ORIGINAL RESEARCH

# Prevalence of Red Cell Antibodies In Antenatal Mothers

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## ABSTRACT

**Introduction:** Hemolytic disease of the newborn can be caused by incompatibility of maternal and fetal erythrocyte for Rh (D) or other blood type antigens. Routine antibody screening is advocated in all antenatal mothers, irrespective of whether they are Rh positive or Rh negative, to look for clinically significant antibodies other than Rh (D) that might cause hemolytic disease of the newborn. The purpose of this study was to assess the Prevalence of Red Blood Cell Antibodies in Antenatal Mothers.

**Methods:** A cross sectional study conducted to screen red cell antibody using11 cell panel among antenatal mothers was undertaken over a period of one year. SPSS ver:17 was used for statistical analysis. Chi-square and frequency was done to compare the data.

**Results:** There were 1225 antenatal mothers included in the study. The prevalence of irregular antibodies in antenatal mothers was 1.1 %. In the overall prevalence the percentages of anti-D and non-anti-D antibodies were 76% in Rh D Negative mothers and 24% in Rh D positive mothers respectively.

**Conclusion:** Prevalence of Red blood cell alloantibody in antenatal mother was 1.1% with Anti-D still being the most common antibody in Rh D negative mothers similar to the findings in others studies conducted in India. This study also included one inconclusive antibody, which needs to be further elucidated with indigenously developed cell panel for present population.

**Keywords:** Red cell Alloantibody, hemolytic disease of the new born, antenatal mothers

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## INTRODUCTION

The antigens present on the red blood cells determine the blood group. ABO blood group was first identified by Karl Landsteiner in 1901 and found that there are some substances present in the blood that induce clumping of red blood cells which is added to those of another type. He identified three groups A,B, and O based on their antigen present to each other, AB was identified a year later by another research team. The International society of blood transfusion now recognizes 302 blood group antigens, most of which belong to one of 29 genetically discrete blood group system. Antibodies to, many of these 302 antigens have the potential to cause Haemolytic Disease of the Fetus and Newborn (HDFN) and they are, therefore, clinically significant. To name a few are Rh MNS, Kell, Duffy and Kidd. These blood group systems were first described after antibodies were identified in patients. Frequently, such discoveries resulted from the search for the explanation of an unexpected unfavorable reaction in a recipient after a transfusion with formerly compatible blood.[1] Maternal alloimmunization occurs when a woman's immune system is sensitized with foreign erythrocyte antigens which helps to stimulate the production of IgG antibodies. Red blood cells destruction among fetus is a direct consequence of mother's alloantibodies. Though the Rh prophylaxix initiated in 1968, hemolytic diseases among fetus and newborn remains a serious problem. Usually alloantibodies are stimulated by exposure to incompatible blood transfusion and fetomaternal hemorrhage during delivery.[2] Although anti-D is the most common cause of HDFN, more than sixty other RBC alloantibodies have been implicated.

Atypical antibodies, other than anti-D can also

cross the placenta and cause HDFN.[1] It is of utmost importance that the blood bank performs an adequate screen on antenatal patients, irrespective of whether they are Rh (D) positive or Rh (D) negative, to look for clinically significant alloantibodies other than Rh (D) that might cause hemolytic disease of the newborn.[2] Proper periodic screening of Red blood cell antibodies in antenatal mothers can alert the doctor to identify potential association with HDFN and plan accordingly. Later in cases of postpartum hemorrhage it alerts the laboratory to possible difficulty in obtaining blood for the obstetric patients, who is always a potential transfusion recipient so that the time taken to find acceptable donor is minimized. The purpose of this study was to assess the prevalence of red cell antibodies in antenatal mothers. Since there are only few such studies conducted in India, it was decided to screen antenatal mothers in Chennai over the period of one year prospectively.

## MATERIALS AND METHODS

A cross sectional study was conducted on 1225 Multigravida women who had attended the antenatal clinic in the outpatient department of Government and corporation Hospital in Chennai, over a period of one year (2010-2011). Only multigravida women in their first trimester coming for antenatal visit were included by using simple random sampling. The Blood group and Rh (D) type along with antibody screening was performed at the first antenatal visit. The pre-tested pro-forma was used to collect the data. Each patient name, age, sex, obstetric history, blood group, husband's blood group, history of Rh anti-D immunoprophylaxis and history of blood transfusions and associated medical illness were recorded prior to collect 5ml of blood sample. ABO blood grouping and Rh typing were performed for each cases and their husbands (wherever possible) by standardized tube technique method. All RhD negative samples by tube method were confirmed for weak D by an indirect anti-globulin test. Red Cell Alloanti-bodies were screened using commercially available three cell antigen panel (Asia -ID-DiaCell I, II, III) by Coombs gel card. Whenever antibody screening was positive, extended eleven cell panel was used for antibody identification. Antibody screening and identification was done by Gel Technique as per the steps given in the Kit Literature. Ethical clearance was obtained from the Institutional Ethical Committee of The Tamilnadu Dr. M.G.R Medical University.

