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Factors associated with alloantibody formation in transfusion dependant Hb E/beta-Thalassemia patients: A hospital-based study in a tertiary care centre of Assam

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ABSTRACT

Background

Interaction of Hb E with beta-thalassemia results in red cell phenotypes with weakened alpha -beta interface causing decreased red cell survival. Severe forms of Hb E/beta-thalassemia on regular blood transfusion are exposed to multiple red cell antigens causing alloantibody formation. India being a resource poor country, majority of the blood centers provide only ABO, Rh D matched blood without antibody screening. Hence, limited data is available on the rate of RBC alloimmunization in the state of Assam, which has highest prevalence of Hb E/Beta thalassemia patients in India[1]. The objective of this study is to analyse the factors associated with RBC alloimmunisation in transfusion dependent Hb E/Beta thalassemia patients.

Materials and Methods

This is a cross-sectional observational study on transfusion dependent Hb E/beta thalassemia patients visiting the blood centre Gauhati Medical and Hospital for a duration of one year. Blood grouping was done by conventional tube technique where as pre transfusion antibody screening and compatibility testing was done by column agglutination technique.

Results

A total of 188 Hb E/beta-thalassemia patients were included in the study. Alloimmunization was seen in 12 cases (6.3%). The age of the patients at the time of study ranged from 1 to 28 years with a mean age of 10.2 years (SD=5.31). Rate of alloimmunisation was lowest (2.36%) in the age group (1-10) years and highest (23%) in the age group (21-30) years. Those who were transfused ≤50 blood units had alloimmunisation rate of 1.71% compared to 20% in those receiving ≥150 units. Three cases (2.7%) who received first transfusion at ≤ 1 years of age developed alloantibody compared to nine cases (11.5%) who received transfusion after 1 year of age.

Conclusion

Factors like age at first transfusion, number of blood units transfused and mean age showed significant correlation with the rate of alloimmunization in transfusion dependent Hb E/beta-thalassemia patients.

Keywords: Hb E/beta-thalassemia patients, alloimmunization, Packed Red blood cell (PRBC), Transfusion Dependent Thalassemia (TDT)

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INTRODUCTION

Haemoglobin E (Hb E) is the most common haemoglobin variant in Southeast Asia and the second most prevalent, worldwide. It results from substitution of glutamic acid by lysine at codon 26 of the beta globin gene[1,2]. As per National Health Mission Guidelines on Haemoglobinopathies in India (2016), Hb E has a carrier frequency as high as 50% in North-Eastern population of India but relatively rare in rest of the country[3].The BODO-KACHARI community of Assam have a gene frequency of 0.50 for Hb E, which is highest anywhere in the world[4]. Hb E disorders may be found in heterozygotes (AE), homozygotes (EE) and compound Heterozygotes form (Hb E/ beta thalassemia, Sickle cell Hb E disease)[5,6,7]

The interaction of Hb E and beta-thalassemia results in thalassemia phenotypes ranging from a mild form to conditions indistinguishable from thalassemia major. An ICMR funded multicentric study in major cities of India by Mohanty D et al [1] found the prevalence of Hb E/ beta thalassemia to be 0.19% in India with Dibrugarh district of Assam having the highest prevalence of 1.44%. Due to weakened alpha -beta interface, these red blood corpuscles have greater instability during oxidative stress leading to decreased red blood cell (RBC) survival and low haemoglobin. The symptoms in these group of patients varied from mild to severe forms. Severe form of Hb E/beta-thalassemia can have hemoglobin as low as 4-5 g/dl and are dependent on regular blood transfusion to prevent complication associated with ineffective erythropoiesis like anemia, fever, skeletal deformity, endocrine abnormality, splenomegaly etc[8]. Hematopoietic stem cell transplantation is the treatment of choice for these patients but it is very costly and getting an HLA matched donor is difficult. Henceforth, majority of these patients are dependent on regular blood transfusion and are termed as transfusion dependent Thalassemia (TDT). These group of Hb E/beta-thalassemia patients, receiving regular blood transfusion are exposed to multiple foreign RBC antigens that might stimulate their immune system to produce antibodies against these antigens leading to alloimmunization[9].

