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A comparative Clinico-hematological profile analysis of HbE beta thalassemia and homozygous HbE disease in adult and children at a tertiary care hospital

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Abstract
Hemoglobinopathies are classified as quantitative or qualitative decrease in production of hemoglobin. Thalassemias result from quantitative decrease of often structurally normal globin proteins. Mutations causing decrease in the synthesis of beta globins cause beta (β) thalassemia. Hemoglobin E is a β chain variant, caused by the structural change at the 26th position where glutamic acid is replaced by lysine in the β globin. It is highly frequent in South-east Asia and is commonly found in India. Hemoglobin E beta thalassemia (HbE β thalassemia), a compound heterozygous state, result in a very variable phenotype ranging from thalassemia trait, Non Transfusion dependent Thalassemia (NTDT), to Transfusion dependent Thalassemia (TDT). Patients with Hemoglobin E disease are usually asymptomatic and result from homozygous presence of Hemoglobin E chains. In this study, fifty cases of HbE disorder involving 43 cases of HbE beta thalassemia and 7 cases of homozygous HbE disease were considered. Their Clinico-hematological highlights and results of high performance liquid chromatography (HPLC) were analyzed. The main concern remain of the Hemoglobin E beta thalassemia, in which it is difficult from HPLC results to interpret whether the patient will behave as a trait, NTDT or TDT.

Keywords: HPLC, hemoglobin E-Beta thalassemia, HbE, hemoglobin E disease

Introduction
Hemoglobin E is a significant hemoglobin variation with an overall conveyance. It's a typical β-globin chain variation resulting from substitution of glutamine by lysine at codon 26 of β-globin gene [1]. Hemoglobin E beta thalassemia (HbE β thalassemia) is a wide spread hemoglobin variation found in India. The most extreme transporter pervasiveness is approximately 27–half in the north eastern states [2, 3, 4]. Patients heterozygous for HbE are clinically asymptomatic. Homozygous HbE disease is an incredibly mild disorder with microcytosis, hypochromia and a few target cells in peripheral smear. But, some of the subjects may have mild splenomegaly [3] and resemble beta thalassemia trait. The compound heterozygous state for HbE and beta thalassemia produces HbE beta thalassemia which varies from a clinically asymptomatic condition to a severe disorder which in many cases is indistinguishable from homozygous beta thalassemia.
The present study was aimed to examine the Clinico hematological aspects in 43 cases of HbE beta thalassemia and make comparative analysis with those of HbE disease i.e. 7 cases.

Material and Methods
All Patients determined to have HbE disorder were evaluated in the Department of Hematology, IMS and Sum Hospital from January 2017–March 2019. A detailed history was recorded covering anemia, jaundice and blood transfusion. Physical assessment was performed in all cases to look for presence of hemolytic facies, growth and development, pallor, icterus, hepatomegaly, splenomegaly. All patients were investigated for complete blood count and red cell parameters on automated analyzers. Wright-Giemsa stained peripheral blood smears were inspected for red cell morphology. Liver Function test, Serum Ferritin and ultrasonography of whole abdomen was done where indicated. Quantitative examination of Hbs A, F, E/A2 was performed utilizing HPLC (Variant, Bio-Rad). Other different reasons for anemia and/or jaundice and splenomegaly, including G6PD deficiency, hereditary spherocytosis and paroxysmal nocturnal haemoglobinuria were excluded using

relevant tests. Family studies were performed where accessible.

Patients who were compound heterozygotes for E and β^0 thalassemia had hemoglobin E 40-60% of total hemoglobin while those who were homozygous hemoglobin E had a percentage ranging from 85-99%. However, Hemoglobin F was 30-40% in Hb Eβ^0 thalassemia. Overall, hemoglobin F was highly variable, from 5%- 87%. When Hemoglobin A is present, it is around 10% of the total hemoglobin, which is very low. Parental screening was done in all cases where both parents were available and also the family members were screened for knowing the status. Family studies could not be performed in 18 of 43 patients. In these patients diagnosis of HbE-beta thalassemia was made on the basis of clinical features of hepatosplenomegaly, requirement for blood transfusion, and HPLC interpretation. Patients found to have HbE trait were not included in this study. Hb Emade up 30% or less of the total hemobglobin in those with HbE trait. Clinical and haematological parameters in patients with HbE beta thalassemia were compared with those of HbE disease using student's t-test and Wilcoxson rank sum test in cases where the data was widely skewed.

