranging from 1010 to 2100 in the transfusion dependent subset as opposed to 671.5 in the non-transfusion dependent group There is a vast phenotypic heterogeneity among patients of HbE- beta thalassemia. The serum ferritin levels were near the upper limit of normal for patients of HbE disease and trait. Splenomegaly was seen in 5 cases of HbE disease ranging from 0 to 3cm.

Mutational Profile of Hemoglobinopathies: Mutational studies were performed in 25 cases. The most common mutation seen in beta thalassemia was IVS1-5(G>C) at Intron 1 which was seen in 10 cases, corresponding to a severe thalassemia variant, however one patient had a compound heterozygous mutation for c.92+5G>C at exon 1 and c-18-122C>T at promoter region which caused a non-transfusion dependent phenotype for the patient. 11 cases had a c.79G>A mutation at exon 1 corresponding to a Hb E variant.

Among these 11 cases, 4 patients had a compound heterozygous mutation at IVS1-5(G>C) at Intron 1 which caused a severe transfusion dependent phenotype of HBE /beta thalassemia. One beta thalassemia patient had a Homozygous mutation at IVS II 1(G>A)(HBBc.315+1G>A), leading to a less severe phenotype. One patient had Homozygous mutation for 619 bp del, causing transfusion dependent beta thalassemia. One child had homozygous mutation c.314G>A at exon 3 of HB A2 causing alpha thalassemia of intermediate severity also known as Hb Sallanches.

Table 1: Genotype-Phenotype Correlation in Thalassemia Patients

Genotype	Phenotype	
MUTATIONAL PROFILE (N = 25) (34.3%)	**TDT (N = 14) (58.3%)**	**NTDT (N = 11) (41.7%)**
HBB, c.79G>A (EXON 1) (N = 11)		
• Homozygous		5
• Heterozygous		2
• Compound heterozygous with IVS 1-5 (G>C)	4 (Severe TDT)	
IVS 1-5 (G>C) (INTRON 1) / c.92+5G>C (N = 10)		
• Compound heterozygous with c.79G>A (EXON 1)	4 (Severe TDT)	
• Compound heterozygous with c.-18-122C>T (PROMOTER)		1
• Compound heterozygous with Codon 30 (G>C)	1	
• Homozygous	3	
• Heterozygous		1
Homozygous for 619 bp Deletion (N = 1)	1	
Homozygous for c.314G>A at HbA2 (EXON 3) (N = 1)		1
Compound heterozygous CAP site +1 (A>C) & Codon 30 (G>C) (N = 1)	1	
Homozygous at c.314G>A at exon 3 of HB A2		1

Discussion

In this hospital based descriptive Cross-sectional study, 70 patients with Hemoglobinopathies presenting to Gauhati Medical College and Hospitalfrom February 2021 to Dec 2022 were included based on the inclusion and exclusion criteria.

In the present study majority of the patients belonged to age group of 12 years &less (60%) paediatric, while 40% of the patients were adults. (Fig 1) 53% of the patients were male and 47% were female.51% of the patients were from rural areas and 49% were from urban. 50% of the study population were Hindu, 37.1% were Muslim and 12.9% were Christian.

The commonest hemoglobinopathy in our study was HbE -Beta thalassemia (44.2%), followed by beta thalassemia major (17.1%). 14.2% had HbE disease and 5(7.1%) had Hb E trait, while 5 (7.1%) had beta thalassemia trait. 4 patients (5.7%) had sickle cell anaemia while 2 patients (2.8%) had sickle cell trait. There was one case of alpha thalassemia diagnosed by genetic studies. (Fig2)

65.7% patients had a variant of HbE disorder. Most of the study population were nontribal (80%), among the tribes, most prevalent were Ahom, Boro, Tea Tribe, and Sonowal each representing 6.67%, 6.67%, 3.33%, and 3.33% respectively. (Fig3)