Alloantibodies are produced after exposure to genetically different, non -self antigens such as different RBC antigens after blood transfusion, pregnancy etc. Development of alloantibodies against RBC antigens also complicate blood cross matching, shortens in vivo survival of transfused cells, delay in providing blood transfusions and may accelerate tissue iron loading. In general, the more is the antigen disparity between the donor and the recipient, the more is the chance of alloantibody formation[10]. Ideally extended phenotype matched, leucodepleted blood must be provided to these TDT patients[9]. India being a resource poor country, majority of the blood center are providing only ABO, Rh D matched blood without antibody screening. Hence, limited data are available on the prevalence of RBC alloimmunization in transfusion dependent Hb E/beta thalassemia patients from North-Eastern part of India, as pretransfusion antibody screening is not routinely performed.In this study, we would analyze the alloimmunization rate and the factors associated with the alloantibody formation in Hb E/beta-thalassemia patients on regular blood transfusion.

METHODOLOGY

This was a cross-sectional observational study, carried out in the blood centre of Department of Pathology, Gauhati Medical and Hospital (GMCH), Assam. An informed consent was taken from all transfusion dependent Hb E/beta-thalassemia patients who had registered in thalassemia clinic, GMCH during a period from July 2015 to June 2016 . Patients enrolled in this study was then interviewed using standard questionnaire to collect data like age, gender, ABO and Rh blood group, history of splenectomy, age at first transfusion and number of blood units transfused. All Hb E/beta thalassemia patients were transfused according to institutional transfusion policy to keep target Hb level 9–11.5 g/dL with a transfusion interval of 2–4 weeks. Antibody screening is done before each and every blood transfusion. All patients under study are given ABO and Rh(D) compatible PRBC by cross matching at antihuman globulin phase (AHG phase)

Inclusion criteria:

Hb E/beta-thalassemia patients in the age group ranging from 1 to 40 years who were dependent on transfusion and had a history of blood transfusion at least once in every month.

Exclusion criteria:

1.Children with Non-Transfusion dependent Hb E/beta-thalassemia and other associated haemoglobinopathies.

2.Patients with background of HIV, HBV, HCV.

3. Patients / Parents not giving consent to participate in the study.

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4.Females with history of alloimmunisation during pregnancy.

It was performed through SPSS software by making the frequency distribution tables and identifying frequency of alloimmunization. Discrete categorical data were presented as (%). Comparisons for categorical data were made by Chi-square test. All reported values are two-sided, with a significance level of 0.05. Sample was collected under aseptic conditions.5ml blood is drawn into an ethylene diamine tetraacetate (EDTA)containing tube, centrifuged at 3000 ×g for 3 minutes to obtain red cells for forward blood grouping and plasma for reverse blood grouping, antibody screening and cross matching.

Blood grouping:

ABO blood grouping (forward and reverse) was done as per departmental Standard Operating procedure and DGHS [11] manual by conventional tube technique.

Screening for alloantibody:

The antibody screening test was done with a combination of 3 sets of commercially available group O red blood cells which have been typed for clinically significant antigens as well as rare antigens. These cell panel are known as Dia – cell I, II, III.

Antibody screening is now done by incubating commercially available three cell panel (Dia -cell I, II, III) with patient's plasma at 37*C for 15 minutes and then centrifuging for 10 minutes on Coombs gel card containing polyspecific antihuman globulin (anti-IgG + C3d). Finally, according to presented antigram pattern of each panel, presence or absence of alloantibody was detected. Auto control is run with each test by similar procedure where patient's own cell is incubated with patient's plasma to rule out autoantibody.

Crossmatching of the recipient's plasma with the donor's red blood cells was too carried out in similar procedure in Coombs Gel card at 37°C to confirm donor-recipient compatibility.

All the tests (Antibody screening and cross match) were performed using column agglutination technology by Dia med ID (Switzerland) and as per manufacturer's guidelines.

Results

A total of 262 transfusion dependent Hb E/beta thalassemia patients visited GMCH blood center for transfusion support during the study period. However only 188 of them were on regular PRBC transfusion. Alloantibodies was detected in 12 cases (6.3%). The alloantibody formed were analysed in relation to gender (Table 1), age (Table 2), blood group (Table 3), age at first transfusion (Table 4), number of blood transfusion (Table 5) and splenectomy status (Table 6).There were 100 (53.1%) male patients and 88(46.9%) female patients (Table 1). The rate of alloimmunization in male and female patients were 6 % (6/100) and 6.8 % (6/88) respectively and was not statistically significant as the p value was more than 0.05(p=0.81).

