Hemoglobin E-beta Thalassemia: Factors Affecting Phenotype

I. Panigrahi, S. Agarwal, T. Gupta, P. Singhal and M. Pradhan

From the Department of Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, U.P., India.

Correspondence to: Dr. Sarita Agarwal, Additional Professor, Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226 014, U.P., India. E-mail: sarita@sgpgi.ac.in

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The phenotype of E-β-thalassemia is affected by several genetic factors. The aim of this study was to analyze severity of E-β-thalassemia and correlate with HbE, HbF, E/F ratios, β - mutation and Xmn I polymorphism. Thirty cases of E-β-thalassemia (23 with childhood onset) were studied. HbE levels were quantitated by HPLC. Xmn1 polymorphism and β-mutations were studied by PCR-RFLP and ARMS respectively. Commonest features were pallor (100%), splenomegaly (74%), and hepatomegaly (65%), 43% (10/23) were on regular transfusions at diagnosis. One case presented with paraplegia. Patients heterozygous for Xmn I polymorphism (±) had later onset (>3yrs) compared to homozygous (-/-) absence (0.5-2.8 yrs). Most (69.6%) showed β-mutation IVS 1-5 (G→C). Negative correlation was found between age of onset and HbE. Thus, presentation is similar to previously reported Thai cases. Heterozygosity of Xmn I polymorphism also delays disease onset. Early diagnosis facilitates appropriate management and prenatal diagnosis.

Keywords: *HbE, HPLC, Mutations, Xmn I polymorphism.*

HEMOGLOBIN E-beta thalassemia is common hemolytic anemia in South-east Asia(1). HbE (β-26 glutamine→lysine) is commonest hemoglobin variant in India with prevalence of 7-50% in Northeastern region and 1-2% in West Bengal(2). HbE may not be of clinical significance, but interaction of HbE and thalassemia produces variable phenotype(1,3). The thalassemia phenotype of HbE is due to activation of cryptic donor splice site by the mutation(1). Manifestations of E-beta thalassemia include refractory anemia, splenomegaly and sometimes, un-explained jaundice(4). In a previous study, no correlation was found between clinical severity and HbF(5). However, HbF is reported to be lower in transfused patients due to suppression of HbF synthesis(6). Genetic factors reported to influence phenotype include co-inheritance of α-thalassemia and homozygosity of Xmn I polymorphism. There are very few reports from India(5-8). This paper discusses correlation of phenotype with HbE, HbF, beta globin mutation and presence of Xmn I site.

Subjects and Methods

The study included patients attending Genetics clinic from 1987-2000. Age of onset at childhood was considered if first symptoms were before 18 years. Patients requiring regular transfusions at presentation were considered more severe compared to those on no/occasional transfusions. Other parameters for judging severity were degree of hepato-splenomegaly and complications like extra-medullary hemopoeitic masses, skeletal deformities, symptoms/signs of endocrine failure and cardiac complications. Indication for regular transfusions included persistently low hemoglobin level <7 g/dL, significant skeletal abnormalities, and marked extra-

medullary hemopoeisis. In those requiring occasional transfusions, transfusion was advised if Hb was <6 g/dL. In those on regular transfusions, patients were advised to maintain Hb above 9 g/dL. The proband (index case) and family members were evaluated.

Blood samples were taken at initial visit, before any transfusion at the Institute. In recently transfused patients, blood for DNA analysis was collected on follow up.

Red blood cell indices were measured on automated Sysmex 800. Osmotic fragility was calculated as per Dacie and Lewis(9). HbA_2 was estimated by column chromatography (10). Electrophoresis was performed on cellogel at pH 8.5 in tris-glycine buffer for 90 minutes. HPLC was performed on anion exchange columns with Gynkotek-High precision pump, Model 300C to separate HbA_2 from HbE and for accurate quantitation of hemoglobins(11).

Amplification refractory mutation system (ARMS) technique(12) was used for β-mutation analysis. Presence of Xmn I polymorphism was detected by PCR-RFLP(13). Splenectomy was advised for high transfusion requirement, huge spleen and evidence of hypersplenism.

Results

Of total of 30 cases of HbE-β-thalassemia, three were from Bihar and rest from UP. Of 23 with childhood onset, onset ranged from 6 months to 16 years. There were 2 affected siblings in three families. Pallor was presenting complaint in all cases. Seven had no history of blood transfusions (BT), 6 were on occasional transfusions, and 10 were on regular transfusions at diagnosis. Most of patients were transfused at other hospitals. Of patients on occasional transfusions, at least 3 were receiving regular transfusions of packed suspended cells on follow up. In those on regular transfusions, interval between BT varied from 3 weeks to 3 months. Seventeen (74%) had clinical evidence of splenomegaly (2-11 cm), 15 (65%) had hepatomegaly and 13 (57%) had jaundice at diagnosis. Thalassemic facies was found in 12/23 (52%), but was less marked than homozygous β-thalassemia.