Result

An aggregate of 50 patients (male 23, females 27) with HbE disorder were analysed, involving 43 cases of HbE beta thalassemia and 7 cases with homozygous HbE disease. The mean age of patient was 34 years (range vaties from 22 to 53) in HbE disease. There was a female preponderance. All patients with HbE disease were asymptomatic besides two patients who had weakness and mild pallor. Physical assessment uncovered jaundice and hepatosplenomegaly in one of them. Mean Hb in patients with HbE disease was 9.6 g/dl (range 6.8–11.8). Peripheral blood smear assessment demonstrated microcytosis, and hypochromia in all and target cells in some of them. There was associated iron deficiency anemia in patients with low hemoglobin with homozygous HbE.

Most patients with Hb Eβ thalassemia were symptomatic and were diagnosed at a mean age of 14.5 years (extend 9 months–40 years). They presented with weakness and pallor and a few had received blood transfusion at local hospital before having a diagnosis of the underlying condition. Splenomegaly was identified in 33 of 43 (76.7%) patients while hepatomegaly was seen in 67.4% and jaundice in 34.9% patients. Other features like chest pain, haemolytic facies and thromboembolic episodes were identified in 16.3% of patients. All patients had a variable level of anemia with a mean hemoglobin level of 6.5 g/dl (extend 2.1–11.1). Thirty of 43 (69.7%) patients had required red platelet transfusion for anemia, of whom 30.2% got frequent transfusion (≥2–3 unit/year). The rest received blood transfusion just occasionally (≤1 unit/year). Thirteen of 43 (30.2%) patients have never been transfused and were able to maintain a mean Hb level of 6.5 g/dl and remained asymptomatic. Patients of HbEβ thalassemia, received Hydroxyurea while those with HbE disease and associated iron deficiency anemia were given haematinics. Serum ferritin was done to indirectly assess iron overload.

Table 1: Clinical features of patients with HbE beta thalassemia

Age of onset of symptoms (years)	n(%)	Mean Hb (g/dl)
≤ 5	10(23.3%)	6.7
>5–15	14 (32.6%)	5.6
>15	19 (44.2%)	7.1
Clinical features		
Pallor/weakness or both	41(95.3%)	
Jaundice	15 (34.9%)	
Splenomegaly	33 (76.7%)	
Hepatomegaly	29 (67.4%)	
Fever	5 (11.6%)	
Growth retardation	5 (11.6%)	
Others	7 (16.3%)	
Blood transfusion		
Frequent (.2–3 unit/year)	13 (30.2%)	
Occasional (#1 unit/year)	17 (39.5%)	
Nil	13 (30.2%)	

In table 1. The clinical features of HbE beta thalassemia are summarized, where 'n' indicates the number of patients. Rheumatic coronary diseases with mitral stenosis, (one patient), thromboembolism with familial hyperhomocysteinemia (one patient), growth impairment (5 patients), bleeding (2 patients) and congestive heart failure (2 patients) were additionally noticed. Total Hb, red cell indices, haematocrit (PCV) and HPLC parameters of patients with HbE beta thalassemia are analysed in table 2 and compared with those of HbE disease.

Table 2: Comparison of haematological and HPLC findings (mean ±SD) in HbE beta thalassemia and HbE disease

	HbE beta thalassemia, (n= 43)	HbE disease (n=7)	Significance
Hb (g/dl)	6.52± 2.1	9.6± 1.6	P< 0.01
HbE/A2 (%)	54.0 ± 17.0	82.6 ± 3.8	P < 0.001
*HbA (%)	12.4 ± 16.1	5.4 ± 5.1	NS
HbF (%)	27.3 ± 15.9	3.4 ± 2.4	P < 0.001
MCV (fl)	65.2 ± 6.9	64.2± 3.8	NS
MCH (pg)	20. ± 0 3.7	21.3± 1.2	NS

MCHC (g/dl)	30.4 ± 4.7	32.9 ± 1.7	NS
RBC (£ 10^2/l)	3.40 ± 0.90	4.59 ± 0.789	P <0.002
PCV	21.6 ± 5.7	28.6 ± 4.5	P <0.003

As expected patients with Hb E disease had higher level of hemoglobin than patients with Hb Eβ thalassemia. Similarly Hb E/A2, TRBC count and PCV were also significantly higher in patients with HbE disease than those with HbE beta thalassemia. However, HbF levels were fundamentally higher in patients with HbE beta thalassemia than HbE disease. The red cell indices, MCV, MCH, MCHC, and levels of HbA were lower than typical and comparable in the two both groups. We could not delineate whether the Hb Eβ thalassemia patients had (β+ or β°) globin genes as the molecular diagnostic facilities were not available.

