



STUDY OF RED CELL ALLOIMMUNIZATION IN MULTI-TRANSFUSED PATIENTS.

Immunohematology

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ABSTRACT

Context: Repeated blood transfusions can result in the production of alloantibodies against one or more red cell antigens, which complicates subsequent transfusions.

Aims & Objectives: The study was done to find incidence of various red cell alloantibodies; to determine the type of alloantibody; to identify the factors such as frequency of transfusion, splenectomy status, donor ethnicity and gender and their association with the development of antibody in repeatedly transfused patients.

Settings and Design: Prospective study in 300 patients.

Methods and Material: This study was carried out in tertiary care centre. Blood sample was taken from the patients of Thalassemia major, Sickle cell disease, Chronic renal failure, post-partum haemorrhage, Aplastic anemia, with more than 10 red cell transfusions. The serum was used for antibody screening and antibody identification test. Three cell antibody screening was performed using AHG gel cards and three cell panel (ID-ReaCell I, II, III). Those with positive antibody screening were analyzed further for antibody identification test using eleven cell panel (ID-ReaCell Panel).

Statistical analysis used: Microsoft Excel 2019

Results: Antibody screening and identification was done in 300 patients which showed, ten (10) patients (3.33%) were alloimmunized.

Conclusions: Alloantibodies should be identified in repeatedly transfused patients and should be given corresponding antigen negative blood unit which will minimize the antibody mediated destruction of transfused red cells.

KEYWORDS

Alloantibodies; antibody identification; antibody screening

INTRODUCTION:

Alloimmunization to red blood cell (RBC) antigens resulting from the genetic disparities between donor and recipient is one of the risks of blood transfusion. Repeated blood transfusions can result in the production of alloantibodies against one or more red cell (RBC) antigens, which complicate subsequent transfusions. Alloantibodies can interfere in the cross-match testing and therefore can cause delay in obtaining compatible blood and also sometimes associated with delayed type of hemolytic transfusion reaction.

The probability of alloimmunization depends on the number and frequency of transfusions, antigen immunogenicity, and recipient's immune response. The influence of ethnic and antigenic pattern differences between donors and recipients has also been reported.^[1]

Various diseases that require repeated red cell transfusions are Thalassemia major, Sickle cell disease (SCD), Aplastic anemia, chronic myeloproliferative disease and other malignancy, Chronic renal failure (CRF).

Antigen matched transfusion would effectively prevent alloimmunization. To do so, the patient's ABO, Rhesus, Kell, Kidd, and Duffy systems should be typed at diagnosis or before the institution of transfusion therapy. Blood to be transfused should always be matched at least with ABO, Rhesus, and Kell system.^[2] Further, using of leukocyte filter during transfusion may prevent alloimmunization due to white blood cells.

Management part of red cell alloimmunization cases include detection, identification of antibodies, and finally providing antigen negative blood for further transfusions.

AIMS AND OBJECTIVES:

- To initiate pretransfusion antibody screening on patient's sample before cross-match to initiate safe transfusion practice
- To find out incidence of various RBC alloantibodies as well as autoantibodies in Multi-transfused patients
- To determine the type of antibody present in multi-transfused patients

- To identify the factors such as frequency of transfusion, splenectomy status, donor ethnicity, and gender and their association with the development of antibody in repeatedly transfused patients.

SUBJECTS AND METHODS:

Prospective study in 300 patients after getting approval by Institutional Ethics Committee meeting held on Dated :11/04/2019.

Patient selection (Inclusion criteria):

- Patients of Thalassemia major, SCD, CRF, Postpartum haemorrhage, Aplastic anemia, those who require blood transfusion at 2–4 weeks interval
- Multi-transfused patients with difficulty in cross-matching.
- Those patients having positive direct Coombs test (DCT) and suspected to have alloantibodies, having increased requirement of blood units.

Patient exclusion criteria:

Patients with known connective tissue disorders were excluded

Clinical transfusion records of the patients who fulfilled the inclusion and exclusion criteria were given a questionnaire and were reviewed for the demographic data, i.e., age, sex, ethnicity, blood group, age at the start of first transfusion, number of packed cell units received, frequency of transfusion, splenectomy status, history of transfusion reactions, if any, use leukocyte filter.

Sample collection:

A volume of 3 to 4 ml of blood was collected in plain and ethylenediaminetetraacetic acid (EDTA) tubes from multi-transfused patients after they met the inclusion criteria. The serum was used for antibody screening and antibody identification test. The red cells were used for ABO grouping, Rh grouping, antigen phenotyping, and DCT.

