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Original Article

Predictors for Transfusion Requirement in Haemoglobin E-β Thalassemia

Prakas Kumar Mandal[1], Pooja Prasad[2], Tanushree Ghosal[3], Suman Kumar Pramanik[4], Tuphan Kanti Dolai[5]

ABSTRACT

Background: Haemoglobin E-β thalassemia is a common haemolytic anaemia in South-East Asia. The patients show remarkable clinical heterogeneity-ranging from asymptomatic anaemia to severe transfusion dependence. There is no specific marker to predict the frequency of blood transfusion (BT) required by these patients.This study aims to find suchfactors like age of first BT, spleen size, levels of haemoglobin E (HbE), haemoglobin F (HbF) and the ratio of HbE to HbF. **Materials and Methods**: Fifty-eight HbE-beta thalassemia patients visiting the Thalassemia OPD and day care centre of a tertiary care hospital were included in the study. They were grouped into 3 groups according to their age of first BT: group1 had patients with first BT below 2 years old, group2 had those with first BT at/above 2 years old; those without any BT were in group3. Hb was estimated by automated cell counter. HbE & HbF levels were quantified by High Performance Liquid Chromatography (HPLC). **Results:** Out of 58 patients, seven patients belonged to group1, 46 to group2 and five to group3. Group1 patients had average spleen size>6 cm, lower levels of steady state Hb and low HbF% whereas group2 had spleen size ≤6 cm, higher steady state Hb levels and a higher HbF%. Significant statistical differences were observed in steady state Hb levels, HbF levels and spleen size. **Conclusion:** The study highlights that MCV, levels of HbE & HbF and the ratio of HbE to HbF can be used as predictors of severity and transfusion requirement in HbE-β thalassemia patients.

Key words: E-β thalassemia, Severity, Transfusion requirement, HbE/HbF ratio, MCV.

Prakas Kumar Mandal[1], Pooja Prasad[2], Tanushree Ghosal[3], Suman Kumar Pramanik[4], Tuphan Kanti Dolai[5]

[1,2,5]Department of Hematology, N.R.S. Medical College & Hospital, Kolkata, India
[3]Department of Pathology, Malda Medical College & Hospital, Malda, India.
[4]Clinical Hematologist, Command Hospital (Eastern Command); Kolkata, India

Correspondence

Dr. Prakas Kumar Mandal,
8C/1/N, Roy Para Road, Kolkata-700050, West Bengal, India.
Mobile no: +91-9433345001.
Email: prakas70@gmail.com.

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INTRODUCTION

HaemoglobinE-beta thalassemia is a common haemolytic anaemia in Southeast Asia.[1] HbE (β-26 glutamine→lysine) is the commonest haemoglobin variant in India with prevalence of 7-50% in North Eastern region and 2.78% in West Bengal.[2,3] The clinical picture shows a wide range of heterogeneity produced by the interaction of various factors. Thus, it ranges from mild asymptomatic anaemia to symptomatic manifestations which include refractory anaemia, splenomegaly and sometimes unexplained jaundice.[4] The frequency of blood transfusion (BT) requirement varies greatly among patients; but no specific marker to predict the BT frequency is available. Several genetic modifiers affect the phenotype, including the type of β-thalassemia mutation, HbF levels, and co-inheritance of α-Thalassemia. The reasons for the extraordinary clinical heterogeneity of HbE/β-thalassemia are not completely understood.[5] The condition may present as a mild and asymptomatic anaemia, or as a life-threatening disorder requiring transfusions from infancy.[6] Attempts to categorize the severity of Hb E/β-thalassemia have included the assignment of patients to "severe" and "mild" disease groups, between which putative genetic and environmental factors were then compared.[7,8] Sripichai O *et al* worked on a scoring system for the classification of beta-thalassemia/HbE **disease severity** based on six independent parameters; haemoglobin level, age at disease presentation, age at first blood transfusion, requirement for transfusion, spleen size and growth and development.[9] They were able to separate patients into three distinctive severity categories: mild, moderate, and severe courses. The present study aims to find out factors that can predict the frequency of BT required by such patients.

