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Clinico-hematological Profile of Hb E- β Thalassemia- Prospective Analysis in a Tertiary Care Centre

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Abstract

Introduction: Hemoglobin E beta-thalassemia is one of the leading forms of severe thalassemia world wide. This disorder is more commonly found in South East Asia including north eastern states of India. Patients suffering from this disorder show marked clinical heterogeneity.

Materials and Methods: Referred cases of hemoglobin disorders from north India were evaluated prospectively. Details of clinical history and haematological findings including HPLC as well as mutation analysis were obtained.

Results: Twenty cases of E beta-thalassemia with widely variable clinical profile were included. The hematological parameters were also extremely variable with a wide range of hemoglobin (1.8-9.9 g/dl). Applying a severity scoring system all patients were classified the patients into mild (n=6), moderate (n=7) and severe (n=7) subclasses. We also correlated red cell Indies with HB E and HB F as well as age of onset of symptoms with HB E and HB F. IVS1-5(G-C) was found to be the most common thalassemia mutation associated with Hemoglobin E beta-thalassemia.

Conclusion: Extremely variable clinical and haematological findings were observed in Hemoglobin E beta-thalassemia patients. These findings are comparable to other Indian studies. Appropriate knowledge of the clinical variability and unpredictable natural history can help better management of this group of patients.

Introduction

Hemoglobin E (HbE) is variant hemoglobin with a mutation in the globin gene causing substitution of glutamic acid for lysine at position 26 of the globin chain. HbE is the second most common structural hemoglobin disorder after sickle cell hemoglobin (HbS).[1] It is common in South-East Asia, with a prevalence of as high as 30-40% in some parts of Thailand and Cambodia and in Laos. Hb E is also commonly found in Sri Lanka, North Eastern India, Bangladesh, Pakistan, Nepal, Vietnam, and Malaysia.[2-4]

HbE may be present in three different forms: Heterozygous state (genotype AE or hemoglobin E trait), Homozygous state (EE or hemoglobin E disease) or Compound heterozygous states such as hemoglobin E/β Thalassemia (E/β thal), sickle cell/hemoglobin E disease (SE genotype).[5]

Hemoglobin E/β Thalassaemia occurs when hemoglobin E trait gets co inherited with either β0 or β+ Thalassemia. Among severely affected thalassemia patients, almost half of the patients are represented by E/β Thalassaemia. The compound heterozygous state is quite common in Thailand, with a rough estimate of 3,000 affected newborns annually and another 100,000 living patients[16]. Higher incidence of Hemoglobin E-beta Thalassemia has been reported from north east part of India including the states of Assam, West Bengal, Orissa, Bihar and eastern part of Uttar Pradesh.[6-12]

Hemoglobin E-beta Thalassemia results in chronic haemolytic anemias with clinical picture similar to that in beta Thalassemia intermedia or major.[1] The considerable phenotypic heterogeneity of Hemoglobin E/β Thalassaemia patients has been attributed to multiple genetic and environmental factors.[1,13] The natural history of this group of patients is also highly unpredictable. So it is necessary to evaluate the phenotypic severity of these patients for proper management and to study the genotype-phenotype correlation. Multiple scoring systems have been developed to grade the patients into mild, moderate and severe subclasses according to the clinical severity.[2,14,15] The current study was conducted to evaluate the clinic-hematological spectrum of Hemoglobin E/β Thalassaemia patients in a tertiary care centre of north India. Moreover we applied a previously designed and validated scoring system to 20 north Indian Hemoglobin E/β Thalassaemia patients.

Material and Methods

This study was carried out in patients with clinical presentation suggestive of hemoglobinopathies from November 2011 to March 2013 in a tertiary referral centre of north India. Patients were included from different parts of UP and neighbouring states as well as from Nepal. Patients with history of recent transfusions (within previous three months) were excluded from the study. Informed written consent was obtained in each case and study protocol was approved by the Ethical Committee of the Institution.

