

Risk Factors for Red Cell Alloimmunisation in Multi-Transfused Patients in a Referral Hospital, North-Eastern India

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Abstract: Introduction: The formation of red cell (RBC) alloantibody in a transfused patient can complicate transfusion therapy and limit the availability of compatible blood for future transfusions. It has a significant negative impact on laboratory and institutional resources. Therefore, identification of patients associated with increased risk of RBC-alloimmunisation is important so as to prioritize the provision of prophylactic extended antigen matched red cell units for those patients. Methods: Our study was conducted for a period of one and half year to determine the prevalence of RBC alloimmunisation and associated risk factors for its development in multi-transfused patients who received ≥ 2 units of packed red cells and/or whole blood. Red cell alloimmunisation was analysed based on the factors i.e. number of units transfused, age of 1st transfusion, disease of transfusion, gender of transfusion and alloantigen specificities. Laboratory tests performed in all study cases were ABO blood group, Rh(D) type, and antibody screen (AS). Direct antiglobulin test (DAT) and antibody identification were carried out when antibody screen was positive. Results: Twelve cases (7.45%) of the study population (n=161) were RBC-immunised, of which RBC alloimmunisation were identified in 9 patients (5.59%). Our study could not identify the specificity of antibody in 3 patients of DAT positive cases and labelled them as 'unidentified' (1.86%). RBC alloimmunisation were more (10.81%, 4/37) among the patients who had transfused ≥ 16 units. There was an association of RBC alloimmunisation and transfusion count. A higher risk of alloimmunisation was also observed (10.53%, 4/38) when 1st transfusions were between 16-30 years of age when compared with the younger and older age groups. The prevalence for RBC alloimmunisation among different diseases/conditions were 14.29% in thalassaemia (n=21), 9.09% in aplastic anaemia (n=11), 7.14% in chronic kidney disease (n=28), 4.35% in trauma (n=23), none were alloimmunised in malignancy and 2.56% in other conditions/diseases (acute bleeding/blood loss from different causes/anaemia of chronic diseases etc.). Females were more alloimmunised (6.59%) than males (4.29%). The distribution of alloantigen specificities were anti-E=4, anti-c=2, anti-D=1, anti-K=1, one each for anti-M and anti-JK^a and unidentified=3. Among the ten (10) identified alloantibodies, maximum (70%) were directed against Rhesus group of antigens. Conclusion: Based on our observations measures to reduce red cell alloimmunisation may include early initiation of transfusion (≤ 2 years), avoidance of 1st transfusion therapy in young adults (16-30 years) and restricted transfusion regime in female gender and disease(s) with increased alloimmunisation risk or transfusion of RBC units that are antigen-matched for the commonly occurring antibodies to RBC antigens such as Rhesus a Kell antigens.

Keywords: RBC alloimmunisation, Antibody screen, Antibody identification

1. Introduction

Patients receiving RBC transfusions are at the risk of forming RBC-alloantibodies to the antigens not present in the recipients. However, there are many factors that can influence an individual's immune system and its response. The probability of the occurrences of alloimmunisation is related to several factors such as age, number of transfusion, phenotypic differences between the donor and receptor, genetic factors related to the antigenic response and recipient's immune status and immune-modulatory effect of blood transfusion. [1],[2] All patients who form any alloantibodies have a significantly higher risk of subsequent alloimmunisation than the general transfused population. There exists a subpopulation of patients ("responders") who respond to RBC antigens to form antibodies at a much higher rate than the general transfused population. [3] The development of anti-RBC antibodies can significantly complicate transfusion therapy and limit the availability of compatible blood for future transfusion. If blood bank could predict who is most likely to make antibodies to RBC antigens, then they could prioritize extended matched RBC units for those patients to avoid RBC alloimmunisation. Therefore, a valid estimate of alloimmunisation and determination of factors associated with increased risk of

alloimmunisation is clinically important to ensure safe transfusion practice. The objective of the study was to determine the rate of red cell alloimmunisation and to identify the risk factors for alloimmunisation in multi-transfused patients.

