

Clinical profile and management of HbE- β thalassemia in children: Experience from a tertiary care center in North India

Nupur Parakh MD ^{1,*}, Afreen Khan MD ¹, Sunita Sharma MD ², Jagdish Chandra MD ¹

1. Department of Pediatrics Kalawati Saran Children's Hospital, New Delhi, India

2. Department of Pathology, Lady Hardinge Medical College, New Delhi, India

*Corresponding Author: Dr Nupur Parakha, Department of Pediatrics, Kalawati Saran Children's Hospital, New Delhi, India. Email: drnupurparakh@gmail.com. ORCID ID: 0000-0001-9817-7602.

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Abstract

Background: E β Thalassemia is characterized by clinical heterogeneity ranging from Non-Transfusion Dependent Thalassemia (NTDT) to Transfusion Dependent Thalassemia (TDT) state, causing management challenges for the clinicians, especially in the pediatric population. Therefore, this study was conducted to give an overview of the clinical profile and management in a tertiary care center.

Materials and Methods: This is a retrospective observational study on the clinical profile of 48 patients with E β Thalassemia, after ethical approval. Clinical and biochemical parameters of enrolled patients were entered in pre-designed proforma. The clinical phenotypes of these patients were classified based on the Sripichai scoring system.

Results: The mean age of subjects at presentation was 3.3 ± 2.8 years (M: F=3.8:1). On presentation, 25 (52.02%) patients had severe disease. Their mean age and initial mean hemoglobin at diagnosis were 2.5 ± 1.3 years and 4.9 ± 0.8 g/dl, respectively. They had a relatively larger spleen ($p=0.16$) and liver size ($p=0.67$). They were treated as TDT. Twenty-three patients were managed as the NTDT group at baseline. During follow-up period 19 out of 23 patients in the NTDT group (82.6%) were continued to be managed as the NTDT whereas the other four out of 23, required regular transfusion for a short duration. Serum Ferritin was <1000 ng/l in 78 % of patients in NTDT group as compared to 48% in TDT group ($p<0.05$). Endocrine complications were present 8% in the TDT group. Correlation of severity of clinical manifestation and laboratory findings were done using Chi-square test and T-test.

Conclusion: In the present study, the proper standardized classification of disease severity, helped in the management of these patients. It was found that detailed clinical knowledge regarding the pathophysiology, genetic modifiers, and complications along with close and careful monitoring of these patients based on clinical scores helps the pediatricians to classify these patients for their appropriate management.

Keywords: Clinical profile, E β -Thalassemia, Genotype, Phenotype

Introduction

E β thalassemia has emerged as one of the major health problems worldwide and accounts for nearly half of all the cases of severe β thalassemia across the globe with the highest frequencies being observed in India, Bangladesh, and throughout Southeast Asia (1). Iyer et al. reported an incidence of less than 1-10.5 % in the general population (2). A multicenter study conducted in six cities of India reported prevalence of β -thalassemia varied from 0 to 10.5 % among the different caste/ethnic groups (3). E β thalassemia results from co-inheritance of a β -thalassemia allele from

one parent and the structural variant HbE from the other. HbE results from the substitution of glutamic acid by lysine at codon 26 of the β globin gene which produces structurally abnormal hemoglobin that activates a cryptic splice site and causes abnormal messenger ribonucleic acid (mRNA) processing (4). The level of normally spliced mRNA β E is reduced because the usual donor site has to compete with this new site, and the abnormally spliced mRNA is nonfunctional because a new stop codon is generated. As a result, HbE is synthesized at a reduced rate and behaves like a mild form of β -thalassemia

