Original Article

Red Cell Alloantibodies in Multiple Transfused Thalassaemia Patients

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Abstract

Background: Thalassaemia major patients require lifelong transfusion support due to which they are prone for alloimmunization to foreign RBCs. Alloimmunization can be prevented by extended phenotype match blood transfusion. The study was conducted to know the extent of problem of alloimmunization and to find important red cell antibodies in thalassaemia patients.
Methods: A cross-sectional study was conducted. A total of 32 thalassaemia patients were enrolled. The specimen was subjected to red cell alloantibody and autoantibody by column gel agglutination technique. $R_1^wR_1$, R_2R_2, rr (papaine and non papain) and 11 cell panel reagent cells were used in screening and identification of alloantibodies respectively.
Result: Six (18.8%) subjects were alloimmunized. All alloimmunized subjects were recipient of more than 20 units of transfusion. Total seven clinically significant alloantibodies were identified. Anti E and anti c were commonest antibodies in four (12.5%) patients.
Conclusion: Red cell alloimmunization is an important risk in thalassaemia patient. 71.4% of alloantibodies were anti E and anti c type. Extended phenotype match blood transfusion for Rh-c and Rh-E antigens or level 2 antigen matching stringency needs to be explored in preventing alloimmunization in thalassaemia patients.

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Key Words : Alloimmunization; Irregular antibodies; Red cell alloantibodies; Thalassaemia

Introduction

Thalassaemia is associated with genetically determined reduction in rate of synthesis of one or more types of normal haemoglobulin (Hb) polypeptide chain. The lack of polypeptide chain results in interference in erythoroid maturation, function and ineffective erythropiosis. β thalassaemia major is characterized by major or total suppression of β (Beta) chain synthesis in the homozygous form of the disease. Lifelong red blood cell (RBC) transfusion is the treatment of thalassaemia major patients which alleviates the anaemia and suppress the compensatory mechanism responsible for clinical disease including deaths in these patients. Bone marrow or stem cell transplantation is the other modality of treatment of thalassaemia, which is out of reach for most of them. Thus RBC transfusion is only treatment available to these patients [1].

Alloimmunization i.e. development of alloantibody against the foreign RBC is one of the important complications of blood transfusions in multiple transfused thalassaemia patients [2,3]. Alloimmunization further complicates the transfusion therapy due to difficulty in getting compatible blood, increased incidence of additional alloantibody and autoantibody (antibody against self RBC antigens) development, delayed haemolytic transfusion reaction (DHTR) and life-threatening hyperhaemolysis syndrome [2-5]. Alloimmunization rate of 5-30% has been reported in thalassaemia patients by various workers [5]. Extended phenotype matched, leucodepleted red cell transfusion is recommended in prevention of alloimmunization [4,6]. There are large numbers of β thalassaemia major patients in India; however, data of alloimmunization is very sketchy. In this background the study was undertaken to find out the prevalence of alloimmunization in thalassaemia patients and to know important alloantibodies in alloimmunization.

Material and Methods

A cross sectional study was undertaken. Patients of thalassaemia major on regular transfusion were enrolled. Information on transfusion history of these patients was recorded. Five ml blood was collected from each subject and plasma or serum was separated. $R_1^wR_1$, R_2R_2, rr (papaine and non papain) 'screening cell' were used as reagent cells for screening irregular antibodies, which was undertaken by column gel agglutination (CGA) technique at 37°C and room temperature (23°C) using LISS Coombs and NaCl gel cards

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(Di Med) respectively. They were also subjected to Direct Coombs test by CGA technique. Specimens found positive for irregular antibody were subjected to alloantibody identification using 11 cells panel by CGA. Panel cells have the known antigram consisting of antigens as- Rh-hr (D,C,E,c,e,C^w), Kell (K, k, Kp^a, Kp^b, Js^a, Js^b), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), Lewis (Le^a, Le^b), P_1, MNS (M,N,S,s), Luth (Lu^a, Lu^b), Xg^a, Bg^a+. Results were interpreted based on cross-out method. Prevalence of alloimmunized with 95% Confidence Interval (CI) was calculated. Chi square test or Fisher extract tests were used to test the association between sex and alloimmunized status. Student's unpaired 't' test was used to compare age, age at first transfusion and duration from first transfusion with respect to alloimmunised status.

