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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: Cross-sectional study.
Place and Duration of Study: Army Medical College and Armed Forces Institute of Transfusion with the collaboration of Pak Emirates Military Hospital, Rawalpindi Pakistan, from Jan to Aug 2018.
Methodology: Blood samples of 75 females with prior history of blood transfusion were collected from Pak Emirates Military Hospital, Rawalpindi Pakistan, selected by a non-probability consecutive sampling technique. After pilot diagnostic tests for ABO and Rh D blood grouping, samples were screened by three cell panels to identify the presence of RBCs alloimmunization. Positive results were further recognized by 11 cell panels.
Results: Seventy-five women with a history of at least one blood transfusion were selected for the screening of red cell alloimmunization. The frequency of alloantibodies in transfused women was 1(1.3%). The only alloantibody identified was anti-e, which was a rare alloantibody.
Conclusion: Study confirmed that Rh alloantibodies are the most prevalent antibodies, regardless of age, ABO blood grouping, Rh grouping and ethnicity. Our study also confirmed that multiple transfusions are associated with the development of red cell alloimmunization.

Keywords: ABO blood grouping, Erythrocyte alloimmunization, Hemolytic Transfusion Reactions (HTR), Transfusion

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INTRODUCTION

Alloimmunization is a process of the development of antibodies in an individual in response to foreign antigens of the 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. There are several structural and functional red blood cell antigens. These antigens are proteins or carbohydrates in nature.[2] To assess the risk of antibody production and its distribution in different ethnic groups, it is necessary to understand the antigens in blood groups and their associations. Many Rh antigens include RhD, RhC, Rhc, RhE, and Rhe.[3] Most blood banks in Pakistan only arrange matched blood against ABO and Rh-D antigens. Therefore the risk of production of alloantibodies against minor blood group antigens stays high.[4,5]

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] Although acute hemolytic transfusion reactions are uncommon, they can occur in high-titer alloimmunized individuals. Red blood cell donors who also have G6PD deficiency or sickle cell trait are more responsible for hemolysis.[9,10]

In our population, knowledge of Rh antigens can also be helpful for blood banks in maintaining their record, finding antigen-negative blood for alloimmunized patients and preparing indigenous red cell panels.

METHODOLOGY

This was a cross-sectional study at Army Medical College and Armed Forces Institute of Transfusion with the collaboration of Pak Emirates Military Hospital, Rawalpindi Pakistan from January to August 2018. Approval from Ethical Committee Review Board (IERB number 3955) was taken. The sample size was calculated using the WHO sample size calculator, taking a confidence level of 95%, a margin of error of 5% and the reported prevalence of alloimmunization was 1%.[11]

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Inclusion Criteria: Women with a history of at least one red cell transfusion, of age ranging from 20 years to 60 years, regardless of the parity and ethnicity were included in the study.

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

All participants included in the 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, a five-millilitre venous blood sample was drawn and transferred in two blood ampoules. Each blood sample was given a specific laboratory code, and the record was kept in a maintained document. Out of 5ml blood, 2ml was transferred in an EDTA bottle for ABO and Rh blood grouping. At the same time, 3ml was transferred in a plane vial. The 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. Screening for erythrocyte alloimmunization was done by three cells diamed panel and erythrocyte allo-identification was done by [11] cells diamed panel.

Data was analyzed on Statistical Package for the social sciences version 23.00 considering Age, Ethnicity, ABO blood grouping, Rh grouping and no. of transfusions as study variables. In addition, frequencies of red cell alloimmunization were calculated. The Chi-square test was applied to detect the association between our variables and red cell alloimmunization.

RESULTS

Seventy-five previously transfused women with at least one unit of red cells were included in our study. The mean age was 38±9years (range 23 to 60). 28(37%) had blood group O, 22(29.3%) had blood group B, 15(20%) women had blood group A, and 10(13%) had blood group AB. 59(78.7%) women were Rh-D positive, and 16 21(35%) were Rh-D negative.

74(98.7%) were screened alloantibody negative, and 1(1.35%) was screened alloantibody positive and was identified as 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 (n=1, 1.3%).