(5). The pathophysiology is the combination of various factors like ineffective erythropoiesis, apoptosis, oxidative damage, and shortened red cell survival (6, 7). Patients with HbE- β thalassemia have wide spectrum of presentation ranging from mild asymptomatic disease to severe disorder requiring regular transfusion and hence can present clinically as Non-Transfusion Dependent Thalassemia (NTDT) to Transfusion Dependent Thalassemia (TDT). The cause of marked clinical heterogeneity largely remains unknown but some studies have suggested that the presence of certain modifying factors is responsible for marked phenotypic heterogeneity like the type of β -thalassemia mutation co-inherited with HbE, co-inheritance of α thalassemia, the polymorphisms associated with HbF production such as homozygosity for Xmn-I restriction site 5' to the γ -globin gene and the BCL11A gene, polymorphism of UDP-glucuronosyltransferase 1A1 (UGT1A1) gene, environmental modifiers, malaria, splenectomy, etc (8-11). E β thalassemia has been studied in adult population and limited literature is present in the pediatric population about the natural history and management of the disease. Regulated management from the beginning will prevent the development of complications in adulthood. Overtransfusion and undertransfusion, both are detrimental to the development. In the majority of centers, no guidelines are being followed and patients are managed inadequately. Therefore, the authors aimed to study the baseline characteristics, natural history, clinical course, management, and follow-up of these patients in the Thalassemia Day Care Centre, at Tertiary Hospital from North India to create a better understanding of the disease in this population.

Materials and Methods

It was a retrospective observational study conducted at Thalassemia Day Care Centre at Tertiary Hospital from North India. Out

of 375 patients with thalassemia syndrome, 48 patients had E β thalassemia. The patients were enrolled after taking informed consent from parents. The diagnosis was made based on the clinical picture, complete blood count with the peripheral smear (CBC with P/S), and high-performance liquid chromatography (HPLC) report of the patients and their parents.

Initial assessment

History and clinical examination:

Detailed clinical history regarding demographic features, symptoms of anemia, age of first blood transfusion, and the number of transfusions until the date was recorded and a complete anthropometric and clinical examination was done. Counseling of the parents for sibling screening and antenatal diagnosis in the next pregnancy was also done.

Investigations: Investigations like hemoglobin levels (Hb level), complete blood counts (CBC) with peripheral blood smear examination for red cell morphology were done by standard methods. Estimation of HbA2 and HbF was done by high-performance liquid chromatography (HPLC). Blood samples were collected before blood transfusion. For Hemoglobin, CBC, and HPLC, the blood was collected in a K₂EDTA vacutainer. CBC sample was analyzed on XN-1000 syemex automated hematology analyzer and wright-stained peripheral smears were examined. The sample for HPLC was stored at 2-4°C and was seen on BIO-RAD variant II.

For serum ferritin, the samples were collected in plain vacutainer, and serum was separated and stored at -40°C in a deep freeze. Serum Ferritin was analyzed by Chemiluminescence immunoassay (Beckman Coulter Unicel DXI' 600 Serum). The patients were also screened for Hepatitis B Surface Antigen (HBsAg) (Plasmatic Laboratory Products-UK), Hepatitis C virus (HCV) antibodies (Plasmatic Laboratory Products-UK), and Human immune deficiency virus (HIV) antibody 1 and 2 (Plasmatic Laboratory

Products-UK). Serum thyroid-stimulating hormone (TSH) and Free T4 were performed using enzyme immunoassay (TOSOH-Japan).

Liver function test, and kidney function tests were done at baseline and in follow up at every three months interval. Deoxyribonucleic acid (DNA) was analyzed for HBB gene mutations whenever possible.

The clinical phenotypes of these patients were classified on the basis of clinical scores calculated using the steady-state hemoglobin (Hb) level, age at presentation, age at first transfusion, transfusion requirements, spleen size, splenectomy, growth, and development as described by Sripichai et al. (12). The patients were classified as mild if the clinical score was between 0-3.5 and severe if the score was between 7.5 to 10.0. The patients with a moderate clinical presentation were scored between 4.0 to 7.0. The patients with the mild and moderately severe disease were not given regular transfusion but were closely observed (NTDT group) whereas patients with severe disease were maintained on regular transfusion therapy (TDT group). Patients in the NTDT group were given regular transfusions for a short duration if hemoglobin declined below 5g/dl, or the patient had growth faltering, or if the spleen enlarged significantly (>3cm/year).

