Phenotypic Diversity and Clinico-Hematological Profile of Hb E-Beta Thalassemic Children

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Abstract Hb E-Beta thalassemia is a disease with marked clinical diversity. In this study, phenotypic diversity of Hb E- β thalassemia children were analysed by studying the clinical and hematological parameters. This was a cross sectional study done in one and a half year period in the department of Pediatrics of a tertiary care teaching hospital. Participants were 62 Hb E- β thalassemic children of age group 1 month to 18 years coming to the Thalassemia day care centre for blood transfusion. Data collected from history, examination findings and investigation reports were analyzed. M:F ratio was 1.07:1; 71% children were above 5 years of age. 90.3% children were Hindu. In 66.1% children, Hb level was below 5 gm/dl at the time of diagnosis. Mean HbF level was $32.6\% \pm 11.2$. Stunting was seen in 64.5%. Average liver and spleen size were 2.5 and 4.4 cm respectively. Beside pallor, most common clinical findings were splenomegaly (93.5%), facial deformity (87%), dusky skin color (82.5%) and hepatomegaly (75.8%). 1.6% children were mild, 43.5% children were moderate and 54.8% children were of severe type. In our study there was no significant correlation between severity of the disease and HbF level ($r = 0.0853$, $p = 0.0509$). Age at the time of diagnosis, hemoglobin level at the time of first transfusion, age at receiving first blood transfusion, requirement of blood transfusion, spleen size and growth, are some factors affecting severity of the disease. But severity cannot be assessed by considering

only one clinical or hematological parameter but by considering several parameters together.

Keywords Children · Clinical parameters · Haemoglobin E- β thalassemia · Haematological parameters · Phenotypic diversity

Introduction

Hemoglobin E-beta thalassemia (Hb E- β thalassaemia) is the genotype responsible for approximately one-half of all severe beta-thalassemia [1] and is the most common severe form of β thalassemia in Asia [2]. It affects at least a million people worldwide [3]. HbE is one of the world's most common and important mutation [3, 4] and the hallmark of Southeast Asia attaining a frequency of 50–60 per cent at the junction of Thailand, Laos, and Cambodia [5]. In the North Eastern region of India, the gene frequency of hemoglobin E is 10.9% [6, 7]. Data on the prevalence of hemoglobinopathies and thalassemias is scarce in India. A multicenter study conducted by the Indian Council of Medical Research (ICMR) in six cities of India, have found the overall incidence of beta thalassemia trait (BTT) to be 2.78% [8, 9]. HbE was mainly seen in Assam (23.9%) and Kolkata in West Bengal (3.92%). Other studies from different parts of India have shown an incidence of BTT to be 4–18.2% [9–11]. The incidence of Hb E- β thalassaemia was 1.44% in general population of this part of the country as reported by ICMR [8] and 1.26% according to the study done by Baruah et al. [9], in 9000 anemic patients from Upper Assam. Though the HbE gene has been detected across all ethnic groups in Assam like the Ahom, Koch, Chutia, Muttok, Deori, Sonowal and Mishang groups, the highest incidence has been detected in the Bodo-Kacharis,

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an ethnic group speaking Tibeto-Burman languages, although all have a common ancestry [8, 12].

Hb E- β thalassaemia results from co-inheritance of a β -thalassaemia allele from one parent and the structural variant Hemoglobin E from the other [1]. HbE is caused by a substitution of glutamic acid by lysine at codon 26 of the β globin gene. This mutation also activates a cryptic mRNA splice site, which results in reduced synthesis of the β E chain and lead to a thalassemic phenotype [3]. Beta-thalassemia is caused by the reduced (beta +) or absent (beta0) synthesis of the beta globin chains of the hemoglobin tetramer [13].

Blood counts, hemoglobin, RBC indices, HPLC and DNA analysis are the various diagnostic modalities which are used worldwide to assess the prevalence of thalassemia and other hemoglobinopathies. HPLC provides effective separation of hemoglobin which gives an accurate quantitation of HbA2/E and HbF, and detects large majority of Hb variants [14].

There is a marked phenotypic diversity of Hb E- β thalassaemia patients, but the reasons for this extraordinary clinical heterogeneity are not completely understood [1]. Genetic factors that influence the clinical course and severity of anemia in Hb E- β thalassemia include type of β thalassemia mutation co-inherited with HbE, the co-inheritance of alpha thalassemia and polymorphisms shown to be associated with increased synthesis of HbF [1]. Several important environmental predictors of severity were noted in the Sri Lankan population over time [3]. Age of presentation, growth rate, splenomegaly, and hemoglobin F levels were stated as prognostic indicators of severity. Although definition of the two clinical extremes (mild and severe courses) is simple, assigning a clear separating cut-off point can be problematic. A novel scoring system that reflects the clinical severity of Hb E- β thalassaemia was proposed by Sripichai et al. [15] that allows separation of patients into three distinctive severity groups: mild, moderate, and severe and, therefore can be used for the study of genotype–phenotype correlation.

