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ORIGINAL ARTICLE

A STUDY OF IRREGULAR ANTIBODIES IN 200 MULTI-TRANSFUSED PATIENTS

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ABSTRACT: BACKGROUND: Alloimmunization is one of the major concern in the management of patients who required repeated blood transfusion as a lifesaving treatment. The knowledge of incidence of such alloantibodies is essential for selecting appropriate red blood cells for transfusion. **AIMS:** This study was carried out to get the frequency and type of unexpected red cell antibodies in the multi-transfused patient at a tertiary level government hospital in South Gujarat. **MATERIALS AND METHODS:** This prospective study was carried out in 200 patients who required multiple blood transfusions. The antibody screening was done with 3 & 11 commercial cell screening & identification panel by column agglutination technique (Matrix Gel System & Matrix Erygen AS-ID, Tulip Diagnostics, India) at saline & anti-human globulin phase. **RESULTS:** The overall prevalence of alloimmunization was 7.0%. The majority of these had a single alloantibody (11 cases, 84.62%) whereas the remaining 2 cases (15.38%) had multiple antibodies. The anti-c and anti-D antibodies comprised the most common alloantibody (27% each both) followed by, anti-N (20%), anti-C (13%), anti-e & anti-M (7%) antibodies. Gender & number of blood units were found to be risk factors of alloimmunization in transfused patients. In our study we found females (79%) are more prone to alloimmunization. Those who were transfused more than 2 units have higher frequency of alloimmunization. The highest incidence of alloimmunization was observed in obstetrics and sickle cell patients. **CONCLUSIONS:** The majority of alloantibodies detected in the current study were clinically significant and of mainly belonging to Rh blood group system. Thus pre-transfusion antibody screening on patients' samples prior to cross-match needs to be initiated in India and we can at-least provide corresponding Rh antigen negative blood to ensure safe transfusion practice

KEYWORDS: Red Cell, Red cell antigen, Alloimmunization, alloantibodies, Indirect Antiglobulin Test.
MESHTERMS: Erythrocyte, Isoantibodies, Coombs test.

INTRODUCTION: Alloimmunization is one of the major concern in the management of patients who required repeated blood transfusion as a lifesaving treatment. In the patients affected with haemoglobinopathies, haematologic diseases, various types of cancers, recipients of organ transplantation, and patients with renal failure, the prevalence of alloimmunization has been reported to be up to 60 per cent.[1] Alloimmunization further complicates the transfusion therapy due to difficulty in getting compatible blood & delayed haemolytic transfusion reaction.[2] The knowledge of such alloantibodies is essential for selecting appropriate red blood cells for transfusion. This study was carried out to get the frequency and type of unexpected red cell antibodies in the multi-transfused patient at a tertiary level government hospital in South Gujarat.

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MATERIALS AND METHODS: The study was performed between the years 2012 to 2014 at blood bank attached to department of Immunohematology & blood transfusion of tertiary level Government Medical College and Hospital of South Gujarat after obtaining ethical committee clearance from the institute and assessed for the presence of alloantibodies. Antibody screening was carried out in 200 multi-transfused patients prior to compatibility testing. A detailed clinical and transfusion history was taken using a set performa which included the name, identification number, age, sex, diagnosis, blood group, transfusions done till date of request, transfusions during the present study period, result of serological testing like direct antiglobulin test and auto control, antibody screen tests in the study period with results and antibody identification results.

The blood requisition for these patients were received along with samples (plain & EDTA) for antibody screen testing and compatibility testing as a protocol. ABO and Rhesus blood grouping tests were done by forward and reverse grouping in all patients so as to confirm the blood group. Subsequent antibody screening was performed on all samples using a commercially available three cell panel (Matrix gel system & Matrix Erygen AS; Tulip Diagnostics, India) by the column agglutination method, using saline, antiglobulin & enzyme phase.

