SCREENING AND IDENTIFICATION OF RED CELL ALLOANTIBODIES IN MULTIPAROUS WOMEN, AN INSTITUTION BASED STUDY

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ABSTRACT

Objective: To screen and identify red cell alloantibodies in multiparous women by red cell panels.
Study Design: Cross sectional study.
Place and Duration of Study: Army Medical College and Armed Forces Institute of Transfusion in collaboration with Pak Emirates Military Hospital Rawalpindi, duration of study was from 1st Jan 2018 to 31st Aug 2018.
Material and Methods: Permission from Institutional Ethical Committee was obtained and blood samples of 200 multiparous women were taken from PEMH Rawalpindi, selected by non-probability consecutive sampling technique. After preliminary workup of ABO and Rh D blood grouping, samples were screened by 3 cell panel to detect presence of red cell alloantibodies and positive results were further identified by 11 cell panel following manufacturer's instructions.
Results: Two Hundreds (200) multiparous women were screened for presence of red cell alloantibodies. The frequency of alloantibodies in multiparous women was 3% (6/200). Most prevalent alloantibody identified was anti-D having frequency of 1.5% (3/6), while other alloantibodies included anti e 0.5% (1/6), Anti K 0.5% (1/6) and insignificant antibody 0.5% (1/6).
Conclusion: Our study confirmed that Rh antibodies were most prevalent alloantibodies, out of which anti D was the most frequent alloantibody in Pakistani population while anti e and anti K were present in relatively lower frequency.

Keywords: Blood group systems, Hemolytic Disease of Fetus and Newborns (HDFN), Hemolytic Transfusion Reactions (HTR), Red cell alloantibodies.

INTRODUCTION

Over 300 different blood group antigens have been recognized by International Society of Blood Transfusion. Most of these blood group antigens belong to 29 distinct blood group systems[1]. Red cell alloantibodies are antibodies that are usually formed as a result of pregnancy or blood transfusion. These alloantibodies may form during pregnancy as a result of natural immune response when mother's blood group is different from baby's blood group[2,3]. Mostly these alloantibodies are IgG in nature and by virtue of their capability to cross placenta, they can cause Hemolytic disease of fetus and new born (HDFN)[4]. HDFN results when red cells of mother cross placenta and hemolysis of fetal red cells can result. It presents as fetal hyperbilirubinemia, fetal anaemia, hydrops fetalis and even intra-uterine death of fetus[5]. Clinically significant antibodies produced against red cell antigens can cause HDFN, acute or chronic hemolytic transfusion reactions (HTR) and reduced lifespan of transfused erythrocytes[2,3].

Most common red cell alloantibodies are ABO, Rh and Kell antibodies[6]. Other minor blood group antibodies include Kidd (jka, jkb), Duffy (fya, fyb), MNS (M, N, S,s), Lutheran (Lu) and Lewis (Le). After ABO blood group system, second most significant blood group system is Rh system[7]. Literature reveals that 98% HDFN are related to Rh and ABO discordancy while 2% are due to irregular antibodies[8]. Significant Rh antigens include D,C,c,E,e[9]. According to published literature most important basis for red cell

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alloimmunization are Rh antibodies[7]. Among all Rh antibodies, RhD cause severe HDFN but non D type antigens may also mediate moderate to severe HDFN and HTR. Immunoprophylaxis against anti-D was introduced in 1970 which resulted in radically reduced number of anti-D alloimmunization from 16% to 1-2%. However, after 50 years of introduction of anti D immunoglobulins, still a significant percentage of females get sensitized[10]. Since no immunoprophylaxis has been introduced against non-D antibodies to date, these irregular antibodies are still problematic[7,10].

Recipient's immune system is exposed to foreign antigens. Immune system of the body produces alloantibodies against these foreign antigens[3]. Multiparous females are the females who have minimum one or more prior child births[11]. There is an increase in alloimmunization with increasing parity, bad obstetric history and previous blood transfusions[11,12]. Multiparous women produce these alloantibodies because of repeated requirement and greater usage of blood transfusions at the time of parturition[13]. Other reasons of development of red cell antibodies comprise occult fetomaternal hemorrhage, transplacental leakages of maternal and fetal blood, inadequatetyping of blood of expectant mothers and errors in transfusion[14].

