



PREVALENCE OF IRREGULAR ANTIBODIES IN BLOOD DONORS IN A TERTIARY CARE HOSPITAL- A ONE YEAR STUDY

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ABSTRACT

Introduction: Most of the irregular antibodies in daily transfusion practices are directed towards the Rh (D,C,E,c,e), Kell (K), Fy (Fya,Fyb), Jk (Jka, Jkb) and MNS (M,N,S,s) blood group systems. Knowledge about the prevalence of these red cell antibodies among blood donors is very important to minimize the risk of alloimmunization.

Aims and Objectives: The study was conducted to estimate the prevalence of irregular antibodies among blood donors.

Material and methods: This was a prospective study carried out over a period of one year with effect from June 2013 to June 2014 in the Department of Blood Transfusion and Immunohaematology of Sher-e- Kashmir Institute of Medical Science. It comprised of voluntary and replacement donors of SKIMS Hospital blood bank who donated blood during the above mentioned period. Donors who fulfill inclusion criteria specified by Departmental SOPs were selected and written informed consent was taken from them. Ethical clearance was obtained from institutional ethical committee.

Results: A total of 11700 blood samples from the donors of all blood groups were typed for the presence of irregular antibodies. The detection was done by indirect coomb's test by conventional tube technique. This study included 11,466 (98%) males and 234 (2%) females. A total of 28 (0.24%) donors were tested positive for irregular antibodies. Among these 26(92.8%) were males and 2(7.14%) were females. Maximum blood donors who had irregular antibodies were in the age group of 18-30 years. O positive blood group donors (28.5%) have more irregular antibodies followed by B positive(25%), A positive (21.4%), AB positive(7.14%), A negative(7.14%), O negative(3.57%), B negative (3.57%) and AB negative (3.57%).

Conclusion: Antibody screening and identification combined with extended phenotyping of blood can help in creating donor data bank and providing proper antigen compatible blood for patients with multiple alloantibodies and also reduce the risk of RBC antigen alloimmunization along with their complications like hemolytic transfusion reactions, hemolytic disease of fetus and newborn.

KEYWORDS : Hemolytic disease of fetus and newborn, Alloimmunization, Irregular Antibodies.

INTRODUCTION

Discovery of the ABO blood group system by the Austrian pathologist Karl Landsteiner together with his colleagues Von Decastello and Sturli, and the introduction of ABO blood grouping tests by Reuben Ottenberg in 1911 greatly reduced the fatalities associated with blood transfusion in early days of transfusion therapy.^(1,2,3) After the introduction of indirect antiglobulin test by Coombs in 1945, which added a new dimension to the safety of blood transfusion, there was a rapid increase in the identification of alloantibodies that causes transfusion reactions or haemolytic disease of the newborn. This has led to the discovery of 245 blood group antigens classified in 29 blood group systems and 38 high and low frequency antigens not yet fulfilling the requirements for classification into the system.^(4,5) Alloimmunisation occurs when an incompatible antigen introduced in an immunocompetent host evokes an immune response. Most of the irregular antibodies are directed towards the Rh (D,C,E,c,e), Kell (K), Duffy (Fya,Fyb), Kidd (Jka,Jkb) and MNSs (M,N,S,s) blood group systems. Of these, D antigen is most immunogenic, resulting in more than 80% of immunocompetent D negative persons becoming alloimmunized after a transfusion of D positive erythrocytes.⁽⁶⁾ The main mechanism for alloimmunization involves the presentation of the donor antigen peptides by APCs to the T-cell receptor (TCR) on recipient CD4 T cells (α T-cell dependent response). The phenomenon of alloimmunization can lead to several problems varying from delay in getting compatible blood to delayed haemolytic transfusion reactions. Knowledge of frequency of irregular antibodies in a population is very helpful in providing antigen negative compatible blood to patients with multiple alloantibodies.

AIMS AND OBJECTIVES:

The aim of our study was to estimate the prevalence of irregular antibodies among blood donors at SKIMS Blood bank.