Table 1:Showing rate of alloimmunisation according to gender

Gender	Number of cases	Alloantibody		P -value
		Present	Absent	
Male	100	6 (6 %)	94	0.81
Female	88	6 (6.8%)	82	
Total	188	12	162	

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The age of the patients (Table 2) at the time of study ranged from 1 to 28 years with a mean age of 10.2 years (SD=5.31). According to age, patients were distributed into three groups. Group (1-10) years had 127cases (67.5%), (11–20) years age group had 48 patients (25.5%) and in (21–30) years age group there were 13 cases (7%). The rate of alloimmunisation was 23 % (3 cases) in the age group 21-30 years ,12.5% (6 cases) in the age group (11-20) years and 2.36% (3 cases) in the age group (1-10) years. There was a strong correlation between alloimmunisation with the age of the patient as the p value was less than 0.05 (p=0.0019).

Table 2: Showing rate of alloimmunisation according to patient's age

Age	Number of cases	Alloantibody		P value
		Present	Absent	
(1-10) years	127	3 (2.36%)	124	0.0019
(11-20) years	48	6 (12.5%)	42	
(21-30) years	13	3 (23.07%)	10	
Total	188	12	176	

All cases were of Rh-positive blood group (Table 3). Most common blood group was O positive in 72 cases (38.3%) followed by A positive in 58 cases (30.8%), B positive in 40 cases (21.3%) and AB positive in 18 cases (9.6%). The rate of alloimmunization was 6.9% (five cases) in O positive,7.5% (three cases) in B positive, 5.17% (three cases) in A positive and 5.5% (one case) in AB positive patients. There was no correlation between blood group and alloantibody formation as the p value was more the 0.05 (p=0.2844)

Table 3: Showing rate of alloimmunisation according to blood group

Blood group	Cases	Alloantibody		P value
		Present	Absent	
A positive	58	3 (5.17%)	55	0.2844
O positive	72	5 (6.9%)	67	
B positive	40	3 (7.5%)	37	
AB positive	18	1 (5.5%)	17	
Total	188	12	162	

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The age at the time of first transfusion (Table 4) was ≤ 1 year in 110 cases (58.5 %) and more than one year of age in 78 cases (41.5%). The mean age for the initiation of transfusion was 1.76years (SD = 1.66). Three cases (2.7%) who received first transfusion at ≤ 1 years of age developed alloantibody compared to nine cases (11.5%) who received transfusion after 1 years of age. The rate of alloimmunisation was statistically significant as the p value was less than 0.05 (p=0.014).

Table 4: Showing alloimmunisation rate based on age at first transfusion

	Criteria	Number of cases	Alloantibody		p-value
			Present	Absent	
Age at first transfusion	≤ 1 year	110	3 (2.7%)	107	0.014
	> 1 year	78	9(11.5 %)	69	
	Total	18	12	176	

Among 12 alloimmunized patients, 1 (1.7%) had received up to 50 units of blood transfusions,3(4.1%) received 51-100 units of blood transfusions,2 (2.04%) received (100-150) units of blood transfusions and 6(20%) had received more than 150 units of blood transfusions. This difference was statistically significant as the p value was less than 0.05 (p=0.005).Spleen was removed in 36 cases (19.1%) and intact in 152 cases (80.9%) (Table 5). Out 36 cases whose spleen was removed, three developed alloantibody where as in the remaining 152 non-splenectomised patients, nine developed alloantibody. The comparison between the rate of alloimmunization among splenectomised and non-splenectomised patients (8.3% and 5.9% respectively) was not statistically significant [P = 0·594].Hence, factors like age at first transfusion, number of blood units transfused and mean age in transfusion dependent Hb E/beta-thalassemia patients showed significant correlation with the rate of alloimmunization where as we found no such correlation with blood group, gender and splenectomy status of these patients.