One case presented with weakness in both lower limbs for 2½ years and inability to walk for 1 year. The patient was operated for spinal extramedullary hemopoietic mass and diagnosis confirmed on post-operative histology.

The hemoglobin of 23 cases at diagnosis varied from 4.3 to 9.4 g/dL. HbA_2 varied from 1.7 to 6.2%. The hematological parameters are summarized in *Table I*. HbE level in carriers of the HbE-trait in families studied was 22.6 to 25.1% (*Table II*). Significant negative correlation was detected between HbE and age of onset (P = 049). No correlation was observed with HbF, E/F ratio and β-mutation. The commonest Indian β-mutation IVS 1-5 (G → C) was present in 16/23 (69.6%). Analysis of Xmn I polymorphism showed homozygous absence (–/–) in 4 and heterozygous state (+/–) in 19 (*Table II*). The average age at diagnosis of homozygous negative (–/–) and heterozygous patients (+/–) was 7.4 years (range 4-13 yrs) and 15.8 years (range 3-35 yrs) respectively. However,

TABLE I–*Hematological Parameters of E-beta Thalassemia Patients.*

Parameter	Values (# 30)
Hemoglobin level (g%)	6.25 ± 1.6 (Mean ± SD)
MCV(fl)	72.85 ± 11.09 (Mean ± SD)
MCH (pg)	20.51 ± 3.32 (Mean ± SD)
HbA(%)	21.9 ± 18.09 (Mean ± SD)
HbA_2(%)	4.37 ± 1.33 (Mean ± SD)

TABLE II–*Levels of HbE, HbF and status of Xmn I polymorphism in E B-thalassemia patients.**

Case No.	Age of onset (years)	HbE (%)	HbF (%)	XmnI site	β-thalassemia mutation
1.	3.0	36.0	16.1	–/–	IVS 1-5 (G-C)
2.	3.0	50.7	35.4	+/–	Co 15 (G-A)
3.	1.5	50.9	36.2	+/–	IVS 1-5(G-C)
4.	3.0	31.0	27.2	–/–	IVS 1- 5(G-C)
5.	3.0	57.1	20.1	–/–	FS41/42(–CTTT)
6.	3.0	54.0	39.7	+/–	IVS 1-5(G-C)
7.	12.0	57.1	13.9	+/–	IVS 1- 5(G-C)
8.	0.5	23. 8	17.1	–/–	IVS 1- 5(G-C)
9.	2.0	51.3	38.7	+/–	FS41/42(–CTTT)
10.	3.0	60.3	29.1	+/–	IVS I-5(G-C)
11.	6.0	39.8	21.7	–/–	IVS I-5(G-C)
12.	11.0	21.0	36.9	+/–	IVS I-5(G-C)
13.	3.5	23.3	69.1	–/+	Co 30 (G-C)
14.	11.5	62.6	22.9	–/–	IVS I-5(G-C)
15.	16.0	35.4	22.1	+/–	Co 30(G-C)
16.	2.8	56.0	31.7	–/–	IVS I-5(G-C)
17.	7.0	38.1	22.8	–/ –	IVS I-5(G-C)
18.	5.0	18.8	13.7	–/–	IVS I-5(G-C)
19.	4.0	60.2	23.9	+/–	IVS I-1 (G-T)
20.	3.0	50.0	24.9	–/–	IVS I-5 (G-C)
21.	3.0	52.0	28.0	–/–	IVS I-5 (G-C)
22.	6.0	67.6	19.4	–/–	IVS I-5 (G-C)

* In one patient sample was not available for detailed analysis.

average age at onset was 4.5 years (0.5-11.5 yrs) and 5.9 years (1.5 - 6.0 yrs). One adult case homozygous positive for Xmn I polymorphism (+/+) had age of onset at 20 years and average Hb of 9.4g%.

In seven untransfused patients, age at diagnosis was 3-18 years, predominant presenting feature was pallor, and average Hb level was 6.2g%. Of these, 2 had significant hepatosplenomegaly with hypersplenism and Hb improved after splenectomy.

Discussion

Hemoglobin E carrier rates of up to 30% are found in Burma, Thailand, Laos, Cambodia, Malaysia and Indonesia(3). In the North Eastern region of India, the gene frequency of hemoglobin E is 10.9%(4). However, the cumulative gene frequency for sickle cell, HbD and HbE is 5.35% in India. Clinical severity of E-beta thalassemia is variable ranging from mild to severe simulating homozygous β-thalassemia (1,3,7,

Key Messages

- Most cases of hemoglobin E-β-thalassemia present in childhood.
- Early diagnosis of E-β-thalassemia facilitates proper management and prevention of occurrence/recurrence in family.