2. Material and Methods

The prospective cross sectional study was conducted in the Blood Bank of Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal for a period of one and a half year from February 2018 to July 2019. Patients requested for blood transfusion in the age group of 2 to 75 years of both sexes with a history of at least two (2) or more units of nonleukoreduced red cell/whole blood transfusion were taken as 'cases'. Patients of autologous transfusions, neonatal/intrauterine transfusions, those suspected with autoimmune diseases and patients with medical record of erythrocyte autoantibody were excluded from the study. Detailed history of previous transfusions was determined by retrospective recall of the patient or from the parents (in case of minors) and hospital records of transfusion. The analysis for the prevalence of RBC-alloimmunisations were based on the parameters such as overall prevalence in multi-transfused patients, number of blood units transfused, age of

first transfusion, condition/disease of transfusion, gender distribution and alloantigen specificities.

Two millilitres of blood sample in EDTA vial for ABO grouping and Rh(D) typing and another five millilitres of nonhemolysed blood sample in plain vial for antibody screening were collected from each patient at the time of blood requisition for transfusion and serum was separated by centrifugation. The laboratory investigations were carried out following the departmental SOPs and strictly following the reagent manufacturer’s instructions. To ensure efficient and accurate working of the equipments at all times annual maintenance contract (AMC) and calibration at regular intervals were done. The facility conducted various internal quality control (QC) measures and participate external quality programs including External Quality Assurance Scheme (EQAS) on samples from CMC Vellore, to ensure quality of laboratory practices.

Laboratory investigations carried out in all the study cases were ABO and Rh (D) grouping by tube method and red cell antibody screen (AS) by tube method using screening panel I-III (DiaCell I+II+III, DiaMed GmbH) at different phases (room temperature and 37 °C) and adding enhancement solutions (enzyme at 37°C, albumin at 37 °C). If antibody screen was positive direct antiglobulin test (DAT) and antibody identification were carried out by using blood samples from EDTA vial and plain vial respectively. Antibody identification was done by tube method using antibody identification reagent cell panels i.e. DiaPanel 1-11 (DiaMed, GmbH) and carried out using the condition under which the antibody in question was originally detected with the highest grade of agglutination/haemolysis.

3. Results

A total of 161 multi-transfused patients in the study transfused 1874 units of nonleuko reduced red cell/whole blood with a mean number of units transfused per patient were 11.64. Twelve cases (7.45%) of the study group were found to be RBC- immunised and remaining 149 cases (92.55%) were non-immunised. Among the 12 immunised patients, 9 patients (5.59%) were identified for red cell alloantibody while in 3 cases (1.86%) that were positive for both AS and DAT, the identity of the antibody could not be determined in our study and were recorded as ‘unidentified’ (1.86%). RBC alloimmunisation was highest (10.81%) among the patients who had transfused ≥16 units and a trend of association for increased RBC alloimmunisation with transfusion count was seen. (Table 1) Patients whose 1st transfusion started in 16-30 years had the highest rate (10.53%) of RBC alloimmunisation (Table 2). A trend of increased alloimmunisation is seen in this age group when compared with the other age groups, based on the age of 1st transfusion. Considering the conditions of transfusion or diseases, RBC alloimmunisation rates were 14.29% (3/21) for thalassaemia, 9.09% (1/11) aplastic anaemia, 7.14% (2/28) in CKD, 4.35% (1/23) in trauma patients, 2.56% (1/39) in other conditions (acute bleeding/blood loss from different causes/anaemia of chronic disease etc) and none were alloimmunised in SCA and malignancy cases (Table 3). Gender distributions for RBC alloimmunisation were 6.59% (6/91) in females and 4.29% (3/70) in males. The distribution of alloantigen specificities among the 12 immunised cases were anti-E=4, anti-c=2, anti-D=1, anti-K =1 and one each for anti-M and anti-JK^a and unidentified=3. Of the 9 identified alloimmunised cases, 8 (88.89%) patients developed single alloantibody and one patient (11.11%) developed multiple alloantibodies (Anti-E & Anti-c).