Detailed present and past history regarding onset, frequency, duration of the presenting symptoms was collected. All the patients were examined for pallor, icterus, haemolytic facies,

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Table 1: Clinical profile and examination findings in E- β thalassemia cases (n=20)

Parameters	E-β Thalassemia cases
Male : female	16:4
Mean age (at diagnosis)	9.2 years (1 -38 years)
Mean age (at onset of symptoms)	3.1 years (8 months-10 years)
Positive family history	10 (50% cases)
H/O consaguinity	None
History of transfusion	13 (65% cases)
Pallor	18 (90% cases)
Jaundice	07 (35% cases)
Failure to thrive	08 (40% cases)
Fever	09 (45% cases)
Generalized weakness	05 (25% cases)
Recurrent infections	04 (20% cases)
Splenomegaly	14 (78% cases)
Haemolytic facies	05 (25% cases)
Dependant on transfusion	07 (35% cases)

skin changes, hepatosplenomegaly, any gross developmental delay or abnormality or any other significant clinical findings related to haemolytic anemias.

Three ml of EDTA anticoagulated blood was drawn from all the patients with proper aseptic procedure. The samples were analysed in fully automated counter (Sysmax XE 2100 Alpha) in the haematology laboratory of Department of Pathology. Multiparameter hemogram, including Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, RBC count, WBC count, platelet count and automated reticulocyte count was done in each case. Manual reticulocyte count, using supravital stain (Brilliant cresyl blue) was done in selected patients. Peripheral blood smears were stained using Leishman stain in all the cases and details of differential count and red cell morphological alterations.

Hemoglobin type and quantification was performed by fully automated cation exchange high performance liquid chromatography using BIORAD VARIANT II (manufactured by BIO-RAD laboratories, USA) by using beta Thalassemia short program. DNA extraction from whole blood was done by standard phenol-chloroform method. Quality and quantity of DNA was checked by spectrophotometer and electrophoresis analysis. The polymerase chain reaction (PCR) technique was used for detecting the polymorphisms of selected genes and for amplification of the gene needed for sequencing analysis.

Table 2: Hematological parameters of E- β Thalassemia cases (n=20)

Parameter	Mean (range)	Hematological abnormality		
HB (g/dl)	6.4 (1.8-9.9)	Anemia	Severe (Hb <7g/dl)	13 (65%)
RBC count (10^6/µl)	3.1 (0.9-4.6)		Moderate (Hb 7-10 g/dl)	07 (35%)
Hematocrit (%)	22.0 (6.4-41.9)	TLC*	Leukocytosis (>12x10^3/µl)	10 (50%)
MCV (fl)	68.0 (56.6-83.1)		Normal Leucocyte count	10 (50%)
MCH (pg)	20.1 (15-29.1)		Leucocytopenia (<5x10^3/µl)	Nil
MCHC (g/dl)	29.6 (24.8-37.9)	* corrected for NRBCs		
RDW (CV %)	31.5 (27.7-37.9)	Platelets	Thrombocytosis (>450x10^3/µl)	Nil
Retic count (%)	5.2 (1.2-15.8)		Normal platelet count	13 (65%)
Absolute reticulocyte count (10^6/µl)	0.17 (0.05-0.28)		Thrombocytopenia (<150x10^3/µl)	06 (30%)

The β globin gene mutations were identified by ARMS-PCR. Multiplex ARMS-PCR system was used to detect five common and five less common β globin gene mutations reported in Indian population.

Statistical analysis was carried out using SPSS statistical package (version 17.0). Descriptive statistics are shown by mean, frequency, % and SD. Correlation of severity of clinical manifestations and laboratory hematological findings were done using statistical methods including Chi Square test and t tests

Results

A total of 20 (16 male and 4 female) patients with wide age distribution (1 year to 38 years) were diagnosed as E-b Thalassemia, on the basis of HPLC and molecular findings. Eleven patients belonged to eastern UP; seven belonged to Bihar and one case each from Assam and Jharkhand. This group of patients had extremely variable clinical severity; most of the patients behaved similar to β-thalassemia intermedia. 10 patients had positive family history. Fifteen patients belonged to pediatric age group. The age of onset ranged from 8 months to 10 years.

Clinical Findings in E – β Thalassemia Cases

Progressive pallor, recurrent fever and failure to thrive were the most common presenting symptoms. Two patients presented with pedal edema and puffiness of face suggestive of congestive cardiac failure. One patient presented with facial palsy and severe anemia. Another patient was severely anemic and had altered sensorium during presentation.

