Alloimmunization In Patients And Donors Visiting Blood Bank of A Tertiary Care Hospital In North India

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Abstract: Objective: Red blood cell (RBC) alloimmunization results from genetic disparity of RBC antigens between donor and recipients and is an immune response against foreign RBC antigens; this generally occurs after sensitization due to blood transfusions and pregnancies. We undertook this study to determine prevalence and specificity of RBC alloantibodies in patients and donor attending blood bank of a tertiary care hospital in North India. Method: This was a prospective observational study carried out at Department of Pathology and Blood Bank of Eras Lucknow Medical College and Hospital, Lucknow. Study was carried out for a duration of six months, from July 2016 to December 2016. Proper approval was taken from the institution Ethics Committee. Antibody screening and indentification was carried out in Total 700 cases including 350 Patients and 350 Donors using the ID SYSTEM (ID – Centrifuge ,37º C Dry Incubator) ID-Dia Cell I-II-III and ID-Dia Panel reagents (Diamed- Biorad, India, Private Limited). ABO and Rh blood groups were done using ID DiaClon ABO/D Grouping cards, containing monoclonal anti-A, anti-B & anti-D suspended in the gel. Results: The incidence of alloimmunization was 0.9% among patients and 0.29% among donors. Among 3 patients detected positive for red cell alloantibody, 2 cases were positive for Anti-D and one was positive for Anti-K alloantibody. The single red cell alloantibody positive donor was positive for Anti-M antibody. [N Gupta, Natl J Integr Res Med, 2018; 9(4):49-54]

Key Words: alloimmunization, red blood cells, antigen, alloantibody.

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Introduction: Red blood cell (RBC) alloimmunization is an immune response against foreign RBC antigens; this generally occurs after sensitization due to blood transfusions and pregnancies[1,2,3,4]Approximately, 400 red blood cell antigens have been identified . These RBC antigens and alloantibodies differ significantly among human populations and ethnic groups.[5]

Hence, alloimmunization after exposure to red cell alloantigens depends on genetic and acquired patient-related factors, dose, and the immunogenicity of the antigens.[6]

Clinically, significant antibodies are capable of causing mild or severe adverse events following transfusion, such as hemolytic disease of the fetus and newborn. In transplantation, RBC alloantibodies may raise the risk of haemolytic reactions, delayed engraftment and pure RBC aplasia.[4] Thus, knowledge of such alloantibodies is essential for selecting appropriate RBC products for transfusion.[7]

Antibodies that may cause hemolysis include those specific to most of the major and the minor blood groups.[8-11] Red Blood Cells 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% .[12-14]

Knowing the prevalence and specificity of RBC alloantibodies for a specific geographic area, race or disease will, therefore, assist in the management of blood transfusions and transplants and in the prevention of hemolytic disease of fetus and newborn (HDFN).

The prevalence of clinically significant alloantibodies has been reported from less than 0.3% to up to 60% of samples depending on the study populations and the test method sensitivity.[15]

Keeping in view, the need to understand the alloimmunization, the present study was carried out with an aim to evaluate the blood donors and recipient patients to determine the prevalence of irregular red cell antibodies in the patients visiting blood bank of our facility.

Method: The present study was carried out at Blood Bank of Era's Lucknow Medical College & Hospital, Lucknow. Duration of study was from July 2016 to December 2016. All patients and blood donors availing the services of Blood Bank of Era's Lucknow Medical College and Hospital, were included . Informed

consent was taken from both donors as well as patients. All cases found positive of viral markers with known alloantibodies were excluded from the study.

Antibody screening and identification was carried out using the ,ID SYSTEM (ID – Centrifuge ,37º C Dry Incubator). ABO and Rh blood grouping - done by ID DiaClon ABO/D Grouping cards -containing monoclonal anti-A, anti-B & anti-D suspended in the gel.Statistical analysis was done using Statistical Package for Social Sciences version 21.0.

Results: A total of 700 subjects were enrolled in the study including 350 donors and patients each. Out of 350 patients enrolled in the study, 3 (0.9%) were found to be positive for presence of alloantibodies. Thus incidence of alloimmunization among the patients was 0.9%. Table no. 1 shows the incidence of alloimmunization according to different characteristics among patients.

Maximum number of patients were in the age group 31-40 years. Age groups 21-30 and 31-40 years together comprised the majority of patients (52.3%). There were only 2 (0.6%) patients in the age group <10 years and 8 (2.3%) in age group 71-80 years. Alloimmunization was seen in only age groups 21-30 years and 31-40 years respectively. In age group 21-30 years and 31-40 years, the incidence of alloimmunization was 2.2% and 1.1%. On evaluating the data statistically, no significant association between alloimmunizaton and age of patients was observed.

Out of 350 patients enrolled in the study, a total of 203 (58.0%) were females and 147 (42%) were males. Incidence of alloimmunizaton was 0.7% among males and 1% among females. Although incidence of alloimmunization was higher in females as compared to males yet the difference was not significant statistically (p=1).

