Short Communication

Frequency of Irregular Red Cell Antibodies in Blood Donor Population

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Abstract

Introduction: Irregular red blood cell (RBC) antibodies may occur in blood donors and potentially can lead to transfusion reactions or decreased survival of transfused RBCs. Therefore, it is necessary to know the prevalence of alloantibodies in donors and their clinical significance. **Aims and Objectives:** The study was aimed to determine the prevalence of RBC alloantibodies in blood donors. **Materials and Methods:** ABO typing and RhD typing were performed using the fully automated immunohematology analyzer (Qwalys 3, Diagast, France). For RhD-negative samples, a "weak D" testing was also performed by tube technique using a blend (immunoglobulin M [IgM] + IgG) of anti-D antisera (Tulip Diagnostics, Goa, India). Antibody screening (3-cell panel) and identification (11-cell panel) were done by gel technique (LISS-Coombs AHG Card, Bio-Rad, Switzerland). The antibody titer was done using the tube technique. **Results:** During the study period, a total of 2310 donor samples were tested. Out of these, 2299 (99.56%) were male and 11 (0.44%) were female. ABO distribution was found to be maximum for blood group B (34.5%), followed by O (33.3%), A (22%), and AB (10.3%). Among the total donors, 2085 (90.3%) were RhD positive. All the RhD-negative samples were negative on "weak D" testing. Antibody screen was positive for only one sample (0.043%); the alloantibody identified was anti-M, which was reactive in anti-human globulin phase as well, and the titer was 1. It was from a male donor who had no history of transfusion. One sample (0.04%) showed autoantibody weak positive (wk+), and there was one ABO discrepancy (0.04%), which was due to weak subgroup of A. **Conclusion:** The prevalence of RBC alloantibodies is 0.043% (1/2310) in our donor population. As the sample size was small, larger studies are needed to determine the actual prevalence of alloantibodies in donors.

Keywords: *Alloantibody, blood grouping, irregular red cell antibody*

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Introduction

All red cell (RBC) antibodies other than naturally occurring anti-A and anti-B are called unexpected or irregular antibodies. They may be present in the form of alloantibodies or autoantibodies. The main stimuli for development of irregular RBC antibodies in healthy donors are previous transfusion and/or pregnancies.[1] A clinically significant red cell antibody is defined as an antibody that is frequently associated with hemolytic disease of the fetus and the newborn (HDFN), with hemolytic transfusion reaction (HTR) or with a notable decrease in the survival of red cells.[2] HTRs due to RBC alloantibodies in donor blood occur rarely. However, when the transfusion recipients are pediatric patients, especially infants, or when given in the setting of massive transfusion, these alloantibodies can cause immune hemolysis and severe transfusion reaction.[1] Therefore, it is necessary to know the prevalence of alloantibodies and their clinical significance.

Alloantibodies also have the potential to cause HTRs in patients who require multiple transfusions, as in those with hematological malignancies, congenital or acquired hemolytic anemia, or blood dyscrasias. Hence, compatibility of the recipients with donor blood

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with respect to minor group antibodies is important in preventing this.[3-5] Warm-reactive antibodies are usually predominantly immunoglobulin G (IgG) and react between 30°C and 37°C. IgG antibodies are important in mature humoral immune effector function and are divided into four subclasses – IgG_1, IgG_2, IgG_3, and IgG_4. The seroprevalence of alloantibodies in blood donors around the world has been estimated as being 0.3% (0.2%–0.8%).[1]

In India, as per the DGHS Technical Manual guidelines under the Ministry of Health and Family Welfare, Government of India, donor blood should be tested for unexpected antibodies by saline albumin/enzyme and anti-human globulin (AHG) tests with screening panel or with pooled fresh O group red cells.[6] However, in most blood banks across the country, the practice varies. In some studies from India, the reported prevalence of alloantibodies was 0.04%–0.09% in donors.[7-9]

Aims and objectives

This prospective study aimed to determine the prevalence of RBC alloantibodies in blood donors and find their specificity and titer.