MATERIALS AND METHODS

A total of 58 consecutive HbE-beta thalassemia patients visiting the thalassemia OPD and day care centre of this Institute from July 2014 to December 2014 were evaluated. Relevant history was taken including age at presentation and diagnosis; age of first BT and frequency of BT. Blood transfusion requirement was determined according to pre-transfusion target Hb level of <70g/L and/or presence of features like facial deformity, stunted growth, endocrine abnormalities and poor school performance. Detailed clinical examination was carried out with special emphasis on pallor, jaundice, bony changes, spleen size and liver size. Growth chart monitoring was done atleast twice for every patient during their follow up at a minimum interval of 6 months. Other causes of hemolytic anemia including G6PD, vitamin B12 and folic acid deficiencies excluded and evaluated for any other comorbidities.

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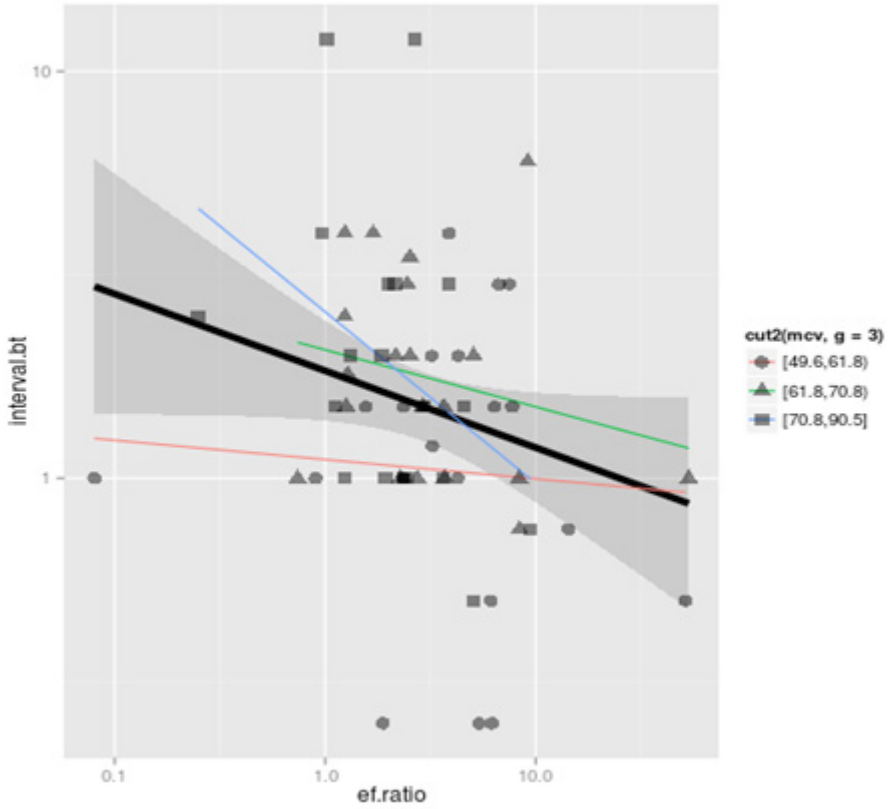

Figure 1: Depicts the relation between EF ratio and interval between two blood transfusions, (both in log scale) across three quartiles of MCV.

Patients were then grouped into the following 3 groups according to their age of first blood transfusion: group 1; age of first BT< 2 years, group 2; age of first BT ≥2 years and group 3; no BT.

The patients were subjected to Complete Blood Count (CBC) and high performance liquid chromatography (HPLC). Complete blood count (CBC) was carried out by Sysmex KX 21 automated cell counter & HPLC by Bio Rad Variant 2 analyzer for β Thalassemia short programme. Haemoglobin (Hb), Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin (MCH) at baseline were recorded. HbE & HbF were obtained from HPLC study. Thereafter, ratio of HbE and HbF was calculated for each case. Data was analyzed using standard statistical methods.

Statistical Methods

R 3.0.2 (citation: R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/was used for statistical analysis.