Table 1: Number of blood transfusion (RBC/ whole blood units) and RBC alloimmunisation

No. of units transfused	No. of patients	Immunisation of the multi transfused patients			
		Non- immunised	Immunised (%)	Unidentified antibody	Allo immunized (%)
2-5 units	61	59	2 (3.8)	1	1 (1.64)
6-10 units	29	28	1 (3.43)	0	1 (3.45)
11-15 units	34	30	4 (11.76)	1	3 (8.82)
≥16 units	37	32	5 (13.51)	1	4 (10.81)
Total	161	149	12 (7.45)	3	9 (5.59)

Table 2: Alloimmunisation status of cases based on age of 1st Transfusuion

Age of 1 st Transfusuion	No. of patients	Immunisation of the multi transfused patients			
		Non- immunised	Immunised (%)	Unidentified antibody	Allo immunized (%)
<2 years	11	11	0	0	0 (0.00)
2-15 years	27	25	2 (7.41)	1	1 (3.70)
16-30 years	38	34	4 (10.53)	0	4 (10.53)
31- 45 years	45	41	4 (8.89)	1	3 (6.67)
> 45 years	40	38	2 (5%)	1	1 (2.50)

Table 3: Diseases/conditions of Transfusuion and RBC Alloimmunisation

Conditions/ Diseases/	Thalassaemia	SCA	Aplastic Anaemia	Hematological Malignancy	Non-Hematological Malignancy	CKD	Trauma	Other Condition
No. of patients	21	3	11	22	14	28	23	39
Immunised (%)	4 (19.05%)	0	1 (9.09)	1	0	2 (7.14)	1 (4.35)	2 (5.13)
Unidentified	1	0	0	1	0	0	0	1
Allo immunized (%)	3 (14.29)	0	1(9.09)	0	0	2 (7.14)	1 (4.35)	1 (2.56%)

SCA= Sickle Cell Anaemia; CKD= Chronic Kidney Disease

4. Discussion

The red cell alloimmunisation rate in the study was 5.59% (9/161) with an overall red cell immunization rate of 7.45% (12/161). In a study by Walker PS, the prevalence of RBC-alloimmunisation ranged 0.3% to 38% depending on the group of patients studied and sensitivity of the test method used. [4] Another study estimated the risk of red cell alloimmunisation at 1 to 1.4% per unit transfused and in multitransfused patients, the incidence of such antibodies ranged from 5 to 30%. [3] The lower rate of alloimmunisation (5.59%) in our study could be due to homogeneity of the populations between the donors and the recipients, resulting in less phenotypic differences of RBC antigens. Most of our patients (cases) were ethnic people of Manipur (93%) and more than 90% of blood donors were ethnic Manipuris. Some studies have shown that transfused patients receiving blood from donors with shared ethnic background may be protective.[20], [21] A low rate of alloimmunisation (5-10%) would be expected if there is less heterogeneity of red cell antigens between the blood donors and recipients.[18]

The limitation of the study was the inability to identify the RBC antibody of the DAT positive cases, as elution and adsorption techniques were not used. So the possibility of an underlying masked alloantibody in the cases of positive DAT (n=3) could not be excluded. It was suggested that alloantibody binding to the RBCs could lead to the conformational changes of the antigenic epitopes that ultimately stimulate production of autoantibodies and a small number (<5%) of RBC alloantibodies were autoreactive as well. [3], [5] Low prevalence (1.86%) of autoreactive RBC antibodies in this study could probably be explained by the exclusion of cases with medical history of positive DAT and autoimmune disease.