Antibody screening was done for antigens of blood groups which include Rh, Kell, Kidd, Duffy, Lewis, P and MNS antigens along with an auto-control. Antibody screen positive samples were further analysed for the specificity of the alloantibody with an eleven cell identification panel (Matrix gel system & Matrix Erygen ID, Tulip Diagnostics, India). Later on compatible blood at anti-human globulin phase was issued for transfusion whenever required. An auto control using the patient's own cell and serum was tested in parallel with each screen to exclude presence of autoantibodies. The criteria for antibody screening and identification were based on the standard recommendations and Manufacturer Company.[3,4]

STATISTICAL ANALYSIS: The patient with positive screen was assessed based on gender, age, and history of transfusion, clinical diagnosis and alloantibody specificity. The two sided chi square t test & odds ratio were performed to determine the difference in antibody rate by gender and no of transfusions. $P<0.05$ was considered significant. The analyses and data management were performed using Epi Info software version.

RESULTS: A total of 200 patients (114 male & 86 female) were included in the present study. Different diagnosis of 200 patients was: 36(18%) of thalassemia, 30(15%) of sickle cell disease, 29(14%) with surgical illness, 23(12%) of other anaemia, 22(11%) with renal disease, 16(08%) of leukaemia, 14(07%) with GIT diseases, 10(05%) with obstetrics condition and 20(10%) with other diseases (Figure 1). Age of the patients included in the study ranged from 2 to 85 years with a mean age of 28.21±16.78 years. Among the alloimmunized cases, the age range was 18 to 35 years with a mean age of 26.61±5.3 years. Among total number of cases, 14(07%) patients were positive for different type of irregular antibodies while remaining 116(93%) patients were negative for alloimmunization. 13 patients were included in study as one patient was having auto antibody.

Among the total 13 number of patients with alloantibodies, three male patients had positive results for antibody which is 2.70% of total male patients and 23.07% of total positive cases while ten female patients were positive for alloantibody which is 13.33% of total female patients and 76.92% of total positive cases.

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Female patients were significantly more positive for irregular antibodies (Chi-square test 2 tailed P value is 0.012). The odds ratio for male and female positivity was 5.4 indicating that female were 5 times more prone to develop alloantibodies in comparison to male patients. Among the positive cases, blood group distribution is shown in Figure 2. Out of 200 patients, 94(47%) patients had received ≤2 units of blood transfusion, among which 01(01.06%) had developed irregular antibodies while 106(53%) patients had received >2 units of blood transfusion, among which 12(11.32%) had developed irregular antibodies which showed significant difference between these two groups (chi-square test 2 tailed P value is 0.008). Among the 13 patients with alloantibodies, 11 patients (84.62%) had a single alloantibody, whereas two patients (15.38%) had multiple alloantibodies.

Among the total 13 patients with alloantibody/alloantibodies, four (31%) patients were having anti-c antibodies, Three (23%) patients were having anti-N antibodies, two (15%) patients were having Anti-D, two (15%) patients were found positive for both anti-D & C, one (08%) each patient was having Anti-e and anti-M (Figure 3). The adsorption & elusion study to find out possibility of Anti G antibody in two patients who were found positive screen for both anti D and anti C was not done. Among the positive cases, four (31%) cases were that of sickle cell anaemia, four (31%) cases of obstetrics, two (15%) cases of anaemia, and one each case of P. vivex (8%), hemolytic anaemia (7%) and Bernard soulier syndrome (8%).

DISCUSSION: It is a routine practice to perform pre transfusion compatibility testing before blood transfusion to prevent immune mediate haemolytic transfusion reactions. The steps of pre-transfusion testing involve reviewing the acceptability of blood sample, checking the ABO group and Rh D type, antibody screening test, determining the specificity of antibodies detected unexpectedly, choosing donor RBC units suitable for recipients, and carrying out cross-match.[3] As blood is routinely matched with respect to major blood group antigens i.e. ABO and Rh D antigen, there is a high probability that the donor will have minor blood group antigens not present in the recipients which will result in alloimmunization. Factors for immunization are complex and involve at least three main contributing elements. This includes RBC antigenic difference between the blood donor and the recipient, the recipient's immune status and immuno-modulatory effect of the allogenic blood transfusions on the recipient's immune system.[5]

The development of red cell antibodies (Allo as well as autoantibodies) occurs in a variable number of multiple 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.[6] Present study is an effort to characterize blood group alloantibody formation in the patient population.