Maximum number of patients had ABO blood group B (n=124; 35.4%) followed by those having blood group A (n=100; 28.6%), O (n=91; 26.0%) and AB (n=35; 10%) respectively. Alloimmunization was seen in blood groups O and B only. The alloimmunization rate was 2.2% in blood group O and 0.8% in blood group B. On evaluating the association between ABO blood group and alloimmunization, it was not found to be significant (p=0.118)

There were 308 (88.0%) Rh positive and 42 (12%) Rh negative patients. Incidence of alloimmunization was significantly higher in Rh negative (4.8%) as compared to Rh positive (0.3%) patients (p=0.038).

Maximum number of cases were from obstetric ward (n=112; 32.0%) followed by Surgery (n=109; 31.1%), Medicine (n=68; 19.4%), Hematology (n=27; 7.7%), Oncology (n=18; 5.1%) and Gynecology (n=16; 4.6%) wards. Alloimmunization was seen in 2 patients from Obstetrics and 1 from Medicine ward. Incidence of alloimmunization was 1.59% in obstetrics and 1.47% in Medicine ward patients. On evaluating the association between alloimmunization and ward from which patients were withdrawn, it was not found to be significant (p=0.099).

A total of 49 (14.0%) patients had a transfusion history. Alloimmunization incidence was 0.3% among those not having transfusion history as compared to 4.1% among those having a transfusion history. On comparing the two groups, the difference was not significant statistically (p=0.053).

Out of 3 positive cases, 2 (66.7%) were positive for anti-D and 1 (33.3%) was positive for anti-K. Both the anti-D positive cases were females. One was aged 35 years, had O negative blood group, was drawn from obstetric ward and had a positive history of transfusion. The other was 34 years old, who had A negative blood,group was drawn from obstetric ward but did not have a positive transfusion history. The single anti-K positive case was a male who was 27 years of age, had A+ve blood, from Medicine ward and had a positive history of transfusion.

Out of 350 donors enrolled in the study, only one was found to be positive for presence of red cell alloantibody. Thus incidence of alloimmunizaton among donors was 0.29%. Table no. 2 shows the incidence of alloimmunization according to different characteristics among donors.

Maximum number of donors were in the age group 21-30 years (n=217;62.0%) followed by those aged 31-40 years (n=67; 19.1%), 41-50 years (n=42; 12.0%) and 11-20 years (n=23; 6.6%). There was only 1 (0.3%) donor aged 51-60 years. The single donor with positive alloantibody status was in age group 41-50 years. Thus, incidence of alloimmunization was 2.38% in age group 41-50 years as compared to 0% among

other age groups. On evaluating the data statistically, the association between age and alloimmunization was not found to be significant (p=0.195).

Only 11 (3.1%) donors were females and rest 339 (96.9%) were males. The single positive donor was male. The incidence of alloimmunization among males was 0.3% as compared to 0% among females. On comparing the data statistically, this difference was not found to be significant (p=1.000). Maximum number of donors had ABO blood group B (n=139; 39.7%) followed by those having blood group O (n=103; 29.4%), A (n=75; 21.4%) and AB (n=33; 9.4%) respectively. The lone positive donor had blood group O. Thus the incidence of alloimmunization was 0.97% in blood group O. On evaluating the data statistically, the association between ABO blood group and alloimmunization was not found to be significant (p=0.493).

Majority of donors were Rh positive (n=334; 95.4%). There were 16 (4.6%) Rh negative donors. The lone positive donor was Rh positive. The incidence of alloimmunization was 0.3% among Rh positive donors as compared to 0% among Rh negative donors. On evaluating the data statistically, the association was not found to be significant (p=1). None of the donors had reported positive transfusion history.

The single donor with alloimmunization was found to be positive for antibody Anti-M. The single Anti-M positive donor was a 45 years old male having O+ve blood and no transfusion history.

Table 1: Incidence of alloimmunization incidence according to different characteristics among patients

Age wise Incidence in Patients			
Variable	**Total number of cases**	**Number of alloimmunized**	**% Incidence**
<10 years	2	0	0.0
11-20 years	34	0	0.0
21-30 years	90	2	2.2
31-40 years	93	1	1.1
41-50 years	54	0	0.0
51-60 years	40	0	0.0
61-70 years	29	0	0.0
71-80 years	8	0	0.0
Sex wise incidence in patients			
Male	147	1	0.7
Female	203	2	1.0
Blood group wise incidence in patients			
A	100	0	0.0
B	124	1	0.8
AB	35	0	0.0
O	91	2	2.2
RhD Status wise incidence in Patients			
Positive	308	1	0.3
Negative	42	2	4.8
Speciality wise incidence in Patients			
Obstetrics	112	2	1.78
Medicine	68	1	1.47
Haematology	27	0	0.0
Surgery	109	0	0.0
Oncology	18	0	0.0
Gynaecology	16	0	0.0
Incidence according to history of transfusion in Patients			
Transfusion history present	49	2	4.1
History Absent	301	1	0.3

