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Prevalence of irregular red cell antibody in healthy blood donors attending a tertiary care hospital in North India

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Abstract:

BACKGROUND: Alloantibodies may be detected in blood donors who have either been transfused previously or female donors with previous obstetric events. These antibodies can occasionally cause severe transfusion reaction, if a large amount of plasma or whole blood is transfused, as in massive transfusions and pediatric patients.

AIMS: The present study aims to assess the prevalence of red cell antibodies in healthy blood donors at a tertiary care hospital-based blood bank in India.

MATERIALS AND METHODS: A total of 82,153 donor samples were screened for irregular red cell antibodies between January 2012 and December 2015 at the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi. Antibody screening was performed by solid phase method using Immucor Capture–R ready screen (pooled cells) on fully automated immunohematology analyzer Galileo Neo (Immucor Inc., Norcross, GA, USA). Positive tests were further confirmed using Capture-R ready screen (4 cell panel). Advanced investigations to identify the antibody/ies were performed on confirmed positive samples. Antibody identification was conducted using various cell panels (Immucor Capture-R Ready-ID, Panocell-10, Ficin Treated). An advanced technique such as adsorption and elution was performed as per requirement.

RESULTS: Screening with pooled cells and 4 cell panel was positive in 227 donors (0.27%), 150 of these donors had autoantibodies, 1 had autoantibodies with underlying alloantibody anti-Jk^{a} (0.001%), and 76 had alloantibodies (0.09%) alone in their plasma. Anti-M was the most common antibody (43 donors) identified, followed by anti-D (21 donors). Anti-N was detected in 4; anti-Jk^{a}, anti-C, and anti-E in two donors each followed by anti-P1 and anti-Le^{b} in 1 donor.

CONCLUSION: Antibodies against red cells can be present in healthy donors detection of which is important in providing safe blood to the patient. The prevalence of red blood cell antibody in healthy donors in this study was found to be 0.27%, while the prevalence of alloantibodies was 0.09%. The majority of alloantibodies were anti-M (56.57%) and anti-D (27.63%).

Keywords:

Alloantibody identification, alloantibody screening, donor screening

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Introduction

Red cell antibody anti-A and anti-B are the naturally occurring antibodies that are found in the human serum. All other antibodies are called "irregular red cell antibodies." There are two types of irregular red cell antibodies: alloantibodies and autoantibodies. Alloantibody is produced against the antigen that is lacking, whereas autoantibody is produced to an antigen that is present. Such irregular alloantibodies/autoantibodies can be encountered in

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healthy blood donors who are either transfused previously or in multiparous females.[1]

The National Blood Policy, India, 2007 (National Aids Control Organization, Ministry of Health and Family Welfare) has laid down the guidelines for the screening of donated blood for the presence of irregular red cell antibodies.[2] The incidence of transfusion reactions due to irregular red cell antibodies in donor blood is rarely seen.[3] However, the presence of such antibodies can occasionally cause severe transfusion reactions if a large amount of plasma or whole blood is transfused as in the cases of massive transfusions or in pediatric population. Only packed red blood cells (PRBCs) should be preferably transfused when irregular red cell antibodies are found.[4] For safe blood transfusion blood donors testing for infectious markers, but also for irregular antibodies should be performed for safe and compatible blood transfusion, especially for previously alloimmunized individuals.

There is a paucity of literature on the prevalence of the irregular red cell antibodies in whole blood donor population. However, among few studies done till date, it is quoted that the alloantibodies are detected in up to 0.8% of the whole blood donors.[5,6] A study by Pahuja *et al*.[7] done on 7756 whole blood donors showed the incidence of irregular red cell antibodies of 0.05% in their donor population. In another study, Garg *et al*.[8] performed on 47,065 whole blood donors found 0.09% prevalence of irregular red cell antibody.

The present study is an overview of the prevalence of irregular red cell antibodies among the whole blood donors who donated at our center.

Materials and Methods

This retrospective observational study was conducted in the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, Sarita Vihar, New Delhi. The data of antibody screening among the whole blood donors who donated blood according to the Directorate General of Health Services criteria at our center from January 2012 to December 2015 were analyzed for the presence of irregular RBC antibodies in whole blood donors. This study was approved by the ethical committee of our institute.

The data which included donors basic profile (age, gender, and address), history of transfusion, drug intake, jaundice, or any clinically significant disease related to autoimmune disorders were retrieved from the donor information, and consent form was filled before blood donation. A detailed obstetrics and gynecology history, especially regarding childbirth and abortion, was taken from all female blood donors.