In this study, clinical and hematological parameters of 62 Hb E- β thalassaemia children were analyzed and their phenotypic diversity and clinical severity were discussed. There are very few studies on Hb E- β thalassaemia from India and no studies from this part of the country, though Hb E is found in a very high incidence.

Materials and Methods

This was a cross-sectional study done in a tertiary care teaching hospital of Assam. 62 children diagnosed by HPLC as Hb E- β thalassemia and attending the day care thalassemia centre of the Department of Pediatrics during

the period of April 2016 to October 2017 were included in the study. After taking informed consent from the parents, data gathered from history, clinical examination, HPLC reports of patients and parents, and other investigation reports were recorded in a predesigned proforma. Patients were contacted more than once if all the information could not be gathered in one sitting. Ethical clearance from the Institutional Ethics Committee was taken for conducting the study.

Severity of the disease was calculated by using the scoring system developed by Sripichai et al. [15]. Parameters used in this system were hemoglobin level (average of all pre-transfusion hemoglobin level or hemoglobin level at the time of first transfusion), age at time of disease presentation, age at receiving first blood transfusion, requirement of blood transfusion, spleen size and growth. Each of these criteria was found to be independently associated with disease severity. Each of the six significant criteria was assigned three order levels and scored as 0, 0.5, 1, or 2 point for increasing severity of phenotype. And the severity scores were grouped into three categories defined as follows: mild, score 0–3.5; moderate, score 4–7; and severe, score 7.5–10.

Data were analyzed by Microsoft Excel 2007. Mean, percentages and p value were used to analyze the variables. For correlation, Pearson correlation co-efficient was used. *P* value of < 0.05 was taken as significant.

Results

Out of 62 children diagnosed as Hb E- β thalassemia, majority of the children (71%) were above 5 years with an average age of 8 years and with a wide age distribution from 1 to 16 years. Male female ratio was 1.07:1. Religion-wise, majority of the children (90.3%) were Hindus. Most of the children (30.6%) belonged to Ahom and Sonowal/Kochari community, 21% children belonged to tea tribe and 11 (17.7%) belonged to Assamese general caste. Most of the cases belonged to the lower socio-economic class. 25 (39.6%) children were diagnosed before 2 years and 37 (59.7%) were diagnosed after 2 years. Minimum age at diagnosis was 2 months and maximum age was 11 years with average age at onset of 3.9 years (Table 1) indicating a significant heterogeneity in clinical presentation. Transfusion requirements were also variable among the patients.

Using the scoring system, we found 1 (1.6%) case of mild severity, 27 (43.5%) children of moderate severity and 34 (54.8%) were of severe variety.

The most common complaints at the time of diagnosis were weakness (94%) followed by loss of appetite (87%), pallor (85.4%) and fever (67.8%). Six children were

Table 1 Baseline variables of the Hb E- β thalassemia cases

	n = 62 (%)
Age (months/years)	
1 month–2 years	7 (11.3%)
2.1–5 years	11 (17.8%)
> 5 years	44 (71%)
Sex	
Male	32 (51.6%)
Female	30 (48.4%)
Religion	
Hindu	56 (90.3%)
Muslim	4 (6.5%)
Christian	2 (3.2%)
Caste/race	
Ahom and Sonowal/Kochari	19 (30.6%)
Tea Tribe	13 (21%)
Assamese (General Caste)	11 (17.7%)
Bengali	8 (13%)
Muslim	3 (4.8%)
Nepali	3 (4.8%)
Bihari	4 (6.5%)
Punjabi	1 (1.6%)
Age at diagnosis	
Birth to 1 year	14 (22.6%)
1.1–2 years	11 (17.7%)
2.1–5 years	21 (33.9%)
> 5 years	16 (25.8%)

diagnosed on screening (family study) and were symptom-free at the time of diagnosis (Table 2).

Under nutrition was seen in 48 (77.4%) cases with severe malnutrition (weight $\leq$ 3SD) in 42 (67.7%) cases.

