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Trends of Erythrocyte Alloimmunization in Transfused Women

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ABSTRACT

Objective: To identify the trends of red cell alloimmunization in multi transfused females using red blood cell panels.
Study Design: Prospective cross sectional study.
Place and Duration of Study: Army Medical College and Armed Forces Institute of Transfusion with collaboration of Pak Emirates Military Hospital Rawalpindi, from Jan to Aug 2018.
Methodology: Consent from Institutional Ethical Committee was acquired. Blood samples of 75 females with prior history of blood transfusion were collected from MH Rawalpindi, selected by non-probability consecutive sampling technique. After pilot diagnostic tests for ABO and Rh D blood grouping, samples were screened by 3 cell panel to identify presence of RBCs alloimmunization. Positive results were further recognized by 11 cell panel following company's directions.
Results: Seventy-five women with previous history of at least one blood transfusion were selected for screening of red cell alloimmunization. The frequency of alloantibodies in transfused women was 1(1.3%). Only alloantibody identified was anti-e, which is a rare alloantibody.
Conclusion: Study confirmed that Rh alloantibodies are most prevalent antibodies, regardless of age, ABO blood grouping, Rh grouping and ethnicity. This study also confirmed that multiple transfusions have strong association of development of red cell alloimmunization.

Keywords: Transfused women, Erythrocyte alloimmunization, Blood group systems, Hemolytic Transfusion Reactions (HTR).

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INTRODUCTION

Alloimmunization is a process of development of antibodies in an individual in response to foreign antigens of same species. These alloantibodies can be produced by blood transfusion, tissue or organ transplantation or incompatible and multiple gestations.[1]

Alloantibodies can cause hemolysis of red blood cells.[2] There are several structural and functional red blood cell antigens. These antigens are proteins or carbohydrates in nature.[3] To assess the risk of antibody production and their distribution in different ethnic groups, it is necessary to have understanding of different antigens in blood groups and their associations with each other. It will help us in searching of blood that is negative for the specific antigen.[4] There are many Rh antigens which include RhD, RhC, Rhc, RhE, Rhe. Most blood banks in Pakistan only arrange matched blood against ABO and Rh-D antigen, therefore risk of production of alloantibodies against minor blood group antigens stay high.[5]

In addition to anti-D alloimmunization, antigens against blood group C/c, E/e, Kell, Duffy, Kidd, Lutheran, Lewis, P and MNS can also cause clinically significant Hemolytic Disease of Fetus and Newborn (HDFN) and Hemolytic transfusion reactions (HTR).[6] HDFN and HTRs have been clinically associated with Rh antibodies.[7,8] Common complications related to erythrocyte alloimmunization comprise.[1] Delay of transfusion due to processing of new alloantibody identification.[2] Difficulty in searching of compatible blood for person who are already highly alloimmunized.[3] Late hemolytic or serological transfusion reactions.[1] Although acute hemolytic transfusion reactions are uncommon but they can occur in high titer alloimmunized individuals. Red blood cell donors who have also G6PD deficiency or sickle cell trait are more responsible to hemolysis.[9,10] Storage hemolysis may also occur in certain ethnic groups.[11]

The knowledge of various antigen frequencies is very important to evaluate the possibility of occurrence of alloimmunization and to help in providing of specific antigen negative blood. It is significantly very constructive when blood is required for a patient having multiple red blood cell alloimmunoglobulins.[12] However in absence of future pregnancies, transplantation or transfusions, alloantibodies are inherently not dangerous.[1] In our population, knowledge of Rh antigens can also be helpful for blood banks in

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maintaining their record, finding antigen negative blood for alloimmunized patients and preparing indigenous red cell panels.[8]

METHODOLOGY

This was a prospective cross sectional study. A structured questionnaire was designed for the study. An approval from Ethical Committee Review Board (IERB approval number 3955) was taken.

Inclusion Criteria: Women who had history of at least 1 red cell transfusion, of age ranging from 20-60 years regardless of the parity and ethnicity at Pak Emirates Military Hospital were included in the study.