Follow-up

The patients were regularly followed up at least once a month. In the follow-up, clinical and laboratory monitoring was carried out during each visit.

- Mean hemoglobin levels, absolute neutrophil count, liver function, kidney function, growth (height, weight), liver and spleen size, and serum ferritin (3 monthly) were monitored.
- Bone age estimation, Tanner's sexual maturity rating, endocrine system evaluation (Oral glucose tolerance test, Thyroid Function Test), 2 D echocardiography, and Magnetic Resonance

Imaging (MRI) were performed after ten years of age.

- Patients whose serum ferritin level reached 1000ng/ml in the TDT group and 800 ng/ml in the NTDT group were started on chelation therapy with Deferasirox or Deferiprone. Deferasirox was started at a dose of 20mg/kg/day and was titrated as per serum ferritin levels up to 40mg/kg/day. Deferiprone was initiated at 75 mg/kg/day and was increased up to 100mg/kg /day. Hydroxyurea was given to all the enrolled patients which were started at 10mg/kg/day and were escalated by 3-5 mg/kg/day every 8 weeks to the maximum tolerated dose not exceeding 20 mg/kg/day. Folic acid supplementation was given to all the patients. Clinical and laboratory monitoring for side effects of chelation therapy was done.
- During the follow-up period, patients were shifted from NTDT to TDT group for a short duration, the reason being the development of significant bony changes, symptoms of anemia, and profound enlargement of the spleen at a rate exceeding 3cm/year, growth failure, pubertal arrest, and delayed puberty.
- In patients with persistently high unconjugated serum bilirubin Gilbert syndrome (GS), mutation analysis was done. GS mutation analysis was done using gene sequencing at TATA box mutation. (Technique- Sanger Sequencing). Genomic DNA extracted from the peripheral and TATA Box region identified following the Polymerase Chain reaction, amplicon collected and sent for Sanger sequencing for the number of TA repeats.

Ethical Considerations

This study was conducted in accordance with the provision of New Drugs & Clinical

Trials Rules 2019, GCP regulations and ICMR guidelines for biomedical research on human participants (2017). It was approved by Institutional Ethics Committee Code No.-LHMC/IEC/2020/57.

Statistical Analysis

The statistical analysis was conducted using SPSS version 22.0 (IBM Corporation, Armonk, New York, USA). Descriptive data were presented as mean, frequency, and standard deviation. Correlation of severity of clinical manifestations and laboratory findings were done using Chi-square test and T-test.

Results

A total of 48 patients (12.8%) out of 375 patients with thalassemia syndromes, were found to have E β thalassemia and were enrolled in the study. The baseline characteristics of these patients are given in Table-I

A total of 23 patients (47.9%) had severe disease based on the Sripichai scoring system. The mean age at diagnosis of these patients was 2.5 ± 1.3 years and the initial mean hemoglobin was 4.9 ± 0.8 g/dl. They had a relatively larger spleen and liver size; the mean size being 7.5 ± 3.6 cms and 3.8 ± 1.9 cms below the costal margin respectively. They were treated as the TDT and their pre-transfusion hemoglobin level was maintained between 9-10.5 g/dl.

Moderately severe disease at presentation was seen in seven patients (Fig I). All these patients except two were managed without regular transfusion. Persistent symptomatic anemia was the reason for requirement of blood transfusion in these two patients.

During the follow-up period; in the NTDT group, four patients (out of 23) required regular transfusion for a short duration in view of increasing spleen size (>3 cm/year) in two patients and the development of growth faltering in other two patients.

Ferritin level was less than 1000 ng/ml in 18 of 23 patients (78%), remaining five patients had serum ferritin >1000 ng/ml (three patients were those who were shifted to the TDT group for a short duration). However, in the TDT group, only twelve patients (48%) had serum ferritin levels <1000 ng/ml. This difference in serum ferritin level was statistically significant ($p < 0.05$).

