Rh Alloantibodies in Rh D Negative Blood Group Pregnant Women - A Regional Transfusion Centre Study

Hussan Bajwa, Maqbool Alam*, Muhammad Ali Rathore, Muhammad Sajid Yazdani**

National University of Sciences & Technology, Islamabad Pakistan, *Bahria International Hospital, Lahore Pakistan, **Armed Forces Institute of Transfusion/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, ***Combined Military Hospital Abbotabad/ National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To determine the frequency of Rh alloantibodies in pregnant women of the Rh-D negative blood group.
Study Design: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Transfusion (AFIT) Rawalpindi, from Jan to Dec 2017.
Methodology: The blood samples of pregnant women received for blood grouping, cross matching and antibodies screening were included in the study. The blood was typed for Rh-D along with ABO blood groups by Column Agglutination Technique (CAT), commonly known as the gel card method. Then, the samples included in the study were subjected to antibody screening by three cell antibody screening panel (Dia Cell, a product of Bio-Rad) by Column Agglutination Technique. The samples with positive antibody screening were further processed by 11 cell antibody screening panel (Dia Cell, Bio-Rad) for Rh antibody identification by Column Agglutination Technique, the same as the indirect antiglobulin test (IAT).
Results: 453 Rh-D negative pregnant women were screened for alloantibodies during the study period. Rh alloantibodies were present in 55 (12.08%) cases. The most common alloantibody identified was anti-D in 48 (87.3%) samples, followed by anti-C in 6 (10.9%) and anti-E in 1 (1.8%) case.
Conclusion: The most prevalent Rh alloantibody identified in Rh-D negative pregnant women was anti-D, while the anti-C and anti-E were less prevalent. However, no case of anti-c and anti-e alloantibody was identified.

Keywords: Allo-antibodies, Indirect antiglobulin test, Rh blood group system.

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INTRODUCTION

The International Society of Blood Transfusion (ISBT) currently recognizes 38 blood group systems, out of which the ABO and Rh blood groups are most important clinically due to the potential of causing hemolytic transfusion reactions (HTR) by their corresponding antibodies. The Rh blood group has 55 antigens encoded by RHD and RHCE genes located on chromosome 1 (at 1p36.11 position).[1,2] The antibodies formed against different blood group systems are either naturally occurring as in the ABO blood group system or formed on exposure to the antigen, called alloantibody, as in the Rh blood group system. An alloantibody is formed in response to pregnancy, transfusion or transplantation.[3] Mainly clinically significant IgG type alloantibodies are produced, reacting optimally at warm temperatures.[4,5] The Rh system antibodies, IgG type and HTR, are strongly associated with hemolytic disease of the fetus and newborn (HDFN), making the Rh system more important clinically in pregnant females. HDFN results in hemolysis of fetal red cells by the antibody in mother serum crossing the placenta and presents in the fetus as hyperbilirubinemia, anaemia, hydrops fetalis and may lead to intrauterine death. The D, C, c, E and e are the most clinically important antigens of the Rh blood group system.[6,7]

D antigen, found in almost 89% of our population, is highly immunogenic and thus a common cause of severe HDFN. Most commonly, the anti-D antibody is produced when the Rh-D negative individual is exposed to Rh-D positive red cells by transfusion or pregnancy of Rh D positive fetus by Rh D negative mother.[6] The c-antigen (referred to as "the little c"), prevalent in almost 80% of the United States population, is next clinically significant after the D antigen, and the anti-c HDFN severity may range from a mild to a severe.[8] The E, e and C antigens are also clinically significant with the variable potential of causing HDFN.[9] These antibodies may also present in combinations with each other, thus enhancing their clinical significance. The Rh antibodies cannot be detected by simple agglutination of saline-suspended red cells with the corresponding antigen because they mostly do not have a major IgM component. Instead, the indirect

Correspondence: Dr Sajid Yazdani, Consultant Haematologist, Combined Military Hospital, Abbotabad-Pakistan
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antiglobulin technique (IAT) has to be employed to demonstrate the presence of these antibodies, with mainly IgG components reacting best at 37°C and enzyme-treated red cells enhancing them.[10] Thus, the screening for Rh antibodies early in each pregnancy is necessary to minimize the risk of HDFN. This study was conducted to determine the seroprevalence of different Rh alloantibodies in Rh-D negative pregnant women, which can have clinical consequences in our local population.

METHODOLOGY

The study was conducted from January to December 2017 at Armed Forces Institute of Transfusion (AFIT) Rawalpindi, after approval of the Ethics Review Committee of the institute (vide letter no 106/Adm dated 2 January 2017). Raosoft® sample size calculator was used for sample size calculation with a confidence interval of 99%, margin of error of 2% and expected frequency of alloantibodies as 2%11. The non-probability consecutive sampling technique did the sampling.

Inclusion Criteria: The pregnant women reporting for blood grouping, cross-matching and antibody screening with Rh D negative blood group were included.

Exclusion Criteria: Rh D positive samples were excluded from the study.

After the consent, the 10 ml blood sample was drawn, and 5 ml was collected, each in ethylene diamine tetra acetic acid (EDTA) and plain glass tubes. The blood samples were typed for ABO and Rh-D by Column Agglutination Technique (CAT), commonly known as the gel card method. The Rh D negative blood group samples were included in the study, while Rh D positive were excluded. The samples included in the study were subjected to antibody screening by three cell antibody screening panel (Dia Cell, Bio-Rad) by CAT. The samples with positive antibody screening were further processed by 11 cell antibody identification panel (Dia Cell, Bio-Rad) for Rh antibody identification by CAT (Figure-1).