Seven patients (35%) had history of recurrent transfusions before presenting to our hospital. Fourteen patients (70%) had increased spleen size and five (25%) patients had haemolytic facies on examination. One adult patient presented with neurological deficit due to spinal cord compression. This patient was found to have a mass at cervico-dorsal spinal column; histopathological findings were suggestive of extramedullary haematopoiesis.

The hemoglobin ranged from 1.8g/dl to 9.9g/dl. Thirteen patients had severe anemia and seven had moderate anemia at presentation. Reduced red cell indices, reticulocytosis and increased RDW were observed in most of the cases. Peripheral smear examinations showed marked anisopokilocytosis, hypochromia, polychromasia, basophilic stippling and variable number of nucleated red cells. Two patients who had already undergone splenectomy showed numerous NRBCs (upto 250 NRBCs/100 WBC) in the peripheral smear. Leukocytosis was observed in ten cases and thrombocytopenia was observed in 6 patients (Table 2).

HPLC and Molecular Findings

HPLC was done in all the cases. The mean HbE was 55.1% (range 28.7-77.3%) and mean HbF was 33.9% (range 13.9-61.2%). Mutation analysis was done in 12 patients with E-beta thalassemia. The details of HPLC and mutation findings of all 20 cases of Hb E beta Thalassemia is shown in table 3.

Correlation between Red Cell Indies with HB E and HB F

Statistical analysis was done to find out correlation between Hb E, Hb F with red cell indices. A significant negative correlation was seen between Hb E and red cell indices like MCV and MCH. Significant positive correlation was also observed between Hb E and RDW. A positive correlation was seen between Hb F and MCV but was not found to be statistically significant. The findings are given in figure 1.

Table 3: HPLC and molecular findings in 20 cases of E- β thalassemia

Case no.	Age of onset (months)	Hb F (%)	Hb E (%)	Hb A (%)	Molecular findings
1	12	24.7	65.5	9.8	IVS1-5(G-C)/E
2	36	61.2	38.8	00	-
3	60	41.7	57.1	1.2	CO 41-42/E
4	24	42.0	40.0	1.8	-
5	24	38.2	52.4	9.4	CO 15/E
6	36	30.1	63.7	6.2	-
7	42	18.6	77.3	4.1	IVS1-5(G-C)/E
8	36	35.0	55.5	9.5	-
9	02	49.3	48.3	2.4	IVS1-5(G-C)/E
10	24	13.9	28.7	57.4	IVS1-5(G-C)/E
11	18	30.1	63.0	6.9	CO 41-42/E
12	12	27.0	40.1	32.9	CO 41-42/E
13	48	18.9	67.8	13.3	-
14	24	44.6	46.9	8.5	CO 8-9/E
15	12	29.9	65.0	5.1	-
16	36	36.0	64.0	00	IVS1-5(G-C)/E
17	18	37.9	43.8	16.5	CO16/E
18	84	22.0	75.8	2.2	-
19	96	51.3	41.0	7.7	-
20	120	57.7	35.6	6.7	CO 41-42/E

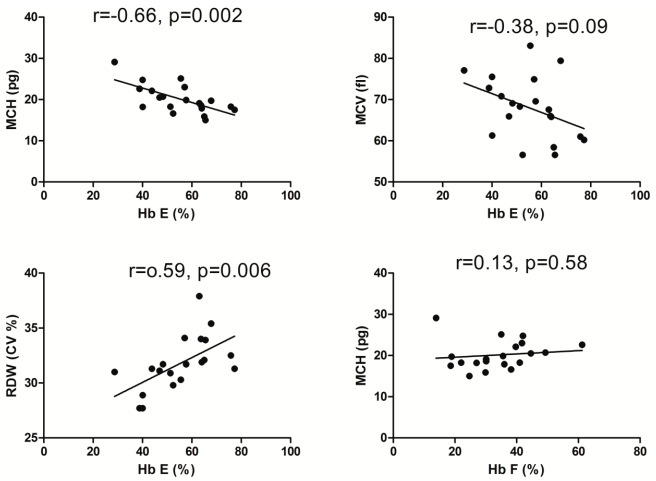

Fig. 1: Correlation between red cell Indies with HB E and HB F in Hb E β thalassemia

Severity Scoring in Hb E Beta Thalassemia Cases

All the patients of E- β –Thalassemia were grouped into mild moderate and severe category according to the scoring system, developed by Sripichai et al[15]. The scoring system included six clinico-hematological parameters (age at presentation, hemoglobin at presentation, age at receiving first transfusion, requirement of transfusion, spleen size and presence growth retardation).

