Original Article

Analysis of red cell alloimmunization in multi transfused patients at a Tertiary care teaching hospital

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Abstract

Background: Red blood cell (RBC) alloimmunization is an immune response against foreign RBC antigens; this generally occurs after sensitization due to multiple blood transfusions and pregnancies. Antibody detection plays a critical role in transfusion medicine as it can detect irregular or unexpected antibodies. This study was done to know the frequency and specificity of unexpected red cell antibodies in the multitransfused patients. **Materials and Methods:** This prospective study was done in the Department of Immuno-Haematology and Blood Transfusion. Antibody screening of 100 multitransfused patients with initial negative antibody screen was carried out prior to compatibility testing and followed for a period of 12 months for each transfusion. Depending on the results, patients were given corresponding antigen-negative blood units. **Results:** In this study, the rate of alloimmunization was 7%. Total number of samples that were positive for irregular alloantibodies were 4 of 54 cases of thalassemia, that is, 7.4%, whereas 3 of 40, that is, 7.5%, cases of solid malignancies developed alloantibodies. None of the patients of chronic kidney disease formed any alloantibody. Anti-K antibody was the most frequent antibody detected in 3 of 7, that is, 42.8% patients. Anti-E was the second most frequent antibody observed in 2 of 7, that is, 28.57%. However, anti-c and anti-M were detected in one each of 7, that is, in 14.28% each. **Conclusion:** It is concluded here that red cell alloimmunization should not be overlooked in multitransfused patients. To avoid the effects of alloimmunization, routine RBC antibody screening at set time intervals after transfusion should be performed.

Keywords: Alloimmunization, antibody screening and identification, multitransfused

Introduction

Blood transfusion is a lifesaving therapy for complications of anemia and for treatment of symptoms and signs of hypoxia. It exposes the patient to numerous foreign antigens that are potential immunogens which can lead to development of antibodies in the recipient within days, weeks or months after transfusion.[1] The process by which the immune system generates antibodies against foreign antigens and yet maintains tolerance to self-antigens is complicated and elegant, with multiple cellular players and intricate regulation.[2]

Red blood cell (RBC) alloimmunization is an immune response against foreign RBC antigens; this generally occurs after sensitization due to blood transfusions and pregnancies.[3] The risk of developing RBC alloantibodies depends on the age, sex,

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and genetic makeup of the patient as well as the number and frequency of transfusions that he or she has undergone.[4,5] In general, women who may have been sensitized by pregnancy are more likely to have alloantibodies than men.[6] Patients who receive multiple transfusions due to various causes such as hemoglobinopathies, hematologic diseases, various types of cancers, organ transplantation, and renal failure have the prevalence of alloimmunization up to 60%.[7]

Alloimmunization may lead to difficulty in cross-matching blood, decrease in RBC survival and an increased transfusion requirement.[8] In addition, downstream effects resulting from RBC clearance can lead to multiple organ failure, electrolyte perturbations, coagulopathy, and in some cases, death.[9]

Antibody detection plays a critical role in transfusion medicine. It is a key process in pretransfusion compatibility testing and is one of the principle tools for investigating hemolytic transfusion reactions and immune hemolytic anemias. The focus of antibody detection method is on irregular or unexpected antibodies as opposed to expected antibodies of the ABO system.[10] This study was done to initiate pretransfusion antibody screening on patient's sample before cross-match for safe transfusion practice and to find out frequency and specificity of various RBC alloantibodies in repeatedly transfused patients.

Materials and Methods

Ethical approval

Study was started after approval from Institutional's ethical and research review board. Ethical approval was taken and approval date -20/2/2016.

Study design: Prospective study

This prospective study was done from May 2016 to September 2017 in the Department of Immuno-Haematology and Blood Transfusion in South West Region of Punjab after approval from ethical committee. A total of 100 patients with history of multiple transfusions and initial negative antibody screening whom we could follow were selected. In the first 4–5 months period patients were enrolled in the study and followed up for 12 months. Every time a blood transfusion requisition for these patients was received; antibody screen was carried out by using column agglutination technology prior to compatibility testing and every patient was followed up for a period of 12 months to find out formation of any new antibody.