RESULTS

Of the 58 patients 34 were male and 22 were female. They had a median age of 23 (range 2.4-55) years. Age distribution of the patients is shown in Table 1.When grouped according to their age of first blood transfusion (BT), 5 (9%) patients belonged to group 1, 46 (79%) belong to group 2 and 7(12%) to group 3. Age distribution across the three groups is shown in Table 2. All patients had a pre-transfusion Hb level of <70 g/L and received blood transfusion for anaemia as well as facial deformity and stunted growth. None of the patients had any other significant co-morbidities. As shown in Table 3, the mean transfusion frequency in group1 was one unit every 45 days as compared to one unit every 75 days in group 2. Majority of patients in group1 had a spleen size 6 cm or greater, group 2 had less than 6 cm whereas group 3 showed mild splenomegaly in general. Among the 3 groups, the mean steady state Hb levels were 65, 73 and 100 g/L in groups 1, 2 and 3 respectively. The mean HbF was 10.8%, 20.65% and 20.9% whereas the mean HbE was 38.3%, 54.96% and 67.3% in group1, group2 and group3 respectively. The ratio of HbE/HbF across the 3 groups (1, 2 and 3) was 3.83, 2.6 and 3.07 respectively.

Data Analysis

All the obtained parameters were analysed by Kruskal-Wallis one way analysis of variance test. Significant statistical difference was found in the average spleen size among group 1 & group 3 (p<0.01). The steady state Hb levels had significant statistical differences between group 1 and group 3 (p<0.001) as well as between group 2 and group 3 (p< 0.05). The statistical differences in HbF% in Group 1 versus group 2 (p<0.01) and group 1 versus group 3 (p<0.05) were also significant. No significant statistical difference was found among group 2 & group 3 (p> 0.05). HbE% and HbE/HbF values did not show any statistical difference among the 3 groups. The summary of the analytical findings in Table 4 shows the difference in log (time to transfusion) per unit increase in continuous covariate, with 95% CI and p value for the same after fitting linear regression model. It shows that baseline MCV, MCH, HbE and HbE/F ratio influences blood transfusion requirement.

On univariate analysis of the effect of different parameters on log of time to blood transfusion requirement, it was found that the difference in log (time to transfusion) per unit increased in continuous covariate. It showed that baseline MCV, MCH, HbE and HbE/HbF ratio influences blood transfusion requirement. The statistical significance for the parameters among the three groups is shown in Table 5.

Then, we ran the multivariate linear regression model (with HbE/HbF ratio and MCV in it) to see the resultsshown in Table 6. On multivariate

Table I: Age distribution of the patients.

Age range (years)	Number of patients	Percentage (%)
0 -10	25	43.2
11 – 20	21	36.2
21 - 30	9	15.5
31 - 40	2	3.4
41 – 50	1	1.7

Table II: Age distribution across the 3 groups.

Parameters	Group 1	Group 2	Group 3
Number of patients	5 (9%)	46 (79%)	7 (12%)
Age Range (years)	3 – 26	3.8 – 43	4 – 55

Table III: Various Parameters across the 3 Groups:

	GROUP 1	GROUP 2	GROUP 3
Total Number	7	46	5
Median Age (Years)	22 (3 – 26)	24 (3.8 – 43)	8 (4 – 55)
Male: Female	2:5	1:1.3	3:2
Average BT Interval (units/no. of days)	1/45 (1/30-60)	1/75 (1/30-360)	No BT
Average Spleen Size (cm)	≥ 6	< 6	Mildly Enlarged
Steady state Hb (g/dL)	65 (30 - 82)	73 (26 – 118)	100 (85 - 120)
MCV (fL)	74.6 (57.7 – 87)	67.6 (53.6 – 90.5)	56.1 (49.6 – 70.8)
MCH (pg)	22.3 (13.5 – 28.3)	19.5 (14.8 – 25.5)	15.9 (13.7 – 22.2)
HbF (%)	10.8 (7.6 – 19.8)	20.65 (12.5 – 48.1)	20.9 (15.8 – 39.3)
HbE (%)	38.3 (20.80 – 77.80)	54.96 (31.7 – 74.4)	67.3 (48.8 – 70.2)
HbE/HbF	3.83 (1.93 – 6.38)	2.60 (0.97 – 6.2)	3.07 (1.24 – 3.23)

Table IV: Univariate analyses of the effect of given covariate on log of time to transfusion.

