Red Cell Alloimmunization and Antibody Specificity in Multigravida Attending Antenatal Clinic at Tertiary Care Centre from Rohilkhand Region

Ankita Singh¹, Milan Jaiswal²

ABSTRACT

Introduction: Red cell alloimmunization is an immune response to foreign red cell antigens which can occur due to transfusion between a donor and recipient, during pregnancy between mother and fetus and also in transplant recipients. Such disparity may result in transfusion reactions or may be associated with hemolytic disease of the fetus and newborn. The current study was undertaken to observe the prevalence of red cell alloimmunization and antibody specificity in multigravida patients with respect to epidemiological (age), obstetrical, major blood group systems and transfusion-related parameters.

Material and Methods: This prospective cross-sectional study was conducted over a period of 1.5 years from October 2018 to April 2020, in the patients at risk of exposure to foreign red cell antigens in multigravida patients, in the blood bank of tertiary care medical institute of North India.

Antibody screening and identification was performed on 1055 multigravida females using commercially prepared O cells (IMMUCOR, USA). Statistical evaluation by Chi-square test was performed wherever applicable. p-value of <0.05 was considered statistically significant at 95% confidence interval.

Results: Prevalence of red cell alloimmunization in multigravida females was 1.99% with anti-D being the most common antibody identified. Other less commonly detected alloantibodies were anti-E, anti-Jk\(^b\) (Kidds) and anti-Kp\(^a\) (Kell). A statistically significant association was found between red cell alloimmunization and increase in gravida status, presence of bad obstetric history, age (>30 years) and Rh-D status. When compared with other independent variables such as parity and ABO blood group of the female, no statistical significant association was observed.

Conclusion: Maternal alloimmunization is associated with anti-D specificity and antibodies against other clinically significant minor blood group antigens such as anti-E and anti-Jk\(^b\) (Kidd) and anti-Kp\(^a\) (Kell). Routine antibody screening of all antenatal females, irrespective of the Rh-D status must be adopted by health care system for detection of clinically significant antibodies that may be associated with HDFN or delayed hemolytic transfusion reaction.

Keywords: Red cell alloimmunization, Multigravida, Red cell antigen, Hemolytic disease of the fetus and newborn.

How to cite this article: Singh A, Jaiswal M. Red Cell Alloimmunization and Antibody Specificity in Multigravida Attending Antenatal Clinic at Tertiary Care Centre from Rohilkhand Region. SRMS J Med Sci. 2023;8(1):1-6.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Maternal alloantibodies, referred as a silent epidemic may result in clinically significant conditions like hemolytic disease of the fetus and newborn (HDFN), delayed hemolytic transfusion reactions and coagulopathies. The prevalence rate of alloimmunization in multigravida women, reported in India by various authors was 1.1, 1.25, 1.5 and 2.0%, respectively with anti-D being the most common antibody whereas studies conducted in western countries reported lower prevalence of alloimmunization as compared to India. Preventive and therapeutic approaches based on appropriate antenatal screening and identification of alloantibodies, timely intervention in alloimmunized cases and proper selection of extended red cell matched blood for transfusion is the need of the hour in every clinical setting of a specific geographic area.

The present study has been undertaken to observe the prevalence of alloimmunization and antibody specificity in multigravida patients; and further, to compare various categories of independent variables pertaining to age, obstetrical, transfusion-related parameters and major blood groups in alloimmunized cases. Results obtained in the study may serve as evidence for bringing amendments to upgrade antenatal care and transfusion-related policies in pregnant women attending antenatal clinic.

MATERIALS AND METHODS

This was a prospective observational study carried out in blood bank, Department of Immunohematology and
Blood Transfusion, conducted over a period of 1.5 years from October 2018 to April 2020 in patients at risk of exposure to foreign red cell antigen in multigravida patients. After meeting inclusion and exclusion criteria, the study population included consecutively selected 1055 multigravida females attending the antenatal clinic of the Department of Obstetrics and Gynaecology. Primigravida females, multigravida with diagnosed autoimmune disorders and Rh-D negative pregnancies with previous history of anti-D immunophylaxis were excluded from the study to avoid false positive reactions due to interfering autoantibodies and passive anti-D following RhIG administration.