## STATSTICAL ANALYSIS

Data entry was done using SPSS version 17. Frequency and chi-square test was done. P value $< 0.05$ is considered to be significant.

## RESULTS

During the study period, 1225 multigravida women were screened for the presence of alloantibodies.

**Table-1:** Antibody screening among the study population

| Antibody | Frequency | Percentage |
|---|---|---|
| **Present** | 13 | 1.1% |
| **Absent** | 1212 | 98.9% |
| **Total** | 1225 | 100% |

The prevalence of red cell alloantibody in antenatal mothers was 1.1% and 98.9% did not have any red cell antibody (Table-1).

Red cell alloantibodies were detected with prevalence of 1.1%. Out of 10 Rh D negative women with anti-D, the husband's blood group in all cases was found to be Rh D positive.(Table-4) Out of three Rh D Positive women who had developed Alloimmunization to Non-Rh D Antigens, their husbands were also belong to Rh D Positive group, which was found to be significant with p value of less than 0.01 ($p<0.01$). A history of blood transfusion was present in 7.6% (1/13) women with Red Cell alloantibodies (P- 0.000. Table-1).

The study found a statistically significant correlation between frequency of alloimmuniza-tion and adverse obstetric history. An adverse obstetric history (abortion or medical termination of pregnancy) was present in 76% of patients with anti-D (10/13) (Table-2).

The Gravida status of women showed a statistically significant correlation with alloantibody formation (p-0.000 Table-3).

**Table-4:** The clinical data of antenatal mothers who were Alloimmunized

| Age | Blood group | Antibody | Previous History | H/O of RhIg Immuno-prophylaxis | Husband Blood group |
|---|---|---|---|---|---|
| 28 | A- | Anti-D | $G_2 P_0 L_0 A_1$ | No | O + |
| 24 | AB - | Anti-D | $G_2 P_0 L_0 A_1$ | No | A+ |
| 26 | A- | Anti-D | $G_4 P_1 L_1 A_2$ | No | O+ |
| 23 | O - | Anti-D | $G_2 P_0 L_0 A_1$ | No | B + |
| 26 | A- | Anti-D | $G_3 P_1 L_1 A_1$ | No | B+ |
| 27 | A- | Anti-D | $G_3 P_0 L_0 A_2$ | No | O+ |
| 30 | AB- | Anti-D | $G_4 P_1 L_1 A_2$ | No | O+ |
| 32 | O + | Anti- c | transfusion | No | O + |
| 34 | O- | Anti-D | $G_5 P_0 L_0 A_4$ | Yes | A+ |
| 28 | O- | Anti-D | $G_5 P_1 L_1 A_3$ | No | B+ |
| 27 | A+ | Unknown | $G_4 P_1 L_1 A_2$ | No | O+ |
| 27 | B- | Anti-D | $G_3 P_1 L_1 A_1$ | No | O+ |
| 23 | B+ | Anti-Le[a] | $G_2 P_1 L_1 A_0$ | No | B+ |

**Table-2:** Antibody Identification Vs Abortion

| Antibody | Abortion | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1st | 2nd | 3rd | 4th | Total |
| **Anti-D** | 0 | 7 | 1 | 1 | 1 | 10 |
| **Anti-c** | 0 | 1 | 0 | 0 | 0 | 1 |
| **Anti-Le [a]** | 1 | 0 | 0 | 0 | 0 | 1 |
| **Unknown** | 0 | 0 | 1 | 0 | 0 | 1 |
| **Total** | 1 | 8 | 2 | 1 | 1 | 13 |
| **p-value** | 0.00 | | | | | |

**Table-3:** Antibody Identification Vs Gravida

| Antibody | Gravida | | | | | Total |
|---|---|---|---|---|---|---|
| | 2nd | 3rd | 4th | 5th | 6th | |
| **Anti-D** | 3 | 3 | 2 | 2 | 0 | 10 |
| **Anti-c** | 0 | 1 | 0 | 0 | 0 | 1 |
| **Anti- Le[a]** | 1 | 0 | 0 | 0 | 0 | 1 |
| **Unknown** | 0 | 0 | 1 | 0 | 0 | 1 |
| **Total** | 4 | 4 | 3 | 2 | 0 | 13 |
| **p-value** | 0.000 | | | | | |

**Table-4:** Association of Rh D Antigen with Alloimmunization

| Rh D | Antibodies not detected | Antibodies detected | p-value |
|---|---|---|---|
| **Positive** | 1092 | 3 | < 0.01 |
| **Negative** | 120 | 10 | |

Among the 130 Rh D negative group, 10 developed antibodies, so the prevalence of alloimmunization in this group was 7.69%.[p-0.01] Table-4. Among the Rh D negative antenatal mothers 10 of them developed anti-D Alloimmunization. Out of 10 positives, nine of them had not been given RhIg immunoprophylaxis which was found to be significant [p<0.01].