Red cell alloimmunization has been studied in various populations in expectant women with prevalence ranging from 0.4 to 2.7%[6]. A study from North India found 2% antenatal females to be alloimmunized[12]. A local study done at Agha Khan Medical University, Karachi Pakistan, frequency of red cell alloantibodies among women of child bearing age was 1.8%[13].

MATERIAL AND METHODS

This cross sectional study was carried out in Army Medical College and Armed Forces Institute of Transfusion from Jan 1st 2018 to August 31 2018 after approval of the Institutional Review Board. Sample size was calculated by WHO calculator. Sample collection was done by non-probability consecutive sampling keeping the Confidence interval at 95% and Anticipated population as 3% with Absolute precision required as 5%.

Relevant history was taken from all women included in the study according to the structured questionnaire. Confidentiality of data was maintained. Samples of two hundreds multi-parous females coming to PEMH Rawalpindi were consecutively taken and screened irrespective of the age. 5ml venous blood was extracted and collected in two different tubes. 2ml blood was collected in EDTA tube for ABO and Rh blood grouping. Remaining 3ml was collected in a tube without anticoagulant and serum was separated for antibody detection and identification. Sample of each patient was given a laboratory number and record was maintained. Blood group was determined by tube method using forward and reverse grouping technique. Rh grouping was performed and D positive and D negative cases were identified. All samples were screened for alloantibodies by tube method irrespective of the blood groups. Antibody screening was done by 3 cell panel and Antibody identification was done by 11 cell panel (Diamed cell panel) by strictly following manufacturer's instructions as shown in fig-1.

Data was analyzed by usign SPSS-23. For qualitative variables frequency and percentages were calculated and for quantitative variables Mean and Standard Deviation (SD) were calculated. The *p*-value of <0.05 was considered statistically significant.

RESULTS

Two Hundred multiparous women were included in our study having parity between 2 to 10 children. Mean age was 38 ± 9 years (range 23 to 63 yrs). Fifty five (27.5%) women had blood type A, 57 (28.5%) women had blood type B, 24 (12.0%) women had blood type AB, and 64 (32.0%) had blood type O. A total of 170 (85%) women were Rh D positive, and 30 (15.0%) were Rh D negative.

Out of 200 multiparous women screened, 194 (97.0%) were negative and 6 (3.0%) were positive for red cell alloimmunization. Out of 6 positive screened alloantibodies, only 1 sample belonged to Rh D positive while rest 5 belonged to Rh D negative multiparous women.

Associated risk factors other than parity were also documented which included still birth, IUD, no. of previous transfusions and Rh status of the women. Among positive screened women 3 women had history of miscarriages, 2 had history of intrauterine deaths and 1 had history of still birth. Only 1 woman had history of seven blood transfusions as shown in table-I.

The frequency of red cell alloimmunization was 3% (6/200), out of which frequency of non-D alloantibodies was 0.5% (3/6) in all multiparous females. Non-D alloantibodies identified in our study were anti-e 1 (0.33%), anti-K 1 (0.33%) and an insignificant IgM antibody 1 (0.33%).

The frequency of anti-D was 0.5% (3/6) in D-negative blood types. After exclusion of prior sensitization due to blood transfusions, overall frequency of red cell alloimmunization remained 2.5% (1.5% in D antibodies while 1% in Non-D antibodies).

Table: Characteristics of positive screened multiparous women.

Antibody specificity	Parity	Bad obstetric history	No. of transfusions	Blood groups
Anti-D	2	Present	-	O-ve
Anti-D	4	Present	-	O-ve
Anti-D	2	Absent	-	A-ve
Anti-e	2	Present	7	A-ve
Anti-Kell	10	Absent	-	O+ve
Insignificant	3	Absent	-	B-ve

Figure: Anantigram of 11 cell panel showing positivity foranti-K alloantibody.