Name, age, bad obstetrical parameters including abortion, stillbirth, postpartum hemorrhage and antepartum hemorrhage, previous history of transfusion, autoimmune disorder and Rh-D immunization was recorded for each case.

The venous blood sample of patients was used to determine the patient’s blood group, including ABO, Rh-D, antibody screening and antibody identification. These samples were received in blood bank from the Department of Obstetrics and Gynaecology in two EDTA vials, each 2 mL in volume. A blood sample was drawn using an acceptable phlebotomy technique. All testing was performed as soon as possible following collection to minimize the chance of false-positive or false-negative reactions due to improper storage or contamination of the specimen. The specimens that were not tested in 24 hours were stored at 2–8°C as soon as possible.

Antibody screening was done by a commercially prepared 3 cell panel of pooled O cells and further identification was done by 16 cell liquid panel by tube technique and 14 cell panel by SPRCA technique. [IMMUCOR, USA]. All positive cases were confirmed using commercially prepared rare antisera. Where antisera was not available, antibody specificity was confirmed on the basis of reaction patterns of the antigram, enzyme enhancement techniques and DTT-treated serum.

The data was analyzed by applying Chi-square test with Yates correction for continuity, wherever applicable. \( p\)-value of <0.05 was considered statistically significant at 95% confidence interval. Statistical analysis was performed using SPSS version 23.

**RESULTS**

The study enrolled 1055 multigravida females after meeting inclusion and exclusion criteria. Twenty-one females were identified with alloantibodies with a prevalence rate of 1.99%.

Alloantibodies against the Rh antigen system were most prevalent (1.80%), with 15 anti-D and 4 cases of anti-E. Only one case each of Anti Jk\(^b\) (Kidd) and anti-Kp\(^a\) (Kell) was detected.

Table 1 represents frequency distribution, prevalence and antibody specificity with respect to age. In the present study, the age of multigravida patients ranged from 19 to 48 years with a mean age of 26.59 ± 5.43 years. The age range of alloimmunized cases was 22 to 48 years, with a mean age of 32.14 ± 8.00 years. A statistically significant association (\( p\)-value=0.00632) was observed when alloimmunization was compared in the age group >30 years (8 alloimmunized, 164 not alloimmunized) and ≤ 30 years (13 alloimmunized and 870 non alloimmunized). The likelihood of alloimmunization in multigravida >30 years was 3 times more than ≤ 30 years \( [\chi^2 = 7.4569, \text{ odds ratio} = 3.2645 (1.332 \text{ to } 8.0007) \text{ at } 95\% \text{ confidence interval}] \)

In the present study, gravida II (G2) constituted 55.26%, gravida III (G3) 31.09%, gravida IV (G4) 6.8%, gravida V (G5) 5.3%, gravida VI (G6) 1.23% while gravida VII (G7) and gravida VIII (G8) constituted only one case each of the total sample population. Out of all antibody positive cases, a maximum number of antibodies was discovered in gravida III (n = 10). Table 2 represents the frequency distribution, prevalence and antibody specificity of alloimmunized cases with respect to the gravida status.

A statistically significant association was observed when alloimmunization was compared between gravida

---

**Table 1: Frequency distribution, prevalence and antibody specificity with respect to age**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Sample population (n)</th>
<th>Alloimmunized patient (n)</th>
<th>Overall prevalence of alloimmunization (n = 1055)</th>
<th>Age specific prevalence of alloimmunization</th>
<th>Antibody specificity (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>73</td>
<td>00</td>
<td>0.00%</td>
<td>0.00%</td>
<td>--</td>
</tr>
<tr>
<td>21–30</td>
<td>810</td>
<td>13</td>
<td>1.20%</td>
<td>1.60%</td>
<td>Anti-D=11 Anti-E=1 Anti-Jk(^b)=1</td>
</tr>
<tr>
<td>31–40</td>
<td>140</td>
<td>05</td>
<td>0.47%</td>
<td>3.57%</td>
<td>Anti-D=2 Anti-E=3</td>
</tr>
<tr>
<td>41–50</td>
<td>32</td>
<td>03</td>
<td>0.28%</td>
<td>9.37%</td>
<td>Anti-D=2 Anti-Kp(^a)=1</td>
</tr>
</tbody>
</table>
Red cell alloimmunization in Multigravida