8,14). Agarwal, *et al.*(5) has earlier reported 21 families of E-beta thalassemia. In an analysis of 50 British E-β-thalassemia patients (Rees, *et al.* 1998), 50% were regularly transfused and 50% required splenectomy(15). In another study by Fouladi, *et al.* on 22 Canadian patients, 30% were regularly transfused and 17% required splenectomy(15). Over 20 case studies till date have reported spinal cord compression in thalassemia, most occurring in mid-thoracic region.

The management of HbE-β-thalassemia is similar to that of homozygous β-thalassemia. In those maintaining Hb of >7 g% without complications, long term folic acid is required. Many may benefit from hydroxyurea therapy(16), which decreases ineffective erythropoeisis and increases Hb with or without increase in HbF. Hormonal complications due to hemosiderosis occur especially in those on transfusions and may require iron chelation and exogenous hormone therapy. A persistently low Hb level <7g%, significant growth retardation, recurrent fractures, significant bony deforinities or complications due to extramedullary hemopoeisis would necessitate regular transfusions. Features of hypersplenism (clinically, on hemogram and/or red cell survival studies), high transfusion requirement (>250 mL packed red cells/kg/year) would warrant splenectomy. Periodic assessment of serum ferritin, calcium, T4, TSH, blood sugar, liver function tests *etc*. aids in proper management. In those with hypogonadism, S. testosterone/estradiol levels and bone mineral density assessment may be required.

The predominant β-mutation in this study was IVS 1-5 G-> C, the same as that found in thalassemia major(17). Co-inheritance of α-thalassemia and homozygosity for Xmn I site polymorphism modify phenotype(7,8,15). Winichagoon, *et al.*(18) found that mild phenotype may be seen even in absence of detectable α-thalassemia and Xmnl +/+. In our study the analysis of Xmn I polymorphism suggested that heterozygosity of Xmn I site might also decrease severity.

We have standardised HPLC technique for separation of HbA_2 from HbE, which is usually considered difficult and HbE and HbA_2 values are reported together. There was significant negative correlation of age of onset with HbE level. We did not find any correlation of HbF, and E/F ratio with age of onset. There may be other undetermined factors affecting phenotype.

It is possible to make diagnosis of E-beta thalassemia even in neonates(19). Early diagnosis would allow better management of the patients. Prenatal diagnosis can be done on chorionic villus samples at 10-12 weeks gestation in the informative couples with identified β-thalassemia and HbE mutations(20).

Conclusions

Our study further supports variable severity of E-beta thalassemia. Heterozygosity for presence of Xmn I site

polymorphism is also likely to influence phenotype. The role of HbE and E/F ratio may be clarified in further studies. It is important to diagnose early to ensure adequate prevention and management of complications. This also facilitates proper counseling of families for future pregnancies, prevent recurrences and reduce burden of disease.

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Avoidance of Food Allergens in Childhood Asthma

S.R. Agarkhedkar, H.B. Bapat and B.N. Bapat

From the Allergy Research Institute, Sinhagad Road, Vadgaon Budruk, Pune, 411 051, India and Department of Pediatrics, MIMER Medical College,Talegaon Dabhade, Pune, India.

Correspondence to :Dr. H.B.Bapat, Allergy Research Institute, Sinhgad Road,Vadgaon Budruk, Pune 411 051, Maharashtra, India. E-mail: kedarbapat@hotmail.com

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Twenty-four patients of moderate persistent perennial asthma with documented aggravation to severe persistent asthma, during monsoon season in the past two years, were put on specific elimination diet during August and September. The diet was based on results of in-vitro allergy tests for a selected food panel. On specific elimination diet, five patients improved to mild persistent asthma and twelve patients improved to mild persistent asthma with occasional exacerbations . Six patients remained at moderate persistent asthma and only one patient deteriorated to severe persistent asthma. These results indicate that food avoidance may help in asthma control in children.

Key words: *Allergy, Asthma, Food-allergens.*

ALLERGIC bronchial asthma is caused by exposure to environmental and dietary allergens. Allergen avoidance is considered to be an important aspect of management of allergic asthma(1). Many studies show the beneficial effect of environmental allergen avoidance(2). Regarding avoidance of food allergens, studies are available only for neonates and infants in relation to later risk of asthma(3). There is paucity of evidence dealing specifically with avoidance of food allergens in the clinical management of childhood asthma.

The purpose of this study was to evaluate possible effect of a specific elimination diet (*i.e.*, avoidance of food items for which serum IgE titers were raised) in pediatric asthmatics having seasonal aggravation.

Subjects and Methods

This study was carried out in pediatric patients of bronchial asthma, attending the out patient department of a general teaching