Mondal UK [11] et al conducted an observational study in the Department of Paediatrics, at Assam Medical College and Hospital, and reported that sickle cell disease was found in 11.2% cases, beta thalassemia major in 10.2%, HbE- Beta thalassemia in 5.8% cases, Hb E disease in 4.8% cases. Pathak et al [12] observed that out of the total 1118 cases , 698 (62.43%) were positive for Hb variants like β-thalassemia minor (16.99%), β-thalassemia major (2.32%), Hb E heterozygous (22%), Hb E homozygous (6.62%), Sickle cell trait (3.04%), Sickle cell disease (1.69%), Compound Hb E-β thalassemia (9.66%) and Compound Hb S-β thalassemia (0.1%).Kalita D et al[13] studied a total of 75 children of thalassemia and reported 58.6% patients were male and 41.3% were female. In their study, the most common haemoglobin disorder was HbE trait 26.6% followed by Beta thalassemia trait 21.3%, Sickle cell trait (18.7%) , E beta thalassemia (9.3%) ,HbE disease 8% and sickle cell disease (Hb SS) 8% respectively. Mondol SK et al[14] evaluated the Prevalence of thalassemia and hemoglobinopathy in eastern India over a period of 10-year and reported that β (beta) thalassemia trait was the commonest abnormality found in 5,488 (4.60%) patients. HbE trait was found in 3,604 (3.02%) patients, β thalassemia major/intermedia in 1,981 (1.66%) cases, and Eβ thalassemia in 1,384 (1.16 %) cases. The mean age was 25.8 years with patients ranging between 5 months and 72 years.

Thalassemia is the most common monogenetic haemolytic anaemia in India. Repeated blood transfusion is needed for the survival of thalassaemic patients. Due to repeated blood transfusion excessive iron get accumulated in the body leading to a condition known as hemosiderosis, which is a major cause of late morbidity and mortality in these patients. Serum ferritin measurement is an easy and cost-effective tool which helps in deciding the ideal timing for starting of iron-chelation therapy.

In the present study, 34 patients were transfusion dependent (48.5%) and out of these, 12 patients (17.1%) had beta thalassemia major. Parental

history of consanguineous marriage was found in 17.6% of patients, which is concordant to the findings by Bhalodiya VR at al [15].

Mean hemoglobin concentration was 7.83g/dl (6.8-9.6). Splenomegaly was present in 83.3% patients, with a mean spleen size of 4.4cm (0-10 cm). Hepatomegaly was present in 55.9% of the patients. Mean liver size was 1.4cm (0-6cm).

The mean age of onset of blood transfusion was 8.8 months, which is similar to the finding by Bhalodiya VR et al. [15] Koreti S et al [16] evaluated 60 patients suffering from beta thalassemia major, and observed that the median age at the first transfusion was six months (range 4–16 months)(Fig 4) with 55.5% having a history of consanguineous marriage of parents. Biswas et al [17] evaluated the quality of life in 328 beta thalassemia major patients and found that 61.3% of them had a palpable spleen with size ranging from 1 to 8 cm. He also observed that the last pre-transfusional Hb level was an important predictor of quality of life. In the present study too, patients maintaining a pre transfusional Hb of≥ 9 g/dl had better physical well-being.

Inadequately transfused patients had poor growth and development, presence of haemolytic facies and endocrinopathies which was statistically significant ($p<0.05$). This was similar to the findings of an Indian study [18] and several foreign studies [19,20,21]. It may be so because the optimal Hb level in the blood at any given point of time directly influences the physical and mental functioning. In the present study, short stature was seen in 12.5% of the poorly transfused individuals, delayed puberty and hypothyroidism was seen in 20.8% and 8.3% of the inadequately transfused patients.

These findings are consistent with the findings by Jain JK et al [22] and Kalita D et al [13]. Jain JK et al studied 48 children of beta thalassemia major and found 41.66% were having endocrinological complications. Most commonly being stunted growth (27%). Delayed puberty was observed in 7 children (14.58%) and hypothyroidism was seen in 4 children (8.33%). Kalita D et al observed that 46.6% of cases of thalassemia were malnourished/stunted and 29.3% children had skeletal changes like frontal bossing, malar prominence, malocclusion of teeth.

The contributing factors for growth retardation in our patients may include chronic hypoxia due to anaemic state, transfusion related iron overload, hypothyroidism, hypogonadism, growth hormone deficiency, undernutrition and psychosocial stress. Hypothyroidism in our study may be due to anaemia, chronic tissue hypoxia, free radical injury and organ siderosis.