Table-I: Frequency of Antibody Screening and Identification in Transfused Women (n=75)

Variables	Frequency (%)
Antibody Screening	1(1.3%)
Antibody Identification Anti-e	1(1.3%)

There was a significant association between the number of red cell transfusions and red cell alloimmunization (*p*-value <0.01). At the same time, 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 the significant *p*-value. The woman who was found to have anti-e alloantibody had a history of 7 units of red cell transfusions, which showed a strong association of red cell alloimmunization with increasing number of transfusions (Table-II).

Table-II: Association of Baseline Characteristics with Red Cell Alloimmunization (n=75)

Baseline Characteristics	Red cell alloimmunization		*p*-value
	Positive (n=1)	Negative (n=74)	
Ethnicity			
Punjab	1 (1.6%)	60(98.4%)	0.972
Sindh	0(0.0%)	1(100%)	
KPK	0(0.0%)	12(100%)	
Kashmir	0(0.0%)	1(100%)	
ABO Blood Grouping			
A	1(4.5%)	21(95.5%)	0.486
B	0(0.0%)	15(100%)	
AB	0(0.0%)	10(100%)	
O	0(0.0%)	28(100%)	
Rh Grouping			
Positive	0(0.0%)	59(100%)	0.053
Negative	1(6.3%)	15(93.8%)	
No. of transfusions			
1	0(0.0%)	36(100%)	0.001
2	0(0.0%)	19(100%)	
3	00(0.0%)	13(100%)	
4	0(0.0%)	3(100%)	
5	0(0.0%)	1(100%)	
7	1(33%)	2(66.7%)	

DISCUSSION

This study was conducted because, in countries like Pakistan, red cell transfusion is very common in women of childbearing 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 than female red blood cells.[12,13] As most blood donors in the Pakistani population are males, females mostly receive transfused red cells from the male population.[14]

A study in Karachi, Pakistan, stated that the risk of sensitization of red blood cells with D antigen in Rh-D-negative ladies was 2.2%.[15] In contrast, not a single case was positive for anti-D in our study. 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 anti-D administration in Rh-D-negative women during and after gestation at tertiary care hospitals like MH.The 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 the frequency of alloimmunization was 2.71%, with antibody specification of anti-D was 0.78%, while other minor antibodies were 0.39%.[16] Similarly, in 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 not commonly found in literature, so it was a rare alloantibody found in the Pakistani population.

Women are more likely to formulate alloantibodies due to the greater need for blood transfusions with more occurrences of bad obstetric histories. Our study also confirmed that with an increased number of blood transfusions, there are increased chances of red cell alloimmunization. Transfusion-associated alloantibodies have strong health impacts relevant to haematology, transplantation, gynaecology, obstetrics, immunology, oncology and other fields.

CONCLUSION

We conclude that with an 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 not commonly found in literature, so it was a rare alloantibody found in the Pakistani population.

Conflict of Interest: None

Author Contribution

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

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

SG & HH: Conception, drafting the manuscript, approval of the final version to be published.

IN & BG: Data acquisition, data analysis, data interpretation, critical review, 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.

LIMITATIONS OF STUDY

In normal settings of blood banks, single antibodies like anti-D, anti-e or nati-K can easily be identified. However, the detection of multiple alloantibodies turns out to be more complex and time-consuming. No "single bead/single antigen" tests are available for red cell alloimmune identification due to the complex structure and variety of red blood cell antigens. Existing blood banking methods in Pakistan do not usually detect low titer antibodies, so they remain unidentified in many cases.

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 a blood transfusion at one hospital and have detected an alloantibody. However, afterwards, the patient receives treatment at any other hospital with no information regarding the antibody. Therefore a study should be done which includes a much larger sample size and multiple blood banks from multiple regions of Pakistan so nationwide data can be maintained for optimal transfusion safety.
- Some drugs also suppress red cell alloimmunization, so pharmacological suppression should also be investigated in multiple healthcare centres. For example, patients receiving maintenance therapy of corticosteroids or chemotherapy have less probability of alloimmunization.

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