Original Article

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Pathology Section

Erythrocyte Alloimmunization and Autoimmunization among Blood Donors and Recipients visiting a Tertiary Care Hospital

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ABSTRACT

Introduction: The ultimate aim of pretransfusion testing is the acceptable survival of donor red cells in recipient's body and antibody detection plays a critical role in achieving the same. The cornerstone of antibody detection method is detecting an unexpected antibody as against the expected antibodies of ABO blood group system. Autoantibodies can also interfere with the detection of clinically significant alloantibodies.

Aim: To study the frequency of alloantibodies and autoantibodies in the healthy blood donors and patient population visiting our hospital.

Materials and Methods: The Column Agglutination Technology (CAT) was used for ABO RhD blood grouping, Direct Antiglobulin Test (DAT), Autocontrol (AC), Indirect Antiglobulin Test (IAT) and red cell antibody screening and the unexpected reactions in any of these tests were recorded for further evaluation. Ethylene Diamine Tetra Acetic Acid (EDTA) blood samples were used for all these tests for both blood donors and admitted patients. The CAT was exercised for the blood grouping (using ABD-Reverse Diluent cassettes) and antibody screening (using 0.8% Surgiscreen, Ortho Clinical Diagnostics Limited, USA and Low Ionic Strength Saline Ortho BLISS with AHG cassettes) on the automated immunohaematology platform ORTHO AutoVue® Innova system (Ortho Clinical Diagnostics Limited, USA).

Results: Among all blood donors (n=6350), seven (0.11%) donors had showed unexpected reaction. Of these, four had positive antibody screen (three having naturally occuring antibodies 2=anti-M, 1=anti-Le^a and 1=inconclusive) and the other three had positive DAT. Of all the patient samples (n=6136) screened for irregular red cell antibodies, four (0.06%) patients were found to have unexpected reaction revealing one (0.02%) with anti-M antibody and the other three (0.05%) had autoantibodies in their serum.

Conclusion: The combined prevalence for both blood donor and recipient population (n=12,486) was found to be 0.11% at our center. The alloimmunisation among patient population was found to be lower than many other studies worldwide as our hospital does not cater to multitransfused or transfusion dependant patients with haematological disorders and majorly elective surgery patients with no history of previous blood transfusions visit our hospital.

Keywords: Alloantibodies, Autoantibodies, Direct antiglobulin test, Red cell antibody screen

INTRODUCTION

Immunology and transfusion medicine histories interweave and efforts in both the fields have uncovered fundamental truths about each other as had been researched very early by Paul Ehrlich [1,2]. Most anti erythrocyte antibodies detected in the transfusion medicine practice are in humoral response to alloantigens encountered through previous exposures via transfusions, pregnancy, and transplantation, needle sharing, following injections of immunogenic material or due to some unknown immunogenic source [3]. Despite there being many foreign epitopes on essentially all transfused allogenic Red Blood Cells (RBC), transfusion is not highly immunogenic stimulus. In response to even multiple transfusions, alloimmunization to alloantigens on transfused RBCs has an overall frequency of approximately 2-6% [4-6]. Moreover, the generation of autoantibodies against self red cell antigens is also among the known autoimmune pathologies which occur due to loss of immunologic tolerance to self tissues. The process can result in a clinically silent autoagglutinin that is detected incidentally by a positive DAT during alloantibody screening but can also result in manifestation of autoimmune haemolytic anaemia if these autoantibodies promote red cell destruction.

Once exposed to an antigen, the immune system can develop antibodies termed as regular antibodies, generated against the antigens of ABO blood group system and irregular or unexpected when against other red blood cell blood group systems. Two types of irregular antibodies are there: alloantibodies and autoantibodies. The alloantibody by definition react only with the allogenic red cells carrying corresponding antigens and inversely the autoantibody reacts with an antigen on the subject's own red cells, whether or not any pathological effects are produced in vivo [7]. Irregular antibody screening test is therefore performed by transfusion centers for reducing the minor incompatibility. At our center, we evaluated all the blood donors and recipients for irregular antibodies who showed unexpected reaction in order to determine the prevalence of irregular red cell antibodies in the population of this region.

