Prevalence of RBC Alloimmunization and Autoimmunization among Thalassemia patients in jaipur

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Abstract:

BACKGROUND: Thalassemia major is a common hemoglobinopathies disorders in India. However, limited data are available on the frequency of RBC alloimmunization and autoimmunization in transfusion dependent Jaipur thalassemia patients. The development of anti-red blood cell antibodies (both allo-and autoantibodies) remains a major problem that is causes blood transfusion reaction.

Aim: To determine the frequency of red cell alloantibodies and autoantibodies in thalassemia patients and to identify the common alloantibodies.

Materials and Methods: A total of 48 samples were collected from thalassaemia patients at SDM Hospital. Laboratory investigations were done by (BioVue cassettes based on Column Agglutination Technology) to all the studied patients including: ABO, Rh blood grouping and Du testing have done, Direct Coomb's Test (DCT), Indirect Coomb's Test (ICT), Antibodies Screening by Ortho Surgiscreen (3 cell panel), finally, specimens found positive for irregular antibody were subjected to alloantibody and autoantibody identification using Ortho Resolve Panel A (11 cell panel). **Results:** 7 patients out of total 48 patients (15%) developed alloantibodies and 4 (8%) autoantibodies. No patients have alloantibodies with autoantibodies. The most alloantibody frequent combination was anti-M (37.5%) which is detected in 3 patients then Anti-D & Anti-E = 25% which are detected in 2 patients and Anti-C was lees one 12.5%. The most common blood groups were AB+ & O+ (30%), eight patients of the total number of patients (11) were blood phenotyped and the most common of Rh+K phenotype were (e &c) 34.5% but Kell was negative. **Conclusion:** Several factors might have contributed to the high alloimmunization and autoimmunization rate observed in this study, including the heterogeneity of the population living in Jaipur, lack of better-matched donors for those patients, and the use of post storage leukodepleted blood. Antibodies screening of patients and antigen detection on RBCs of donor specially (Rh and Kell antigen) have to do to avoid development new alloantibodies.

Keywords: Alloimmunization; Autoimmunization; Thalassemia Major; Multiple Transfusions.

Introduction:

Alloimmunization to erythrocyte antigens is the most one of the major complications of multiple blood transfusions, especially in patients who is regular transfused.[1, 2] The reported frequency of antibody formation is highly variable in different parts of the world ranging from 1.13% to 40.4%.[3] In similar studies in different states in India. Thalassemia majore is the most common inherited haemoglobinopathies in the world that results from a reduced rate of one or more of the globin chains. Severe clinical manifestations of Beta-thalassemia

major such as anemia and delayed growth are apparent in the first years of life.[2, 3] Moreover; RBC transfusion regimen is confronted with numerous complications. In almost every patient, the transfusion requirement slowly increases over the years. Various factors which contribute towards this increased requirement include hypersplenism, alloimmunization, chronic infections, foliate deficiency, and iron overload , aplastic crises and hemolytic crises.[4,5] In the absence of stem cell transplantation, partially in poor country the disease is treated by life-long red blood cell (RBC) transfusion to keep the hemoglobin (Hb) level between 9 and 12 g/dL.[6] Therefore, routine pre-transfusion testing is one of the important safety measures to detect the unexpected red cell antibody in the patient's serum to prevent the immediate and delayed haemolytic transfusion reactions.[7] In addition, donor feasibility and the high cost of RBC matching, antigen profile (phenotype), antibodies screening and antibodies identification affects the approach of private and public medical centers. Therefore, there is limited data on the RBC phenotypes and the extent of alloimmunization and autoimmunization among poor countries. We studied the prevalence of RBC alloimmunization and autoimmunization among thalassemia patients who received regular transfusions at SDM Hospital and analyzed the factors, which may be responsible for development of these antibodies so that serious hazards because of immunization may be avoided by screening these patients for alloantibodies and autoantibodies.