## DISCUSSION

Antenatal services in India are fragmented and not uniform and there is a limited amount of published data on red cell alloimmunization among pregnant women in India. Although guidelines for screening have been laid down by Directorate General of Health Services, India[3] for screening alloantibodies primarily for Rh D negative women presenting with an adverse obstetric history, it has not been carried out as a routine in many of the centres. Blood grouping and Red cell alloantibody screening though is heavily biased towards the prevention and management of the continuing problems of HDFN, it also gives an advanced warning for adequate compatible blood supply for emergency. This latter point is especially relevant to the urgency of blood demands late in pregnancy and the fact that 16 to 22% of women have blood cross matched during pregnancy.[4] The overall prevalence of red cell alloantibodies among antenatal mothers in present study was 1.1%. Screening and identification of red cell alloantibodies showed 10 cases of Rh D, 1 case each of anti-c, anti-Le[a] and inconclusive antibodies in 1225 mothers during first trimester antenatal visit. This is in accordance with Queenan et al,[5] Helen Howard et al[6] and Sangeeta Pahuja et al[7] studies, which had reported prevalence of 1 to 2% red cell alloimmunization in antenatal mothers.

In the present study, the percentage of red cell alloimmunization in RhD negative antenatal mother was 7.6%. There is a wide variation in

alloimmunization rates among Rh-negative women. Out of 130 Rh D Negative antenatal mothers 78 had RhIg Immunoprophylaxis and 52 had no RhIg Immunoprophylaxis. Out of 52 antenatal mothers who had no history of RhIg Immunoprophylaxis 9 (17.31%) developed red cell alloimmunization. This is in line with a study done by Deka D et al who also observed that failure to administer postnatal anti D prophylaxis was responsible for RhD alloimmunization in more than 50% of cases, followed by failure to administer Anti D after MTP (10%).[9] Another factor also has to be taken seriously as previous studies have shown that there is a complex genetic factor which plays an important role in production of antibodies. The risk of immunization is only about 6 to 9% for an RhD negative mother after an RhD positive pregnancy if RhIg is not administered.[10] In this study, out of 78 RhD Negative antenatal mothers who had RhIg Immunoprophylaxis 1 (1.2%) developed red cell alloimmunization. This is similar to the study done by J.M. Koelewijn et al[11] and Shanthala A.M et al, which states that despite use of anti–D immunization, 1to2% of the cases are still sensitized, indicates that transplacental or fetomaternal hemorrhage may occur during pregnancy or at delivery and can lead to alloimmunization. Half of the failures of anti D immunoglobulin prophylaxis were due to increased fetomaternal hemorrhage.[12,13] In present study, out of 1195 RhD Positive antenatal mothers 3(0.25%) developed red cell alloimmunization against c, Le (a+b) and Unknown antigen. The development of anti-c antibody in one of the 3 patients in this study was due to the previous history of multiple blood transfusions for anaemia in earlier pregnancy. This is in an agreement with other studies such as those of Leif Kornstad[14] who showed a significant proportion of mothers had previously received blood transfusion developed anti-c. In India, Thakral B et al[15] and Shilpa Singla et al[16] reported severe hydrops in an infant born to Rh D positive mother due to anti c antibodies who have received multiple blood transfusions. The percentage of anti-Le$^a$ antibody in this study was 8% (1/13). The development of anti-Le$^a$ antibody in these studies could be explained by the over expression of auto antibodies in pregnancies.[17] The percent- age of an Unknown antibody after 11 cell panel identification in present study was 8 % (1/13). The current study found a statistically significant correlation between frequency of alloimmuni- zation with adverse obstetrics history and gravid status of the women. To conclude, With the advent of advanced IUT (Intrauterine transfusion) technologies and non-invasive investigative procedures like middle cerebral artery peak systolic velocity to predict intrauterine hemolysis, it is imperative to do antenatal red cell antibody screening to save not only fetal/newborn but also alloimmunized mothers who may be in dire need of suitable compatible blood during delivery.

## CONCLUSION

In the present study the prevalence of red cell antibodies in antenatal mothers in Chennai was 1.1%, which is similar to other studies conducted in India. However, large numbers of samples have to be studied to arrive at a conclusive number and type of the antibody prevalent in present population.This study also included one inconclusive antibody, which needs to be further elucidated with indigenously developed cell panel for present population. Present study on red cell alloimmunization in antenatal mothers showed definite spresentce of antigenicity such as husband's incompatible blood group, gravid status, bad obstetric history and previous history of blood transfusion.

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