Results

A total of 32 β thalassemia major subjects were enrolled for the study. They were regular recipients of blood transfusion at interval of 3-5 weeks. They received ABO Rh D match homologous, non leucodepleted whole blood or packed RBC. None of them underwent splenectomy. Six subjects were found positive for RBC alloantibodies; none was positive for autoantibody. Thus the prevalence of alloimmunized was 18.8% with 95% CI 5.3-32.3%. A total of 22 (68.75%) study subjects were male while 10 (31.25%) were female, amongst them 18.2% (4/22) male and 20% (2/10) female were alloimmunized. There was no significant association between sex and alloimmunized status (Fisher exact two tail p value >0.05). The age of study subjects ranged from 1 to 18 years. Mean age of alloimmunized subjects was 12.0 ± 3.8 year as against 8.2 ± 3.4 year in non alloimmunized; the difference in mean was statistically not significant by Student's 't' test (p>0.05, NS). Age distribution of thalassaemia subjects is presented in Table 1. Mean age at first transfusion of study subjects was 2.7 ± 1.3 years; which was 2.6 ± 1.3 and 3 ± 0.9 year in non-alloimmunized and alloimmunized subjects respectively (p> 0.10, NS). Mean duration from first transfusion (transfusion dependence) in alloimmunized and non-alloimmunized thalassaemia patients was 8.5 ± 4.0 and 5.6 ± 2.5 years respectively, the same was statistically not significant by Student's 't' test (p> 0.5, NS). ABO blood group distribution revealed that 19 (59.4%), 6 (18.8%), 4 (12.5%) and 2 (6.3%) subjects were of O, B, A and AB blood group respectively; amongst them 4 (21.1%), 1 (16.7%), 1 (25%) subjects of O, B and A blood group respectively were alloimmunized. Only two subjects were Rh D negative and none were alloimmunized. The transfusion history revealed that 29 patients received more than 20 units of transfusion, one patient received 2 units while two received 15-19 units. All alloimmunized patients received more than 20 units of blood transfusion.

A total of seven alloantibodies were detected in six patients i.e. five subjects with one while one subject with two alloantibodies. Anti E followed by anti c with the frequency of three (42.9%) and two (28.6%) respectively were the most prevalent alloantibodies, however, anti Le^b and anti Jk^b was found in one (14.3%) subject each. One subject had both anti E and anti c antibodies. Profile of alloimmunized thalassaemia patients is given in Table 2.

Table 1

Age distribution of thalassemia patients

Age group (year)	Non-ALLO* (%)	ALLO* (%)	Total studied (%)
0-4	5 (15.6%)	-	5 (15.6%)
5-9	13 (40.6%)	2 (6.3%)	15 (46.9%)
10-14	8 (25%)	3 (9.4%)	11 (34.4%)
15-19	0	1(3.1%)	1 (32.2%)
Total	26 (81.2%)	6 (18.8%)	32 (100%)

* ALLO-alloimmunized

Table 2

Profile of alloimmunized thalassaemia patients

Sex	Age (year)	Blood group	Units tx	Age at first tx years	Duration of tx years	Alloantibody specificity
F	18	O+	>20	4	14	Anti Le^b
F	14	A+	>20	4	10	Anti c
M	8	O+	>20	2	4	Anti c, Anti E
M	8	B+	>20	3	4	Anti JK^b
M	13	O+	>20	2	11	Anti E
M	11	O+	>20	3	8	Anti E

tx:- Transfusion

Discussion

We reported 18.8% alloimmunization prevalence in thalassaemia patients. High alloimmunization in thalassaemia patient was reported from Taiwan (37%) [7], Arab (30%) [8], and Asian descent (22%) [9]. Compared to this lower alloimmunization was reported in thalassemia patients from Iran (5.3%) [10], Pakistan (9.2%) [11], (6.8%) [12] and Malaysia (8.6%) [13]. Very few studies from India reported alloimmunization in multiple transfused patients including thalassaemia patients. A study from North India reported alloimmunization rate of 3.4% in multiple transfused patients; they screened 531 patients [14]. Shukla et al [15] from Lucknow reported RBC alloimmunization rate of 9.8% in chronic renal failure patients undergoing haemodialysis. The differences in alloimmunization were attributed to at least three contributing factors: the RBC antigenic difference between the blood donor and the recipient, the recipient's immune status and the

Table 3

Antigen matching stringency levels [23]

Level	Antigen set	Antigen number
1	AB (O), Plus; {Ag-neg}	3+ Ag Neg*
2	Level 1 Plus: c, C, e, E; K	+5
3	Level 2 Plus: Fy^a, Fy^b; Jk^a, Jk^b; S, s	+6
4	Level 3 Plus: k; M, N; Do^a, Do^b, Hy, Jo^a; Lu^a, Lu^b	+9

* Ag –Neg- of corresponding alloantibody (ies) identified.