Materials and Methods

This was a prospective prevalence study on donor samples collected from whole blood donations done at the blood donation center of our institute over a period of 4 months (February to May 2015). Ethics: The study was approved by the Institute Ethics Committee (Letter No. Histo/15/IMEC/42 dated January 12, 2015). As it was a nonfunded study, the target samples size was kept at around 4%–5% of our annual collection (50,000 units approximately). ABO typing and RhD typing were performed using the fully automated immunohematology analyzer (Qwalys 3, Diagast, France). The equipment is based on "erythrocyte-magnetized" technology, and the configuration included typing with anti-A, anti-B, and anti-D in forward grouping and A1 and B cells in reverse grouping (Hemalys, A1, B cells, Diagast, France). For RhD-negative samples, a "weak D" testing was also performed by tube technique using a blend (IgM + IgG) of anti-D antisera (Tulip Diagnostics, Goa, India). The testing was done till the AHG phase, and the sample was considered as RhD negative only after the "weak D" testing was negative. Antibody screening and identification were done using 0.8% (± 0.1%) commercial preparation of O group red cells, a 3-cell panel (ID-DiaCell I-II-III, Bio-Rad, Switzerland) and an 11-cell panel (ID-DiaPanel, Bio-Rad, Switzerland), respectively. The testing was done by column agglutination (gel) technique (LISS-Coombs ID-Card, Bio-Rad, Switzerland) where each microtube contained polyspecific AHG (rabbit anti-IgG and monoclonal anti-C3d, cell line C139-9) within the gel matrix. After adding 50 µL of the red cell suspension of the antibody screening/identification cells to each microtube, 25 µL of donor's plasma was added. It was then incubated at 37°C for 15 min in a dedicated incubator (ID-Incubator 37 S I, Bio-Rad, Switzerland), followed by centrifugation at 1030 rpm (85 g) for 10 min (ID-Centrifuge 12 S II, Bio-Rad, Switzerland).[10] Subsequently, the results were graded from 0 (negative) to 4+, and interpretation regarding the possible alloantibody was done using the respective antigen tables provided with the antibody screening and identification cell panels. An autologous control was also run with each antibody screen using the RBC and plasma from the same donor. Antibody titer was done using the tube technique with endpoint titer as the reciprocal of the highest dilution at which 1+ agglutination was observed.[10] Any sample found to be reactive or positive on transfusion-transmissible infection screening was excluded from the study. Statistical analysis was carried out using the Statistical Package for the Social Sciences, version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as mean, median, and percentage.

Results

During the study period, a total of 2310 donor samples were tested. Out of these, 2299 (99.5%) were males and 11 (0.48%) were females. The maximum number of donors was of the age group of 18–29 years (n = 1233, 53.4%), while there were only eight donors in the age group of 60–65 years (0.3%) [Figure 1]. There were 753 (32.6%) donors in the age group of 30–39 years, 260 (11.3%) in the age group of 40–49 years, and 56 (2.4%) in the age group of 50–59 years. The mean age of the donors was 30.3 years, and the median age was 30 years. The state-wise distribution

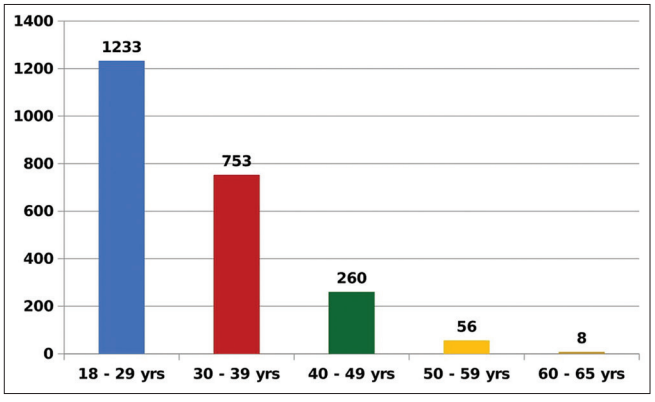

Figure 1: Number of donors in different age groups (n = 2310)