DISCUSSION

Worldwide, many studies have been published regarding red cell alloimmunization in pregnant women but there was limited data related to multiparous women especially in developing countries like Pakistan. Multiparous women develop these antibodies because of frequent need and greater use of blood transfusion at the time of delivery[13]. Other reasons for formation of red cell antibodies

include occult fetomaternal hemorrhages, transplacental leaks of maternal and fetal blood, errors in typing of blood of pregnant women and errors in transfusion treatments[14].

In this study the frequency of red cell alloantibodies in multiparous women was 3% which is slightly higher than another study done in Southern Pakistan which showed 1.8% alloanbodies in pregnant females[13]. In a study done in North India overall frequency of erythrocyte alloimmunization was 2%[12] which is lower than our study. In another study from South India frequency of red cell alloantibodies was 1.48%[15] which is also lower than our study. A study from Kingdom of Saudi Arabia showed prevalence of 1.03% in alloimmunized red cells in pregnant women[16]. Overall prevalence of erythrocyte alloimmunization rate in antenatal females ranged from 0.4% to 2.7%[12,13].

The frequency of non-D alloantibodies was 1.5% in our study which was similar to the other study done in Pakistan which found non-D antibodies to be 1.6%[13].

The frequency of non-D alloimmunization varies in different studies from the West ranging between 0.15% to 1.15[7]. In a study done in Australia overall frequency was 0.73% with incidence of 0.08% in D antibodies and 0.65% in non-D antibodies[4] which is also significantly lower than our study. A study from Turkey found prevalence of non-D antibodies to be 1.2%[17] which was almost similar to our study.

The most frequent alloantibody in our study was anti-D followed by anti-e and anti-K which also correlates with published literature that Kell is the second most common antibody after Rh antibodies[17]. A study from Canada showed anti-E to be most prevalent antibody followed by anti-c and anti Jka. Similarly a study from United States found anti-K to be most frequent alloantibody[5].

A study done in Australia found that frequency of anti-D alloantibodies has decreased to 0.8% in 2013[4] but our study showed 1.5% prevalence of anti-D antibody which was slightly higher. The study done in southern Pakistan found prevalence of anti-D to 2.2%[13] which was higher than this study that might be due to their large sample size (n=1000). The high prevalence rate of anti-D in our population might be due to low socioeconomic status of our general population, non-affordability of anti-D immunoprophylaxis, inadequacy of health education, poor reach to medicare, inappropriate dosage or late administration of anti-D immunoprophylaxis. Many women living in peripheries still don't have direct access to secondary and tertiary care hospitals and are less aware of their Rh-D status, their babies' blood group status and anti-D immunoprophylaxis.

The women who developed anti-K was a grand multiparous (para 10) having no associated risk factor except that of grand multiparty. The women who developed anti-e had 2 alive issues, history of 7 blood transfusions, 1 IUD and 1 still birth.

With increasing age and parity, a women is more prone to develop alloantibodies. It can be due to increased need of blood transfusions and more incidence of bad obstetrical histories[12,18]. Associated risk factors in our study included number of previous transfusions, parity, intrauterine deaths and still births. We found that alloimmunization increases when the parity of a women increases (p<0.001). It also correlated with another study done in India which says that production of red cell alloantibodies is directly relatedto a woman's parity[18].

In our study, 5 out of 6 positive screened samples were Rh D negative and 1 was Rh D positive which shows that alloantibodies can be produced in Rh D positive as well as D negative blood groups but their chances are higher in Rh D negative status.

RECOMMENDATION

1) Rh pregnant ladies should be identified in the antenatal checkup and there should be provided proper anti-D prophylaxis.

2) With increasing parity of women, considering the associated risk factors and cost

effectiveness, at least Rh and Kell antigens should be matched in both Rh D positive as well as Rh D negative women because there is increased risk of occurrence of HDFNand a good number of these patients are expected to receive blood transfusions.

3) Women having clinically significant erythrocytealloantibodies should be given an adequate written as well as verbaldetails of antibody specificity sothey can acquire antigen negative blood whenever they require blood transfusion in future.

CONCLUSION

1) We conclude that multiparous women have a significant risk of being alloimmunized against red blood group antigen.

2) Our study also confirmed that anti-D was most prevalent alloantibody in Pakistani population while anti-e and anti-K are relatively lower in frequency.

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CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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