Laboratory methods:

Using column agglutination technology (CAT), serum was analyzed for detection of red cell alloantibodies.

Antibody screening was performed using antihuman globulin (AHG) gel cards and three-cell panel (ID-ReaCell I, II, III). Those with positive antibody screening were analyzed further for antibody identification test using eleven-cell panel (Set ID-Reacell Panel).

Criteria for completion of test/further testing:

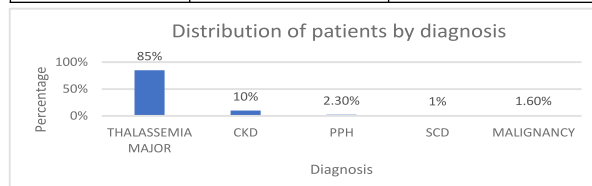
- The panel results are consistent with one or more clear-cut antibody specificities satisfying the conditions for identification
- The patient types negative for corresponding antigen
- The direct antiglobulin test (DAT) is negative.

RESULTS:

Out of total 300 patients, there were 180 males and 120 females who received regular blood transfusions. The age range of the patients was from 1 year to 80 years. There were 237 Hindu, 61 Muslim, 2 Jain patients.

Table-1 Distribution of Patients by Diagnosis

Disease	Number of Patients	Percentage
Thalassemia Major	255	85%
CRF	30	10%
PPH	7	2.30%
SCD	3	1%
Malignancy	5	1.60%
Total	300	100%



In present study maximum no. of patients were from Thalassemia major (255 patient-85%), followed by Chronic Renal failure patients on dialysis (30 patient-10%).

The packed cell units transfused were between 10 and 828 units (mean – 178.97 units). Only 10 (3.33%) patients were using leukocyte filter during blood transfusion. The low rate of using leukocyte filter during blood transfusion can be due to lack of awareness and cost-effectiveness. 51 (17%) patients had transfusion reactions in the form of mild itching, fever, chills, rigors, and breathlessness. 10–550 transfusion episodes were tracked to capture transfusion reaction. In the present study, 36(12%) out of total 300 patients had splenectomy, but only 2(5%) were alloimmunized.

Result of antibody screening and identification:

A total of 12 RBC alloantibodies were detected in 10 out of total 300 patients.

A total of 10(3.33%) samples showed to have alloantibody, 10(80.33%) belonged to Rhesus system (3 Anti-D, 3 Anti-E, 3 Anti-c, 1 Anti-e), 1(8.33%) belongs to Lutheran (1 Anti-Lu^a) and 1(8.33%) from MNSs (1 Anti-S) blood group system.

Out of 180 males, 5 (2.77%) developed alloantibodies, and out of 120 females, 5(4.16%) developed alloantibodies

Table-2 Red Cell Alloantibody and blood group antigen system

Alloantibody	Number of patients	Percentage	Blood Group System	Percentage
Anti-D	3	25%	Rhesus	83.33%
Anti-E	3	25%		
Anti-c	3	25%		
Anti-e	1	8.33%		
Anti-Lu ^a	1	8.33%	Lutheran	8.33%
Anti-S	1	8.33%	MNSs	8.33%

Anti-D, Anti-E, Anti-c was noted to be the most common type of alloantibody, followed by Anti-e, Anti-Lu^a, Anti-S

Table-3 Alloimmunization in various disease

Disease	Total number of patients	Alloantibody positive patients	Percentage
Thalassemia Major	255	7	2.74%

CRF	30	1	3.33%
PPH	7	1	14.28%
SCD	3	1	33.00%
Total	300	10	3.33%

Rate of alloimmunization in Thalassemia major patients was 2.74%, in CRF 3.33%, in PPH 14.28% and in SCD 33.00%.

Number of packed cells transfused in alloimmunized patients was between 11 and 264 units (mean – 100.3 units)

Out of 10 alloimmunized patients, 5 patients (50%) were DCT positive for IgG+C3d, while 5 (50%) were negative both for IgG and C3d.

DISCUSSION:

The red cell alloantibodies develop in a variable number of multi-transfused patients. In such circumstances, transfusion therapy may become significantly complicated. Effects of alloimmunization may include difficulty in finding compatible RBC units because of the presence of clinically significant RBC antibodies, transfusion reactions, or platelet refractoriness. The present study is an effort to characterize blood group alloantibody formation in the patient population.

Rate of alloimmunization:

The present study showed low rate of alloimmunization 3.33% which can be explained by homogeneity between the donor and the recipient population.^[3]

In the present study, rate of alloimmunization was consistent with other studies done internationally and various studies from India.