MATERIAL AND METHODS:

This was a prospective study carried out over a period of one year with effect from June 2013 to June 2014 in the Department of Blood Transfusion and Immunohaematology of Sher-e-Kashmir Institute of Medical Science. This study comprised of voluntary and replacement donors of SKIMS Hospital blood bank who donated blood during the above mentioned period. Donors who fulfilled the inclusion criteria specified by Departmental SOPs and national guidelines were selected and written informed consent was taken from them. Samples from blood donors were collected. About 3 ml of blood sample was taken in anticoagulant vial at the time of blood collection for blood phenotyping and irregular antibody screening. ABO, Rh D blood grouping and screening was done by conventional tube technique. The Rh D negative donors were further confirmed by doing weak D testing. Irregular antibody screening was done by indirect antiglobulin technique.

RESULTS

A total of 11700 blood samples from the donors of all blood groups were typed for the presence of irregular antibodies. The detection was done by indirect coomb's test by conventional tube technique. This included 11,466 (98%) males and 234 (2%) females. A total of 28 (0.24%) donors were tested positive for irregular antibodies. Among these 26(92.85%) were males and 2(7.14%) were females (Table 1).

Table 1. Gender distribution of Blood Donors and prevalence of Irregular antibodies

Gender	Number (N)	Percentage(%)	Prevalence of irregular antibodies(N)	Percentage (%)
Males	11,466	98	26	92.85%
Females	234	2	2	7.14%
Total	11,700	100	28	100

Out of total donors, 10647 (91%) were replacement donors and 1053(9%) were voluntary donors(Table 2)

Table 2. Distribution according to type of blood donors

Type of donor	Number (N)	Percentage(%)
Replacement donor	10647	91
Voluntary donor	1053	9
Total	11700	100

Age of the donors ranges from 18-60 years with a mean age of 25 years. Maximum donors were in the age group of 18-30 years 5616(48%) followed by 31-45 years 4095(35%) and 46-60 years 1989(17%). Irregular antibodies prevalence was more among age group 18-30 years (64.28%) (Table 3).

Table 3. Age wise Distribution of blood donors and prevalence irregular antibodies

Age (years)	Number (N)	Percentage (%)	Prevalence of irregular antibodies(N)	Percentage(%)
18-30	5616	48	18	64.28
31-45	4095	35	9	32.14
46-60	1989	17	1	3.57
Total	11700	100	28	100

In our study, 76% donors were first time donors and only 24% were repeat donors (Table 4).

Table 4. Distribution of blood donors as per history of previous donation

Donors	Number(N)	Percentage(%)
First time donors	8892	76
Repeat donors	2808	24
Total	11700	100

Frequency of O positive blood group was more in our study compared to other groups i.e 34% followed by B positive 30.8%, A positive 21.8%, AB positive 6%, O negative 2.7%, B negative 2.5%, A negative 1.3% and AB negative 0.9% (Table 5).

Table 5. Blood group wise distribution of donors and prevalence of irregular antibodies

Blood group	Number (N)	Percentage (%)	Prevalence of irregular antibodies(N)	Percentage (%)
A +	2551	21.8	6	21.4
A-	152	1.3	2	7.14
B+	3604	30.8	7	25
B-	293	2.5	1	3.57
AB+	702	6	2	7.14
AB-	105	0.9	1	3.57
O+	3978	34	8	28.5
O-	315	2.7	1	3.57

O positive blood group donors (28.5%) have more irregular antibodies followed by B positive(25%), A positive (21.4%), AB positive(7.14%), A negative(7.14%), O negative(3.57%), B negative (3.57%) and AB negative (3.57%).

DISCUSSION

Red cell alloimmunization results from the genetic disparity between red cell antigens of the donor and recipient or from mother to fetus.⁽⁷⁾Transfusion of incompatible blood may lead

to an immune mediated haemolytic transfusion reaction, and to avoid such cases, pretransfusion compatibility testing is carried out.^(8,9)The steps of pretransfusion testing involve reviewing the acceptability of blood sample, checking the ABO group and Rh D type, antibody screening tests, determining the specificity of antibodies detected unexpectedly, choosing antigen negative compatible unit for the recipient.⁽⁹⁾The intention of improving blood safety by performing antibody screening in all prospective patients is a great step towards reducing adverse reactions caused by transfusion .⁽¹⁰⁾However, the use of screening cells prepared from foreign donors still leaves a possibility where the antibodies especially the ones against minor antigens may go undetected.⁽¹¹⁾

Alloimmunization complicates the transfusion therapy due to difficulty in getting the compatible blood for transfusion which increases the risks of additional alloantibody and autoantibody formation further leading to haemolytic transfusion reactions, haemolytic disease of fetus and newborn and life threatening hyperhemolysis syndrome.^(12,13,14,15) The rate of alloimmunization in our general donor population was 0.24%. The reason for this could be that our study population comprised general donor population and not the high risk groups like multitransfused patients.