Exclusion Criteria: Male gender, Non-transfused women, patients with any known autoimmune disease, thyroid disorder and diabetes were excluded from the study.

Sample size was calculated using WHO sample size calculator taking confidence interval 95%, margin of error 5%, and reported prevalence of alloimmunization was 1%. The estimated sample size came out to be 75 transfused women.

All participants included in study were informed about the study and written consent was taken for record. History was taken according to a designed questionnaire. Data was kept confidential and used only for academic purposes. Carefully, 5 mililiter venous blood sample was drawn and transferred in two different blood ampoules. Each blood sample was given a specific laboratory code and record was kept in a maintaining document. Out of 5ml blood, 2ml blood was transferred in EDTA bottle for ABO and Rh blood grouping. While 3ml was transferred in a plane vial. Serum was separated for alloantibody screening and alloantibody identification. Blood grouping was done by both forward and reverse grouping techniques. Rh blood grouping was also performed and D positive and D negative cases were recognized and listed. All samples were screened by tube methods intended for alloantibody identification regardless of their blood groups. Strictly following manufacturer's instructions by Diamed cell panel, screening for erythrocyte alloimmunization was done by 3 cell diamed panel and erythrocyte allo-identification was done by 11 cell diamed panel.

Data was analyzed on SPSS version 23 considering Age, Ethnicity, ABO blood grouping, Rh grouping and no. of transfusions as study variables. Frequencies of red cell alloimmunization were calculated. Chi-square test was applied for detection of association between our variables and red cell alloimmunization. *p*-value less than 0.05 was considered significant with 95% confidence interval.

RESULTS

75 women who were previously transfused with at least one unit of red cells were included in our study. Mean age was 38±9 years (range 23 to 60). 28(37%) had blood group O, 22(29.3%) had blood group B, 15(20%) women had blood group A, 10(13%) had blood group AB. A total of 59(78.7%) women were Rh-D positive, and 16(21.35%) were Rh-D negative.

About 74(98.7%) were screened alloantibody negative and 1(1.35%) was screened alloantibody positive and was identified a non-D alloimmunized woman (Table-I). The red cell alloimmunization was 1.3%, which was a non-D alloantibody. Non-D alloantibody identified in our study was anti-e 1(1.3%).

Table-I: Frequency of Antibody screening and identification in transfused women.

Variables	n=75(%)
Antibody Screening	1 (1.3%)
Antibody Identification Anti-e	1 (1.3%)

There was significant correlation between number of red cell transfusions and red cell alloimmunization (*p*-value <0.00), while there was no association of red cell alloimmunization with age, ethnicity and ABO blood type. The asymptotic significance of Rh grouping and red cell alloimmunization was 0.053 which is also very close to significant *p*-value (Table-II).

The woman who was found to have anti-e alloantibody had history of 7 units of red cell transfusions which showed strong association of red cell alloimmunization with increasing no. of transfusions (Table-II). Overall prevalence of red cell alloimmunization in our study remained 1.3%.

DISCUSSION

This study was conducted because in countries like Pakistan, red cell transfusion is very common in women of child bearing age due to multiple pregnancies, high rate of C/sections, poor dietary iron in females, low compliance of gestational iron intake and less awareness of hazards of blood transfusions especially in lower setups. Studies have also shown

that male donors red blood cells are more liable to storage related breakdown, oxidative hemolysis and osmotic fragility as compared to females red blood cells.[11-13] As most blood donors in Pakistani

Table-II. Chi-square test

Baseline Characteristics	Study Groups n=75	*p*-value
Age		
Mean 38 yrs Range (23-63 yrs)		0.758
Ethnicity		
Punjab Sindh KPK Kashmir	61(81.3%) 1 (1.3%) 12(16%) 1(1.3%)	0.972
ABO Blood Grouping		
A B AB O	22(29.3%) 15(20.0%) 10(13.3%) 28(373%)	0.486
Rh Grouping		
Positive Negative	59(78.7%) 16(21.3%)	0.053
No. of transfusions		
1 2 3 4 5 7	36(48%) 19(25%) 13(17.3%) 3(4%) 1(1.3%) 3(4%)	0.000

population are males, females are mostly receiving transfused red cells from male population.

