Original Article

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Pathology Section

# Prevalence and Characterisation of Irregular RBC Antibodies in Antenatal Females from a Tertiary Care Centre in Northern India: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Haemolytic Disease of the Foetus and Newborn (HDFN) is widely known to be caused by the presence of irregular Red Blood Cell (RBC) antibodies in pregnant females. To prevent HDFN, it is crucial to identify these antibodies in pregnant women.

**Aim:** To evaluate the prevalence of irregular red cell antibodies in pregnant women in a tertiary care hospital.

**Materials and Methods:** A cross-sectional retrospective study was conducted in the Department of Transfusion Medicine at Indraprastha Apollo New Delhi, from November 2009 to December 2017. A total of 8,217 prenatal females were examined for irregular red cell antibodies. Antibody screening and blood grouping were performed. The Capture-R ready screen was used to confirm screen-positive tests. Adsorption, elution, and other advanced techniques were utilised as needed. Data was collected from patient records and entered into a Microsoft excel spreadsheet. The statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) software version 14.0 (USA). Statistical tests, including the Chi-squared and Fisher's exact test, were performed, with a p-value less than 0.05 considered statistically significant.

**Results:** Out of the 8,217 prenatal females examined, 105 (1.27%) had positive screening results with the four cell panel. Red cell alloimmunisation was observed in groups that were RhD negative (n=93/105; 88.57%) and positive (n=12/105; 11.42%). The most prevalent alloantibody among RhD negative individuals was alloantibody anti-D (n=83/93; 89.24%), followed by alloantibody anti C+ anti D (n=13/93; 13.97%). Anti-D was the most prevalent alloantibody overall (n=83/105; 79.04%).

**Conclusion:** Alloimmunisation rates were notably high among women lacking the Rh D antigen. Individuals with a history of adverse obstetric outcomes showed a statistically significant association with alloimmunisation. Therefore, it is recommended to screen such individuals for alloantibodies to enable early detection and improve the management of HDFN.

**Keywords:** Alloimmunisation, Antibody screening, Haemolytic disease of the foetus, Red blood cell

## INTRODUCTION

Red cell alloimmunisation in pregnancy occurs due to differences in maternal and foetal RBC antigens [1]. These maternal alloantibodies pass through the placenta into the foetal circulation. If the foetus is positive against maternal red cell antigens, it can lead to the haemolysis of foetal erythrocytes. This can result in a decrease in red cell count and death from heart failure (hydrops foetalis) [1]. The destruction of foetal red cells into the maternal circulation can occur during pregnancy or due to trauma, amniocentesis, cordocentesis, abortion, and other manipulations [1].

The worldwide rate of alloimmunisation ranges from 0.4% to 2.7% [2-4]. The introduction and utilisation of Rh-immunoglobulin prophylaxis, appropriate monitoring, and intervention have successfully reduced alloimmunisation [5]. The most common alloantibody that can lead to HDFN is against Rh (D), with others including Rh (E, e, C, c), Kell, Duffy, Kidd, and MNSs [6,7].

Alloantibodies cause red cell destruction, resulting in severe foetal anaemia. Various strategies are available to treat foetal anaemia, such as Intrauterine Transfusions (IUTs) during pregnancy and exchange transfusions after birth. IUT is a safe procedure for treating foetal anaemia, with an overall perinatal loss rate of approximately 1 to 3% and a significant survival rate of over 80% [8]. Developed countries have their guidelines for antenatal antibody screening to prevent complications related to alloimmunisation. The British Committee for Standards in Haematology (BCSH) has recommended a type and screen policy for all pregnant females to check for the presence of irregular red cell antibodies in the early gestational period [9].

In developing countries like India, the implementation of a type and screen policy during pregnancy to prevent alloimmunisation remains a significant challenge for obstetricians and transfusion practitioners due to the lack of evidence-based guidelines. Regular antenatal screening for irregular alloantibodies may help identify potential complications that can be managed in a timely manner. Based on red cell screening results and the presence of any significant alloantibody, clinicians can be more vigilant for the development of HDFN. Specialised care may be arranged, such as IUT, exchange transfusion, phototherapy, or close monitoring post-delivery. Evidence-based protocols will guide the transfusion centre to ensure the availability of compatible blood for the patient, especially in cases of rare antibodies. Limited data is available from India on red cell alloimmunisation among pregnant women or on other antigens responsible for alloimmunisation. Moreover, knowledge about treatment and follow-up of other clinically significant antibodies, other than anti-D, is lacking in most centres across India.