Antibody screening was performed using antihuman globulin gel cards (ID-Card LISS/Coombs) and three-cell panel (ID-DiaCell I, II, III-Asia). Those with positive antibody screening were analyzed further for antibody identification test using eleven-cell panel (Set ID-Dia Panel). Bio-Rad ID microtyping system was used for performing these tests. The results of antibody screening and antibody identification were analyzed using the Diamed 3 cell screen and 11 cell identification panel key available with the reagent cells. The antibody was confirmed by use of corresponding antisera. If the patient was negative for that particular antigen, antibody was confirmed.

Crossmatched compatible antigen negative PRBCs were issued to patient with positive antibody for each transfusion. All other patients with no antibody formation were given crossmatched compatible PRBCs till AHG Phase.

Patient selection

Patients included in the study were as follows:

- Thalassemia patients, patients with history of solid malignancies such as breast cancer, cervical cancer, ovarian cancer, colon cancer, and patients of chronic kidney disease (CKD) on hemodialysis with initial negative antibody screening.

Patient exclusion criteria included the following:

- Patients in whom second transfusion was scheduled within 72 h of previous transfusion.
- Patients who have known antibodies against RBC antigens.
- Patients with any autoimmune disease such as systemic lupus erythematoses, lupus nephritis, and idiopathic thrombocytopenia.

A detailed clinical and transfusion history was taken using a proforma including name, identification number, age, sex, diagnosis, and blood group of the patient. The transfusion history included indication for transfusion, number of blood units transfused, record of any out-of-group transfusion, and transfusion of any other blood components. Age at splenectomy if applicable and drug history was also included.[11]

Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software program, version 17.0. Student's *t* test, one-way analysis of variance (ANOVA), and Chi-square test were used. Mean (±standard deviation, [SD]) was used for normally distributed continuous data. Charts and tables were prepared in Microsoft Excel sheet.

Results

This study included 100 multitransfused patients, of which 64 were females and 36 were males with initial negative antibody screen. Diagnostic group included 54% thalassemia, 40% solid malignancies, and 6% were of CKDs on regular hemodialysis. Patients included in the study were in the age group of 1–73 years with mean age of 28.01 years. In these patients, number of transfusions ranged from 3 to 570 with mean 57.72 transfusions.

As shown in the Figure 1, four patients of thalassemia (7.40%) and three patients of solid malignancies (7.5%) developed alloantibodies. However, no alloantibody was detected in patients with CKD.

Table 1: Details of patients alloimmunized

Age in years	Sex	Blood group	Diagnosis	Total no. of transfusions till date	Antibody formed after (*n*) no. of transfusions	Antibody specificity
11	F	B + ve	Thalassemia	132	120	Anti-K
2	F	B + ve	Thalassemia	14	10	Anti-K
12	F	A + ve	Thalassemia	202	180	Anti-K
3	F	O + ve	Thalassemia	59	50	Anti-E
50	F	AB + ve	CA cervix	3	2	Anti-c
42	F	B + ve	CA ovary	14	9	Anti-E
65	F	B + ve	CA cervix	5	3	Anti-M