In our study, overall rate of alloimmunization among donors was 0.24%. 92.85% donors who had alloantibodies were men and 7.15% were women. On the other hand, considering the total number of males and females tested, 26 out of 11466 men (0.23%) and 2 out of 234 women (0.85%) had alloantibodies. A higher rate of alloimmunization was observed in females than in males.A similar observation was made by Hoeltge et al⁽¹⁶⁾. Rh and Kell antibodies have been reported to be the most common clinically significant alloantibodies.^(17,18)Garg N et al⁽¹⁹⁾ conducted a study on voluntary blood donors in Delhi population and showed a prevalence of 0.09% of alloantibodies in their serum and also showed a higher prevalence of alloantibodies in females than in males similar to our study. A similar study was conducted by Pahuja S et al⁽²⁰⁾ and found that the prevalence of alloantibodies was 1.25%. Pahuja S et al⁽²¹⁾ conducted a study on 7756 healthy blood donors and a total of 4 donors (0.05%) showed presence of alloantibodies in their serum and all were males. Zhu et al⁽²²⁾ in 2007 found a prevalence of 0.279% among donor in Shaoguan area. The frequency of irregular antibodies in females was higher than that of males similar to our study. The reported incidence of erythrocyte alloantibodies in the donor population varies from 0.32% to 2.4%.^(22,23,24,25,26,27)(Table 6)

Table 6. Comparison of studies on prevalence of alloimmunization among blood donors

Studies	Frequency (%)
Sallander et al ⁽²⁸⁾	0.7%
Myhre et al ⁽²³⁾	0.34%
Giblett ⁽²⁴⁾	0.32%
Winter et al ⁽²⁵⁾	0.89%
Ameen et al ⁽²⁶⁾	General population: 0.49% Blood donors:2.3%
Zhu et al ⁽²²⁾	0.279%
Tormey et al ⁽²⁷⁾	2.4%
Pahuja et al ⁽²¹⁾	0.05%
Present study	0.24%

The frequency of irregular alloantibodies in our study population is lower than most of the studies except study conducted by Pahuja et al. Female donors have higher percentage of irregular alloantibodies considering total number of females as denominator than males similar to other studies conducted by Ameen et al, Zhu et al, and Giblett who

also found a significant higher percentage in female donor group. This might be due to past pregnancies.

Prevalence of irregular antibodies can be of significance if large amount of plasma is to be transfused as in massive transfusion and in pediatric patients as the plasma components has the potential to cause immune hemolysis in recipients. It is recommended that only packed red blood cells should be issued and transfused when irregular antibodies are found in the blood.

In recent years, medical tourism to India has increased largely due to affordable medical facilities available here. In context of Transfusion Medicine, this trend would mean that a large number of international patients would be receiving blood transfusion from Indian donors. The differences observed in antigen frequencies among different ethnic groups can lead to development of irregular antibodies.

Although our tertiary care centre caters patients from all across the state but most of the donors were from the North of the state. The gene pool of people from other parts of the state may be different so a multicentric study in hospitals located in different regions should be carried out that would be valuable to provide information regarding the frequencies of various blood group antigens. Antibody screening and if required identification combined with extended phenotyping of donors should be carried out to provide antigen negative compatible blood to the recipients.

CONCLUSION

Provision of safe blood for transfusion does not imply only testing for infectious markers, but also protection from haemolytic transfusion reactions that results from transfusion of blood that has antibodies against the red cell antigens. Thus alloimmunization due to foreign RBC antigens or antibodies is a life threatening adverse effect of blood transfusion.