Chirico V et al [23], observed that patients with ferritin values above 1800 µg/L experienced a significantly faster development of endocrinopathies. In our study, a ferritin value of 1640 µg/L was associated with development of endocrinopathies, which was statistically significant. Hence, Serum Ferritin represents a prognostic marker for transfusion dependent thalassemia patients and a predictive factor for progression to endocrine dysfunctions.

Our study has shown that as the patients of transfusion dependent beta thalassemia grown older, their blood requirement increases. This observation is consistent with the findings by Bhalodiya VR et al [15] and Koreti S et al [16]. The mean serum ferritin concentration in the study subjects was 1513.3µg/l (range 1220-1900 µg/l). The mean ferritin values in various national and international studies as compared to our study. We can see that it is consistent with the findings by Cunningham et al [24] but significantly lower as compared to other studies by other national and south east Asian authors.

In the present study, serum ferritin levels did not show a positive corelation with increasing age and with the number of transfusions per year as opposed to the findings by Bhalodiya VR et al [87] and Koreti S et al [16]. This may probably be due to prompt initiation and regular patient compliance to oral iron chelation therapy.

In our study, there was only one well transfused (pre transfusional Hb>9), well chelated (s. ferritin-950µg/l) transfusion dependent Hb E beta thalassemia patient. This patient did not have any hemolytic facies, endocrinopathy, no hepatomegaly, however had mild splenomegaly of 1cm. This emphasizes the importance of adequate chelation. Various studies [25,26,27] have shown that chelation therapy helps to prevent iron overload state and thus reduce morbidity and mortality induced by iron toxicity.

In the present study,51.4% patients had a non-transfusion dependent phenotype. The mean Hb concentration in beta thalassemia trait patients was 8.98g/dl. 80% of the beta thalassemia trait patients never had a history of blood transfusion, however had a higher serum ferritin level of upto 800µg/l. This is similar to the findings by Shah R et al [28] and Musallam KM et al [29].

Ferritin levels, which serve as an indicator of iron stores in the body, exhibited variations among the different groups. Patients with HbE Beta Thal had the highest average ferritin level of 671.56 ng/mL, followed by beta thalassemia trait.

The observed differences in ferritin levels could be attributed to the complex interactions [9,29] between iron metabolismand the specific

hemoglobinopathies, as well as other factors such as iron supplementation and inflammation. This disproportionate increase in serum ferritin is due to increased intestinal absorption of iron.

Current models for iron metabolism in beta (β)-thalassemia intermedia patients suggest that suppression of serum hepcidin results in increased iron absorption and release of iron from the reticuloendothelial system, leading to depletion of macrophage iron, relatively low levels of serum ferritin, and liver iron loading. The consequences of iron overload in patients with non-transfusion dependent thalassemia are multifactorial and include endocrinopathy, bone disease, thromboembolism, pulmonary hypertension, cerebrovascular and neuronal damage, liver fibrosis or cirrhosis, and increased risk of hepatocellular carcinoma [9,29,30].

The variations in haemoglobin levels, spleen size, and ferritin levels among different subgroups highlight the heterogeneity of these disorders. This information can contribute to the understanding of disease progression, management strategies, and the development of targeted interventions for patients with non-transfusion dependent hemoglobinopathies.

The North Eastern Region of India particularly Assam is a rich reservoir of hemoglobinopathies and thalassemia because of the migration of various races over a period of time. It has been shown by various authors that HbE is prevalent in the inhabitants of Assam, having cultural and linguistic associations with the population of South East Asian countries.[31] Though the HbE gene has been detected across all ethnic groups in Assam like the Ahom, Koch, Chutia, Muttock, Deori, Sonowal and Mishing groups, the highest incidence has been detected in the Bodo-Kacharis, an ethnic group speaking Tibeto-Burmanlanguages[6,31,32].