Universal precautions were taken during all sample handling, processing, and testing. As per the departmental protocol, all donor samples were collected from the donor during blood donation in EDTA (Ethylenediaminetetraacetic acid) vials for blood grouping and antibody screening. Blood grouping was performed on fully automated immunohematology system Neo (Immucor Inc., Norcross, GA, USA) using commercially available antisera's (Immucor Inc., Norcross, GA, USA). Antibody screening was performed using solid-phase red cell adherence (SPRCA) technology on fully automated system Neo (Immucor Inc., Norcross GA, USA) using pooled "O" cells (Capture-R ready screen).

Direct antiglobulin test (DAT) and autocontrol (A/C) were also performed simultaneously on the immunohematology system. Any sample with positive antibody screen result using pooled "O" cells was subjected to antibody screening and identification using commercially available cell panels from Neo (Immucor Inc., Norcross, GA, USA). In cases with positive auto control and DAT, complete blood units were discarded. In screen positive cases, plasma was discarded and ABO, Rh-matched PRBCS was given. Adsorption and elution were conducted according to the American Association of Blood Banks technical manual for the cases with autoantibody to rule out the presence of any underlying alloantibody.

Statistical analysis

The data were retrieved and entered into Microsoft excel sheet and analysis was performed with SPSS SOFTWARE (SPSS version 17).

Results

A total number of 82,153 donors donated at our center during the study [Table 1]. Of these, 227 (0.27%) were positive for antibody screening using pooled "O" cells. Among them, 93.40% were males and only 6.60% were females [Tables 2 and 3]. Donors within the age group of 26–30 years showed maximum number (n = 69; 30.39%) of antibody screen positivity. The results showed statistically a higher prevalence of RBC alloantibodies in males than females (P = 0.000037). On identification, 150 (0.18%) donors were screen positive

Table 1: Profile of the donors tested for antibody screening

	Male	Female	Male: female	Total
Total donors	81,193	960	84.57:1	82,153
Alloantibody positive donors	63	13	4.84:1	76
Autoantibodies	148	2	74:1	150
Auto + alloantibody	1	0	1	1

with autoantibody, 1 (0.001%) had autoantibody with an underlying alloantibody, namely, anti-Jk[a]. Seventy-six had alloantibodies (0.09%) alone in their plasma. Anti-M (n = 43; 56.57%) was the most common antibody identified, followed by anti-D (n = 21; 27.63%) [Table 4].

Discussion

Several studies have reported that the rate of alloimmunization in blood donors varies from 0.32% to 2.4%.[9,10] This large variation may be due to the different screening method used, and characteristics of the population studied. The prevalence noted in the present study is 0.09%, which is comparable with the similar two studies conducted from the same region.[7,8] Pahuja *et al*.[7] showed the prevalence of 0.05% among 7756 whole blood donors. Garg *et al*.[8] reported a prevalence of 0.09% among 47,450 whole blood donors. On the contrary, Giblett[9] had reported 0.32% incidence of irregular RBC antibodies in blood donors. However, the incidence went down to 0.04% after the donors with any history of blood transfusion or pregnancy were excluded from the analysis making the reports comparable. Similarly, Winters *et al*.[6] in 2001 reported 0.89% prevalence among blood donors of Olmsted county, Minnesota that consisted of donors who were previously transfused and pregnant women in their study, which probably explains the higher percentage of alloantibodies.

Table 2: Age wise distribution of donors with positive antibody screen results

Age group (years)	Number of positive donors
18-25	57
26-30	69
31-35	47
36-40	32
>40	22

Table 3: The characteristics of donors with positive antibody screen results

Group	*n* (%)
Total	227
Gender	
Male	212 (93.40)
Female	15 (6.60)
ABO blood group	
A	36 (15.85)
B	89 (39.20)
O	70 (30.84)
AB	32 (14.09)
Rh (D) group	
Negative	31 (13.65)
Positive	196 (86.35)

Table 4: Frequency of alloantibody among antibody screen blood donors

Alloantibody	Frequency (%)
Anti-M	43/76 (56.57)
Anti-D	21/76 (27.63)
Anti-N	4/76 (5.26)
Anti-Jka[a]	2/76 (2.63)
Anti-C	2/76 (2.63)
Anti-E	2/76 (2.63)
Anti-P1	1/76 (1.31)
Anti-Leb[b]	1/76 (1.31)