The maximum number (4/9) of alloimmunisation based on transfusion (unit) count was among the patients who transfused ≥ 16 units with a prevalence rate of 10.81%. A trend of increased RBC alloimmunisation is seen with increased in transfusion count. (Table 1) Rosse WF et al mentioned that the primary determinant of the risk of alloimmunisation is the number of transfusion (units) that were administered. [6] However, another study opined that differences in alloimmunisation risk are unlikely to be strongly determined by transfusion count. [3], [19]

This study shows a higher alloimmunisation risk when ages of 1st transfusion were between 16-30 years than the younger or older age groups. It has been observed that patient's age at the start of transfusion management may influence the development of RBC alloantibodies. An earlier start of transfusion may impart immunotolerance in some patients whereas immune function decreases as age increases. Infants who are less than 6 months old usually do not produce alloantibodies, but newborn may have passive antibody of maternal origin. It is also shown that long lasting immunological tolerance can be induced either by introducing into an embryo a graft that survives throughout life or by giving repeated injection of cells. [1], [7], [8]

Considering the conditions of transfusion or diseases, RBC alloimmunisation was highest in patients with thalassaemia (14.29%) followed by aplastic anaemia (9.09%). In CKD patients the rate was 7.14%. Other studies reported RBC alloimmunisation rates of 18.6% in patients with sickle cell anaemia, 11.3% in thalassaemia, and 7.69% among the CKD patients. [6], [5] RBC alloimmunisation rates in transfused patients with malignancy were significantly lower in other studies suggesting that the immunosuppressive nature of these disorders and/or their associated therapies may play a role in limiting blood group antibody development.[10,13] Mariette A et al in their study on 18,750 transfused patients with haematological and oncologic malignancies the RBC alloimmunisation rates were under 2%. [13] The alloimmunisation rate in trauma (4.35%) and other bleeding conditions (2.56%) were lower than the overall average and these were not the risk factors for increased RBC-alloimmunisation in our study.

In our study, red cell alloimmunisation was more frequent in female (6.59%, 6/91) than in male patients (4.29%, 3/70), as suggested in other studies. [6],[10] Studies in India, for red cell alloimmunisation on 5347 antenatal women, the prevalence rate was 1.48% which was higher than the prevalence rate of irregular antibodies (0.27%) in female blood donor population (n=86,507).[15], [16] Another study in western India on 154,387 general population (87,707 males and 86,507 females) the rate alloimmunisation in male patient was 0.19% and in female patient was 0.39%.[17] Mixing of blood between the mother and foetus can occur due to miscarriage, ectopic pregnancy, ante-partum bleeding, abdominal trauma and procedures like amniocentesis/cordocentesis. These could be the risk factors for increased sensitisation and development of RBC alloantibodies in female patients of our study, as 41 female patients had history of pregnancy.

Among the 9 alloimmunised patients, 8 (88.89%) developed single alloantibody and one patient developed multiple alloantibodies (Anti-E & Anti-c). The specificities of the ten (10) identified alloantibodies were highest (7/10) in Rhesus group (70%) of antigens and the incidence of the alloantibodies were anti-E (40%), anti-c (20%), anti-D (10%), anti-K (10%) anti-M (10%), and anti-JK^a (10%) respectively. However, the anti-D development in one multi-transfused female patient in this study had history pregnancies with two Rh (D) positive issues. The possibilities for developing this alloantibody could be from the fetomaternal haemorrhage during pregnancy or childbirth or from the Rh Immune globulin (RhIG) that was received during postnatal period or transfusion of donor red cells of weak-D type. Most of the alloantibodies identified (80%) were against Rhesus and Kell group as found in other studies. [3], [7], [11] Inclusion of strategies like extended phenotype matching for Rhesus and Kell antigens between the donor and recipient would significantly reduce the development of alloantibody.

5. Conclusion

Alloimmunisation has a significant negative impact on laboratory and institutional resources. In view of the prevention of adverse transfusion outcome, it is important to

minimize RBC alloimmunisation through extended antigen matching in all patients. However, prophylactic antigen matching decreases the total availability of compatible RBC units, increases turnaround time for transfusion recipients and further increases laboratory expenses. Therefore, blood bank may adopt a strategy of either extended antigen-matched RBC units in patients at the increased risk of RBC-alloimmunisation. Based on our observations measures to reduce alloimmunisation may include early initiation of transfusion (≤ 2 years), avoidance of 1st transfusion therapy in young adults (16-30 years) and restricted transfusion regime in female gender and disease/conditions with increased alloimmunisation risk or transfusion of RBC units that are antigen-matched for the commonly occurring antibodies to RBC antigens such as Rhesus a Kell antigens.

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