Table 2: Incidence of alloimmunization incidence according to different characteristics among donors

Age wise Incidence in Donors			
Variable	**Total number of cases**	**Number of alloimmunized**	**% Incidence**
11-20 years	23	0	0.0
21-30 years	217	0	0.0
31-40 years	67	0	0.0
41-50 years	42	1	2.38
51-60 years	1	0	0.0
Sex wise incidence in Donors			
Male	339	1	0.3
Female	11	0	0.0
Blood group wise incidence in Donors			
A	75	0	0.0
B	139	0	0.0
AB	33	0	0.0
O	103	1	0.97
RhD Status wise incidence in Donors			
Positive	334	1	0.30
Negative	16	0	0.0

Discussion: Alloimmunization is an adverse consequence of exposure to red blood cell (RBC) antigens through transfusion, pregnancy, or transplantation. The development of alloantibodies

can significantly complicate transfusion therapy and results in difficulties in the cross-matching of blood.[16]
In present study, Out of a total of 350 patients screened, a total of 3 (0.9%) were found to be positive for alloantibodies. Alloimmunization rate among hospital patients has shown a tremendous variability among different studies. Ameen et al. in their study at a facility in Kuwait reported alloimmunization to be 0.49%.[17] Similarly, Xu et al in their study reported this rate to be 0.50%.[18] Alloimmunization rate close to this stydy has been reported by Promwong et al .and Reyhaneh et al. in their study.[19,20] These variations signify ethnic diversities.

In their study Tiwari et al.after assessment of 32,560 patients reported the alloimmunization rate of only 0.12% .[21] while Makroo et al.in a study conducted among 49,077 patient samples reported alloimmunization rate of 0.49% only.[22]

None of the studies reviewed by us report an association between age and alloimmunization. In present study, alloimmunization rate was higher in females (1%) as compared to males (0.7%), however, this difference was not significant statistically. Similar to present study Zaman et al. in their study also found a higher risk of alloimmunization in females (100/4662; 2.1%) as compared to males (57/6573; 0.9%) and reported it to be clinically significant too.[16] The higher risk of alloimmunization among females could be attributed to alloimmunization during pregnancy and associated transfusion rates.[1-4,22]

In present study, among patients alloimmunization occurred only in patients with blood group B (n=1/124; 0.8%) and blood group O (n=2/91; 2.20%), however, no significant association was observed between ABO blood group and alloimmunization rate. Compared to this, Kaur et al.in their study found single patient positive for alloantibody having blood group A.[23] No significant association between positive alloantibody screen and ABO blood group has been shown in any of the previous studies reviewed by us. In present study, a significant association of alloimmunization incidence was observed with RhD status. It was observed that RhD negative patients had significantly higher alloimmunization rate (4.8%) as compared to RhD positive cases (0.3%). A number of studies have reported a positive association between RhD negative status and alloimmunization.[24-29] In pregnant women the global literature shows a prevalence ranging from 0.58% (Hasan et al.) to 12.1% (In exclusively Rh negative women in a study from Uganda by Mbaibulha et al..[26,30] The alloimmunization rates among pregnant women in present study (1.78%) are close to that reported by Karim et al. (Karachi, Pakistan) (1.8%) and Suria et al. (Malaysia) (1.3%).[25,31]

In present study, alloimmunizations rates were much higher in patients having a transfusion history (4.1%) as compared to those not having a transfusion history (0.3%). Foudoulaki-Paparizos et al. in their study showed that past history of blood transfusion was more common in alloimmunized patients (6/39; 15.38%) as compared to non-immunnized (81/4329; 1.87%) patients.[32] In present study, out of 3 positive cases in patients, 2 (66.7%) were positive for anti-D and 1 (33.3%) was positive for anti-K. Anti-D alloanitibodies have been stated to be most commonly identified alloantibodies in different hospital based studies.[33-35] In studies conducted among hospitalized patients in general, Zaman et al. found anti-E and anti-D to be the most common alloantibodies (36.3% and 16%) but found reported anti-K in only 4.5% cases.[16]

Among donors, in view of a single positive case, the findings of present study are more incidental. Our study showed the incidence of alloimmunization to be 0.29%. Kaur et al. in their study found it to be only 4/6350 (0.06%) donors[23],Tiwari et al. reported it as 0.009% only.[21]

The high incidence of alloimmunization among donors in present study could be an incidental finding which needs to be verified further. In present study, the single alloantibody was found to be anit-M alloantibody. Similar to present study, Kaur et al. who could identify alloantibody in 3 out of 4 alloimmunized cases and 2 out of these 3 to be anti-M positive. [23] Tiwari et al. too in their study found anti-M in two out of three donors positive for alloantibody.[21]
Given the low incidence, a universal screening for alloimmunization among patients is not recommended, however, a universal screening among pregnant women can be recommended. Keeping in view the heavy adverse consequences associated with alloimmunization, despite low incidence, a universal screening policy among donors is recommended in order to maintain the high standards of patient care in the facility.

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