The highest frequency of alloantibodies was identified in blood donors aged between 26 and 30 years in our study. Similar results were reported by Pahuja *et al*.[7] In the present study, we found a predominance of male donors compared to female donors, which is comparable with a study done by Garg *et al*.[8] Retrospective analysis of RBC alloimmunization among 179,045 Kuwaiti patients, pregnant women, and allogeneic blood donors was conducted for 1992–2001 by Ameen *et al*.[10] in 2005. The overall incidence of alloimmunization among the general population was 0.49% and in blood donors was 2.3%. The incidence of alloimmunization among female patients and donors was >3 times higher than among male subjects. In this study, however, the prevalence of irregular RBC alloantibody in males (n = 63; 82.90%) was higher than females (n = 13; 17.10%). This finding was consistent with the reports of Pahuja *et al*.[7] but not in agreement with Ameen *et al*.[10] This can be attributed to the predominant donor population in our analysis with positive antibody screen consisted of males (n = 212; 93.40%).

In our study, the most frequent alloantibodies identified were from the MNS blood group system followed by Rh blood group system. The frequency of anti-M and anti-N were found to be 56.57% and 5.26%, which was clinically significant. Anti-M and anti-N are generally naturally occurring alloantibody which do not react at 37°C, and are not clinically significant for transfusion but can cause a problem in pretransfusion testing. It is clinically significant when detected at 37°C, wherein, cross-match compatible antigen negative blood should be given to prevent any hemolytic transfusion reaction.[11]

The Rh blood group is one of the most complex blood groups known among blood group system. D antigen is considered to be the most immunogenic of all antigens and has the potential to cause clinically significant Hemolytic disease of fetus and new born (HDFN) and transfusion reactions. Anti-C and anti-E, do not often cause HDFN, and when they do, it is usually mild.[11]

The frequency of anti-D in our study was found to be 27.63%. Of the donors with anti-D (n = 21), 13 were females and 8 were males. Eleven of the 13 female donors had a history of previous lower segment cesarean section

and blood transfusion, the remainder had unknown transfusion history. Five of the 8 males gave a history of previous blood transfusion, whereas the rest three did not remember about any previous transfusions. The frequency of minor antigens anti-E and anti-C was found to be 2.63%. In comparison with the study by Garg *et al*.[8] reported a frequency of anti-M, anti-D found in our study was high and frequency of anti-N was less. This difference could be due to the different techniques used for antibody screening and identification. In our study, antibody screening and identification was performed by SPRCA technique, whereas, they used column agglutination technique. The sensitivity of antibody detection by SPRCA is higher as compared to column agglutination.[12]

Other alloantibodies found in our study are anti-Jk^a (3.94%), anti-P1 (2.63%), and anti-Le^b (1.31%). However, the results were remarkably different from a study done in Chinese blood donors by Zhu *et al*.,[13] where the frequency anti-P1 and anti-Le^b were 7.1% and 2.3%, and no anti-Jk^a was found in their donor population. This difference can be attributed to the heterogeneity of the population, and differences in the prevalence of antigens according to the ethnicity.[13]

Anti-Jk^a is of IgG type; hemolytic transfusion reactions are very common because Kidd antibodies are often not detected in pretransfusion testing as their levels in plasma drop below the detectable level and they show dosage effect.[11] Anti-P1 and anti-Lewis both are of IgM type and occurs naturally; they are often detected as a weak, room temperature agglutinin. In rare cases, they are reactive at 37° C or shows *in vitro* hemolysis. Both are of IgM type, do not cross the placenta and do not cause HDFN.[11]

In our study, hundred and fifty (n = 150/82,153; 0.18%) had autoantibodies (DAT positive) which is much higher than reported by Tiwari *et al*. (0.04%) and Kaur *et al*. (0.05%).[14,15] This could be because of the fact that solid-phase testing has increased sensitivity and may detect weak autoantibodies that other method may miss.[12] As per our institutional policy, DAT-positive blood units were discarded.

Red cell antibody screening of the donors is a simple test, adds a layer of safety in transfusion and reduces the need for minor cross matching. In addition, we recommend that in cases where the antibody is found in blood donor, they should be informed, so that in future if they require any transfusion they can inform the blood bank prior.

Conclusion

We found that the overall prevalence of irregular RBC alloantibodies was 0.09% with anti-M, anti-D being the most frequently identified alloantibodies in blood donors at our center. To the best of our knowledge, this is the largest study from India on screening and identification of irregular RBC antibodies among blood donors using SPRCA technology.

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Conflicts of interest

There are no conflicts of interest.

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