Table-4 Rate of Alloimmunization in various studies

Sr. No.	Author	Year	Total Numbers of Patients	Allo-immunized Patients	Rate of Allo-immunization
1	Sirchia et al. ^[4]	1985	1432	74	5.20%
2	Roopam et al. ^[7]	2009	96	5	5.21%
3	Sood et al. ^[11]	2013	306	13	4.24%
4	Bhuva DK et al. ^[20]	2013	300	9	3%
5	Prajapati et al. ^[21]	2019	100	5	5%
6	Present Study	2020	300	10	3.33%

Rate of red cell autoantibodies:

Out of total 300 patients, 5 patients (1.6%) were DCT positive for IgG+C3d but negative for C3d. There was no evidence of autoimmune hemolytic anemia in these patients. Positive DAT did not interfere in finding compatible blood. Positive DAT may indicate alloantibodies in a recipient's circulation, reacting with antigens on recently transfused donor red cells.

Gender:

In the present study, out of 300 patients of repeatedly transfused, there were 180 males and 120 females.

In the present study, the rate of alloimmunization was higher in females (4.16%) comparable with males 180(2.77%) which is similar to other studies.^[3,7,11]

Age:

In the present study showed that out of 300 patients of repeatedly transfused, 10 showed presence of alloantibody. The age of alloimmunized individuals ranged between 3 years to 40 years (mean – 16.6 years) showed results similar to other studies.^[7,13]

Religion and caste:

In the present study, 10 patients were alloimmunized 6 Hindus, 4 Muslims. Hence, alloimmunization rate was more in Hindu as compared to Muslim and Jain patients.

Age at the first transfusion:

In the present study, alloimmunized patients received the first transfusion ranged from 5 months to 26 years (mean – 6.8 years), and results were similar with other studies.^[15,16]

Number of packed cells transfused:

In the present study, number of packed cell units transfused in alloimmunized patients ranged from 11 to 264 units (mean – 100.3 units). All alloimmunized patients received more than ten transfusions

correlating with other studies.^[7,12,13,14,16]

Splenectomy:

Splenectomy may enhance or promote immune reactions as there is absence of an efficient filtering system for removal of damaged RBC.

Singer *et al*^[18] found from their study that patients who had a splenectomy had a higher alloimmunization rate.

In the present study, 10 patients were alloimmunized, but only 2 had splenectomy. Out of total 300 patients, 36 had splenectomy. No association between alloimmunization and splenectomy ($P = 0.34$) was found out in the present study similar to other studies.^[9,16]

Leukocyte filter:

Another important aspect that has emerged is the role of contaminating leukocytes of the allogeneic blood transfusion in causing immunomodulatory effects in the recipient. Contaminating leukocytes downregulate T-helper cell Type 1 immune responses and drive the recipient toward T-helper cell Type 2 responses. Such skewing toward Type 2 immunity may enhance alloantibody formation.^[19]

In the present study, only 10 (3.33%) patients were using leukocyte filter, none of them were alloimmunized.

In the present study, however, it was not established that leukocyte filter would prevent alloimmunization probably due to a small number of patients, in whom leukocyte filters were used during transfusions.

CONCLUSION:

It is concluded here that red cell alloimmunization should not be overlooked in repeatedly transfused patients. It should always be considered if the patient repeatedly suffers from hemolytic transfusion reactions, difficulty in finding compatible blood during cross-match, or patients not able to maintain hemoglobin at desired level in spite of regular transfusions.

It is also concluded here that regular screening for development of alloantibodies in repeatedly transfused patients would add toward better management of these patients. With the screening and identification technique, the alloantibodies should be identified and patients should be given corresponding antigen negative blood unit which will minimize the antibody-mediated destruction of transfused red cells.

Several factors might contribute to red cell alloimmunization such as heterogeneity of population, difference in age at first transfusion, antigenic difference between the donor and the recipients, recipient's immune status, immunomodulatory effects of allogeneic blood transfusion on recipient's immune status, and splenectomy.

Obtaining RBC antigenic phenotype on all repeatedly 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.

The present study also recommended extended phenotype-matched blood transfusion for antigen against Rhesus, Lutheran, MNSs and Kell blood group system. This will minimize the antibody-mediated destruction of transfused red cells that result in reduction of transfusion needs for the patients. Less number of transfusions reduces the psychological and financial burden on the family and will increase the patient compliance.

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Conflicts of interest:

Nil.

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