The likelihood of alloimmunization in pregnant females with gravid ≥G5 was 3 times more than those with gravid status < G5 \( \chi^2 = 10.6376, \) odds ratio 3.05

The most frequent blood group observed in the sample population was B (n = 385, 36.49%), followed by O (n = 312, 29.57%), A (n = 258, 24.45%) and AB (n = 100, 9.48%). Out of all blood groups maximum prevalence amongst alloimmunized females was observed for blood group AB (4%) followed by O (1.92%), B (1.82%) A(1.55%).

Table 2: Frequency distribution, prevalence and antibody specificity with respect to gravidity status

<table>
<thead>
<tr>
<th>Gravida</th>
<th>Total number of cases (n)</th>
<th>Alloimmunized patient (n)</th>
<th>Overall prevalence in the sample population ( (n = 1055) ) (%)</th>
<th>Group specific prevalence (%)</th>
<th>Antibody specificity (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>583</td>
<td>05</td>
<td>0.47</td>
<td>0.86</td>
<td>Anti-D(4) Anti-E(1)</td>
</tr>
<tr>
<td>G3</td>
<td>328</td>
<td>10</td>
<td>0.95</td>
<td>3.05</td>
<td>Anti-D(6) Anti-E (3) Anti-Jk(1)</td>
</tr>
<tr>
<td>G4</td>
<td>72</td>
<td>02</td>
<td>0.19</td>
<td>2.78</td>
<td>Anti-D(2)</td>
</tr>
<tr>
<td>G5</td>
<td>56</td>
<td>03</td>
<td>0.28</td>
<td>5.36</td>
<td>Anti-D(2)</td>
</tr>
<tr>
<td>G6</td>
<td>13</td>
<td>00</td>
<td>0.00</td>
<td>0.00</td>
<td>--</td>
</tr>
<tr>
<td>G7</td>
<td>01</td>
<td>01</td>
<td>0.09</td>
<td>100</td>
<td>Anti-D(1)</td>
</tr>
<tr>
<td>G8</td>
<td>01</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>--</td>
</tr>
<tr>
<td>G9</td>
<td>01</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 3 (a): Prevalence of specific alloantibody with respect to major blood group system

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Total number of cases</th>
<th>Anti-D (%)</th>
<th>Anti-E (%)</th>
<th>Anti-Jk-b (%)</th>
<th>Anti-Kp-a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>258</td>
<td>1.16</td>
<td>0.39</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>B</td>
<td>385</td>
<td>1.04</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>O</td>
<td>312</td>
<td>1.60</td>
<td>0.32</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AB</td>
<td>100</td>
<td>3.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3 (b): Frequency distribution, prevalence and antibody specificity with respect to blood group

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Total number of cases (n)</th>
<th>Alloimmunized cases (n)</th>
<th>Overall prevalence in sample population (%)</th>
<th>Group specific prevalence (%)</th>
<th>Antibody specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>258</td>
<td>4</td>
<td>0.38</td>
<td>1.55</td>
<td>Anti-D=3 Anti-E=1</td>
</tr>
<tr>
<td>B</td>
<td>385</td>
<td>7</td>
<td>0.66</td>
<td>1.82</td>
<td>Anti-D=4, Anti-E=1, Anti-Jk-b=1 Anti-Kp-a=1</td>
</tr>
<tr>
<td>O</td>
<td>312</td>
<td>6</td>
<td>0.57</td>
<td>1.92</td>
<td>Anti-D=5 Anti-E=1</td>
</tr>
<tr>
<td>AB</td>
<td>100</td>
<td>4</td>
<td>0.38</td>
<td>4.00</td>
<td>Anti-D=3 Anti-E=1</td>
</tr>
</tbody>
</table>

in the present study, with \( p-value =0.495 \) at \( p <0.05 \) significance level.