Table 2 Presenting complaints at the time of diagnosis

Presenting complaints	n = 62 (%)
Weakness and fatigue	58 (94%)
Loss of appetite/refusal to take feed	53 (87%)
Pallor	52 (85.4%)
Fever	42 (67.8%)
Abdominal mass	26 (42%)
Repeated ARI	17 (29%)
Miscellaneous	15 (25.8%)
Icterus	14 (24.1%)
Abdominal pain	10 (17.7%)
History of recurrent jaundice	6 (9.7%)
Severe anemia with CCF	5 (8.1%)
Bone pain	1 (1.6%)
No complaints	6 (9.7%)

Stunting was seen in 39 (63%) cases with severe stunting (height $\leq$ 3SD) in 28 (45.2%) cases. Upper segment lower segment ratio was normal in 84.8% of cases and immature in 15.2% of cases (Table 3). Sexual maturity rating was done in children above 10 years and was immature according to age and sex in all children.

Average liver size was 2.5 cm ranging from 0 to 10 cm and mean spleen size was 4.4 cm ranging from 0 to 18 cm. There was significant positive correlation of age with hepatomegaly and splenomegaly (p value 0.008747 and 0.00001 respectively). Splenectomy was done in 7 patients.

All cases showed pallor at the time of examination. Splenomegaly (93.5%), facial deformity (87%), dusky skin color (82.5%) and hepatomegaly (75.8%) were the next common findings.

Patients were managed in the centre with demand/regular transfusions and with deferasirox when serum ferritin level exceeds 1000 ng/ml.

There was wide variability in hematological parameters in our children. In maximum number of patients (66.1%) hemoglobin level was below 5 gm/dl at the time of diagnosis. Mean HbA level was $10.1\% \pm 13.4$ ranging from 0.7 to 45.4%, mean HbE level was $50.7\% \pm 14.7$ ranging from 29 to 80.5% and mean HbF level was $32.6\% \pm 11$ ranging from 10.8 to 54.3%. There was no significant correlation between Hb F level and severity of the disease ($r = 0.0853$; $p = 0.5098$). All cases showed microcytic hypochromic picture with average MCV of $61.7 \text{ fL} \pm 6.3$, MCH of $18.4 \text{ pg} \pm 2.3$ and MCHC of $29.3 \text{ gm} \pm 3.8$. Average RDW was high with an average of 27.6 ± 5.4 (Table 4).

Parental studies revealed β thalassemia trait (BTT) in one parent and HbE trait or Hb EE in the other (Fig. 1). In one patient, one parent was Hb E trait and the other parent was Hb E- β thalassemia (diagnosed on parental study) and

Table 3 Important history and clinical findings at the time of examination

Clinical findings	n = 62 (%)
H/O major disease	5 (8.1%) (TB-3, SLE-1, Aplastic anemia-1)
H/O malaria	3 (1.65%)
H/O fractures	3 (1.65%)
H/O gall stone	3 (1.65%)
H/O leg ulcers	0
Splenomegaly	58 (93.5%)
Facial deformity	53 (87%)
Dusky skin color	51 (82.5%)
Hepatomegaly	46 (75.8%)
Undernutrition	48 (57.4%) with severe undernutrition in 42
Stunting	39 (62.9%) with severe stunting in 28

Table 4 Hematological parameters of the cases

Hb level at the time of diagnosis (gm/dl)	n = 62
1.9–5	29 (46.8%)
5.1–7	25 (40.3%)
> 7	8 (13%)
Level of Hb A (%)	0.7–45.4 (mean 10.1 ± 13.4)
Level of Hb A2 (%)	29–80.5 (mean 50.7 ± 14.7)
Level of Hb F (%)	10.8–54.3 (mean 32.6 ± 11.2)
MCV (fL)	47–73 (mean 61.7 ± 6.3)
MCH (Picogram)	13.9–24 (mean 18.4 ± 2.3)
MCHC (gm/100 ml)	20.1–37.9 (mean 29.3 ± 3.8)
RDW	4–38.6 (mean 27.6 ± 5.4)

Patient report

Bio-Rad DATE: 03/01/2019
D-10 TIME: 02:34 PM
S/N: #DC3D787408 Software version: 4.20-2
Sample ID: 15306
Injection date: 09/15/2016 02:11 PM
Injection #: 16 Method: HbA2/F
Rack #: --- Rack position: 8

Peak table - ID: 15306

Peak	R.time	Height	Area	Area %
A1a	0.20	3544	17736	0.8
A1b	0.28	3270	12368	0.6
F	0.42	2101	16699	< 0.8 *
LA1c/CHb-1	0.66	1771	14109	0.7
A1c	0.85	4748	47183	4.8
P3	1.50	8982	97665	4.6
A0	1.72	271351	1253407	58.5
A2	3.13	24941	682828	41.8 *
Total Area:			2141994	