Patients over 10 years were closely followed up for endocrine complications and transfusion iron overload. There were sixteen and six patients in the TDT and the NTDT groups, respectively. None of the patients in the NTDT group developed any endocrine complications, however, four patients in the TDT group developed these complications (two patients developed sub-clinical hypothyroidism, one clinical hypothyroidism, and one developed impaired glucose tolerance). MRI heart and liver was performed to assess the iron overload. The MRI T2 * could be performed in fourteen patients in the TDT group and two patients in the NTDT group to look for the severity of iron overload. Of the fourteen patients who underwent MRI in the TDT group, nine patients had severe iron overload whereas five had moderate iron overload in the liver but none had iron overload in the heart. In the NTDT group, none of the patients had cardiac and hepatic iron overload in MRI. Mutation analysis for Gilbert syndrome could be performed in seven patients out of eleven who had persistent unconjugated hyperbilirubinemia on follow-up. Homozygous TA7/TA7 repeats were noted in six patients, while one patient had TA7/TA6 repeats. Both the NTDT and the TDT groups had three patients with each of the above mutations. One patient in the NTDT group with homozygous Gilbert mutation (TA7/TA7) had symptomatic gall stones and was managed surgically.

Table I: Baseline characteristics of enrolled patients of E- β thalassemia

Total patients (n)	48
Present Age (years) (mean \pm SD)	9.4 \pm 4.25
Age at diagnosis(years)(mean \pm SD)	3.3 \pm 2.8
Sex: (n (%))	
Male	38 (79%)
Female	10 (21%)
Baseline Hemoglobin (g/dL) (mean \pm SD)	6.1 \pm 1.4
Hemoglobin F(%) (mean \pm SD)	29.2 \pm 13.8
Hemoglobin A2(%) (mean \pm SD)	47.4 \pm 18.0
Initial liver size (centimeters below coastal margin)(mean \pm SD)	3.2 \pm 1.7
Initial spleen size (centimeters below coastal margin) (mean \pm SD)	5.4 \pm 3.8
Symptoms of Anemia (n (%))	17(35.41%)
Received blood transfusion prior to presentation(n (%))	22(46%)
Hemolytic facies (n (%))	10(20.83%)
Growth –Height in percentile(n (%))	
>25 th	20(41.6%)
3-25 th	10(20.8%)
<3 rd	18(37.5%)
Sripichai Clinical score (n (%))	
Mild (<4)	18(37.5%)
Moderately severe (4-7)	7(14.5%)
Severe (>7)	23(47.9%)
Received Transfusion (n (%))	
Regular transfusion	25(52%)
Independent/Intermittent transfusion	23(48%)
Mutation analysis (n)	
HBBG*:	
Compound heterozygous for IVS 1;5 and codon 26(G/A)	9
Nucleotide TA repeats :	
Homozygous for TA7/TA7 repeats	6
Heterozygous for TA7/TA6 repeats	1

*Hemoglobin subunit Beta gene

Table II: Comparison of NTDT and TDT group

	TDT	NTDT	p-value
Present Age (y) (mean \pm SD)	10.8 \pm 3.5	7.8 \pm 4.5	0.49
Children > 10 yrs of age (n)	14	6	
Age at diagnosis	2.5 \pm 1.3	4.2 \pm 3.6	0.50
Initial Hemoglobin (g/dL) (mean \pm SD)	4.9 \pm 0.8	7.4 \pm 0.5	0.47
Hemoglobin F(%) (mean \pm SD)	32.4 \pm 13.8	25.8 \pm 11.2	0.39
Hemoglobin A2(%) (mean \pm SD)	47.9 \pm 18.0	47.0 \pm 10.2	0.92
Initial liver size (cms below coastal margin) (mean \pm SD)	3.6 \pm 2.1	2.7 \pm 1.2	0.67
Present liver size (cms below coastal margin) (mean \pm SD)	3.3 \pm 1.9	3.0 \pm 1.3	0.90
Initial spleen size (cms below coastal margin) (mean \pm SD)	7.5 \pm 3.6	3.0 \pm 2.5	0.16
Present spleen size (cms below coastal margin) (mean \pm SD)	7.1 \pm 3.8	3.2 \pm 2.7	0.20