In our study, HbE beta thalassemia was most common among the muslims (22.92%), followed by Biharis (4.17%), Boro (2.08%), Ahoms (2.08%) and Bengalis (2.08%). Hb E disease was more common among the Boro (4.17%) and Sonowal (2.08%) while Hb E trait was seen among the Ahoms (2.08%) We studied clinical features in 70 patients of hemoglobinopathy of which, 46 patients (65.7%) had a variant of Hb E disorder. 31(44.2%) had HbE beta thalassemia, which is similar to the finding of Baruah A et al (58.5%) [32] Phenotypic variability of HbE-β thalassaemia, the limited understanding of its natural history, make the management of Hb E-β thalassaemia particularly challenging. Marked clinical variability ranges from a mild and asymptomatic anaemia to a life-threatening disorder requiring transfusions from infancy.[7]

In our study, 22 (70.9%) cases were behaving as β thalassemia major or severe phenotype and remaining 29% cases were behaving as thalassemia intermedia or moderate phenotype. Among those having a severe phenotype, 50% of the patients became transfusion dependent by the first year of life with an average of 8 months, while 27.2% of the patients started requiring blood transfusion at a mean of 2.3 years of age and 22.7% of the patients developed a severe phenotype by 5.8years of age (4-9 years), previously being non transfusion dependent. These findings are similar to the study by Baruah Aditi et al [33] wherein the clinical features of 62 children with HbE -beta thalassemia was evaluated in a hospital-based study in Dibrugarh, and it was seen that 65.4% had a severe phenotype while 34.6% had a moderate phenotype.

The cause for this changing phenotype of HbE-beta thalassemia is not completely understood.[7] A modified ―natural historystudy of Hb E-β thalassaemia in children in Sri Lanka highlighted the instability of phenotype over the first 10–15 years of life, during which there was a variable, and changing pattern of anaemia and erythroidexpansion [7].

This study attempts to study the underlying genetic mutations responsible for this phenotypic heterogeneity. There is a widely disparate range of clinical and haematological parameters in patients with Hb E-β thalassaemia [7,33,34]

In our study, average Hb level at the time of diagnosis was 8.9 gm/dl ranging from 6.3 to 15.2 gm/dl. Fucheroen et al. [35] stated that the steady-state haemoglobin levels in Hb E-β thalassemia range widely between the different phenotypes, from 3 g/dl or less to as high as 11 g/dl. In the present study, mean serum ferritin was 1568, ranging from 1010 to 2100 in the transfusion dependent subset as opposed to 671.5 in the non-transfusion dependent group. These findings are consistent with the study by Das et al [36], where cases of HbE Beta thalassemia on regular blood transfusions had a higher ferritin (1309.75) than those with occasional transfusions. (715 ng/ml) In our study, the average Hb was 12.1 and 13.9 in Hb E disease patients and HB E trait patients respectively, which is similar to the findings by Bhargava P et al [38], wherein the average value for haemoglobin was 12.2 in Hb E trait and 11.2 in Hb E disease. It is also consistent with the findings of Hmar L et al. [38]

Splenomegaly was found in 2 cases of HbE disease in the study by Bhargava P et [37] al, while in the present study, it was found in 5 cases. In a study by Vichinsky[39], HbE disease patients were occasionally found to have splenomegaly. Clinically HbE is a mild type of disorder both in homozygous and heterozygous states therefore Hb

E individuals are minimally anaemic and asymptomatic.[40]. Lachant et al[40] described HbE homozygous to have on an average of 1g/dl less haemoglobin concentration than that seen in heterozygotes; which is elucidated in the present study. Several studies have shown in the past that the HbE trait and disease patients usually do not develop a state of iron overload. In the study by Aggarwal et al [41], and Bhargava P et al [37], iron profile was normal in all patients with HbE trait and disease. In our study alsoiron profile was normal in all patients which was consistent with the findings of above studies. HbE disorders can coexist with alpha or beta thalassemia and iron deficiency anaemia.

