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# Red cell alloimmunization among antenatal women attending tertiary care center in Jamnagar, Gujarat, India

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**Abstract:**

**BACKGROUND:** The following study was conducted to measure the presence of alloantibodies of Rh and other blood group antigens produced due to fetomaternal hemorrhage in all antenatal women as well as those leading to hemolytic disease of fetus and newborn; presenting to a tertiary care center, G.G. Government Hospital, Jamnagar, Gujarat, India, between April 2014 and March 2016 (2 years).

**MATERIALS AND METHODS:** All multiparous women irrespective of their period of gestation or obstetrics history were included whereas those having taken anti-D immunoprophylaxis or with a history of blood transfusion were excluded. Antibody screening and identification were done using Bio-Rad ID microtyping system.

**RESULTS:** Out of total 8920 multigravida females, 8488 were D-antigen positive whereas 432 were D-antigen negative. A total of 126 antibodies among 117 females (1.31%) were found; out of them, 33 were found in D-antigen positive females (0.39%) and 84 in D-antigen negative ones (19.44%) looking at overall frequency of other antibodies such as anti-C: 9, anti-c: 9, anti-E: 13, anti-Cw: 1, anti-M: 5, anti-S: 8, anti-Fya: 3, and anti-D: 78; it was found that anti-D is the most common.

**CONCLUSION:** The rate of alloimmunization in D-antigen negative women was found to be very high as compared to other studies in western region; hence, strict follow-up of immunoprophylaxis of all Rh D-negative women needs to be taken care of. Apart from this, D-antigen-positive women also show alloimmunization against various antigens giving the prevalence of 0.39%; hence, it should be mandatory that there should be one standard universal protocol for screening of all antenatal women.

**Keywords:**

Alloimmunization by fetal red cells due to fetomaternal hemorrhage, blood groups, hemolytic disease of fetus and newborn

## Introduction

Landsteiner,[1] with his colleagues von Castello and Sturli,[2] discovered ABO blood group system. Fatal rates related to blood transfusion were greatly reduced when blood grouping tests were introduced by Ottenberg.[3] The concept of existence of irregular antibodies by giving possibility of agglutination reaction between donor cells and patient serum was discussed by Unger[4] in 1921. Identifying alloantibodies by test introduced by Coombs *et al*.[5] added a new dimension to the blood transfusion safety. All these led to the discovery of 346[6] blood group antigens classified in 35 blood group systems[7] and 38 high- and low-frequency antigens which do not fulfill the requirements for classification system.

An immune response is evoked by invasion of incompatible antigen to immunocompetent host leading to alloimmunization. Immune responses are of two types:

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a. Thymus independent: Consisting mostly of carbohydrate antigens producing IgM antibodies either naturally occurring or derived from the environment; their interaction leads to hemolysis mostly of intravascular type
b. Thymus dependent: Consisting mainly of protein antigens producing IgG antibodies whose interaction leads to extravascular hemolysis.

Out of various blood group systems discussed, most important antibodies leading to alloimmunization are RH (anti-D, -C, -E, -c, and -e), KEL (anti-K), FY (anti-Fya and anti-Fyb), JK (anti-Jka and anti-Jkb) and the MNS (anti-M, -S, and -s). Out of them, anti-D is considered to be the most immunogenic. After transfusion of D-positive erythrocytes, about 80% of immunocompetent D-negative people become alloimmunized.[7,8] Hence, in order to prevent this, testing of anti-D before transfusion is mandatory.

The major cause of hemolytic disease of the fetus and newborn (HDFN) was anti-D occurring in pregnant Rh-negative women; the incidence of which has drastically reduced from 12%–13% to 1%–2% due to introduction of anti-D prophylaxis worldwide.[9] However, HDFN due to other minor blood group antigens such as Kell, Kidd, Duffy, and MNS blood group systems and anti-c, anti-E were left out. Availability of wider screening tests has made it possible to detect these antigens also, but in developing countries like India, routine screening of these irregular antibodies may not be possible. Antibodies to s, p, Kidd, and Duffy are known to cause mild-to-severe degree of HDFN whereas those to S, I, k, Lutheran, and Kell are known to cause mild-to-moderate degree of hemolysis.[10,11]

Maternal sensitization or isoimmunization occurs in response to foreign erythrocyte surface antigens leading to the formation of IgG type antibodies. The most common routes of alloimmunization are through blood transfusion, fetomaternal hemorrhage due to delivery, other interventional procedure, ectopic pregnancy, or abortion. During pregnancy, antibodies produced due to alloimmunization cross placenta and cause hemolysis of fetal red cells and thereby leading to anemia. More severe stage due to deranged fetal metabolism leads to hydrops fetalis. With anti-D immunoprophylaxis, the frequency of alloimmunization of the mother has severely reduced resulting in better fetal outcome and prognosis. Moreover, due to recent advancement in fetal surveillance, outcomes of such conditions have improved to a great extent. The present study reviews pathophysiology, diagnosis, and management of red cell RhD alloimmunization and also includes discussion of various rare erythrocyte antigens.

The present study has been done with the aim to determine the prevalence and trends of RhD negativity among pregnant women who attended Outdoor Patient Department of Obstetrics and Gynecology at present institute, to analyze antenatal Rh and other red cell antigen alloimmunization by fetomaternal hemorrhage, and to determine the presence of other uncommon antibodies in antenatal women leading to hemolytic disease of newborn.

## Materials and Methods

This prospective study was carried out for the duration of 2 years from April 2014 to March 2016 in Department of Institute of Himalayan Bioresource Technology at the present institute. Consent was taken from each patient. The study includes all the multiparous pregnant women irrespective of their period of gestation and obstetric history. History of having received anti-D immunoprophylaxis (in the current pregnancy) and history of blood transfusion were excluded from the study. For each patient, name, age, sex, obstetric history, and blood group were noted.