MATERIALS AND METHODS

A retrospective study was conducted in the Department of Transfusion Medicine at a tertiary care corporate hospital in North India from January 2013 to December 2015. We routinely perform ABO RhD blood grouping and 3-cell antibody screen on all the patients and donors visiting our hospital. DAT is not routinely done for the blood donors at our centre.

Sampling, routine testing and technology: EDTA blood samples were used for all the tests including ABO RhD blood grouping, DAT, IAT, AC and antibody screening in both blood donors and admitted patients. For antibody identification (ABID), the blood samples were sent to the Immunohaematology Reference Laboratory (at

Gurgaon, National Capital Region, India) maintaining the cold chain through out. The CAT was exercised for the blood grouping (using ABD-Reverse Diluent cassettes) and antibody screening (using 0.8% Surgiscreen, Ortho Clinical Diagnostics Limited, USA and Low Ionic Strength Saline Ortho BLISS with AHG cassettes) on the automated immunohaematology platform ORTHO AutoVue® Innova system (Ortho Clinical Diagnostics Limited, USA). Repeat testing and cross matching for patient's compatibility testing were done on semi-automated working station BioVue® (Ortho Clinical Diagnostics Limited, USA) with the use of respective cassettes. Unexpected reaction in any of these tests was recorded for further evaluation. The antibody titration and thermal amplitude were checked using conventional test tube technique. Informed consent and the history of previous blood donation, blood transfusion, pregnancy, medication and prolonged illness/hospitalization were obtained through blood donor questionnaire and patient transfusion requisition forms for donors and patients, respectively.

RESULTS

During the study period (January 2013 to December 2015), a total of 6,350 donors had donated whole blood and blood transfusion request was obtained from 6,136 patients. Of the total population studied, the prevalence of autoantibodies and alloantibodies in them was observed as 0.05% for each and the DAT positivity among blood donors was also observed as 0.05% [Table/Fig-1]. No unexpected reaction was observed in female blood donor or patient and all the 11 cases studied at our center were males.

The details of the 11 cases that showed unexpected reactions are as follows [Table/Fig-2]:

CASE 1: A 42-year-old male, first time, replacement blood donor, B RhD positive, IAT positive, positive 3-cell antibody screen (SC I, II, III - 2+, 2+, 1+), DAT and autocontrol negative, and ABID suggested anti-M antibody with dosage, IgG (1:4) and IgM (1:2) both, with wide thermal amplitude (4ºC-22ºC-37ºC). Appropriate antigen typing suggested donor was negative for 'M' red cell antigen. Enzyme treatment showed decreased reaction. The donor was advised to refrain from plasma donation (or can be shunted for plasma fractionation) in future and in case, he is ever warranted a red blood cell transfusion, then he must be transfused M antigen negative packed red cell unit.

CASE 2: A 46-year-old male, repeat, replacement blood donor, AB RhD positive, IAT positive, positive 3-cell antibody screen (SC I, II, III - N, 2+, N), DAT and autocontrol negative, and ABID suggested anti-Le^a antibody of both IgG and IgM type with titre 1:2 and thermal amplitude 22ºC -37ºC. Appropriate antigen typing suggested donor was negative for 'Le^a' red cell antigen. Special treatment- Neutralization showed decreased reaction. The donor was advised not to donate plasma in future (or can be shunted for plasma fractionation) and if red cell transfusion need arises, then to get Le^a antigen negative packed red cell transfusion only.

CASE 3: A 21-year-old male, repeat, replacement blood donor B RhD positive, IAT positive, positive 3-cell antibody screen (SC I, II, III - 2+, N, N), DAT and autocontrol negative, and ABID suggesting anti-M antibody of both IgG and IgM type with titre 1:2 and thermal amplitude 22ºC-37ºC. Appropriate antigen typing suggested donor was negative for M red cell antigen. Enzyme treatment showed decreased reaction. The donor was advised not to donate plasma (or can be shunted for plasma fractionation) in future and if need arises, to get M antigen negative packed red cell transfusion only.