Discussion

Hemoglobinopathies like sickle cell disease and thalassemias have a high prevalence in the states of Eastern India and also in Odisha. This results in a major health problem and has considerable morbidity and mortality. Proper diagnosis and management along with counseling regarding the inheritance of the disease is the key to reduce the disease burden in the society and the state. The co-inheritance of beta-thalassemia (β-Thal) mutations and Hemoglobin E gives rise to a compound heterozygous condition hemoglobin Eβ thalassemia with avaried phenotypic presentation as described by Silverstroni E et.al [1].

In the present study, 13 of 43 patients, diagnosed with HbE-beta thalassemia had negligible symptoms of pallor and jaundice. Mild to moderate liver enlargement was seen in 5 and splenomegaly (size<5cm) observed in 6.The mean haemoglobin level was 6.5 g/dl and they had never required transfusion. However, HbF levels were high in these patients ranging from11.6 to 53.5% (mean 35.8%). Of these only seven patients were found to have a positive family history of beta thalassemia trait. All of them had anaemia requiring blood transfusion (≥2–3 unit/year). Eleven of 13 patients had moderate to marked splenomegaly (size >5–10 cm) and in 2 splenic enlargement was slight (0.5 cm and 1.0 cm). One of 13 patients had growth retardation and another two had repeated chest infections. Similar cases have also been described by Chernoff *et al*. [5] The mean haemoglobin in patients with HbE disease was 9.6 g/dl and HbF levels were in the range of 0.9–7.2% (mean 3.4%).Findings are comparable with other studies [5] except for the presence of marked splenomegaly which was present in one of our cases. This patient came from the state of Assam where the prevalence rate for malaria and kala azar is high and could have been a contributing factor.

The phenotypic presentations of HbE beta thalassemia and homozygous HbE disease can help differentiate the two conditions clinically which can be confirmed on HPLC by the levels of HbA2+E and HbF as described earlier [6, 7]. The present study showed higher HbA2+E levels (mean 82.6%, range 77.2–86.7%) in homozygous HbE disease than in HbE beta thalassemia (mean 54.0%, range 12.0–84.4%), (Table 2). However, there is a wide variation in individual values [6, 7]. Six of these 8 patients had severe anemia requiring blood transfusion (≥2 unit/year). So also, a greater part of cases with HbE beta thalassemia had fundamentally more significant levels of HbF (mean 27.3%) than those of HbE disease (mean 3.4%). There are some genetic modifiers which contribute significantly to the varied phenotypic expression of HbEβ thalassemia which have not been elaborated or mentioned in guidelines till now. A lot of research work are ongoing in this field to understand the heterogeneity of the disease. HPLC should be interpreted in caution. In case of any discrepancy, the results should be analysed only after family and parental screening and if available with molecular studies. Since HbA2 and HbE elute in the same region in HPLC, one should be aware of the values and ranges to give a proper diagnosis of the underlying condition. In addition, recent blood transfusion can interfere with HbF and HbA2 levels and hence, patient should be asked to repeat the test after 3 months or undergo a parental screening or molecular testing [6].

The determination of HbE disorders in neonates can be especially difficult where the significant hemoglobin is HbF and just an exceptionally modest quantity of HbE (3.9–14.9%) [8, 9] is available. The practical option is to obtain a complete family study which may help in diagnosing whether the neonate is homozygous for HbE disease or a compound heterozygous for HbE beta thalassemia. This isn't constantly conceivable and family studies may once in a while have traps [8, 9]. In areas with high incidence of thalassemia, universal screening of neonates is recomeended for both α- and β- thalassemia disorders. Krishnamurti *et al*. [8] reported a case where the infant was determined to have HbE trait dependent on HbF and HbE+A2 levels after birth while both parents had a normal hemoglobin level with microcytic hypochromic indices. However, further evaluation showed that father had HbE beta thalassemia and had co-inherited a deletional type of alpha thalassemia. Hence, it is always advised to do the parental screening and corroborate with molecular studies whenever feasible before diagnosing a case of hemoglobinopathy.

Conclusion

The phenotypic expression of thalassemia is largely dependent on ability of the bone marrow to compensate for the accelerated destruction of red cells. Thus, patients with HbE-beta thalassemia may be asymptomatic to those requiring regular blood transfusions like thalassemia major for sustaining life. As a result, proper diagnosis, treatment and follow up is required for management of these cases. Patients should be counseled regarding blood transfusions, need to monitor iron overload and initiate iron chelation therapy when required to minimize complications and allow proper growth and development and improved Quality of life. Splenectomy should be advised only when indicated. Public awareness and education, public surveillance and population screening, extended family screening of first born child, premarital screening and genetic counseling, prenatal diagnosis and family planning are among the strategies which must be sincerely implemented to reduce the burden of thalassemia in the state. Research acitivities should be carried out to find out the genetic modifiers in a particular region or cohort for the better understanding of the disease.

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