The aim of the present study is to determine the prevalence of red cell alloimmunisation in antenatal females and to underscore the paramount importance of antibody screening in antenatal women of childbearing age. To make new recommendations, there is an urgent need for data analysis of red cell alloimmunisation during the antenatal period in the present study population.

## MATERIALS AND METHODS

A cross-sectional retrospective study was conducted in the Department of Transfusion Medicine at the tertiary care centre

Indraprastha Apollo New Delhi, India between 1st November 2009 and 31st December 2017. Informed consent was obtained from each of the 8,217 prenatal females whose data was analysed.

**Inclusion criteria:** The study included all multiparous pregnant women, regardless of their gestational period or obstetric history. Women with a previous history of abortions, foetal deaths, stillbirths, early neonatal deaths, intrauterine growth restriction, and congenital anomalies were included in the group with bad obstetric history.

**Exclusion criteria:** Women who had received anti-D prophylaxis in their current pregnancy were excluded from the study.

### Procedure

The data was collected from the patients' medical records, which included details such as the patient's name, age, previous obstetric history, blood group, history of Anti-D Immunoprophylaxis administration, and history of blood transfusions.

In the antenatal clinic, 3 mL blood samples were collected in 2 Ethylene Diamine Tetra-Acetic Acid (EDTA) vials for an immuno-haematological work-up. Blood grouping and red cell antigen phenotyping for "C," "c," "E," "e," and "K" were performed on a fully automated immunohaematology system using Solid Phase Red Cell Adherence technology (SPRCA) on Galileo (Immucor Inc., Norcross, GA, USA) with commercially available antisera (Immucor Inc., Norcross, GA, USA). Antibody screening and identification were carried out on a fully automated immunohematology analyser (Galileo, Immucor Inc., USA) using cell panels and SPRCA technology (Capture-R Ready-ID, Immucor Inc., Norcross, USA). The capture technology for antibody screening uses microplate test wells precoated with intact reagent RBCs. Patients' serum or plasma and Low Ionic Strength Saline (LISS) were added to the RBC-coated microwells and incubated at 37ºC. Unbound red cell antibodies and other plasma proteins were washed off, and an indicator anti-IgG coated RBCs was added. The microwells were then centrifuged, and a positive test was indicated by adherence of indicator RBCs to the bottom of the well.

All laboratory investigations were conducted in accordance with the department's Standard Operating Procedures (SOPs) and the guidelines from the American Association of Blood Banks (AABB) technical manual [10]. Infants were assessed for HDFN using clinical criteria (jaundice, pallor) and laboratory criteria (anaemia and increased serum bilirubin) [11].

## STATISTICAL ANALYSIS

Data was analysed with the help of SPSS version 14.0 (IBM, Chicago, USA) software. Mean and percentages were calculated for quantitative data. The Chi-squared and Fisher's exact tests were applied. A p-value of <0.05 was considered statistically significant.

## RESULTS

In total, 8,217 prenatal females were examined for the presence of irregular antibodies during the study period. The majority blood group found in antenatal females was B positive with 36.68% (n=3014/8217). There were 92.55% (n=7605/8217) D antigen-positive women and 7.44% (n=612/8217) D antigen-negative [Table/Fig-1].

| Blood group | No. of women (n=8217) (%) |
|---|---|
| A | 2195 (26.17) |
| B | 3014 (36.68) |
| AB | 710 (8.46) |
| O | 2298 (27.96) |
| $Rh^{+ve}$ | 7605 (92.55) |
| $Rh^{-ve}$ | 612 (7.44) |
| Total | 8217 (100) |

**[Table/Fig-1]:** Distribution of blood groups in antenatal females.

A total of 105 prenatal females tested positive in antibody screening, resulting in an overall prevalence of alloimmunisation of 1.27% (105/8,217). Among the 105 alloimmunised females, 93 (88.57%) were RhD negative. Anti-D (n=83/93; 89.24%) and anti-C+D (n=13/93; 13.97%) were the two most prevalent alloantibody types among RhD negative individuals [Table/Fig-2].

Among the 105 alloimmunised females, 12 (11.42%) were Rh(D) positive [Table/Fig-3]. Out of the 105 females, 83 (79.04%) had a single alloantibody and 22 (20.95%) had multiple alloantibodies. Anti-D was the most prevalent single alloantibody (n=61/83; 73.49%) as well as in combination with other alloantibodies (22/22; 100%). Anti-C+D was the most prevalent multiple alloantibody (n=13/22; 59.09%), followed by anti-D+ Anti-E (n=2/22; 9.09%). Anti-D (n=83/105; 79.04%) was the most prevalent alloantibody overall (alone or in combination). The prevalence of alloimmunisation in the D antigen-negative group was 15.19% (n=93/612). The prevalence of alloimmunisation in the D antigen-positive group was 0.15% (n=12/7605). Within the D antigen-positive group, 4/12 (33.33%) of the antibodies were anti-C (alone), and 3/12 (25.0%) had Anti-E [Table/Fig-3].