Table 4 represents distribution prevalence and antibody specificity with respect to adverse obstetric history and past history of transfusion. Among 1055 enrolled multigravida females, adverse obstetric history was present in 120 cases, of which only 8 (6.7%) were detected positive for alloantibodies and anti-D was the only significant red cell antibody identified. A statistically significant association was observed between the adverse obstetric history and alloimmunization with \( p-value = 0.00098 \) at \( p <0.05 \) significance level. Alloimmunization was five times more likely to occur in multigravida with adverse obstetrical history \( (\chi^2 = 15.762, \) odds ratio = 5.06)  

Past history of transfusion was present in 24 multigravida females of which only 3 (12.5%) were detected positive for alloantibody. Anti-D was the only clinically significant alloantibody detected in these three cases. A statistically significant association was observed between multigravida females with transfusion history and red cell alloimmunization, compared to those without transfusion history \( (p-value = 0.0016) \). Rate of alloimmunization was 8 times more likely in Multigravida with transfusion history. \( (\chi^2 = 13.90, \) odds ratio = 8.03)
DISCUSSION

Clinically significant red blood cells antibodies are immunoglobulin which reacts against the antigen present on the surface of red cells. These antibodies can be naturally occurring or can be acquired from foreign red cells, forming alloantibodies or auto-antibodies.\(^7\) The alloimmunization risk is proportional to the exposure and immunogenicity of the host against foreign red cells.\(^8\)

The British Committee Standard in Hematology (BCHS) guidelines for antenatal antibody screening recommends screening of antibodies of all antenatal cases, irrespective of their Rh-D status on the first visit and the next screening to be done at 28 weeks.\(^3\) Further the Drug controller general of India also recommends antibody screening of all antenatal women.\(^4\) Literature has enough evidence that only anti-D but alloantibodies against other red cells antigens of the minor blood group system also leads to adverse outcomes in the form of HDFN. Due to cost constraints and lack of resources at many centers and unawareness of the role of minor blood group system in alloantibodies the international and national recommended protocols have not been implemented successfully in this region and therefore this study was undertaken to evaluate the red cell alloimmunization in 1055 multigravida of Rohilkhand and nearby areas.

The overall prevalence of alloantibodies in Multigravida was 1.99%, similar to a study from Jammu (North India) that reported a prevalence of 2.0%.\(^4\) Other studies conducted in India observed a prevalence rate between 1.1 to 2.27%.\(^1,4,10\) Western countries have reported a slightly lower prevalence rate of alloimmunization. The probable explanation to these findings could be better and successful implementation of the anti-D immuno-prophylaxis program and provision of extended red cell antigen-matched blood for transfusion, reducing the chances of foreign red cell antigenic exposure in individuals.\(^11,12\)

The Rh blood group system was observed to be the most frequent cause of red cell alloimmunization contributing 90% amongst alloimmunized females \((n = 21)\) followed by Kidd and Kell constituting 5% each which was similar to the other studies done in India who also reported Rh as the most common blood group antigen. Alloimmunization against Rhesus (Rh) blood group system was most prevalent as it is known that Rh is the most immunogenic blood group system next to the ABO system.\(^13\)

In some of the studies conducted outside India, anti-K (Kell antibody) was found to have higher frequency as compared to Indian studies as the incidence of Kell antigen in Indian population is only 1.97%, which is lower as compared to Caucasian’s where the incidence is 4.5%.\(^14\)

In this study, anti-D was the most prevalent alloantibody amongst multigravida females, followed by anti-E alloantibody. The probable explanation to this finding is that Rh-D antigen is most immunogenic amongst other Rh and minor blood group antigens.\(^8\) Further, lack of standardization in the anti-D immuno-prophylaxis program, especially in rural settings, could explain the current prevalence of anti-D alloantibodies in pregnant females.\(^15\)

However, studies from abroad have reported anti-E as the most commonly occurring alloantibody followed by anti-D; this could be explained by the successful implementation of anti-D immuno-prophylaxis and better antenatal protocols in managing alloimmunized pregnant females.\(^15\)

**Age and Alloimmunization**

In the present study rate of alloimmunization was observed maximum between the age range of 21 to 30 years which was similar to the study done by Das et al.\(^16\) who also reported maximum cases between 21–30 years of age, whereas Sidhu et al.\(^4\) and Suresh et al.\(^1\) observed maximum numbers of cases within the age range 23–36 years.