Few studies of multiple transfused patients in India had revealed rate of alloimmunization ranging from 3 to 13% as mentioned in Table 1. The rate of present study was 07% which is similar to the studies conducted by J Shukla et al (9.87%), Pradhan et al (08%) and Gupta et al (9.48).[7,9] The studies done by Pahuja et al (3.7%) and Dhawan et al (5.64%) had lower rate while study of V Sangole et al had higher rate of 13.04 %.[10,12]

Females have been observed to be more prone to development of alloimmunization than males probably due to the fact that females, especially in developing countries, are anaemic and pregnancy is an important risk factor for alloimmunization.[13]

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In present study, 11(79%) out of 14 alloimmunized patients were women, with significant association in chi-squares test (P value <0.05). This finding of the present study is in agreement with the study done by Alick et al while studies done by Bhaskar S et al & Makroo et al did not show such association with gender.[13,15] In the present study, female were five times more prone to develop alloantibodies in comparison to male patients. Clinical diagnosis of the study group may lead to a vulnerable immune status which may predispose to altered or increased immune response to various antigens. In our study, significant number of sickle cell anaemia patients developed alloantibodies. Out of 30 sickle cell cases, alloimmunization was found in four (13.33%) cases, which is comparable to study by Elliott et al (30%) and Murao et al (9.9%).[16,17]

Though antigen typing before transfusion of people with sickle cell disease and providing antigen negative units is now widely employed by sickle cell centers, the alloimmunization rate remains quite high in contemporary sickle cell populations and may be due in large part to transfusions received at institutions not providing extended matching. Two out of 21 patients of chronic anemia developed alloantibodies (9.52%), which is comparable to study by Elliott et al (05%).[16] Out of 5 patients of obstetrics, 4 (80%) patients developed alloantibodies. (Figure 3).

The specificity of most alloantibodies detected in the present study was against Rh system (85%) due to their high immunogenicity, which is similar to previous reports of Thakral et al (61%), Hmida et al (59%) and Dhawan et al (52%).[11,18,19] Anti c was detected in four patients, Anti-D in four, Anti-N in three, anti-C in two and Anti-M, Anti e in one patient each. Anti-c and anti-D (27%) were the most common antibodies in our study, which is comparable to Thakral et al (38.8%).[18] Hence, the transfusion of blood matched for Rh could prevent alloimmunization resulting in a significant difference in the alloimmunization rates, but the potential to form RBC alloantibodies to unmatched antigens will exist.[20] In our study, majority of the patients with anti-D (either singly or in combination) were multiparous females who might have formed anti-D due to previous pregnancies or transfusions. (Figure 3)

In the present study, there was an absence of anti-Kell antibody in all subjects which was similar to the findings of the study done by Thakral et al while other studies found anti Kell antibodies.[11,13,15,18] This could be due to differences in blood group antigen frequencies in different populations. According to the study on blood donors of the South Gujarat, Kell antigen positivity was found to be 6%.[21] Thus, the lower frequency of Kell antigen in donated blood might be the reason behind the lesser risk of alloimmunization from transfusion of a Kell antigen positive unit and the result was absence of anti Kell antibody in present study.

In the present study we detected single antibody in 84.62% of cases and multiple antibody in 15.38% of cases, which is comparable to study by Alick et al who found single antibody in 78.6% and multiple antibody in 21.4%.[14] Similar results was also found by Dhawan et al (22%cases had dual allo antibodies).[11] Since pre-transfusion antibody screening in patients' samples is not a routine practice in India, these patients might have received antigen mismatched blood leading to formation of multiple alloantibodies.

