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Prevalence of red blood cell antibodies in whole blood donors: A single-centre experience in north India

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***Background & objectives*: Blood transfusion therapy involves multiple steps to ensure selection of safe blood component for transfusion. This includes testing for infectious markers, full ABO compatibility, free from any clinically significant red cell antibodies and acceptable donor's red cell survival rates without destruction of recipient's red cells. The red cell antibodies present in healthy blood donors can cause severe haemolytic transfusion reaction, especially in massive blood transfusion recipients and paediatric patients. Hence, screening of red cell antibodies in donor blood is important to provide compatible blood products and to avoid haemolytic transfusion reactions in susceptible patient population. This study was planned to assess prevalence, aetiology and type of unexpected red cell antibodies in a large number of whole blood donor population in north India.**

***Methods*: This three-year prospective observational study included blood donor samples for antibody screening from January 2015 to December 2017. A total of 166,803 healthy blood donors including 156,128 (93.6%) males and 10,675 (6.4%) females were screened.**

***Results*: The prevalence of red cell antibodies was 0.17 per cent in our donor population. Of the total 286 donors with red cell antibodies, 248 (86.7%) had alloantibodies, 30 (10.5%) had autoantibodies and eight donors (2.8%) showed positive antibody screening with inconclusive results.**

***Interpretation & conclusions*: Alloimmunization to red cell antigens is a challenging task for current transfusion practices. The antibody screening in blood donors may improve the quality and safety of blood transfusion in the recipients. It also reduces the risk of complications from incompatible blood transfusions.**

Key words Alloantibody identification - antibody screening - blood transfusion safety - haemolytic transfusion reaction - red blood cell panel - red cell alloimmunization - whole blood donors

The International Society of Blood Transfusion[1] recognizes 360 red cell antigen specificities, of which 322 belong to one of the 36 blood group systems. A single gene or cluster of two or three closely-linked homologous genes represents each blood group system. There are four categories of blood group antigens: blood group systems (322 antigens in 36 blood group systems); 700 series low prevalence (17 antigens); 901 series high prevalence (7 antigens) and 200 series collections (14 antigens in 5 collections)[1]. Normally, anti-A and anti-B are the only red cell antibodies present in human plasma and known as

naturally occurring antibodies. All other antibodies are known as unexpected red cell antibodies because these require immune exposure for development (pregnancy, transplant or blood transfusion)[2]. The clinical significance of an unexpected antibody must be assessed. It is defined as an antibody associated with haemolytic transfusion reaction or with haemolytic disease of foetus and newborn or with shortened red cell survival post transfusion. Screening of donated blood for the presence of unexpected red cell antibodies has been laid down in the guidelines of the National Blood Policy, India[3]. The prevalence of red cell alloantibodies varies in males and females (multiple pregnancies) and individuals with past history of transfusion and/or transplant. Red cell alloantibodies have been found in up to four per cent of blood donors[4-11]. Among patient populations, the prevalence of alloantibodies has been reported to be 2-9 per cent in multitransfused patients and 9-40 per cent in sickle cell anaemia and thalassaemia patients[12-14]. The alloantibodies can occasionally cause severe transfusion reaction as in case of massive transfusions and paediatric patients if a large quantity of plasma is transfused. Thus, screening of red cell antibodies in donor blood is important to provide compatible blood products and to avoid haemolytic transfusion reactions. Only packed RBCs (PRBCs) should be transfused when red cell antibodies are found, as these antibodies present in plasma are capable of causing immune-mediated haemolysis in recipients[15]. With this background, this study was planned to analyze prevalence, aetiology and type of unexpected red cell antibodies in general donor population at a multispeciality hospital in north India.

Material & Methods

This prospective observational study was conducted in the department of Transfusion Medicine, King George's Medical University, Lucknow, India, after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all blood donors included in the study. All the blood donors were screened after filling blood donor questionnaire form. This form included basic profile of donors (name, age, sex, address, *etc*.), any history of blood transfusion, trauma, surgery, drug intake, jaundice, high risk behaviour and any other clinically relevant illness. From all female blood donors, detailed gynaecological and obstetrical history was taken. Blood donor samples for red cell antibody screening were tested from January 2015 to December 2017. During the study period, a total of 166,803 healthy blood donors including 156,128 (93.6%) males and 10,675 (6.4%) females were screened for the presence of red cell antibodies.