Six patients were classified as mild; seven as moderate and seven patients were classified as severe category. Patients with severe disease show earlier age of presentation, more dependency on transfusion, larger spleen size and lower hemoglobin at presentation in comparison to mild and moderate category. However there was no statistically significant difference in other hematological parameters between the three groups. The clinical and hematological parameters compared are presented in table no 4.

Correlation between Age of Onset of Symptoms with HB E and HB F

Statistical analysis was done to correlate the age of onset of symptoms with Hb E, Hb F and Hb E/Hb F. No significant correlation was observed. Again we tried to correlate the clinical severity of patients with Hb E and Hb F by applying a clinical severity scoring system. No significant correlation was found between clinical severity and Hb E as well as Hb F (p value 0.849 and 0.152 respectively.

Discussion

Hemoglobin E; a variant structural hemoglobin is the most common hemoglobin variant in South East Asia and the second most hemoglobin variant worldwide.[16] In India previous studies has shown that HB E is mostly confined to North East part of India including West Bengal.[17] However sporadic cases of various hemoglobin E disorders have been reported from other states as well. HB E can be present mainly in three forms, heterozygous state (HBE trait), homozygous state (HBE disease) and most importantly compound heterozygous states (HBE- β Thalassemia and HBE-S disease). Both heterozygous as well as homozygous states of HBE disease are clinically silent, whereas the compound heterozygous states show variable clinical severity, ranging from β Thalassemia trait to Thalassemia major[5]

HBE β Thalassemia patients show extremely variable clinicohematological profile. Agarwal et al[18] divided 26 patients of HBE β Thalassemia into two groups according to their clinical severity and compared the hematological profile of the two groups. By using a previously developed scoring system[15] we classified the 20 patients of HBE β Thalassemia, into mild (30%), moderate (35%) and severe (35%) category.

Sixty five percentage of patients in the current study had severe anemia (HB<7g/dl) at the time of presentation whereas 35% of patients had history of recurrent transfusions. A previous study by Tyagi et al[19] which included 43 cases of E β Thalassemia showed that only 30% patients required recurrent transfusion. In another study by Aggarwal et al,[16] 20 (59%) patients out of 34 patients had severe disease and they required regular transfusion. The presenting complaints of patients with E β Thalassemia in the current study are similar to that of the study conducted by Panigrahi et al[20] (Table 5). In the current study, a patient presented with a spinal extramedullary hemopoietic mass. A similar case was described by Panigrahi et al[20] previously from the same centre. Our findings further support the variable severity of E β Thalassemia.

The mean hemoglobin of all the patients with E β Thalassemia in the current study was 6.4 g/dl which is comparable to other Indian studies. (Table no. 6). Markedly reduced MCV, MCH and increased RDW were observed in most of the patients in the current study; which is again similar to previous studies. Peripheral smear examination revealed marked anisopoikilocytosis with numerous target cells, polychromatophilic red cells and nucleated red cells. Similar blood picture was observed in previous studies (Aggarwal et al[16] and Patne et al[17]). The % of HBE and HBF were also comparable.

Kishore et al[26] described existence of a negative correlation between levels of HbA2/ Hb E and RBC indices including the MCV and MCH in cases