CASE 4: A 22-year-old male, repeat, replacement donor, A RhD positive, with no history of blood transfusion, showed positive DAT (3+), IAT (3+), Autocontrol (3+) and panpositive (3+) 3-cell antibody

Cases	Male	Female	Total	Unexpected Reaction	ALA*	ALA Specificity	AUA** or DAT +	Non Specific Antibody
DONORS	6172 (97.2%)	178 (2.80%)	6350 (100%)	07 (0.11%)	03 (0.047%)	Anti-M (0.024%) Anti-Lea (0.008%)	03 (0.047%)	01(0.02%)
RECIPIENTS	4290 (69.91%)	1846 (30.08%)	6136 (100%)	04 (0.06%)	01 (0.016%)	Anti-M (0.016%)	03 (0.049%)	-

[Table/Fig-1]: Demographic data for the study population.
*ALA-alloantibody; ** AUA- autoantibody, DAT -Direct Antiglobulin Test

S.No.	Case	Age/ Sex	Blood Group	H/o BT	IAT	DAT	AC	A/B Screen	ABID	A/B Type	T.A (ºC)	Titers	Treatment
1	DONOR	42/M	B+	No	2+	Neg	Neg	2+/2+/1+	Anti-M	IgG, IgM	4-22-37	IgG-2 IgM-4	Enzymes- decreased reaction
2	DONOR	46/M	AB+	No	1+	Neg	Neg	N/2+/N	Anti-Le a	IgM, IgG	22-37	IgG-2 IgM-2	Neutralization- AB plasma
3	DONOR	21/M	B+	No	2+	Neg	Neg	2+/N/N	Anti-M	IgM, IgG	22-37	IgG-2 IgM-2	Enzymes- decreased reaction
4	DONOR	22/M	A+	No	4+	CAT † 3+ CTTNeg	CAT4+ CTT-Neg	CAT Pan3+, CTT ‡ Neg	ND	Incon clusive	-	-	
5	DONOR	50/M	B+	No	ND	1+		ND					
6	DONOR	22/M	B+	No	ND	2+		ND					
7	DONOR	26/M	O+	No	ND	2+		ND					
8	PATIENT	60/M	A+	No	3+	Neg	Neg	2+/2+/3+	Anti-M	IgG, IgM	22-37	IgG-2 IgM-4	
9	PATIENT	60/M	A+	No	1+	Neg	4+	Pan+ (4+)	Pan+	Cold agglutinin		1:128 (4º); 1:1(RT) [CTT]	
10	PATIENT	50/M	A+	No	1+	Neg	1+str.	Pan+ (1+str.)	PRA §	-			
11	PATIENT	80/M	B+	Yes	Neg	2+	2+	Neg	ND	ND	ND	ND	

[Table/Fig-2]: Cases- observations and results.
†- Column agglutination technology, ‡ - Conventional test tube technology, §-Pan reactive antibody; A/B- Antibody, T.A.-Thermal amplitude, DAT -Direct antiglobulin test, AC-Auto control, BT- Blood Transfusion, Str- strong

screen with column agglutinated technology. But, the conventional test tube technique showed negative DAT, IAT, Autocontrol and antibody screen (both IS and AHG). No clinically significant antibody was found and the donor blood components were quarantined in view of non specific reaction of donor blood with the gel card ingredients. The donor was suggested to get AHG compatible packed red cell unit in case a need of blood transfusion is warranted in future.

CASE 5-7: Three donors were incidentally found to be having positive DAT (1+, 2+& 2+) after showing incompatible crossmatch tests. Two of them were repeat whole blood donors while one was a first time donor. None of them had any history of previous blood transfusion in the past and none of them could visit us back for further investigations on recall. One of the donors, 50-year-old male is a regular voluntary blood donor with other blood bank in the city and currently his DAT is negative as confirmed telephonically from his last blood donation records.

CASE 8: A 60-year-old male patient, A RhD positive, was admitted with acute myocardial infarction with moderate LV dysfunction, Type 1 respiratory failure and anaemia (HB-6.9g/dl) under evaluation (HPLC awaited) with no history of previous blood transfusion. Upper gastro-intestinal endoscopy was done and patient was further posted for colonoscopy to rule out cause for anaemia and two units of packed red cells were requisitioned by the treating clinician. On crossmatching, it was found that one of the two units put for compatibility testing using CAT gel cards was incompatible. Patient's serum was further tested for the presence of any unexpected antibody wherein he had positive IAT (3+), positive 3-cell antibody screen (I, II, III –2+, 2+, 3+), DAT and autocontrol were negative, and ABID suggested presence of anti-M antibody in his serum. The patient's red cell and the compatible unit's phenotyping revealed absence of M antigen in both. The compatible unit was issued to patient and was transfused uneventfully. The patient was advised and was handed over an immunohaematological report informing about the presence of anti-M in his serum and he must receive M antigen negative packed red cell for blood transfusion need in future.