Initially, antibody screening of all blood donors was performed using commercially available single vial of two pooled donors on a fully automated platform [Diagast, qwalys, hema-screen, a fully automated walk-away random access immunohaematology analyzer based on erythrocyte magnetized (EM) technology, 251 Avenue Eugène Avinée, France]. Positive serum samples were further investigated to identify their specificity by commercially available three and 11 red cell panels along with auto-control (Diagast, Hemaident, Avenue Eugène Avinée, France) as per the manufacturer's instructions. These pre-magnetized panels expressed homozygous antigen profiles of 23 clinically significant antigens including Rh, Kell, Kidd, Duffy, Lewis, MNS, Lutheran, PI blood group systems. The antigens in 11 cell panel were D, C, E, c, e, C^w, K, k, kp^a, kp^b, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P1, M, N, S, s, Lu^a and Lu^b. Antibody detection results were considered positive depending on the reactivity observed in the form of agglutination or haemolysis. The clinical significance of an antibody was determined by its reactivity at different temperature (4, 22 and 37°C, thermal amplitude) and at different phases such as normal saline or in antihuman globulin serum (AHG) phase. The antibodies reacting at 37°C or in AHG phase were considered clinically significant. In the present study, antibodies against M, N, Le^a, Le^b and P1 were not clinically significant as were of IgM type, reacting in saline phase and not reacting at 37°C or in AHG phase. A total of 120 alloantibodies belonged to the above blood groups. The rest 128 alloantibodies were clinically significant and reacted at 37°C. The antibody specificity was confirmed by using PRBCs of having positive homozygous expression of RBC antigens. The donor's antigen phenotype and the probability of antibody positivity were also taken into account for the final interpretation of results. A direct antiglobulin test (DAT) was performed in all cases with positive auto-control. Adsorption and elution were performed according to the American Association of Blood Banks technical manual[2] with autoantibody positive donors to rule out any underlying alloantibody. However, no alloantibody was found. As per our institutional policy and standard operating procedure to handle these blood units, the plasma and platelet concentrate of blood donors positive for clinically significant alloantibodies were discarded and PRBC was given to ABO- and Rh-matched recipients. Thus, 128 plasma

units and 120 platelet concentrates were discarded. If a unit had autoantibody (positive DAT and auto-control), no blood components were prepared and the unit was discarded as a whole blood.

Statistical analysis: The data were collected from blood bank records. Data processing was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Chi-square test was used for statistical analysis and $P<0.05$ was considered significant. Odds ratio was also calculated to assess the increased likelihood of alloimmunization in female blood donors as compared to male blood donors.

Results

Antibody screening was done in 166,803 healthy blood donors including 156,128 (93.6%) male and 10,675 (6.4%) female donors during the study period. Of these, 31 per cent (n=51,709) were voluntary donors and 69 per cent (n=115,094) were related or replacement donors with age groups ranging from 18 to 65 years. Antibody screening results were positive in 286 cases including 244 male donors and 42 female donors. The overall prevalence of red cell antibodies was 0.17 per cent. Of the total 286 cases, 248 donors (211 males and 37 females *i.e.*, 86.7%) had alloantibodies, 30 donors (26 males and 4 females *i.e.*, 10.5%) had autoantibodies and eight (7 males and 1 female *i.e.*, 2.8%) showed positive antibody screening with inconclusive results (Table I). Among immunized donor group (286), 16 (11 females and 5 males) had a history of previous blood transfusions. In non-immunized donor group (166,517), none had or gave past history of blood transfusion. Thirty nine (39/42) and 688 female donors (688/10,633) had a history of pregnancies in immunized and non-immunized groups, respectively (Table II). No donor had both allo- and autoantibodies. The rate of alloantibodies formation in males was 0.14 per cent (211/156,128) and in female blood donors rate of alloantibodies formation was 0.35 per cent (37/10,675). The frequency of alloantibodies was significantly higher in female blood donors [0.35% OR: 0.40; 95% confidence interval (CI): 0.29-0.55, $P<0.001$] compared to male blood donors. Thus, the most common aetiology for red cell antibody formation was pregnancy followed by blood transfusion and of unknown aetiology in many of the blood donors in the study. For inconclusive antibody screening results, the reason could be very low titter, low affinity, in developing stage or may be directed against the antigens not provided in the identification panel.

Antibodies against the MNS blood group system were most common, followed by Rh system. Among the MNS blood group system, the most common alloantibody identified was anti-M (20.5%) followed by anti-N (11.0%). In the Rh blood group system, the most common antibody was anti-D (7.2%) followed by anti-E (4.0%) (Table III).