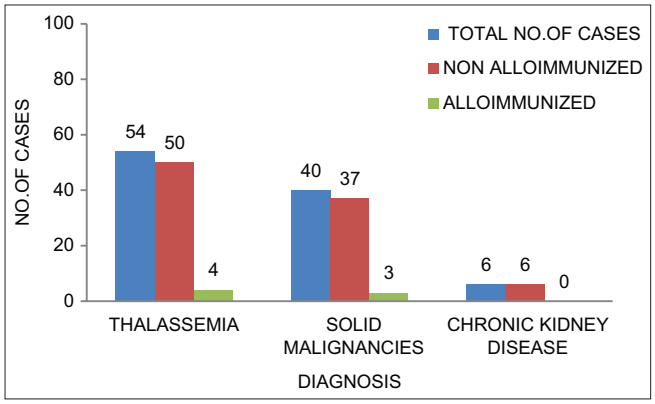

Figure 1: Frequency of antibody formation as per diagnosis

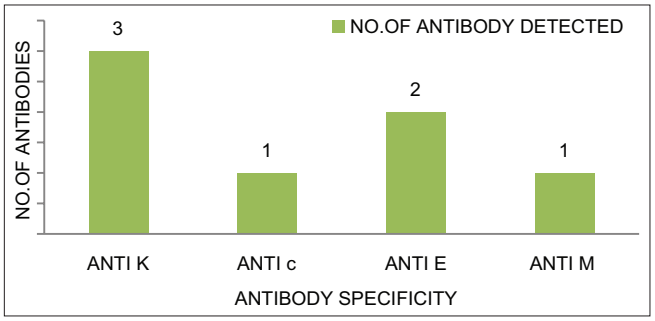

Figure 2: Specificity of antibodies detected in patients

As shown in Table 1 and Figure 2, anti-Kell antibody was the most frequent antibody detected, that is, 42.80%. Second most frequent antibody observed was anti-E, that is, 28.57%. Anti-c and anti-M were present in one of 7, that is, 14.28%.

Discussion

RBC alloimmunization is an immune response against foreign RBC antigens that generally occurs after sensitization due to blood transfusions and pregnancies. It is one of the major concerns in the management of patients who requires repeated blood transfusions as a lifesaving treatment. This prospective study was conducted after approval from thesis and ethical committee to analyze the red cell alloimmunization in multitransfused patients.

Gender and alloimmunization

In this study, there were 100 patients of multiple transfusions, of which 36 were males and 64 females. In our study 7 of 100 were alloimmunized, all of these were females. *P* value between gender and alloimmunization was statistically significant (*P* 0.040). Females have been observed to be more prone to develop alloimmunization than males probably due to the fact that females, especially in developing countries are anemic and pregnancy is an important risk factor for alloimmunization.[11]

Pimpaldara *et al.*[4] and Philip *et al.*[12] also observed significant association between gender and alloantibody formation. This finding is in concordance with our study.

However, in the studies by Sood *et al.*[11] and Bhuva *et al.*[13] no statistically significant association between gender and alloimmunization was observed.

Rate of alloimmunization

A number of multiple transfused patients in India had revealed that rate of alloimmunization is ranging from 4% to 10% as given in Table 2. In our study rate of allomunization was 7%. Rate of alloimmunization was in concordance with the study done by Pimpaldara *et al.*[12] in which rate of alloimmunization was 7%.

However, studies such as Pahuja *et al.*[14] reported a very low rate of alloimmunization which was 3.79%. This low rate of alloimmunization can be explained by homogeneity between the donor and the recipient population.

Rate of alloimmunization in thalassemia was 7.4%, that is, 4 of 54 patients developed alloantibodies. Similar results were observed by Pradhan *et al.*,[15] that is, 8% and Gupta *et al.*,[16] that is, 9.48% in studies on thalassemia patients.

In our study, rate of alloimmunization was 7.5%, that is, in 3 of 40 patients of solid malignancies. This is in concordance with study done by Mohison *et al.*,[17] who found 6% RBCs alloantibodies in non-hematological malignancies.

No alloantibody was found in CKD patients as the study population was very less.

RBCs alloimmunization is complex and involves at least 3 main contributing elements that include RBC antigenic difference between the blood donor and the recipient, recipient's immune

Table 2: Rate of alloimmunization in various studies across India

Authors	Place	Year	Rate of alloimmunization	Most specific antibody
Pradhan *et al.*[16]	Mumbai	2001	8%	Anti-D
Gupta *et al.*[15]	Delhi	2011	9.48%	Anti-E
Dhawan *et al.*[18]	Chandigarh	2010	5.64%	Anti Kell
Sood *et al.*[11]	Delhi	2013	4.24%	Anti E
Roopam *et al.*[19]	Gujarat	2010	5.21%	Anti-Kell
Philip *et al.*[4]	Western India	2013	5.5%	Anti-E
Datta[20]	Eastern India	2014	5.6%	Anti-c
This study	Punjab	2017	7%	Anti-Kell

status and immuno-modulatory effect of the allogeneic blood transfusions on the recipient's immune system.[11]