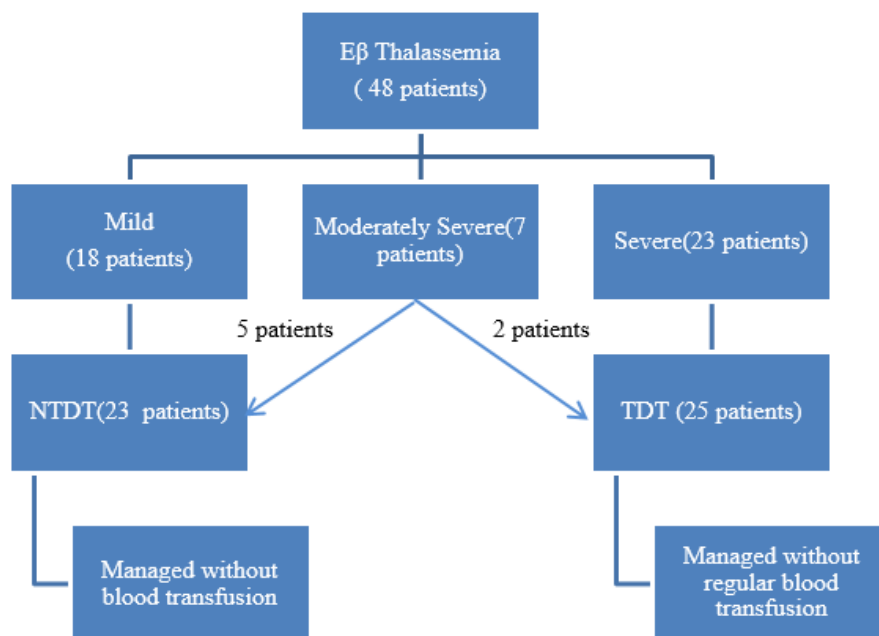


Figure 1. Management flow chart of patients. Out of 48 patients of E β Thalassemia 18 patients had mild, 7 had moderate and 23 had severe disease based on Sripichai scoring system. Out of 7 patients with moderately severe disease only 2 required regular transfusion. During follow up period; in NTDT group nineteen out of 23 patients remained asymptomatic and hence they were kept in the same group. However, four patients (out of 23) required regular transfusion for short duration.

Discussion

E β thalassemia is an important variant of thalassemia with the highest frequency observed in South East Asian countries. Though the exact prevalence of this disease in India is unknown and is known to vary in different parts of India (13-16). In the present study, nearly 12.8 % of thalassemia syndromes were E β thalassemia.

E β Thalassemia is characterized by marked clinical heterogeneity. The reason for this variable presentation is still not completely understood. Oliveri et al. tried to elucidate the factors contributing to this phenotypic diversity and they concluded that Hb-F concentration, coexistence of α thalassemia, HbG2 promoter polymorphism may be the possible modifiers (1). Panigrahi et al. did not find any correlation between Hb F and E/F ratio with the age of onset in patients with E β thalassemia, but XMN-1 polymorphism has been shown to influence the phenotype (17).

Symptomatology of E β thalassemia is quite variable and it is very difficult to elicit a detailed history of symptoms in the pediatric population as compared to adults who generally presents pallor and weakness. Other symptoms include abdominal pain, abdominal mass, jaundice, and fever. On clinical examination, spleen and liver enlargement may be seen (17, 18). In the present study, nearly 35% of patients presented with symptoms of anemia, 46% had already received blood transfusion prior to enrolment from outside. Pallor was the presenting symptom in nearly 90% of patients in a study by Pani et al (19).

Despite its high prevalence, no proper management guidelines have been established and are often managed haphazardly by random blood transfusion. For proper management of these patients, it is prudent for clinicians to classify them according to the severity of the disease. It has been shown that only one or two parameters cannot reliably classify these

patients. A novel scoring system was described by Sripichai et al. which was based on six parameters namely-hemoglobin levels, age at receiving a first blood transfusion, spleen size, growth and development, age at presentation, and requirement for transfusion. They categorized the patients in three distinctive groups - mild, moderate and severe (12). In this study, the above-mentioned scoring system was used and results showed that 37.5% of patients had the mild phenotypic presentation and 23 patients (47.9%) were managed in the severe category. Similar results were also documented by Italia et al. with 37% were mildly symptomatic and 46% had clinically severe disease (20).