Table 5: Showing rate of alloimmunisation based on splenectomy status

Splenectomy Status	Number of cases	Alloantibody		P-value
		Present	Absent	
Yes	36	3 (8.3%)	33	0.59
No	152	9 (5.9%)	143	
Total	188	12	176	

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DISCUSSION

Regular Blood transfusion is the mainstay of care for individuals with severe Hb E/beta thalassemia. However, once started, the transfusion-related complications like alloimmunization, haemolytic transfusion reactions etc become a major source of morbidity.The rate of alloimmunization among 188 Hb E/beta thalassemia patients receiving regular blood transfusion at GMCH blood Centre was 6.3%(12cases) which was similar to the studies done in Eastern India by Datta SS et al [12](5.39%) and Northern India by Dhawan HK et al[13] (5.64%) in thalassemia patients. However, it was much lower than the Singer ST et al[14] (22%) and Amin M et al[15] (30%) who attributed the higher rates of alloimmunization to heterogenous population in their study group. The lower alloimmunization rate in our study may be attributed to lower antigenic disparity between the recipient and the blood donor and also because majority of the patient's received first transfusion before the 1 year of age (58.5%). Floss A[16] et al. reported that infants even on exposure to many red and white cell antigens, do not produce alloantibodies.Majority (67.5%) of our patients receiving blood transfusion were from the youngest age group but the rate of alloimmunization was more (23.07%) in the oldest age group. It was probably because the older age group was dependent on blood transfusion for a longer duration of time and hence exposure to a greater number of blood units. This was consistent with studies[1,17,18] which showed increase in alloimmunisation with increase in the number of blood transfused. The lower rate of alloimmunization in paediatric age group (2.36%) might be due to immune tolerance to form alloantibody on exposure to foreign red cell antigens[19].Based on number of blood units transfused, the rate of alloimmunisation varied between 1.7% to 20 %. It was highest (20%) in those who had received ≥ 150 blood transfusion and lowest (1.7%) in those who received ≤ 50 blood transfusion. This difference was probably due to repeated exposure of recipient immune system to multiple red cell antigens. Spanos T et al[20] reported earliest sensitization after approximately 10 blood transfusions and Dogra A et al[21] found it after 12 units of PRBC transfusion. Hence, pre transfusion antibody screening should be done before each transfusion for early detection of alloantibodies as it may develop following any random blood unit transfused.In our study, the rate of alloimmunization was higher (11.5%) in those who received blood transfusion after one year of age compared to those received first transfusion before one year of age (2.7%). According to Michail-Merianou V et al [22] and Spanos T et al[20], transfusion at an early age (1-3 years old) may offer some immune tolerance and protection against alloimmunization.Alloimmunisation was higher in female (6.8%) compared to males (5.3%) which was similar to a study done by Datta SS et al[12] (6.32 versus 4.65) in Eastern India but was not statistically significant. Verduin EP et al[23] states that females might have higher rate of alloimmunisation following exposure to foreign antigens during pregnancy and this should not be considered as a risk factor. Besides this, alloimmunisation rate was not related to the blood group of the patients.The prevalence of alloantibodies was more in splenectomised patients (8.3%) compared to non-splenectomised patients (5.9%) but was not statistically significant similar to other Indian studies[21,24].However majority of other studies have shown good correlation between splenectomy and increase in the alloantibody formation[14,17,25].The lower rate in our study may be due to less antigenic disparity in the population under study and blood donors decreasing the overall effect of splenectomy in causing alloimmunisation.

CONCLUSION

In our study, factors affecting alloimmunization were age at first transfusion, number of blood units transfused and the age of the patient. The lower alloimmunization rate in our study could be attributed to homogeneity between the blood donor and the recipient or may be because majority of our recipients started blood transfusion before one year of age. This study re-emphasizes the need for antibody screening before each blood transfusion for early detection of alloantibody and provide phenotype matched blood to prevent alloimmunisation in multi-transfused patients. Early institution of transfusion therapy after diagnosis is another means of decreasing alloimmunization as it may help in developing immune tolerance. All these measures will help in decreasing the incidence of RBC alloimmunization and hence the morbidity in Hb E /Beta Thalassemia patients on regular transfusion support.

Table 5: Showing rate of alloimmunisation based on number of PRBC transfusion

Number of blood units transfused	Number of cases	Alloantibody		P-value
		Present	Absent	
<50	57	1 (1.7%)	56	0.005
51-100	72	3 (4.1%)	69	
101-150	29	2 (6.8%)	27	
>150	30	6(20%)	24	
Total	188	12	176	

**PRBC: Packed Red Blood Cells*

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