SL. no.	Parameter (ID)	EFFECT	CI.95.LL	CI.95.UL	p.val
1	Age of presentation	0.006	-0.011	0.023	0.493
2	Sex	0.158	-0.245	0.562	0.436
3	Age of Diagnosis	0.005	-0.015	0.026	0.620
4	Age of 1st.BT	-0.000	-0.022	0.021	0.967
5	Growth defect	0.274	-0.132	0.680	0.182
6	Liver enlargement	0.026	-0.152	0.204	0.773
7	Spleen enlargement	-0.004	-0.071	0.063	0.906
8	Hb	0.041	-0.039	0.122	0.306
9	MCV	0.022	0.001	0.044	0.048
10	MCH	0.073	0.005	0.141	0.036
11	HBE	-0.017	-0.031	-0.003	0.017
12	HBF	0.010	-0.005	0.025	0.188
13	EF ratio	-0.020	-0.041	0.002	0.069
14	Ferritin	-0.000	-0.000	0.000	0.508

Table V: Parameters showing statistical significance between the different groups

PARAMETER	Group I vs Group II	Group II vs Group III	Group I vs Group III
SPLEEN SIZE	p < 0.01	p = 0.68	p = 0.73
STEADY STATE Hb	p < 0.01	p = 0.54	p < 0.01
HbF	p=0.002	p = 0.03	p = 0.64

Table VI: Results of multivariate analysis

Covariate	Coefficient	Std Error	p value
EF ratio	0.89	0.92	0.34
MCV	0.04	0.02	0.05
EFratio and MCV (interaction term)	-0.016	0.014	0.26

analysis, it was found that as the HbE/HbF ratio increases the requirement for blood transfusion also increases (Figure 1). We found that only MCV was significantly associated with BT requirement. Patients with lower MCV had more transfusion requirement.

DISCUSSION

Patients with haemoglobin E-beta thalassemia represent approximately 50% of those affected with severe phenotype.[1] The highest frequencies are observed in India, Bangladesh and throughout Southeast Asia, including Thailand, Cambodia and Laos.[10] In the North Eastern region of India, the gene frequency of haemoglobin E is 10.9%.[2,3] There is a wide range of clinical and haematological parameters in patients with HbE-beta thalassemia.[5,6] HbE-beta thalassemia results from co-inheritance of a beta-thalassemia allele from one parent and the structural variant HbE from the other. HbE results from a G→A substitution on codon 26 of the beta globin gene. This mutation not only produces structurally abnormal haemoglobin, but also activates a cryptic splice site that causes abnormal messenger RNA processing. Because the usual donor site has to compete with this new site, the level of normally spliced mRNA is reduced, and the abnormally spliced mRNA is non-functional because a new stop codon is generated. As a result, HbE is synthesized at a reduced rate, and behaves like a mild form of beta-thalassemia.[11] The pathophysiology of HbE-beta thalassemia is complex and related to many factors. It is characterized by different clinical manifestations at particular stages of development. Despite its frequency, little is known about its natural history, the reasons for its clinical diversity, or its optimal management, and is often managed in an ill-defined and haphazard way, usually by demand transfusion. The reasons for its phenotypic variability are not completely understood.[5]There is an emerging understanding of the genetic and environmental factors that may influence the clinical course and severity of anaemia. Genetic factors include the type of beta thalassemia mutation co-inherited with HbE, the co-inheritance of alpha thalassemia, and that of polymorphisms shown to be associated with an increase in HbF synthesis.[7,12]The first important step in the study of genotype–phenotype interaction is the accurate definition of phenotypes. It is known that the severity of thalassemia intermedia cannot be judged using only one or two parameters such as the haemoglobin level and/or the transfusion history.[9,13]Several investigators have attempted to categorize disease severity with assignment of patients to severe and mild groups, between which genetic and environmental factors could be compared.[9,14]In 2003, Phadke *et al*[13] devised a novel scoring system based on six independent parameters, haemoglobin level, age at disease presentation, age at receiving first blood transfusion, requirement for transfusion, spleen size, and growth and development, which was able to separate patients into three distinctive severity categories: mild, moderate, and severe course . The scoring system included 6 phenotypic variables, each divided into 2 to 4 possible severities. According to the authors, their score appears to correctly classify cases of anaemia presenting at any age and also mild anaemia patients can be picked up by observant paediatricians. Their scoring system can be of practical utility and can be used to define treatment guidelines for the subgroups.