Discussion

The overall prevalence of red cell antibodies was 0.17 per cent, and the frequency of alloimmunization was 0.15 per cent. Among male donors, the frequency of alloimmunization was 0.14 per cent (211/156,128) and in female blood donors, the frequency of

Table I. Profile of normal and red cell antibody positive whole blood donors

Blood donors	Male (%)	Female (%)	Total
Total whole blood donors	156,128 (93.6)	10,675 (6.4)	166,803
Alloantibody-positive donors	211 (85.0)	37 (15.0)	248
Autoantibody-positive donors	26 (86.6)	4 (13.4)	30
Donors with inconclusive results	7 (87.5)	1 (12.5)	8

Table II. Donor characteristics with positive antibody screening results (n=286)

Donors	n (%)
Gender	
Male	244 (85.3)
Female	42 (14.7)
ABO blood group	
A	70 (24.5)
B	102 (35.7)
AB	22 (7.7)
O	92 (32.1)
Rh blood group	
D positive	264 (92.3)
D negative	22 (7.7)
History of blood transfusion	
Male	5/244 (2.0)
Female	11/42 (26.2)
History of pregnancy in female donors	39/42 (92.8)

Table III. Frequency of red cell alloantibodies in whole blood donors among different blood group systems

Blood group system	Alloantibody	Frequency (%)	Antibody specificity in male donors with history of blood transfusion	Antibody specificity in male donors without history of blood transfusion	Antibody specificity in female donors with pregnancy and blood transfusion	Antibody specificity in female donors with a history of pregnancy but no blood transfusion
MNS	Anti-M	51 (20.5)	-	51	-	-
	Anti-N	28 (11.0)	-	28	-	-
	Anti-S	8 (3.2)	-	6	1	1
Rh	Anti-D	18 (7.2)	2	3	4	9
	Anti-C	8 (3.2)	-	2	1	5
	Anti-E	10 (4.0)	-	9	-	1
	Anti-C^w	9 (3.7)	-	8	-	1
	Anti-c	6 (2.6)	-	4	-	2
Kidd	Anti-Jk^a	10 (4.0)	-	9	1	-
	Anti-Jk^b	11 (4.4)	1	8	-	2
Duffy	Anti-Fy^a	13 (5.3)	1	12	-	-
	Anti-Fy^b	10 (4.0)	-	9	-	1
Kell	Anti-K	10 (4.0)	1	7	1	1
	Anti-Kp^b	7 (2.9)	-	6	-	1
Lutheran	Anti-Lu^b	5 (2.1)	-	5	-	-
P	Anti-P1	10 (4.0)	-	10	-	-
Lewis	Anti-Le^a	23 (9.3)	-	23	-	-
	Anti-Le^b	8 (3.2)	-	6	-	2
Multiple antibodies	Anti-D+K	2 (0.9)	-	-	2	-
	Anti-D+E	1 (0.5)	-	-	1	-
	Total	248	5/211	206/211	11/37	26/37

alloimmunization was 0.35 per cent (37/10,675). The higher rate of alloimmunization in females could be attributed to antigenic exposure during pregnancies. Other studies on healthy blood donors showed frequency of alloimmunization from 0.05 to 4.0 per cent[4-11]. In our study, most frequent alloantibodies identified were of MNS blood group system. M and N antigens are the oldest blood antigens known after the ABO system[16]. Rare examples of anti-M and anti-N have been reported in the literature to cause immediate or delayed haemolytic transfusion reactions and severe haemolytic disease of the foetus and newborn (HDFN)[16]. Anti-M is a common antibody usually cold-reacting IgG and is enhanced by serologic testing in an acidic environment[16]. Thus antibodies to antigens in the MNS blood group system are mostly naturally occurring, usually do not react at 37°C, and can be overlooked during pre-transfusion testing. In Rh blood group system, Rh (D) antigen is the most potent immunizing antigen after A and B blood group antigens and capable of causing clinically significant HDFN[17]. In our study, the most frequent antibody other than D was anti-E (4.0%) in Rh blood group system. Anti-E does not usually cause HDFN, and if does so, it is mild in nature[17]. Lewis antibodies are naturally occurring and usually IgM in nature, and reactive at or below room temperature. Lewis antibodies may be clinically relevant if causes *in vitro* haemolysis during serologic testing and in such cases antigen-negative blood should be selected for transfusion. One study conducted among healthy blood donors in Delhi, showed a prevalence of 0.09 per cent and the most frequent alloantibodies identified were from the MNS blood group system[5]. A study conducted in Southern Thai population[8] showed

the following antibodies, frequency-wise as most common - anti-Mi^a, anti-E, anti-Le^a, anti-c and anti-Le^b. In the present study, only 30 donors (0.018%) had autoantibody as also reported earlier by Kaur *et al* (0.5%)[6] and Tiwari *et al* (0.04%)[18]. In the present study, though we took detailed history about the aetiology of antibody formation, many alloantibodies were found in male blood donors. It has also been reported that in some cases the immune antibodies are found in non-transfused, healthy, male blood donors[19].

Alloimmunization to red cell antigens is a challenge being faced in transfusion practices. The antibody screening in blood donors as well as in multi-transfused patients is necessary to improve the quality and safety of blood transfusion in the recipients. Additional large-scale studies are required to assess the need of antibody screening in healthy blood donors and to assess the clinical significance of the same.

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Conflicts of Interest: None.

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