HPLC study can diagnose HbE beta thalassemia but not alpha thalassemia. Genetic study is required for further analysis of such cases. Mutational studies were performed in 25 cases. This data is important for understanding the genetic basis of thalassemia and HbE disorders and their implications for disease severity. Studies conducted in various parts of the country have identified common as well as some rare mutations in the population. In general, the mutations IVS I-5 (G→C), IVS 1-1(G→T), Codon 41/42 (−TCTT), 619-bp deletion and Codon 8/9 (+G) make up for the 5 most common mutations in India. [42,43]

The most common mutation seen in our patient population was c.79G>A (p.Glu27Lys) which is a missense SNP mutation where guanine is replaced by adenosine at exon 1,causing a change in single amino acid glutamic acid to lysine.(Table1) This corresponds to a Hb E variant which was seen in 11 patients. c.79G>A at exon 1 is the most common mutation seen in HB E variants as shown in various studies by Flatz G et al [44], Nakatsuji T et al [45]. Among these 11 cases, 4 patients had a compound heterozygous mutation at IVS1-5(G>C) at Intron 1 which caused a severe transfusion dependent phenotype of HBE /beta thalassemia, as supported by a study by Shah PS et al [46]. The most common mutation seen in beta thalassemia was IVS1-5(G>C) at Intron 1, which is similar to the findings of Saikia M [47] et al who evaluated beta thalassemia mutations in 105 cases in Northeast India and reported 63.09% were positive for IVS1-5(G>C) mutation. One beta thalassemia patient had a Homozygous mutation at IVS II-1(G>A) (HBBc.315+1G>A), leading to a less severe phenotype probably due to higher HBF production as supported by the study by Oppenheim et al. [48]

One child had a rare homozygous mutation c.314G>A at exon 3 of HB A2 causing alpha thalassemia of intermediate severity also known as Hb Sallanches, with only few isolated case reports being reported of the same. [49,50]

It is worth noting that the sample size in this study was relatively small (N=25), which may limit the generalizability of the results. Additionally, the study focused on a specific population, and the prevalence of specific mutations may vary in different ethnic groups or regions. Further research with larger sample sizes and diverse populations would be beneficial to validate and expand upon these findings.

Strength of the study was that it included various age groups, with a particular emphasis on the paediatric population. This approach provides insights into the distribution and impact of hemoglobinopathies throughout different stages of life, highlighting the importance of early detection and intervention. The study covers both rural and urban areas, ensuring a diverse representation of the population. This inclusiveness strengthens the applicability of the findings to a broader range of individuals. By examining the transfusion requirements and complications associated with transfusion therapy, the study addresses an essential aspect of disease management. This information can guide healthcare providers in optimizing transfusion strategies and chelation therapy to minimize complications and improve patient outcomes These strengths collectively enhance the robustness and applicability of the study's findings, making it a valuable resource for healthcare professionals, policymakers, and researchers involved in the management and understanding of hemoglobinopathies

Limitations of the study was that the sample size of each subgroup is relatively small that limit the generalizability of the findings and the burden of hemoglobinopathies can be correctly estimated only by carrying out large population-based studies. As it was a single centre study,the milder phenotypes of thalassemia may have been missed. The true incidence and their follow up can only be ascertained in a population-based study. Genetic studies could be performed in only 25 patients due to logistic reasons. A larger sample size is needed to validate the impact of genetic analysis on the phenotype of hemoglobinopathies.

Conclusion

HbE-Beta thalassemia was the most common subtype followed by HbE disease and HbE trait. The majority of patients with HbE beta thalassemia disease were transfusion-dependent and showed poor growth and development, delayed puberty, presence of hemolytic facies, and endocrinopathies. Their mean serum ferritin concentration was much higher than normal limit. Other hemoglobinopathies included beta thalassemia major, beta thalassemia trait, sickle cell anemia, and sickle cell trait, were observed in smaller proportions.Associated iron deficiency anaemiawas

also seen in few non-transfusion dependent thalassemia cases.The most common mutation seen was c.79G>A (p.Glu27Lys) which corresponded to an HbE variant. Co inheritance of IVS1-5(G>C) at Intron 1, is responsible for a severe transfusion dependent phenotype.