of donors according to their place of residence is given in Table 1. ABO distribution was found to be maximum for blood group B (796; 34.5%), followed by O (769; 33.3%), A (508; 22%), and AB (237; 10.3%). RhD testing showed that 2085 (90.3%) donors were RhD positive and 225 (9.7%) were RhD negative. All the RhD-negative samples were negative on "weak D" testing. Only 1 out of 2310 donor samples (0.043%) was found to be positive for antibody screening; the alloantibody specificity was found to be anti-M antibody, which was reactive in AHG phase, and the titer was 1 in AHG phase, negative on immediate spin. One donor sample (0.04%) was weak positive (wk+) on autocontrol. There was one ABO discrepancy (0.04%), which turned out to be weak subgroup of A, as resolved by the adsorption-elution technique.

Discussion

RBC alloantibodies are known to occur in general population. Those of clinical significance may result in HTR, HDFN and/or decreased survival of the transfused RBCs in the recipients. The prevalence of alloantibodies was found to be 0.043% (1/2310) in our donor population. The only screen-positive sample belonged to a 21-year old male replacement donor from Uttar Pradesh. From the details given in the donor registration form, it was found that he had no history of transfusion in the past. The alloantibody was identified to be anti-M which was reactive only in the AHG phase and not in the immediate spin saline phase and thus was probably an IgG type of alloantibody with a low titer (1). Anti-M is a relatively common naturally occurring antibody in adults, which reacts optimally at 4°C and weakly or not at all at 37°C and thus may not be clinically significant. At times, the naturally occurring anti-M may exist as IgG type only.[1] When anti-M is encountered to be active at 37°C, it may cause acute or delayed HTRs and rarely HDFN.[10] Table 2 enlists various studies on frequency of RBC alloantibodies in donor population.

Table 1: State-wise origin of blood donors (*n*=2310)

State	*n* (%)
Punjab	874 (37.8)
Haryana	579 (25.1)
Chandigarh	487 (21.1)
Himachal Pradesh	185 (8.0)
Uttar Pradesh	77 (3.3)
Jammu and Kashmir	39 (1.7)
Rajasthan	15 (0.6)
Bihar	10 (0.4)
Jharkhand	4 (0.2)
Uttarakhand	27 (1.2)
Chhattisgarh	2 (0.1)
Delhi	9 (0.4)
Gujarat	2 (0.1)

Tormey *et al.*[13] observed a prevalence of RBC alloantibodies as 2.4% in a population of male military veterans. It was a relatively higher prevalence despite the fact that, in their study population, there was a male predominance and a lack of pregnancy-related alloimmunization. They hypothesized that the stimulus for alloimmunization in this population could be the combat-related transfusion. Ameen *et al.*[16] also found that the prevalence of RBC alloantibodies was quite high in their blood donors (2.3%), whereas it was 0.49% in Kuwaiti general population with frequency of alloantibodies being three times higher in females as compared to males. Zhu *et al.*[15] too found a higher frequency of alloantibodies in female donors with overall prevalence of 0.279% in donors of the Shaoguan area. The authors recommended that antibody screening must be performed for all female donors to ensure the safety of the corresponding plasma recipients like in case of massive transfusion and infants.