Antibody specificity (type of antibody)

The specificity of most alloantibodies detected in this study was against Rh and Kell antigen systems due to their high immunogenicity which is similar to previous reports of alloimmunization.[15,20]

In our study, Rhesus and Kell blood group consisted of majority of alloantibodies with 42.85% each followed by anti-M with 14.2%. Among Rh blood group, anti-E was 66.66%, the most common followed by anti-c with 33.33%.

However in thalassemia, anti-K comprised major antibodies three of four, that is, 75% followed by anti-E which showed similar results with Roopam *et al.*[20] with 80% incidence of anti-K. Higher rate of anti-K alloantibodies could be explained on basis of higher number of Kell positive antigen in donor population of our belt.

In patients with solid malignancies, antibodies against Rh blood group were in two out three, that is, 66.66% followed by anti-M in 1 of 3, that is, 33.33% which was comparable with Mohsin *et al.*[17] who found 55.55% patients with non-hematological malignancies developed alloantibodies against Rh system. Genetic factors and ethnicities effect the development of alloantibodies which might have influenced the higher incidence of developing anti-Rh antibodies observed in our study.

Hence, transfusion of blood matched for Rh and K antigens could prevent alloimmunization resulting in a significant difference in the alloimmunization but the potential to form RBC alloantibodies to unmatched antigens will exist.

Number of packed cells transfused

In this study, number of packed cell units transfused ranged from 3 to 570 units with mean number of 57.72 units. In thalassemia patients mean number of transfusions were 99.45. However, in solid malignancies mean number of units transfused were 8.85. In alloimmunized patients, mean no. of units transfused were 60.29 units ranging from 3 to 202 units. There was no statistically significant association ($P = 0.935$) between number of packed cell units transfused and alloimmunization.

Bhatti *et al.*[11] and Sood *et al.*[21] showed no correlation between the number of transfusions and the alloimmunization rate. Study by Pimpaldara *et al.*[12] has found a strong correlation between the number of blood units transfused and alloantibody formation.

In our study, we found an interesting fact that in thalassemia patients, mean no. of transfusions were 99.45 and rate of alloimmunization was 7.4%, that is, 4 of 54. However, in solid malignancies mean number of units transfused were only 8.85 and rate of alloimmunization was 7.5%, that is, 3 out 40. A significant difference was found between mean no. of transfusions and alloimmunization rate in both the categories. This could be due to individual immune competence which determines the potential to respond to RBCs antigen challenge. So, association of number of packed cell units transfused as a risk factor for alloimmunization was not established in our study.

In normal practice, blood banks usually give only ABO and Rh (D) antigens crossmatch compatible units, so the risk to form alloantibodies against minor blood group antigens becomes high. Obtaining RBC antigenic phenotype on all multi-transfused patients, providing leukodepleted blood-matched for antigens of ABO, Rhesus and Kell systems in patients who have a lifelong transfusion dependency could be effective against RBC alloimmunization at primary level.

In multitransfused patients who receive blood units at districts levels should carry blood phenotype reports and antibody screening results which enables the safe and timely supply of blood units to such patients.[22,23]

Conclusion

It is concluded here that red cell alloimmunization should not be overlooked in multitransfused patients. To avoid the effects of alloimmunization, routine RBC antibody screening at set time intervals after transfusion should be performed. Regular screening for development of alloantibodies in multiple transfused patients would add toward better management of these patients. After antibody screen and identification, corresponding antigen negative blood should be given to the patient. This will minimize the antibody-mediated destruction of transfused red cells.

This study recommends regular antibody screening for already alloimmunized patients to check for the disappearance of old antibodies or development of new alloantibody. These measures will help in decreasing the incidence of RBC alloimmunization and delayed hemolytic transfusion reactions in these multi-transfused patients.

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Conflicts of interest

There are no conflicts of interest.

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