A statistically significant association \((p = 0.00632)\) was observed when alloimmunization was compared between the two age groups >30 years and ≤ 30 years at a significance level \(p < 0.05\). Increased prevalence in the higher age group in the current study may be due to multiple confounding factors such as pregnancies and feto-maternal hemorrhage leading to multiple exposures to foreign red cell antigens. Comparative analysis between various studies cannot be done on this observation as limited data is available in literature.

**Gravida Status and Alloimmunization**

In the present study, higher prevalence of alloimmunization was observed with increasing gravida status and maximum prevalence was seen in G7 females. A statistically significant association was observed between...
gravid status and alloimmunization which was also reported by Pahuja et al. ⁷ (p value<0.001) and Sidhu et al. ⁴ (p-value=0.0296) respectively. The underlying cause of increased risk of alloimmunization with subsequent pregnancies could be due to increased exposure to fetal red cells carrying antigens, foreign to maternal red cells.¹

**ABO and Alloimmunization**

Amongst 1055 multigravida, ABO type B was the most frequent blood group comprising 36.49% of the study population whereas the alloimmunization rate was maximum in the AB type blood group. Similar trend was observed in the studies by Pahuja et al.,² Sidhu et al.,⁴ and Zaman et al.¹⁴

There was no statistical significant association between the major blood groups and alloimmunization in the present study (p-value= 0.495).

**Adverse Obstetric History and Alloimmunization**

When alloimmunization was compared between pregnant females with and without adverse history a statistical significant association was observed between the two groups at p < 0.05 significance level (p = 0.0001). A significant correlation was also observed in the studies done by Pahuja et al.² and Suresh et al.¹ with p-values, < 0.001 and 0.160, respectively. The probable explanation to these observations could be increased risk of exposure to foreign red cell antigens due to feto-maternal hemorrhage during pregnancy which exposes the mother to larger volume of blood carrying fetal red cell antigens.¹⁵

Feto-maternal hemorrhage could be associated with delivery, trauma, abortions that could be spontaneous or induced, ectopic pregnancy and various procedures. Since our hospital mostly caters to the medical needs of rural population of the Rohilkhand region and nearby areas, anti-D being the only specific type of alloantibody identified in the eight cases presenting with bad obstetric history reflects that standardized anti-D immunoprophylaxis protocols are not followed strictly in this region.

**Transfusion History and Alloimmunization**

In the present study, the risk of alloimmunization in multigravida with transfusion history was 8 times higher than in multigravida without history of blood transfusion. Higher rate of alloimmunization was also observed by studies done by Pahuja et al.,² Sidhu et al.,⁴ and Das et al.¹⁰ Whether alloimmunization in these cases is due to pregnancy or transfusion, cannot be commented upon. However, greater exposure to multiple foreign red cell antigens in pregnant females with past history of transfusion may be a risk factor. In contrast to these observations, Suresh et al.¹ reported no alloantibody in 0.7% females with history of blood transfusion.

Observations in the present study are similar to various other studies conducted in India with only slight difference in the overall prevalence of alloantibodies and there specific types in various geographic regions. Detection of alloantibodies other than anti-D supports the view that mandatory screening of all antenatal women, irrespective of their Rh-D status should be performed to prevent alloimmunization-related pregnancy outcomes in the fetus and newborn; and also to prevent untoward reactions during transfusion. Early detection of such cases will prompt early interventions and management of mother and fetus, preventing adverse outcomes such as HDFN.

**Limitations**

Impact of maternal red cell alloimmunization on clinical, hematological and biochemical parameters of the fetus or the newborn were not included in this study which could be beneficial in understanding the clinical significance of antibody screening and identification in antenatal females.

**CONCLUSION**

In the present study, prevalence of unexpected alloantibodies in Multigravida was 1.99% with anti-D being the commonest. maternal alloimmunization to Rh antigens other than D (E) as well as minor blood group antigens Jk⁰ (Kidd) and Kp⁺ (Kell) were also observed. Therefore, the authors recommend that antibody screening be implemented as a routine investigation for all pregnant females, irrespective of their Rh –D type.

**REFERENCES**

5. Rossi EC. Transfusion in the New Millennium. In : Simon LT, Synder EL, Solheim BG, Stowell CP, Strauss RG, Petrides