Concentration:	%
F	< 0.8 *
A1c	4.8
A2	41.8 *

1

Patient report

Bio-Rad DATE: 03/01/2019
D-10 TIME: 02:34 PM
S/N: #DC3D787408 Software version: 4.20-2
Sample ID: 15307
Injection date: 09/15/2016 02:18 PM
Injection #: 17 Method: HbA2/F
Rack #: --- Rack position: 9

Peak table - ID: 15307

Peak	R.time	Height	Area	Area %
A1a	0.19	3916	19226	1.0
A1b	0.28	3292	10621	0.5
F	0.43	2840	18850	< 0.8 *
LA1c/CHb-1	0.66	2224	17800	0.9
A1c	0.84	6329	62954	5.0
P3	1.50	11750	93384	4.7
A0	1.71	334474	1615118	82.1
A2	3.10	6272	129282	8.6
Total Area:			1967235	

Concentration:	%
F	< 0.8 *
A1c	5.0
A2	8.6

2

Patient report

Bio-Rad DATE: 03/01/2019
D-10 TIME: 02:33 PM
S/N: #DC3D787408 Software version: 4.20-2
Sample ID: 13947
Injection date: 10/26/2015 03:43 PM
Injection #: 25 Method: HbA2/F
Rack #: --- Rack position: 2

Peak table - ID: 13947

Peak	R.time	Height	Area	Area %
Unknown	0.13	8613	18235	1.0
A1b	0.27	27952	186836	10.1
LA1c/CHb-1	0.67	66512	882265	47.5
P3	1.45	6347	67875	3.7
A0	1.70	8417	47846	2.6
A2	3.26	27818	653810	44.6 *
Total Area:			1856868	

Concentration:	%
A2	44.6 *

3

Fig. 1 Chromatograms of a family showing 1: Father with Hb AE (E:41.8%), 2: Mother with BTT (A2:8.6%) and 3: the Child with E-Beta Thal having HbE 44.6% and HbF 47.5% moving in the LA1c/CHb-1 peaks

the child was of severe type. 22 subjects had no other siblings; 27 subjects had 1–3 siblings who had either E trait or BTT. In 12 subjects, a severe type patient had siblings who also had severe or moderate type, or a moderate type

patient had siblings with either moderate or severe type. The single patient of mild severity did not have any other sibling.

Discussion

The North Eastern Region of India particularly Assam is a rich reservoir of hemoglobinopathies and thalassemias because of the migration of various races over the ages and hence home to an assortment of socio-cultural, linguistic and ethnically diverse people [12]. It has been shown by earlier authors that a high gene frequency for HbE is prevalent in autochthonous inhabitants of Assam, having cultural and linguistic affiliation with the population of South East Asian countries [12]. We studied clinical features and hematological parameters of 62 children of Hb E- β thalassemia over a period of one and a half years. It is said that world-wide approximately 50% of severe β thalassemia major patients are of Hb E- β thalassemia [1]. In our study, the number of E- β thalassemia patients was 62 against 106 total β thalassemia major cases i.e. 58.5%. In the study done by Baruah et al. [9], the number of E- β thalassemia patients was more than 3 times the number of beta thalassemia homozygous (114 patients of E- β thalassemia against 32 beta thalassemia homozygous).

In our study, 47.7% children were from Assamese community, out of which 30.2% were from Ahom or Sonowal Kochari tribe. Tea tribe, a group of population brought to Assam by the British colonial tea planters from central, eastern and southern India during mid-nineteenth century [16] contributed to 22.2% and the remaining 30.2% children were from Bengali, Nepali, Muslim and Bihari community.

Phenotypic variability of Hb E- β thalassaemia, an emerging awareness that its phenotype may evolve over time, and the limited understanding of its natural history, combine to make the management of Hb E- β thalassaemia particularly challenging [1]. Marked clinical variability ranges from a mild and asymptomatic anemia to a life-threatening disorder requiring transfusions from infancy [1]. Vichinsky E stated that severe anemia with symptoms that begin between 6 and 12 months indicated a thalassemia major phenotype. A stable clinical condition at 5 years generally indicated a thalassemia intermedia phenotype, at least through early adulthood [3]. In our study, 26 (41.9%) cases were diagnosed within 2 years of age and out of them, 17 (65.4%) cases were behaving as β thalassemia major or severe phenotype and remaining 34.6% cases were behaving as thalassemia intermedia or moderate phenotype. Children diagnosed after 2 years showed variable severity. 15 (71.4%) cases were behaving as thalassemia major and 6 (28.6%) cases as intermedia. And out of 15 children diagnosed after 5 years, 13.3% were behaving as thalassemia major, 80% were behaving as intermedia and 6.7% were behaving as minor or mild variety. Hence significant positive association was seen