There is a dearth of knowledge in the literature regarding the management and follow-up of these patients. In the present study, the proper standardized classification of disease severity helped in the management of these patients. Nearly 40% of patients were managed without any blood transfusion and their mean hemoglobin was 7.4 ± 0.5 gm/dl in this study. This result collaborated with those of Premawardhena et al. who prospectively observed 109 patients of age 1-51 years over five years and found that nearly 60% of patients with HbE- β thalassemia can be managed without any blood transfusion (8). All patients in the severe group required blood transfusion.

Regular follow-up is prudent for these patients to monitor them for signs and symptoms of anemia, extra-medullary hematopoiesis, growth, and development. As shown in Table II, the majority of patients who were managed without blood transfusion did not show any significant difference in growth and development throughout the study. This was similar to the study by Premawardhena et al. (8). However, Fucharson et al. showed that nearly 75% of patients with HbE- β thalassemia presented with growth retardation (10).

As patients with mild to moderately severe disease can be managed without regular blood transfusion, the complication rates of iron overload were also less. Serum ferritin levels were <1000 ng/ml in nearly 12 patients in the NTDT group in the present study.

Patients with E β thalassemia may have persistent unconjugated hyperbilirubinemia which may be due to the mutation in the gene for UDP-glucuronosyl transferase1 enzyme. Premawardhena et al. reported that the UGT*1 genotype is of importance in the genesis of gallstones (8). In the present study, eleven patients had persistent unconjugated hyperbilirubinemia; however, mutation analysis could only be done in seven patients of which six had homozygous TA7/TA7 repeats.

Limited literature is available on the natural course of HbE thalassemia in children. A longitudinal study in Sri Lanka showed that phenotypic instability was more common among children and there were thirteen changes of patients' designation during the observation period (8). In the present study, four patients required change in their management, hence it is prudent for pediatricians to regularly follow up these children.

The limitation of this study was that the primary, secondary, and tertiary modifiers like XMN polymorphism, associated α mutations, Gilbert mutations could not be assessed in all the patients due to the lack of facility of molecular laboratory in this setup.

However, the investigators found that the detailed clinical knowledge regarding the pathophysiology, genetic modifiers and complications, and also close and careful monitoring of these patients based on clinical scores can help the pediatricians to classify these patients and their adequate management. These E β patients need a timely diagnosis, accurate stratification, and regular follow-up to get the best outcome of management.

Since nearly 50% of E β patients in this study were transfusion-dependent,

therefore prevention should be the ultimate goal to decrease the incidence of this disease and the development of future complications which can arise due to the repeated transfusions and iron overload mediated damage to vital organs. Screening of all siblings and family members whose family members are affected with thalassemia syndrome to detect carrier state, counseling of the parents and family members regarding an antenatal diagnosis for next pregnancy, increasing awareness among the general population about the disease, and screening of all marriageable population before marriage or before conception are some important steps for prevention of the disease. Hence, with the more accurate simple screening measures and antenatal diagnosis in a high-risk pregnancy, physicians can handle this disease with its dreaded complications, in a more precise and easy way.

Conclusion

In the present study, the proper standardized classification of disease severity, helped in the management of these patients. It was found that detailed clinical knowledge regarding the pathophysiology, genetic modifiers, and complications along with close and careful monitoring of these patients based on clinical scores helps the pediatricians to classify these patients for their appropriate management.

Conflict of interest

The authors declared no conflict of interest.