CASE 9: Another 60-year-old male patient, A RhD positive, admitted with coronary artery disease for CABG and four units each of packed red cells, Fresh Frozen Plasma (FFP) and Platelet Concentrates (PCs) were requested for this patient for the surgery. During compatibility testing, it was found that all the red cell units were incompatible with the patient's serum. Immunohaematological (IH) work up showed negative DAT, positive autocontrol (4+), panreactive (4+) antibody screen and ABID, indicating presence of an autoagglutinin which reacted strongly at 4ºC with titres of 1:128 (CTT) and thermal amplitude of 4ºC-30ºC while the autoantibody did not react at 37ºC. There was no evidence of alloantibody as ruled out by negative antibody screening after alloadsorption (R1R1, R2R2 and rr cells). Since the patient was posted for cardiac surgery demanding hypothermia, the surgery was with held and the patient was put on steroids by treating clinician to suppress the antibody titres as other modalities of treatment (intravenous immunoglobulin, rituximab or therapeutic plasma exchange) were denied by the patient. The patient's preoperative complete haematological profile was normal and there was no evidence of haemolysis. After four weeks of steroid therapy, the cold agglutinin titre came down to 1:2 and his haemoglobin was maintained at 12.5 gm/dl. The patient was planned up for cardiac surgery under normothermic conditions. The patient was operated using two units of best compatible A+ packed red cells postoperatively with no evidence of haemolysis and he was discharged in healthy condition.

CASE 10: A 50-year-old male patient, A RhD positive, admitted with fracture right tibia and fibula with haemoglobin of 12.0 gm/dl for open reduction under orthopaedic unit of our hospital. CAT showed incompatible crossmatch results with the PRBC units tested. IH work up showed negative DAT, positive autocontrol (1+strong) and panreactive (1+strong) antibody screen. The differential adsorption (using R1R1, R2R2 and rr cells) was performed and antibody screen and identification were negative with adsorbed plasma indicating absence of any alloantibody in patient's serum and confirming presence of only an autoagglutinin. The best compatible A+ PRBC was selected and reserved for the patient to be used for the surgery.

CASE 11: An 80-year-old male patient, B Rh D positive, admitted with carcinoma right maxillary sinus and underwent total maxillectomy with microvascular flap surgery and was hospitalized for more than two months postoperatively in view of wound complication and sepsis. He was transfused 13 units of PRBCs, 19 units of FFP, 33 units of random donor platelet concentrates and two Single Donor Apheresis Platelets (SDAP) during his course of hospitalization. Each time during his compatibility testing, antibody screen was negative and fully compatible units were issued and transfused. On one occasion, at day 60 of hospitalization, his DAT and AC were found to be positive but antibody screen was negative. Patient was being managed in intensive care unit and his blood culture was positive for *E. coli*, *Pseudomonas, Acenetobacter baumanni* and later for *Candida albicans* as well. He was on injection noradrenaline, vasopressin, sodabicarbonate, solumedrol, immunoglobin and culture sensitive antibiotic and antifungal drugs. He finally succumbed to death due to neutropenic sepsis with DIC.

DISCUSSION

The incidence of RBC alloimmunization depends largely upon the demography of the population studied. The prevalence of red cell allo- and autoantibodies has been reported in several study populations including hospital based patients, transfusion dependent patients with chronic haematological disorders, pregnant females and blood donors, and the incidence of alloantibodies detected worldwide is 0.2%-0.9% in healthy blood donors, 2%-9% in patients with a history of blood transfusion, 9%-30% among chronic transfusion dependant patients, 0.5%-1.9% among antenatal women and 0.5%-1% of red cell autoantibodies among transfused patients [8-14].