Bibliography

1. WHO-Executive Board EB118/5, 118th Session Report by the Secretariat on thalassemia and other hemoglobinopathies: Prevalence of hemoglobinopathies. 11 May 2006. p. 1-8.
2. Balgir RS. Spectrum of hemoglobinopathies in the state of Orissa, India: A ten years cohort study. J Assoc Physicians India 2005; 53:1021-6.
3. Dolai TK, Dutta S, Bhattacharyya M, Ghosh MK. Prevalence of hemoglobinopathies in rural Bengal, India. Hemoglobin 2012; 36:57-63.
4. Jain BB, Roy RN, Ghosh S, Ghosh T, Banerjee U, Bhattacharya SK. Screening for thalassemia and other hemoglobinopathies in a tertiary care hospital of West Bengal: Implications for population screening. Indian J Public Health 2012; 56:297-300.
5. Lukens JN. The abnormal hemoglobins: General principles. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, editors. Wintrobe‘s Clinical Hematology. 10th ed. Baltimore: Lippincott Williams & Wilkins; 1998. p. 1329-45.
6. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, et al. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. J Community Genet 2013;4:33-42.
7. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta-thalassaemia: A common & clinically diverse disorder. Indian J Med Res 2011; 134:522-31.
8. Aessopos A, Farmakis D, Deftereos S, Tsironi M, Tassiopoulos S, Moyssakis I, et al. Thalassemia heart disease: a comparative evaluation of thalassemia major and thalassemia intermedia. Chest. 2005;127(5):1523-30.
9. Taher AT, Porter J, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, et al. Deferasirox reduces iron overload significantly in nontransfusion-dependent thalassemia: 1-year results from a prospective, randomized, double-blind, placebo controlled study. Blood. 2012;120(5):970-7.
10. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica. 2004;89(10):1187-93.
11. Mondal UK, Dowerah P, Mukherjee R. Hemoglobinopathy in pediatric population: A cross sectional study at tertiary care center in Assam. Indian J Med Sci 2021;73:327 30.
12. Pathak MS, Borah MS. Genetic Hemoglobin Disorders Among the People of Assam A Tertiary Care Hospital Based Study. International Journal of Medical Research & Health Sciences January 2023,12(1):65-68.
13. Kalita, Dulal et al. ―Original Research Article: Clinical Profile and Pattern of Hemoglobinopathies and Thalassemias Among Children Admitted in a Tertiary Care Hospital, Assam, India.‖ (2019).
14. Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016 Jan-Jun;10(1):105-10. doi: 10.4103/0973-6247.175424. PMID: 27011683; PMCID: PMC4782486.
15. Bhalodiya VR, Valiya LG, Mehta NA, PadhariyaBB. Correlation of serum ferritin level in transfusion-dependant thalassemia major patients: a study at a medical college affiliated hospital in Gujarat region. Int J ContempPediatr2023;10:330-3.
16. Koreti S, Gaur B.K, Das G, Gaur A. Study of Serum ferritin levels in β-Thalassemia major children. Int J Pediatr Res. 2018;5(6):308-313.doi:10.17511/ijpr.2018.i06.02.
17. Biswas B, Naskar N N, Basu K, et al. (March 29, 2023) An Epidemiological Study of the Quality of Life of Children With Beta-Thalassemia Major (βTM) and Its Correlates in Kolkata, West Bengal, India.
18. Cureus 15(3): e36888 Saha R, Misra R, Saha I: Health related quality of life and its predictors among Bengali thalassemic children admitted to a tertiary care hospital. Indian J Pediatr. 2015, 82:909-16.
19. Thavorncharoensap M, Torcharus K, Nuchprayoon I, Riewpaiboon A, Indaratna K, Ubol BO: Factors affecting health-related quality of life in Thai children with thalassemia. BMC Blood Disord. 2010, 10:1.
20. Ismail M, Chun CY, Yusoff NA, et al.: Quality of life among thalassaemia children, adolescent and their caregivers. Sains Malays. 2013, 42:373-80.
21. Mevada ST, Al Saadoon M, Zachariah M, Al Rawas AH, Wali Y: Impact of burden of thalassemia major on health-related quality of life in Omani children. J Pediatr Hematol Oncol. 2016, 38:384-8.
22. Jain JK, Vaishnav JG, Vohra AS, Shah M, Goyal A, Kansagara M, et al. Study of endocrinological complications in children