Table 4: Clinical severity scoring in cases of E-β thalassemia

Clinico-hematological parameters	Mild (A)	Moderate (B)	Severe (C)	P value
Number of cases	6	7	7	-
Male : female	5:1	6:1	5:2	-
Mean Age of presentation (mths)	48 ±21.4	47 ± 42.7	21±14.1	0.180
Age at 1st transfusion (years)	NA	3.2±3.4	1.9±1.1	0.374
Mean spleen size (cm below LCM)	4.5 ±2.5	4.4±5.4	6.4±4.9	0.662
Mean Hb (gm/dl)	7.0 ±1.4	6.2±2.6	6.0±1.7	0.656
Mean MCV (fl)	68.4±8.7	68.4±4.9	64.5±9.6	0.950
Mean MCH (pgm)	19.7v2.6	19.6±3.8	18.8±4.0	0.764
Mean RDW (CV %)	31.2±3.0	31.2±1.4	32.1±3.1	0.787
Mean reticulocyte (10^6/µl)	6.7±4.4	4.7±2.1	4.3±1.7	0.303
Mean HB F (%)	36.3±15.2	33.1±10.4	32.8±10.3	0.849
Mean HB E (%)	59.3±12.9	47.5±11.5	59.2±12.3	0.152
Mean score	2.8	5.4	8.1	

Table 6: Hematological parameters in Hb E β thalassemia in various studies

Parameters	Sharma et al[21]. (n=25)	Agarwal et al[18] (n=26)	Panigrahi et al[20] (n=23)	CS et Al[22] (n=25)	Jha et al[23]. (n= 5)	Moiz et al[24] (n=14)	Uddin Et al[25] (n=81)	Present study (n=20)
HB (g/dl)	7.3	6.7	6.2	6.9	6.6	5.0	6.1	6.4
HCT (%)	33				24.2		22.1	22.0
MCV (fl)	60.7	64.9	72.8	67.2	65	68.1	64.8	68.0
MCH (pgm)	19.3	19.6	20.5	19.7	17.3	21.0	17.8	20.1
MCHC (g/dl)	31.8	29.5		31.9	26.6		27.5	29.6
RDW (CV%)	35.7			26.6	25.6			31.5
HbE (%)	54.2	48.5	45.3	54.8	57.6	25.9	45.6	55.1
HbF (%)	31.9	23.3	27.7	31.3	26	36.3	41.5	33.9

Table 5: Clinical parameters in Hb E β thalassemia in various studies

Presenting complaints	Panigrahi et al[20] (n=23) (%of cases)	Current study (n=20) (% of cases)
Pallor	100	90
Jaundice	57	35
Splenomegaly	74	75
Hepatomegaly	65	40
Haemolytic facies	52	25
Dependant on transfusion	33	35

with hemoglobin E disorders. A similar correlation was found in a study by Sharma et al[21] from north India. Positive correlation between RDW and Hb E as well as between MCV and Hb F was also observed in that study. Similar correlations of MCV, MCH and RDW with Hb E were found in the present study. However we did not find any significant correlation between Hb F and MCV (Figure 1).

The disease severity of Hb E β Thalassemia patients is affected by multiple genetic factors. Two studies (panigrahi et al[20], agarwal et al[18]) had been done previously on the genetic determinants of disease severity in Hb E β Thalassemia on Indian patients from the same centre. The role of Hb E, Hb F, E/F ratios, β Thalassemia mutations and Xmn I polymorphism was evaluated in those two studies. A negative correlation was observed between age of onset and Hb E level by Panigrahi[18] et al. However no such significant correlation was observed in the present study. Moreover we tried to evaluate the significance of levels of Hb E, Hb F and E/F ratio with disease severity by applying a clinical scoring system in 20 patients of Hb E β Thalassemia. Early age of disease onset, more dependency on transfusion was observed in severe subgroup of patients. However no significant differences in hematological parameters were observed in mild, moderate and severe disease category. There may be other undetermined factors affecting phenotype.

Mutational analysis was done in 12 patients. IVS1-5(G-C) was found in maximum number (5 cases, 42%) of cases followed by CO 41-42 (4 cases). This finding is similar to that of previous two studies (Panigrahi et al[20] and Agarwal et al[16]) from the same centre which showed IVS1-5(G-C) as the single most common mutation (77% and 50% respectively) found in this part of the country.

Limited numbers of patients were evaluated in the present study. A larger study including more patients is necessary to validate the current scoring system[15] in Indian patients. Moreover we didn't evaluate the genetic modifiers (Xmn I polymorphism, α thalassemia mutations etc) in our patients.

Conclusions

Patients with Hb E β thalassemia have extremely variable clinical and hematological profile. Proper phenotypic stratification of these patients helps in genotype-phenotype correlation, developing treatment guidelines for subgroups and also trying newer modalities of treatments.

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