References

1. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/ β thalassemia: basis of marked clinical diversity. *Hemat Oncol Clin N Am* 2010; 24:1055-1070.
2. Iyer S, Sakhare S, Sengupta C, Velumani A. Hemoglobinopathy in India. *Clin Chim Acta* 2015; 444: 229-233.
3. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, et al. Prevalence of β -thalassemia and other hemoglobinopathies

- in six cities in India: a multicentre study. *J Community Genet* 2013; 4: 33–42.
4. Tubsuwan A, Munkongdee T, Jearawiriyapaisarn N, Boonchoy C, Winichagoon P, Fucharoen S et al. Molecular analysis of globin gene expression in different thalassaemia disorders: individual variation of $\beta(E)$ pre-mRNA splicing determine disease severity. *Br J Haematol* 2011; 154: 635-643.
 5. Das R, Sharma P. Disorders of abnormal hemoglobin. In: Kumar D. *Clinical Molecular Medicine Principles and practice*. London :Academic Press Elsevier; 2020; 327-339.
 6. Datta P, Basu S, Chakravarty SB, Chakravarty A, Banerjee D, Chandra S, et al. Enhanced oxidative cross-linking of hemoglobin E with spectrin and loss of erythrocyte membrane asymmetry in hemoglobin E beta-thalassemia. *Blood Cells Mol Dis* 2006; 37:77–81.
 7. Lathanatudom P, Leecharoenkiat A, Wannatung T, Svasti S, Fucharoen S, Smith DR. A mechanism of ineffective erythropoiesis in β -thalassemia/Hb E disease. *Haematologica* 2010; 95:716-723.
 8. Premawardhana A, Fisher CA, Olivieri NF, Silva S, Arambepola M, Perera W, et al Haemoglobin E β thalassemia in Sri Lanka. *Lancet* 2005; 366:1467–1470.
 9. Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. *Cold Spring Harb Perspect Med* 2013; 3(1):a011643-a11647.
 10. Fucharoen S, Weatherall DJ. The hemoglobin E thalassemias. *Cold Spring Harb Perspect Med* 2012; 2(8):a011734-a011745.
 11. Azman NF, Abdullah WZ, Hanafi S, Diana R, Bahar R, Johan MF, et al. Genetic polymorphisms of HbE/ β thalassemia related to clinical presentation: implications for clinical diversity. *Ann Hematol* 2020; 99:729-735.
 12. Sripichai O, Makarasara W, Munkongdee T, Kumkhaek C, Nuchprayoon I, Chuansumrit A. A scoring system for classification of beta thalassemia/ Hb E disease severity. *Am J Hematol* 2008; 83:482-484.
 13. Madan N, Sharma S, Sood SK, Colah R, Bhatia LH. Frequency of β -thalassemia trait and other hemoglobinopathies in northern and western India. *Indian J Hum Genet* 2010; 16:16-25.
 14. Kalantri SA, Ray R, Choudhuri S, Roy S, Bhattacharyya M. Key determinants of phenotypic heterogeneity of Hb E/ β Thalassemia: A Comparative study from Eastern India. *Indian J Hematol Blood Transfus* 2020; 36:123-128.
 15. Kumar R, Sharma DC, Kishor P. Hb E/ β -thalassemia: the second most common cause of transfusion-dependent thalassemia in the Gwalior-Chambal region of Central India. *Hemoglobin* 2012; 36:485-490
 16. 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; 10:105-110.
 17. Panigrahi I, Agarwal S, Gupta T, Singhal P, Pradhan M. Hemoglobin E β thalassemia Factors affecting phenotype. *Indian Pediatrics* 2005; 42:357-362.
 18. Tyagi S, Pati HP, Choudhry VP, Saxena R. Clinico-hematological profile of Hb E syndrome in adults and children. *Hematology* 2004; 9:57-60.
 19. Pani K, Sharma S, Muraari M, Yadav M, Phadke S, Agarwal S. Clinico-hematological profile of Hb E β Thalassemia -prospective analysis in a tertiary care centre. *J Assoc Physicians India* 2018; 66:42-45.
 20. Italia K, Dabke P, Sawant P, Nadkarni A, Ghosh K, Colah RB. Hb E β thalassemia in five Indian states. *Hemoglobin* 2016; 40: 310-315.