Among the studies done on blood donors in India, Pahuja *et al.*[7] (Delhi, 2012) reported a prevalence

Table 2: Studies on frequency of alloantibodies in blood donors

Authors	Year	Region/country	Sample size	Frequency (%)	Antibody specificity (ies)
Keokhamphoui *et al.*[11]	2014	Laos	1181	3.2	Anti-P1, -Le^a, -Le^b, -M, -P1, -Le^a, -Le^{a+b}
Promwong *et al.*[12]	2013	Thailand	65,781	0.7	Anti-Le^a, -Mi^a, -Le^b, -P^1
Tormey *et al.*[13]	2008	Yale, New Haven, USA	18,750	2.4	Anti-K, -E, -D, -Le^a, -Fy^a, -c, -C, -P^1, -Jk^a, -Le^b
García *et al.*[14]	2012	Columbia	60,539	0.73	Anti-Le^a, -Le^b, -D, -E, -K, -M, alloantibodies against low frequency antigens
Zhu *et al.*[15]	2007	Shaoguan area, China	15,033	0.279	Anti-D, -E, -cE, -C, -Le
Ameen *et al.*[16]	2005	Kuwait	179,045 (donors and patients)	0.49 (overall); 2.3 in donors	Anti-D, -E, -K, -Le^a, -Le^b (overall)
Garg *et al.*[8]	2014	Delhi, India	47,450	0.09	Anti-N, -M, -D, -Le^a, -E+K, -E, -S, -C, -c, -E+K +S, -Le^a, -Le^b, -K, -C^w
Tiwari *et al.*[9]	2014	Haryana, India	31,367	0.009	Anti-K, -M
Pahuja *et al.*[7]	2012	Delhi, India	7756	0.05	Anti- C, -Le^a, autoantibody
Present study	2015	North India	2310	0.043	Anti-M

of 0.05% (4/7756). The alloantibody specificities in these 4 donors were anti-C (in 2) and anti-Le^a (in 1), while the fourth donor had an autoantibody. All of them were male donors. One of the donors with anti-C (titer: 4) had a history of transfusion 4 years back, and the alloantibody was reactive at 37°C in AHG phase, while the anti-Le^a was IgM type (titer: 1) with lack of reactivity at 37°C, thus clinically not significant. Garg *et al*.[8] (Delhi, 2014) screened 47450 donors and found a prevalence of 0.09% (46 donors). The frequency of alloantibodies was higher in females (male-to-female ratio of 8.25:1) and was statistically significant ($P < 0.0001$). Amongst the five alloimmunized females, three had a history of single uncomplicated pregnancy. They found that the alloantibodies to MNS blood group system (47.8%; 22/46) was the most common followed by those of Rh blood group system (39.1%; 18/46). In an another study by Tiwari *et al*.[9] (2012), the cumulative incidence of RBC alloantibodies was 0.12% and 0.009% among patients (n = 32,560) and donors (n = 31,367), respectively. In donors, anti-M was the most common (66.6%) alloantibody.

The frequency of alloantibodies in the present study is low as compared with studies done in the West but similar to those reported from the studies in North India. The reasons for this could be multifactorial. Most of the studies from India had a lower percentage of female donors, as was in our study as well (0.48% females), who are more likely to be alloimmunized, possibly due to previous pregnancy(ies). Female donors who qualify for donation and may have a history of pregnancy tend to have received transfusions, and the incidence of alloantibodies in them is higher.[4,17] The present study also included a heterogeneous group, with donors being included from 12 states in North and North West India.

Strength and limitations of the study

Although the sample size was small, the majority (2125/2310; 92%) of the donors in this study were representative of the donor population of this region of North India. Nevertheless, the prevalence of irregular antibodies was similar to those reported in other studies from our country. However, the sample size in our study was not large enough to reflect the true prevalence of alloantibodies in blood donors of our country as more heterogeneous population across various regions needs to be included for determining the same.

Conclusion

The prevalence of RBC alloantibodies was found to be 0.043% (1/2310) in our donor population. Thus, it appears that the overall prevalence of irregular antibodies in donors is quite low in our region of the country. However, further studies from different regions of the country are required for knowing the true prevalence, which would also help to derive policy to prioritize the available resources across blood centers in the country.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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