The risk of developing alloimmunization was very clearly associated with the number of transfusions received. In our study 11.32% patients of patient group who received more than 2 units of blood transfusion were developed alloantibodies and it is significant statistically (P<0.05). This finding is supported by some of the earlier studies done by Alick et al, Dhawan et al, Vishinski E et al & Jensen LS et al who have found a strong correlation between the numbers of blood units transfused and alloantibody formation.[11,14,20,22]

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CONCLUSION: By considering the results of present and other reference Indian studies, blood banks of India should go for universal type and screen policy for finding the prevalence of alloantibodies in general patient and donor population. The indigenous development of local cell panels would be a better option to ensure adequate supplies of reagent red cells and introduction of type and screen policy for all the patients. Patients with identified alloantibodies can be flagged in a database and the information can be shared between institutions and shared with the patient in the form of report issuing to the concern person as well as patient education if possible. To avoid the effects of alloimmunization, after antibody screen and identification, corresponding antigen negative blood should be given to the patient.

The other approach to avoid alloimmunization in regularly transfused patients like sickle cell disease & thalassemia is to allot a group of 10-15 donors to such single patient. Whenever the transfusion required, donor will be selected from this group. In this way we can minimize alloimmunization as well as better safety in terms of transfusion transmitted infections also.

STUDY LIMITATION: The adsorption & elusion study to find out possibility of Anti G antibody in two patients who were found positive screen for both anti D and anti C was not done. The limitations of this study was follow up data was not available due tovarious reasons & phenotyping of each & every donor was not possible so only cross match compatible blood were issued.

Tables:

Sl. No	Studies	No. of Cases	% of Positive Cases
1	J Shukla et al	81	9.87
2	Pradhan et al	100	8
3	Pahuja et al	211	3.79
4	Gupta et al	116	9.48
5	Dhawan et al	319	5.64
6	V Sangole et al	46	13.04
7	Present study	200	7

Table 1: Incidence of Alloimmunization in Multi-Transfused Patients in various Studies

Studies	Most common Antibody	%
Satyam arora et al	anti kell	35
J Shukla et al	anti C & anti E	50
R Gupta et al	anti E	36.4
B Shenoy et al	anti C & anti kell	43
R Makroo et al	anti E	37
Thakral et al	anti c	39
Present study	anti c and anti D	27

Table 2: Major Antibody type in various Studies

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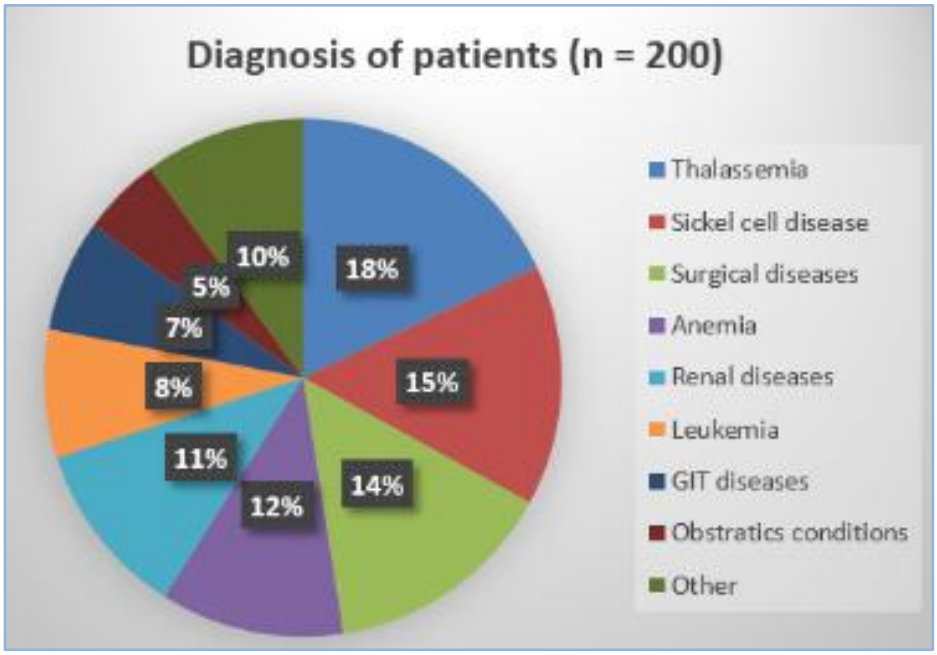

Fig. 1: Diagnosis of Patients (n = 200)