| D antigen- Negative antibodies (N=93) | Frequency | % |
|---|---|---|
| Anti-D | 61 | 65.59 |
| Anti-C | 08 | 8.6 |
| Anti-C+D | 13 | 13.97 |
| Anti-Lea, Anti-D | 02 | 2.15 |
| Anti-D and Anti E | 02 | 2.15 |
| Anti-Cw | 01 | 1.07 |
| Anti-D+Anti-G | 01 | 1.07 |
| Anti-D, Anti-C, Anti fyb | 01 | 1.07 |
| Anti-D, Anti-C , Anti-S | 01 | 1.07 |
| Anti-D, Anti-E, Anti-K and Anti-S | 01 | 1.07 |
| Anti-D, Anti-C, Anti-E | 01 | 1.07 |
| Autoantibody with underlying alloantibody | 01 | 1.07 |

**[Table/Fig-2]:** Distribution of antibodies among D antigen- positive alloimmunised women.

| D antigen- Positive antibodies (N=12) | Frequency | % |
|---|---|---|
| Anti-M | 1 | 8.3 |
| Anti-Mia | 1 | 8.3 |
| Anti- C | 4 | 33.33 |
| Anti- Leb | 1 | 8.3 |
| Anti-jka | 1 | 8.3 |
| Anti-fya | 1 | 8.3 |
| Anti- E | 3 | 25 |

**[Table/Fig-3]:** Distribution of antibodies among D antigen- positive alloimmunised women.

In the present investigation, alloantibodies were discovered in 5.95% (n=85/1427) of pregnant women with a poor obstetric history and 0.29% (n=20/6695) of pregnant women without a poor obstetric history (p<0.001) [Table/Fig-4]. Reviewing the demographics of the 105 alloimmunised pregnant women reveals that the mean maternal age was 31.2 years (range: 24-40), 93.33% of them (n=98/105) were multiparous, and the remaining 6.66% (n=7/105) were primigravida. According to [Table/Fig-5], parity ranged from 0 to 8, whereas gravidity ranged from 1 to 7.

| Obstetric history | Antibodies detected | Antibodies not detected | Chi-square test |
|---|---|---|---|
| Poor obstetric history present (n=1512) | 85 (5.62%) | 1427 (94.37%) | 277.6125 (p-value <0.001) |
| Poor obstetric history absent (n=6715) | 20 (0.29%) | 6695 (99.70%) | |

**[Table/Fig-4]:** Association of poor obstetric history with alloimmunisation.

| Maternal characteristics | Values in mean or % |
|---|---|
| Maternal age mean (years) | 35 (25-45) |
| Gravidity mean (n) | 4 (1-7) |
| Multiparous women, (%) | 93.33% |
| Primigravida, (%) | 6.67% |
| Parity mean (n) | 4.5 (0-8) |

**[Table/Fig-5]:** Maternal demographics.

## DISCUSSION

The RBC antibody screening is a common prenatal procedure with the goal of identifying maternal RBC antibodies that can pass the placental barrier and destroy foetal RBCs, potentially leading to foetal morbidity and mortality [1]. Although the Drug Controller General of India has established guidelines for screening, the majority of alloantibody testing is done to check for anti-D antibodies, even though antibodies against more than 50 other RBC antigens have been linked to haemolytic disease in foetuses and newborns [12].

A total alloimmunisation rate of 1.27% was discovered in this study (n=105/8217) among pregnant women. A comparison of the published worldwide rates of alloimmunisation in pregnancy is shown in [Table/Fig-6] [4,13-18]. Dholakiya SK et al., in their study reported an alloimmunisation rate of 1.3%, while Suresh B et al., reported an alloimmunisation rate of 1.1% [13,14]. Basu S et al., reported an alloimmunisation rate of 2.3% in all pregnancies [15]. In contrast, Gottvall T and Filbey D found an alloimmunisation rate of 0.5% in all pregnancies, with clinically significant alloimmunisation in 0.16% of pregnancies [4]. Both Pahuja S et al., and Varghese J et al., reported prevalence rates of 1.25% and 1.5%, respectively [16,17]. In contrast, Gothwal M et al., observed an alloimmunisation rate of 3.2% [18]. The variability of the participating populations, the many screening procedures, and the various methods for antibody identification may all contribute to the high prevalence of alloimmunisation.