Materials and Methods:

A total of 48 samples were collected from thalassaemia patients at SDM Hospital. Two blood samples with volume of 4 ml were collected from each patient for standard tube EDTA and plan. Laboratory investigations were done to all the studied patients including: ABO, Rh blood grouping and Du testing have done by (BioVue cassettes based on Column Agglutination Technology), Direct Coomb's Test (DCT) Poly specific (BioVue AH G Polyspecific cassettes) then (DCT) mono specific, Acid Elution, In cases of a positive DAT, further investigation using specific reagents to detect IgG, IgM, or a complement were carried out. When an antibody was detected, eluates were prepared and tested against common sample erythrocytes. In some cases polyethylene glycol was used to enhance the reactivity.

Indirect Coomb's Test (ICT) was done on patient blood sample to detect the irregular antibodies (BioVue cassettes), Antibodies Screening by Ortho Surgiscreen (3 cell panel in Ortho BioVue AHG polyspecific cassettes), finally, specimens found positive for irregular antibody were subjected to alloantibody and autoantibody identification using II cells panel by Ortho Resolve Panel A (11 cell panel in BioVue AHG polyspecific cassettes) based on Column Agglutination Technology. The study was carried out from June 2016 to July 2017.

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), Dnffy (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'+. Results were interpreted based on cross-out method. Adsorption methods were used in patients presenting with a new autoantibody.

Results:

Table 1 show out of 48 patients of thalassaemia 29 (60.5%) were males and 19 (39.5%) were females. The age range was from 1 year to 28 years with the mean age of 8.4 year. Male: female ratio of 1.3:1. Mean age o f patients developing autoantibodies was 5 years, which is significantly higher than the patients without the presence of autoantibodies. Mean age o f patients developing allantibodies was 7 years. Seven patients (15%) of the total number of patients (49) were found to have alloantibodies to RBCs and 4 patients (8%) of the total number of patients (49) were found to have autoantibodies (figure: 1), of these patients, 6 Six patients (85%) developed single antibody while one (15%) patients developed dual alloantibody in their sera (Table 1). Alloantibodies development in patients at ≤ 5 years (n= 4 / 57%) more than ≤10 years (n=2 / 28%) because the patients at ≤ 5 years need to more units of blood.

Out of 8 alloantibodies, the most alloantibody frequent combination was anti-M (37.5%) which is detected in 3 patients while 62.5% belonged to Rh blood group system (Anti-E = 25%, Anti D = 25%, Anti-C= 12.5%) demonstrated in (Table 2 and Figure 2).

The most common blood group among our patients was AB positive and O positive (30%), eight patients of the total number of patients (11) were blood phenotyped and the most common Rh+K phenotype were (e &c) 34.5% but Kell was negative.

Figure 1: Distribution of Alloantibodies and Autoantibodies:

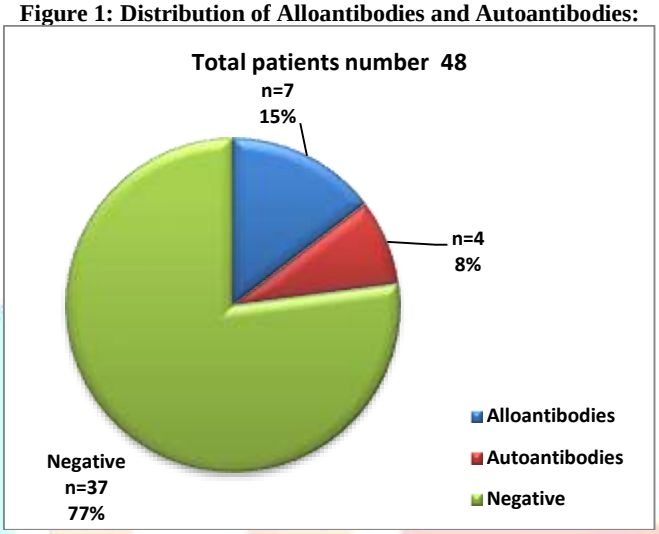


Table 1: Distribution of Age, Alloantibodies, Autoantibosies and Sex:

Age	No. of Patients	Allo-Abs	Mean of age	M	F	Auto-Abs	M	F
1-5	6	4	57 %	1	3	2	1	1
6-10	4	2	28.5 %	0	2	2	2	0
28	1	1	14.5 %	0	1	0	0	0
Total	11	7	100 %	1	6	4	3	1

Allo-Abs- Alloantibodies/ Auto-Abs- Autoantibodies

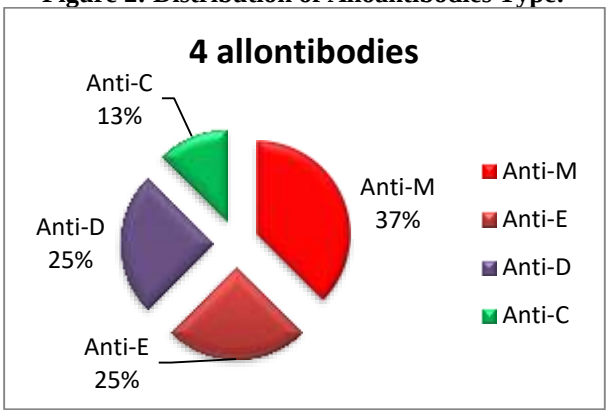
Table 2: Frequency of different alloantibodies:

Type of antibody detected	Frequency of antibody detected	Percent
Anti-E	2	25
Anti-M	3	37.5
Anti-D	2	25
Anti-C	1	12.5
Total	8	100

Table 3: Distribution of blood group and Rh+K phenotype:

Blood group	No.	Percent	Rh+K Phenotype	No.	Percent
AB+	3	30	C	6	26
O+	3	30	c	8	34.5
O-	1	10	E	1	5
B+	1	10	e	8	34.5
A+	1	10	-	-	-
A-	1	10	-	-	-
Total	10	100	Total	23	100

Figure 2: Distribution of Alloantibodies Type:



Discussion:

Only a few studies, mostly in non-Indian transfused thalassemia patients, have investigated the frequency and causes of alloimmunization and autoimmunization. In the present study we examined these elements and defined the common RBC phenotypes among Rajasthani, which have not been previously described. Alloimmunization to red cell antigens is an immune response usually stimulated by the transfusion of blood products or through pregnancy and is the most one of the complications of RBC transfusions.

The previous studies have reported quite variable rate of alloimmunization ranging from 3.1% to 37% in patients of different ethnic origin.[6] A High rate of alloimmunization in our study may be expected when there is no homogeneity of RBC antigens between the blood donors and recipients.

In our study, rate of alloimmunization was high in patients at ≤5 years of age may be because high requires to transfusion of blood components in this age. And rate of alloimmunization in patients at 6-10 years old more than ≥11 years old.

Development of alloantibodies, we found association of gender female (n=6 /85%) of the total number of patients (7) while male patient only one (n=11 / 15%). In comparison to literature, the previous study have reported a significant association between alloimmunization and gender, as they found alloimmunization to be associated more with female patients while on the other hand there study found more association in male patients. However, some studies showed that gender was not a significant factor in the development of alloimmunization. [6] In the meantime we found association of gender to development of autoantibodies with male more than female.

In the present study, the most frequently detected alloantibodies belonged to MNs blood group system (37.5.17%) and Rh blood group system (Anti-D=25% ,Anti-E=25% , Anti-C=12.5%).

Conclusion:

In all blood banks, we have to do routine tests of pre-transfusion (Antibody screening of patient) for already alloimmunized patients to check for the disappearance of old antibodies or development of new alloantibody and avoid hemolytic transfusion reaction. Extended phenotype matched, leucodepleted red cell transfusion is recommended in prevention of alloimmunization. Antigens detection on RBCs of donor specially (Rh and Kell antigen) have to do to avoid development alloantibodies.

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