Premawardhena *et al*[14] in 2005 studied 109 patients of HbE-beta thalassemia to assess its natural history and the reason for its clinical diversity. The authors clinically graded the patients into 5 groups on the basis of age of presentation, transfusion requirement, growth and development including sexual maturity and quality of life. They found a strong correlation between concentrations of erythropoietin and haemoglobin (p=0·0001) and a decline in erythropoietin response with age. They have emphasized the remarkable instability of the phenotypes, particularly in the early years, during which there are changing patterns of anaemia and growth retardation. They concluded that age at presentation, γ gene promoter polymorphisms, α thalassaemia, and *UGTA1A* promoter polymorphisms are useful prognostic indicator which could serve as environmental and genetic modifiers[14]In 2008, Sripichai *et al*[9] examined the phenotypic diversity of 950 beta-thalassemia/HbE patients in an attempt to construct a system for classifying disease severity. They evaluated 14 parameters including age, gender, age at disease presentation, age at receiving first blood transfusion, requirement for transfusion, spleen size, liver size, growth and development, ferritin level, haemoglobin level, haemoglobin F level, percentage of HbF, beta thalassemia mutation and alpha thalassemia co-inheritance. It was concluded that the severity classification of the patients should be defined before the first decade of life, so that an effective individual management such as regular blood transfusion and adequate chelation therapy can be applied to prevent a severe clinical course. The score can be calculated easily and may help to identify those patients with a low score who do not need aggressive treatment.

In the present study, we grouped patients of HbE-beta thalassemia into 3 groups according to the age of first blood transfusion and then evaluated the significance of various clinical parameters. Although the age at first receiving blood transfusion and the requirement for transfusion correlate well with disease severity, these are subjective assessments based on the physicians' judgment. The patients who had first BT before 2 years old were grouped into group 1 having a severe phenotype, and were found to have a spleen size ≥ 6 cm in majority of cases, lower levels of mean steady state Hb (65g/L), low Hb F (10.8%) by HPLC & lesser BT interval. On the other hand, patients who had first BT at or after 2 years old (group 2- Intermediate phenotype), had spleen size <6 cm, higher mean steady state Hb (73g/L) levels, a higher HbF% (20.65) & greater BT interval. The patients in group 3 (no transfusion requirement/NTDT)

had mildly enlarged spleen and maintained mean steady state haemoglobin level of 100 g/L.

Between the three groups, significant statistical difference for steady state Hb level was found for group 1 & 2 and between group 2&3. The amount of HbF was also found to be statistically significant between group 1&2 and group1 &3. HbE % and HbE/HbF values did not show any statistical difference among the 3 groups. Thus, statistical analysis between the three groups based on age of first BT showed that baseline Hb level and Hb F% are the factors that can help determine the required transfusion frequency.

On univariate analysis, it was shown that baseline MCV, MCH, HbE and HbE/HbF ratio influences blood transfusion requirement. However, on multivariate analysis, only MCV at baseline was found to be significantly associated with transfusion requirement.

On initial evaluation, HbE, HbF, HbE/HbF ratio and MCV seem to have some role in prediction of transfusion requirement. These findings may improve our current understanding of the biology of these haemoglobinopathies and may shed some light to the phenotypic variability in HbE-beta thalassemia. However, further studies including more number of cases need to be done to formulate a meaningful predictive model for BT requirement.

CONCLUSION

There is wide phenotypic heterogeneity in HbE-beta thalassemia and affected patients have a disparate range of clinical and haematological parameters. The lack of a standardized classification of disease severity is a major impediment to the complete understanding of the clinical spectrum. The severity scoring used in our study may help to predict the transfusion requirement in these patients. However, other parameters still need to be analyzed for formulating effective management guidelines.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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ABBREVIATIONS USED

Hb: Haemoglobin; BT: blood transfusion; HbE: haemoglobin E; HbF: haemoglobin F; HPLC: High Performance Liquid Chromatography; CBC: Complete Blood Count; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; EF ratio (HbE/HbF): haemoglobin E and haemoglobin F ratio; NTDT: non transfusion dependant thalassemia; Std error: Standard error.

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