| Study | Country | Year of study | Year of publication | Alloimmunisation rate |
|---|---|---|---|---|
| Dholakiya SK et al., [13] | Gujrat, India | 2014 to 2016 | 2021 | 1.3 |
| Suresh B et al., [14] | Tirupati, India | 2012 to 2013 | 2015 | 1.1 |
| Basu S et al., [15] | Karnataka, India | 2013 to 2015 | 2011 | 2.3 |
| Gottvall T and Filbey D [4] | Sweden | 1992 to 2005 | 2008 | 0.5 |
| Pahuja S et al., [16] | New Delhi, India | 2008 to 2009 | 2011 | 1.3 |
| Varghese J et al., [17] | Vellore, India | 2008 to 2009 | 2013 | 1.5 |
| Gothwal M et al., [18] | Rajasthan, India | 2019 to 2021 | 2022 | 3.2 |
| Present study | New Delhi, India | 2009 to 2017 | 2023 | 1.27 |

**[Table/Fig-6]:** Comparative study of the prevalence of red cell alloantibodies in antenatal women [4,13-18].

In the present study, the authors observed that women with poor obstetric histories had a higher incidence of alloimmunisation, with rates of 5.95% (n=85/1427) compared to those without such histories, where the rate was 0.29% (n=20/6695). The alloimmunisation rate in the D antigen-negative group was 15.19% (n=93/612). Anti-D (n=83/93; 89.24%) and anti-C+D (n=13/93; 13.97%) were the two alloantibody types that were most prevalent among Rh(D)-negative individuals in the present study group. These antibodies are highly immunogenic and clinically significant, as they can cause severe Haemolytic Disease of the Newborn (HDFN) in neonates and haemolysis in patients if antigen-positive blood is transfused.

A similar alloimmunisation rate of 10.4% (41/394) among Rh-negative women was reported by Pahuja S et al., [16] while Al-Ibrahim NA and Al Saeed AH found a higher prevalence of 7.1% in Saudi Arabia [3]. Lurie S et al., discovered a low alloimmunisation rate of only 0.9% in Israel [19]. In women who were Rh-negative, the alloimmunisation rate was reported to be 2.98% by Solola A and Mason JM [20]. In contrast to research conducted in the West, the present study found a significantly higher rate of alloimmunisation in Rh-negative women, which may result from a lack of standardised protocols.

The Rh-positive group in the present study had an alloimmunisation rate of 0.15%. Lurie S et al., and Adeniji AA et al., reported alloimmunisation rates of 0.2% and 0.15%, respectively, in Rh-positive women [19,21]. In the Rh-positive group, RBC alloimmunisation occurred at a rate of 0.7% (7/1000) each year, according to Sankaralingam P et al., from India [22]. The prevalence of non Rh D alloimmunisation was reported to be 1.7% (34 out of 2001) in a similar study from Uganda, where anti-M, anti-Lea, anti-Fyb, anti-K, anti-Jk a, anti-Lua, and anti-Kp antibodies were discovered [23]. In the present study, anti-C antibodies were found to be the most prevalent, followed by anti-E antibodies (25%). Therefore, the majority of the alloantibodies were against the Rh system antigens and were clinically significant.

Antenatal red cell screening is not universally available in all healthcare centres across India. Accessibility to screening tests is limited, particularly in rural areas or resource-constrained settings. Extensive research on pregnant women is required to gather enough information to develop recommendations for red cell screening in pregnant women.

Red cell screening during pregnancy is a crucial step in ensuring the health and well-being of both the mother and the foetus. It is important to establish protocols to ensure that screening is conducted in a timely manner, allowing enough time for appropriate interventions if necessary. In a resource-poor country like India, where adequate health infrastructure is not available, the implementation of universal antenatal antibody screening may not be feasible. In light of this scenario, atleast screening of Rh-negative pregnant women with poor obstetric history should be implemented for better outcomes. In future studies, the authors recommend studying the correlation of these alloantibodies with neonatal outcomes.

### Limitation(s)

In the current study, the lack of long-term follow-up of these antenatal cases to assess the severity of red cell alloimmunisation. However, there is a need for large-scale studies in pregnant women to gather enough data to develop guidelines for testing and intervention methods for alloimmunisations during pregnancy.

## CONCLUSION(S)

It was observed that the prevalence of alloantibody anti-D among pregnant females, emphasising the importance of implementing adequate Anti-D prophylaxis to mitigate complications associated with anti-D alloimmunisation in the foetus. The authors recommend the adoption of routine antenatal alloantibody screening for all multiparous women. Additionally, given in the present findings, it is imperative to conduct antibody screening in pregnant women with a history of obstetric complications.

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