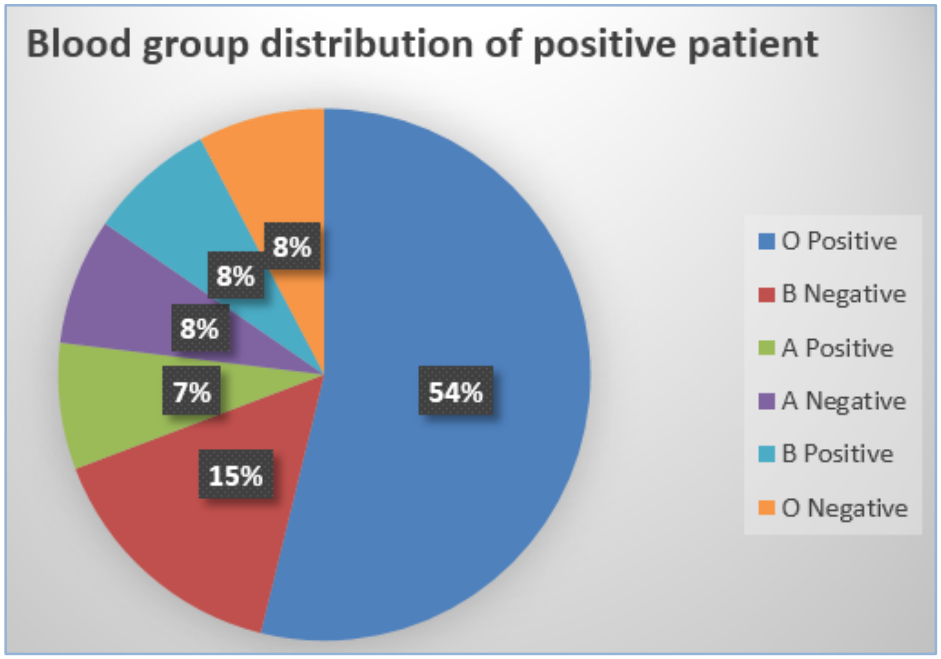

Fig. 2: Blood Group Distribution of Positive Patient

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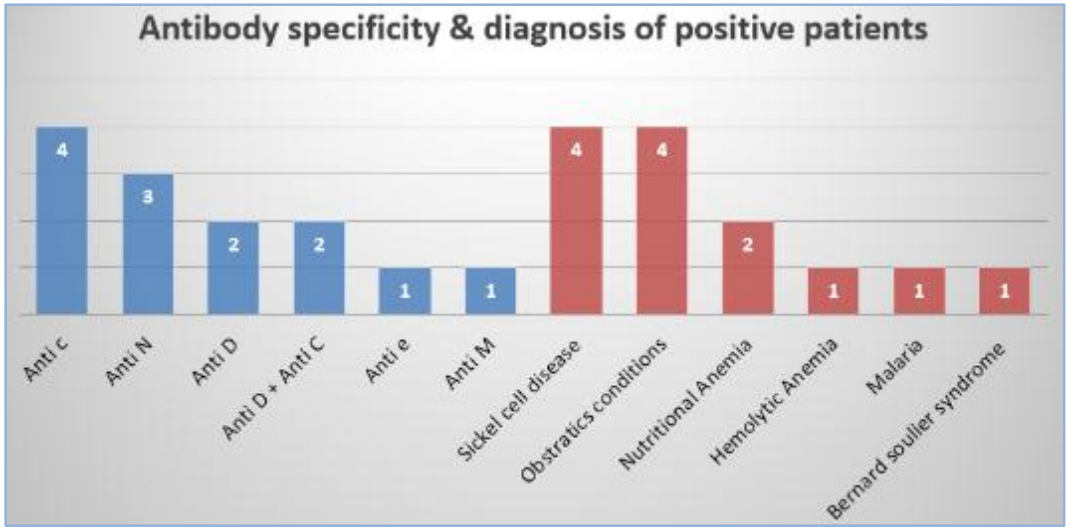

Fig. 3: Antibody specify & Diagnosis of Positive Patients

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