It is a topic of high debate as to what all red cell immunohaematological tests should be made mandatory for donor and patient testing as per serological testing is concerned. Opting out or mandating such tests clearly depend on the kind of population visiting a centre and the type of hospital setting one has. Previously transfused, multiple transfusions, transfusion dependent patients, multigravida female donors/patients obviate the need of mandating such tests. Ours is a corporate superspeciality hospital where male:female patient ratio is 2.3:1 and 67.81% of blood component consumption is by surgical units and 32.19% by medical specialities [15]. The prevalence of alloimmunization among blood donor population at our centre was 0.05% (n=3/6350) similar to Pahuja S et al., (0.05%), lower than Garg et al., and higher than Tiwari AK et al., [16-18]. Alloimmunization was less among patients (0.02%, n=1/6136) since our hospital does not cater to transfusion dependant patients with haematological disorders and majorly elective surgery patients with no history of previous blood transfusions are visiting.

Contrary to few studies in the past [19, 20] where female dominance was observed, the female blood donors at our centre constituted only 2.8% of all donors and none of them was immunized. As far as specificities of the antibodies were concerned, commonest was anti-M (0.024%) followed by anti-Le^a (0.008%) similar to findings as observed by Garg N et al., [17], unlike other studies where antibodies to Rh blood group system were most common than MNS and Lewis blood group systems [18-20]. All the cases had clinically significant naturally occurring alloantibodies with both IgM and IgG component, wider thermal amplitude and titres varying from 1:2 to 1:4. This clearly commands the use of AHG compatible corresponding antigen negative packed red cell unit in need of future transfusions for such candidates as recipients and discarding their plasma component as donors.

The prevalence of autoimmunization was 0.05% in our study population which is much lower than Makroo RN et al., and Cruz

RD et al., as 0.39% and 20% respectively [21,22]. Only one of the four patients with autoantibodies, needed blood transfusion who had cold agglutinin of wide thermal amplitude and was issued AHG crossmatched best compatible blood units after cardiac surgery. For cold antibodies, the distinction between harmless and harmful depends solely on the maximum temperature at which they are active. Cold autoantibodies that are harmless, because they are active only up to a temperature of about 25°C, may nonetheless be very troublesome in the laboratory, especially if tests are carried out at room temperature or the antiglobulin test is carried out in an albumin-containing solution or in a low-ionic-strength medium. The titre of normal autoagglutinins at 0–2°C does not usually exceed 64 using a tube technique with a 2% cell suspension and reading the results microscopically while our case had titre of 1:128 at 4ºC. It is a matter of great emphasis that the clinical significance of a cold antibody is determined wholly by its ability to combine with RBCs at, or near, body temperature rather than by its titre at some lower temperature [23].

The fact that an apparently normal donor has a positive DAT is often first discovered when the donor's red cells are used in crossmatching. We found three blood donors (0.05%,n=3/6350) whose donated blood units were found incompatible during AHG phase compatibility testing despite patient's negative antibody screen. The donors DAT (polyspecific; IgG and C3D both) were positive, and as per our institutional policy, those three blood units were discarded. In view of potential risk of haematological malignancies among blood donors with positive DAT as demonstrated by Rottenberg Y et al., donors must be called for follow up at regular intervals as DAT positivity may presage the clinical detection of carcinoma by months or years [24].

About 8%-15% of hospitalized patients are reported to have a positive DAT in the absence of haemolysis. A positive DAT may occur due to immunoglobulin or complement binding to the RBCs in vitro or in vivo; due to nonspecific uptake of plasma IgG during RBC storage in donated blood or can be associated with antiphospholipid antibodies healthy donors. In hospitalized patient, may be because of certain drugs activating complement binding to red cells or patients receiving ABO non identical whole blood derived platelet concentrates or the patients who receive intravenous immune globulin can have positive DAT {and that could be the reason for DAT positivity in our patient (case 11)} [25].

LIMITATION

The hospital does not cater to multitransfused or transfusion dependant patients and hence, the prevalence of alloimmunisation in the patient population is observed as low.

The female blood donor or recipient population was quite low in our study group.

CONCLUSION

It is imperative to determine policies in the management of blood donors with unexpected immunohaematological results found incidentally during pretransfusion compatibility at a blood center. Also, re-emphasis can be given for type and screen policy at blood centers with low prevalence of alloimmunization among patients which would allow widening of blood inventory instead of holding blood units after AHG crossmatch compatibility testing and depriving the emergency patients of specific blood units. It is, therefore, quite sagacious to continue the use of antibody screening for blood donors and recipients on a routine basis to avoid cost, time and labour intensive testing for provision of compatible blood unit in an alloimmunized patient.

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