with beta thalassemia major. Int J ContempPediatr2022;9:1063-7.
23. Al Jaouni SK. Survival and disease complication of thalassemia major: Experience of 14 years at king Abdulaziz University Hospital, Jeddah, KSA. JKAU Med Sci 2010;17(1):19–28.doi: 10.4197/med. 17-1.3. 103).
24. Cunningham MJ, Macklin EA, Neufeld EJ, et al. Thalassemia Clinical Research Network. Complications of beta-thalassemia major in North America. DOI:10. 1182/blood-2003-09-316717.
25. Piga A, Galanello R, Forni GL, Bertrand Y, Foschini ML, Bordone E, Leoni G, Lavagetto A, Zappu A, Longo F, Maseruka H, Hewson N, Sechaud R, Belleli R, Alberti D. Randomized phase II trial of deferasirox (Exjade, ICL670), a once-daily, orally-administered iron chelator, in comparison to deferoxamine in thalassemia patients with transfusional iron overload. Haematologica. 2006;91:873–880.
26. Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, Aydinok Y, Kattamis A, Kilinc Y, Porter J, Capra M, Galanello R, Fattoum S, Drelichman G, Magnano C, Verissimo M, Athanassiou-Metaxa M, Giardina P, Kourakli-Symeonidis A, Janka-Schaub G, Coates T, Vermylen C, Olivieri N, Thuret I, Opitz H, RessayreDjaffer C, Marks P, Alberti D. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with β-thalassemia. Blood. 2006;107(9):3455–3462 106).
27. Porter J, Galanello R, Saglio G, Neufeld EJ, Vichinsky E, Cappellini MD, Olivieri N, Piga A, Cunningham MJ, Soulières D, Gattermann N, Tchernia G, Maertens J, Giardina P, Kwiatkowski J, Quarta G, Jeng M, Forni GL, Stadler M, Cario H, Debusscher L, Della Porta M, Cazzola M, Greenberg P, Alimena G, Rabault B, Gathmann I, Ford JM, Alberti D, Rose C. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. Eur J Haematol. 2008;80(2):168–176.
28. Shah R, Trehan A, Das R, Marwaha RK. Serum ferritin in thalassemia intermedia. Indian J Hematol Blood Transfus. 2014 Dec;30(4):281-5. doi: 10.1007/s12288-013 0267-y. Epub 2013 Jun 6. PMID: 25435728; PMCID: PMC4243403.
29. Musallam KM, Cappellini MD, Wood JC, Taher AT. Iron overload in non transfusion-dependent thalassemia: a clinical perspective. Blood Rev. 2012 Apr;26 Suppl1:S16-9. doi: 10.1016/S0268-960X(12)70006-1. PMID: 22631036. 109).
30. Fiorelli G, Fargion S, Piperno A, Battafarano N, Cappellini MD. Iron metabolism in thalassemia intermedia. Haematologica. 1990 Sep-Oct;75 Suppl 5:89-95. PMID: 2086386.
31. Deka R, Reddy AP, Mukherjee BN, Das BM, Banerjee S, Roy M, et al. Hemoglobin E distribution in ten endogamous population groups of Assam, India. Hum Hered. 1988;38:261–266. doi: 10.1159/000153796.
32. Baruah AB, Bharali R. Prevalence of hemoglobinopathies in the ethnic groups of assam. Indian Journal of Applied Research. Volume - 10 ,Issue - 9 :September – 2020. 112).
33. Baruah A, Baruah MK. Phenotypic Diversity and Clinico-Hematological Profile of Hb E-Beta Thalassemic Children. Indian J Hematol Blood Transfus. 2020 Jan;36(1):117-122. doi: 10.1007/s12288-019-01150-5. Epub 2019 Jul 4. PMID: 32158093; PMCID: PMC7042447.
34. Pani K, Sharma S, Murari M, Yadav M, Phadke S, Agarwal S. Clinico-hematological profile of Hb E- β Thalassemia prospective analysis in a tertiary care centre. JAPI. 2018;66:42–45.
35. Fucharoen S, Weatherall DJ. The Hb E thalassemias. Cold Spring Harb Perspect Med. 2012;2(8):a011734. doi: 10.1101/cshperspect.a011734.
36. Das U, Kakati S, Gogoi GN. A clinical study of hemoglobin E with special reference to serum ferritin level. Journal of the Scientific Society. 2015;42(3):170.
37. Bhargava P , Datta D , Bhattacharjee S. AClinico-Haematological study of hemoglobin E disease and trait. International Journal of Medical Research and Review.September, 2016/ Vol 4/Issue 9.
38. Hmar L, Barman S, Deka J. Study of hemoglobin disorders in college students. International Journal of Research and Review. 2018;5(8):92-96.
39. Vichinsky E: Hemoglobin E syndromes. ASH Education Book January 1, 2007 vol. 2007 no 1 79-83
40. Lanchant NA. HbE an emergency haemoglobinopathy in US.Am J. Haematol. 25,499-562,1987.
41. Aggarwal S, Saluja S, Bhasin S, Sharma M, Gupta DK, Gupta B, et al. HbE variants–retrospective analysis in a tertiary carecentre. JIACM 2011;12:263–5.
42. Kimura EM, Grignoli CRE, Pinheiro VRP, Costa FF, Sonati MF. Thalassemia intermedia as a result of heterozygosis for β0-thalassemia and αααanti-3.7/αα genotype in a Brazilian patient. Braz J Med Biol Res Rev Bras PesquiMédicas E Biológicas Soc Bras Biofísica Al. 2003;36:699–701.