between the age at the time of diagnosis and severity of the disease ($\chi^2 = 12.73$; $p = 0.0017$). This finding correlated with the study done by Vichinsky E in Sri Lanka [3]. But the reason for these children presenting late and behaving as intermedia up to 5 years and then behaving as major after diagnosis was difficult to explain. Oliveri et al. [1] also stated that the cause of rapidly changing phenotypes in younger patients is not completely understood. A modified “natural history” study 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 anemia and erythroid expansion [1]. In a series of children with Hb E- β thalassemia in Sri Lanka, observed over 15 years, at least 20 cases changed from mild to a more severe phenotype during the first 15 years of life [17]. Using the scoring system of Sripichai [15], we found it easier to classify patients into the three groups of mild, moderate and severe. This scoring system was used earlier by other authors also with similar experience [1, 17].

In the study of the siblings of patients affected by E-Beta Thalassaemia which were seen in only 6 families, we found that the siblings of a severe type of patients were severe or moderate type, and vice versa, the siblings of a moderate type had siblings of either moderate or severe. Study of a bigger sample size would be necessary to comment on the veracity of this finding.

There is a widely disparate range of clinical and haematological parameters in patients with Hb E- β thalassaemia [1, 18]. In our study, average Hb level at the time of diagnosis was 5.3 gm/dl ranging from 1.9 to 9 gm/dl. Fucheroen et al. [17] stated that the steady-state hemoglobin 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.

The mean level of Hb F in 200 patients from Sri Lanka was approximately 28%, ranging from less than 10–50%; even higher levels of Hb F have been reported from Thailand [17]. In our study mean Hb F level was $32.4\% \pm 14.5$ ranging from 10.8 to 54.3%. There was no significant correlation between HbF level and severity of the disease ($r = 0.0853$; $p = 0.5098$). Olivieri et al. [1], Vichinsky [3] and Fucheroen et al. [5, 17] however stated that Hb F level appears to be the most important factor in phenotypic modification. That there was no correlation between HbF level and the severity of the Hb E-Beta Thalassaemia was also found by other Indian studies [6, 19].

There was significant positive correlation of age with severity of hepatomegaly and splenomegaly (p value 0.008747 and 0.00001 respectively). If we consider splenomegaly alone then it showed no association with severity of the disease ($\chi^2 = 1.787$; $p = 0.4092$) though Vichinsky [3] mentioned that splenomegaly was a prognostic indicator of

severity. In our study, severity of the disease was significantly related to growth retardation ($\chi^2 = 0.41683$, p value = 0.00001). So growth retardation can be taken as an indicator of severity as mentioned by Elliot Vinchinsky [3].

Conclusion

Hb E- β thalassemias are a heterogeneous group of disorders that are rapidly increasing worldwide and very common in this part of the country. There is a marked clinical diversity that is known in no other disease and the exact etiology for this diversity is still unknown. In our study, severity of the disease was associated with growth retardation and age at the time of diagnosis but not with Hb F level or severity of splenomegaly alone. These patients, specially the intermedia group, can thrive normally in relatively low hemoglobin level; so growth status, general wellbeing and status of extramedullary hematopoiesis should be considered while giving regular blood transfusions in children and not solely on hemoglobin level.

Being a hospital based study, the milder phenotypes of HbE Beta Thalassaemia has been missed. The true incidence and their follow up can only be ascertained in a population based study and such studies should be done in all individuals especially in communities known to be at risk. Standard treatment with repeated blood transfusions with effective iron chelation is not very easy for economically backward classes. Bone marrow transplantation, the definitive treatment, is impossible for most of the patients, for its high cost and donor non-availability. Prevention is the ultimate goal to decrease the incidence of this disease. Awareness among the population about the disease, screening of all marriageable population before marriage or before conception, counseling of the parents and family members, prenatal diagnosis are some important steps for prevention of the disease.

Genetic studies, which have not been done in this study, could show the genetic pleomorphism responsible for the phenotypic diversity in these patients.

Author's Contribution AB concept, implementation, data collection, analyses and drafting of the manuscript. MKB supervision and analyses of data collection and manuscript writing.

Compliance with Ethical Standards

Conflict of interest They authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration and its later amendments or comparable ethical

standards. Approval was taken from Institutional Ethics Committee (H), Assam Medical College, Dibrugarh (Reg. No.ECR/636/Inst/AS/2014).

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