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Nil

Conflict of interest

None

REFERENCES

- Landsteiner K. Ueber Agglutinationserscheinungen normalen menschlichen Blutes. *Wien Klin Wochenschr* 1901; 14:1132-1134.
- Von Castello A and Sturli A. Uber di iso-agglutinine im serum gesunder und kranker menschen. *Munch Med Wochenschr* 1902;49:1090-1095.
- Ottenberg R. Studies on isoagglutination. Transfusion and the question of intravascular agglutination. *J Exp Med* 1911;13:425-438.
- Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and incomplete Rh agglutinins. *Br J Exp Pathol* 1945;26:255-266.
- Daniels GL, Fletcher A, Garratty G et al. Blood group terminology from the international society of blood transfusion committee on terminology for red cell surface antigens. *Vox Sang* 2004;87:304-316.
- Levine P, Stetson RE. An unusual case of intragroup agglutination. *JAMA* 1939;113:126-127.
- Schonewille H. Red blood cell alloantibodies after transfusion. Leiden University Press ;2008.
- Roback JD, Combas MR, Grossman BJ et al. American association of Blood Banking, Technical Manual 16th edition; 2008.
- Shulman IA, Maffei LM, Downes KA. North American pretransfusion testing practices, results from the college of American pathologists. Interlaboratory comparison program survey data. *Arch Pathol Lab Med* 2005;129:984-9.
- Wallis JP. Is it time to give up the cross match. *J Clin Pathol* 2000;53:673-5.
- Oberman HA, Barnes BA, Steiner EA. Role of cross match in testing for serological incompatibility. *Transfusion* 1982;22:12-6.
- Eder AF, Chambers LA. Noninfectious complications of blood transfusion. *Arch Pathol Lab Med* 2007;131:708-18.
- Schonewille H, Van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusion in a non haematological alloimmunized patient cohort: is it time to take precautionary measures. *Transfusion* 2006;46:630-5.
- Schonewille H, Haak HL, Van Zij IAM. Alloimmunization after blood transfusion in patients with haematological and oncological diseases. *Transfusion* 1999;39:763-71.
- Zumberg MS, Procter JL, Lottenberg R et al. Autoantibody formation in the alloimmunised red blood cell recipient and laboratory implications. *Arch Intern Med* 2001;22:285-90.
- Hoeltge GA, Domen RE, Rybicki LA et al. Multiple red cell transfusions and alloimmunization. Experience with 6996 antibodies detected in a total of 159,262 patients from 1985-1993. *Arch Pathol Lab Med* 1995; 119:42-45.
- Singer ST, Wu V, Mignacca R et al. Alloimmunization and erythrocyte alloimmunization in transfusion dependent thalassemia patients of predominantly Asian decent. *Blood* 2000;96:3369-73.
- Pahuja S, Pujani M, Gupta SK et al. Alloimmunization and red cell alloimmunization in multitransfused thalassemics of Indian origin. *Hematology* 2010;15:174-7.
- Garg N, Sharma T, Singh B. Prevalence of irregular red blood cell antibodies among healthy blood donors in Delhi population. *Transfus Apher Sci* 2014;50(3):415-7.
- Pahuja S, Gupta SK, Pujani M et al. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. *Blood Transfus* 2011;9(4):388-393.
- Pahuja S, Kushwaha S, Sethi N et al. Screening of blood donors for erythrocyte alloantibodies. *Hematol* 2013;17(5):302-305.
- Zhu JY, Lan JC, Luo HQ. Screening analysis of irregular antibodies from random donor population in Shaoguan area. *J Exp Haematol* 2007; 15(3):630-1.
- Myhre BA, Greenwalt TJ, Gajewski M. Incidence of irregular antibodies occurring in healthy donor sera. *Transfusion* 1965;5:350-4.
- Giblett ER. Blood group alloantibodies: an assessment of some laboratory practices. *Transfusion* 1977;17:299-308.
- Winters JL, Pinedo AA, Gorden LD, Bryant SC, Melton LJ 3rd, Vamvakas EC, et al. RBC alloantibody specificity and antigen potency in Olmsted County, Minnesota. *Transfusion* 2001;41:1413-20.
- Ameen R, Eyaadi OA, Shemmari SA, Chowdhury R, Bashir AA. Frequency of red blood cell alloantibody in Kuwaiti population. *Med Princ Pract* 2005;14:230-4.
- Tormey CA, Fisk J, Stack G. RBC alloantibody frequency, specificity and properties in a population of male military veterans. *Transfusion* 2008;48:2069-76.
- Sallander S, Shanwell A, Aqvist M. Evaluation of a solid phase test for erythrocyte antibody screening for pregnant women, patients and blood donors. *Vox Sanguinis* 1996;71:221-5.