43. Shrivastava M, Bathri R, Chatterjee N. Mutational analysis of thalassemia in transfusion-dependent beta-thalassemia patients from central India. Asian J Transfus Sci. 2019 Jul-Dec;13(2):105-109. doi: 10.4103/ajts.AJTS_115_18. Epub 2019 Dec 3. PMID: 31896916; PMCID: PMC6910036.
44. Flatz G, Sanguansermsri T, Sengchanh S, Horst D, Horst J: The 'hot-spot' of Hb E [beta26(B8)Glu-->Lys] in Southeast Asia: beta-globin anomalies in the Lao Theung population of southern Laos. Hemoglobin. 2004 Aug;28(3):197-204. 121).
45. Nakatsuji T, Kutlar A, Kutlar F, Huisman TH. Haplotypes among Vietnamese hemoglobin E homozygotes including one with a gamma-globin gene triplication. Am J Hum Genet. 1986 Jun;38(6):981-3. PMID: 3728469; PMCID: PMC1684863.
46. Shah PS, Shah ND, Ray HSP, Khatri NB, Vaghasia KK, Raval RJ, Shah SC, Rao MV. Mutation analysis of β-thalassemia in East-Western Indian population: a recent molecular approach. Appl Clin Genet. 2017 May 11; 10:27-35. doi: 10.2147/TACG.S127531. PMID: 28546763; PMCID: PMC5436775.
47. Saikia M, Bhattacharya P, Mauchumi S. A Hospital Based Study of Hb Variant and Beta Thalassaemia Mutational Pattern Characterization Among the People of Northeast Region of India. Annals of Pathology and Laboratory Medicine. 2016Aug, 10-3 124).
48. Oppenheim A, Yaari A, Rund D, Rachmilewitz EA, Nathan D, Wong C, Kazazian HH Jr, Miller B. Intrinsic potential for high fetalhemoglobin production in a Druz family with beta-thalassemia is due to an unlinked genetic determinant. Hum Genet. 1990 Dec;86 (2):175-80. doi: 10.1007/BF00197701. PMID: 1702403.
49. Warang P, Nair S, Nadkarni A, Ghosh K, Colah RB. Hb H disease due to homozygosity for a rare alpha2-globin variant, Hb Sallanches. Hemoglobin. 2010;34(1):45-8. doi: 10.3109/03630260903547526. PMID: 20113287.
50. Dash S, Harano K, Menon S. Hb Sallanches [alpha104(G11)Cys-->Tyr, TGC-->TAC (alpha2)]: an unstable hemoglobin variant found in an Indian child. Hemoglobin 2006;30(3):393-6. doi: 10.1080/03630260600755872.