There were 75 women included in our study at MH Hospital Rawalpindi who had history of minimum one red blood cell transfusion. The most frequent ABO blood group antigen observed among our study groups was O seen in 28(37%) of women followed by B in 22(29.3%), A in 15(20%) and AB in 10(13%). This result was similar to a study conducted in Kashmir which detected O blood group (35.6%) to be the most prevelant blood group amongst their population followed by blood groups B (32.1%), A (24.1%) and AB (8.2%).[14] Another study conducted at AFIT Pakistan showed that B blood group was the most prevalent blood group in Pakistan (33.53%) followed by O blood group (29.65%), while AB blood group (9.76%) was the least prevalent.[8] That difference might be was due to small sample size included in our study.

A study done at Karachi, Pakistan stated that the risk of sensitization of red blood cells with D antigen in Rh-D negative ladies was 2.2%.[15] while in our study not a single case was positive for anti D. This difference again might be because of our small sample size and discrepancy in different ethnic groups of Pakistan. This difference might also be due to administration of anti-D in Rh-D negative women during and after gestation at tertiary care hospitals like MH.

Frequency of red cell alloimmunization in our study was 1.3% (1/75) which was anti-e. In India, a bicentric study was carried out on 258 transfused patients where frequency of alloimmunization was 2.71% with antibody specification of anti-D was 0.78%, while other minor antibodies were 0.39%.[16] Similarly, a study conducted in North India on 531 transfused patients, total alloimmunization was 3.4% (18/531), while anti-c with 38.8% specificity was the most common antibody in their study. Anti-E was 22.3% and anti-M was 11.1 %.[17] A similar study was also conducted in Malaysia including 263 transfused persons, erythrocyte antibody was detected with 0.76% prevalence. The most frequent suspected alloantibody was anti-Mia (30.4%) followed by anti-E 18.6% and anti-D 13.7%.[18] In our study Anti-e was the only alloantibody which is not commonly found in literature, so it was a rare alloantibody found in Pakistani population.

Women are more likely to formulate allo-antibodies due to greater need of blood transfusions with more occurrence of bad obstetric histories.[19,20] As literature shows, our study also confirmed that with increased number of blood transfusions, there are increased chances of red cell alloimmunization. Transfusion associated alloantibodies have strong health impacts with relevance to haematology, transplantation, gynaecology, obstetrics, immunology, oncology and other fields.

CONCLUSION

We conclude that with increasing number of red cell transfusions, there are increased chances of development of red cell alloimmunization irrespective of age, ABO blood grouping, Rh grouping and ethnicity. Our study also found that Anti-e was the only alloantibody which is not commonly found in literature, so it was a rare alloantibody found in Pakistani population.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SL & SP: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

MI & TA: Data acquisition, data analysis, approval of the final version to be published.

KMB & JAS: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

LIMITATION OF STUDY

In normal settings of blood banks, single antibodies like anti-D, anti-e or nati-K can easily be identified but detection of multiple allo-antibodies turn out to be more complex and time-consuming. At present, there are no "single bead/single antigen" tests available for red cell allo-immune identification due to complex structure and variety of red blood cells antigens.

Existing blood banking methods in Pakistan does not usually detect antibodies that are low in titers, so they remain un-identified in many of cases.

It was a small sample sized, single center study.

RECOMMENDATIONS

Careful blood transfusions or avoidance of unnecessary blood transfusions can prevent erythrocyte alloimmunization.

There is no national registry of blood groups and alloantibodies, consequently a patient may get blood transfusion at one hospital and have detected an alloantibody but afterwards he receives treatment at any other hospital which has no information regarding his antibody.[21] Therefore a study should be done which include much larger sample size, and also include multiple blood banks from multiple regions of Pakistan, so a nationwide data could be maintained for optimal transfusion safety.

Some drugs also suppress red cell allo-immunization, so pharmacological suppression should also be investigated in multiple health care centers. For example in patients receiving maintainance therapy of corticosteroids or chemotherapy have less probability of alloimmunizion.[22]

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