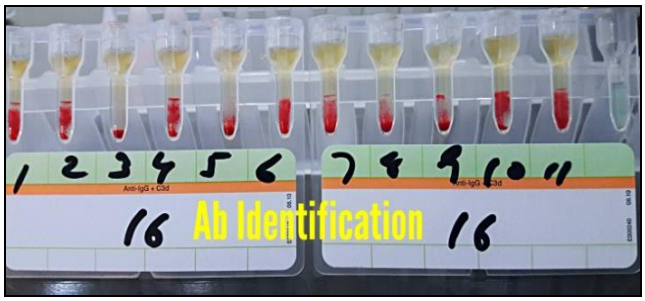

Figure-1: Gel card showing positive and negative results (11 cell panel).

The results were interpreted according to the manufacturer's instructions, and the data was analyzed using Microsoft Excel. The frequencies and percentages of alloantibodies identified were calculated for the study population.

RESULTS

A total of 453 Rh-D negative group pregnant women reported during the study period were screened for Rh alloantibodies. The age ranged from 19 to 48 years with a mean of 28 + 4.5 years. Out of 453, Rh alloantibodies were present in 55 (12.1%) of these women screened. Among ABO blood group distribution of Rh alloantibodies positive cases, the predominant blood group was B in 155 (34%), followed by O in 140 (31%), A in 113 (25%) and AB in 45 (10%).

The antibody specificity in 55 Rh alloantibodies positive samples was anti-D in 48 (87.3%), anti-C in 6 (10.9%) and anti-E in 1 (1.8%), as depicted in Figure-2. Anti-c and anti-e antibodies were not identified in any sample.

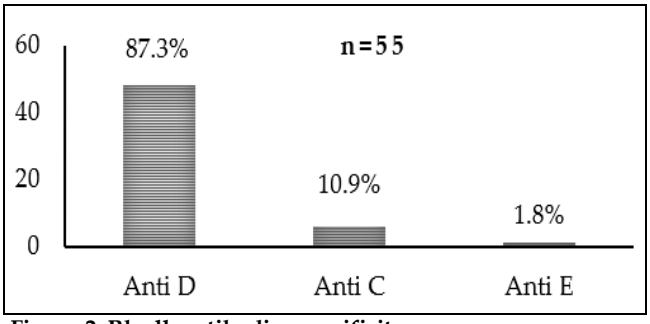

Figure-2: Rh alloantibodies specificity.

DISCUSSION

Rh alloantibodies in pregnant women can lead to a significant risk of HDFN and HTR.[11,12] In our study, 12.1% (55/453) of Rh-D negative pregnant women were positive for alloantibodies. Of these, anti-D was found in 87.3% (48/55), anti-C in 10.9% (6/55) and anti-E in 1.8% (1/55). This could be due to previous sensitization with transfused blood or exposure to Rh-D-positive fetus-carrying pregnancies.

In contrast to our results, a study in China showed a 0.74% prevalence of alloantibodies in hospitalized patients. Among alloantibodies, anti-c and anti-E were most commonly detected; however, only three cases of anti-D (1.8%) were observed.[13] This was probably due to the inclusion of fewer child-bearing age females irrespective of their Rh blood group. Another reason may be the population's high prevalence of Rh D antigen. Another study from Kuwait

observed the lowest number of antibody-screened positive cases (0.49%), again in contrast to our study. However, the most prevalent alloantibody they detected was anti-D (27.3%), followed by anti-E (18.5%), similar to ours.[14] Similar studies from North West and Southern region of Pakistan showed low prevalence (3.0% and 5.5%) of alloantibodies, with anti-D being the most frequent.[11,15] Low prevalence (0.9%), with anti-E being the most common alloanti-body identified in a Malaysian study, also disagrees with the current study.[16] Our results also match the previous study by Ghaffar *et al*, in the same region where anti-D was found to be the most prevalent antibody.[17] Although it was done in all pregnant women irrespective of Rh D status.

In our study ABO blood group distribution of Rh allo-antibodies positive cases, the predominant blood group was B (34%), followed by O (31%), A (25%) and AB (10%), similar to the normal distribution as shown in a study from northern Pakistan.[18] We can infer from this finding, although not our primary objective, that the Rh alloantibodies formation is independent and is not affected by ABO blood group antigens. However, further targeted studies may be required. The sample size variability, antibody screening methods and geographical distribution are important factors for the varied prevalence of Rh alloantibodies in different studies. The risk of transfusion-associated health hazards and HDFN can be minimized by antibody screening in pregnant females, as highlighted by our study. Most of the pregnant ladies in our country either do not attend the antenatal clinics or report to primary healthcare setups without proper transfusion facilities, which is a great challenge for timely diagnosis and management of Rh alloantibodies-related complications. The history of transfusion and pregnancy is also important as these antibodies are formed due to previous exposure. The significance of routine administration of anti-D prophylaxis in D-negative females of child-bearing age is further highlighted by our study.

Limitations of Study

The limitation of our study was the selection of women who reported to the tertiary care centre, so the prevalence in the general population may vary. It is suggested to conduct further studies on peripheral health care setups on a larger scale.

DISCLAIMER

This manuscript was extracted from the research thesis of the first author (Hussan Bajwa), which was mandatory for fulfilment of the requirement of BSc Hons MLT.

CONCLUSION

The anti-D was the most common alloantibody in Rh-D negative pregnant women. The relatively less prevalent antibodies were anti-C and anti-E. Identifying the Rh alloantibodies early in pregnancy is essential for clinical decision-making to minimize the associated complications. The high prevalence of anti-D in pregnant ladies signifies the routine prophylactic anti-D administration in females of the Rh D negative blood group.

Conflict of Interest: None.

Authors' Contribution

HB: Performed the study, MA: Overall supervision of project, MAR: Technical support and review of manuscript, MSY: Idea conception, supervision of study and manuscript writing.

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