Blood sample was taken from antenatal patients mostly in the first trimester or whenever the patient first presented to the institute. After they met the inclusion and exclusion criteria, the sample was collected in ethylenediaminetetraacetic acid (EDTA) and plain vacuettes.

Using standard blood bank methods, red cells from EDTA are used for forward grouping and serum from plain tube was used for reverse grouping. The presence of agglutination and its grade was noted and results were interpreted accordingly.[12] After confirmation of ABO and Rh group of females, plasma/serum was screened for the presence of antibodies which was performed using antihuman globulin gel cards (ID-Card LISS/coombs) and three cell panel (ID-DiaCELL I, II, III-Asia). Those with positive antibody screening were analyzed further for antibody identification test using eleven cell panel (Set ID-Dia Panel). The entire test was done using Bio-Rad ID microtyping system. Results were read according to the grading system.

Analytical criteria of antibody identification panels for detection of antibody are as follows:
1. Three antigen positive cells are reactive
2. Three antigen negative cells are nonreactive
3. Clinically significant and common antibodies are "Ruled Out" – Rh (anti-D, -C, -c, -E, and -e), Kell (anti-K and anti-k), Duffy (anti-Fya and anti-Fyb), Kidd (anti-$Jk^a$ and anti-$Jk^b$), MNSs (anti-M, -N, -S, and -s), Lewis (anti-$Le^a$ and anti-$Le^b$), P system (anti-P1)

4. The patient lacks the corresponding antigen; data were entered in Microsoft excel 2007 and data were analyzed using EPI INFO 7.0 for Windows for the statistical analysis of the association between red blood cell alloantibody and adverse obstetric history and gravida status.

## Results

### Blood group distribution among multigravida women

During the study period, 8920 multigravida women were screened for the presence of alloantibodies. With regard to the major blood group systems (ABO and Rh), the most common phenotype was B positive. There were 8488 D-antigen-positive women (95.16%) and 432 D-antigen-negative women (4.84%) 0. A total of 126 antibodies were detected in 117 patients, giving an overall prevalence of alloimmunization of 1.31% (117/8920).

### Association of D-antigen with alloimmunization

Among the 432 women in the D-antigen-negative group, 84 developed antibodies, so the prevalence of alloimmunization in this group was 19.44% [Table 1].

Within the D-antigen negative group, 78/84 (92.85%) of the antibodies were anti-D (alone or in combination with anti-C), 9/84 (10.00%) were anti-C (in combination with anti-D), 03/84 (3.57%) were anti-E, and 01/84 (0.84%) was for each anti-Fya, anti-S, and anti-c.

Of all 126 antibodies detected in this study, 33 were found in D-antigen-positive women, giving the prevalence of alloimmunization in the D-antigen-positive group of 0.39% (33/8488) [Table 2].

### Frequency of alloantibodies according to blood group systems

Within the whole study group ($n$ = 8920), antibodies to Rh blood group system were most common, out of them anti-D (61.91%) was most frequently encountered whereas least common alloantibodies were that of Duffy blood group, out of them anti-Fya (2.39%) was found to be the culprit [Table 3].

### Adverse obstetric history and alloimmunization

In the present study, alloantibodies were found in 5% (89/1778) of antenatal females with an adverse obstetric history and in 0.39% (28/7142) of antenatal women without an adverse obstetric history [Table 3].

An adverse obstetric history (any history of stillbirth, abortion, or medical termination of pregnancy) was present in 71.91% of patients with anti-D (alone or in combination with anti-C, 64/89).

**Table 1: Association of D-antigen with alloimmunization**

| | Antibodies not detected (%) | Antibodies detected (%) |
|---|---|---|
| D-antigen positive | 8455 (99.6) | 33 (0.38) |
| D-antigen negative | 348 (80.56) | 84 (19.45) |

**Table 2: To compare rate of alloimmunization in Rh-negative and Rh-positive women in various studies**

| Study | Alloimunization in Rh negative (%) | Alloimmunization in Rh positive (%) |
|---|---|---|
| Lurie *et al*. | 0.9 | 0.2 |
| Al-Ibrahim *et al*. | 7.1 | 0.15 |
| Solola *et al*. | 2.98 | - |
| Pahuja *et al*. | 99.8 | 0.12 |
| Varghese *et al*. | 58.2 | 41.8 |
| Present | 19.44 | 0.39 |

### Antibody formation in relation to gravida status

The data relating to antibody formation and the number of pregnancies are presented in Table 4.

## Discussion

Limitation exists on data of alloimmunization in India. All pregnant females with adverse obstetrics history or RhD-negative status are only screened despite several other screening guidelines laid out by Drug Controller General, India.[13] In case of Rh-negative women, the rate of alloimmunization among different studies varied widely as shown in Table 2.[14-18] Comparison between rates of alloimmunization of the present study with those of other studies is shown in Table 5.[15,17-21]

As there is lack of implementation of anti-D immunoprophylaxis, this ratio of the present study is higher. Out of total alloimmunization, about 61.91% were due to anti-D. Despite immunoprophylaxis, there are several studies that support our finding like Gottvall and Filbey[19] has 60% prevalence of anti-D alloimmunization whereas Lenkiewicz and Zupańska,[22] Howard *et al*.[23] and Pahuja *et al*.[17] had 45.5%, 40.98% and 78.4% respectively.

In case of Rh-positive women, alloimmunization rate was low. In the present study, it was 0.39%, Lurie *et al*.,[14] Adeniji *et al*.,[24] and Pahuja *et al*.[17] had 0.2%, 0.15%, and 0.12%, respectively.

In the present study, we found a statistically significant correlation between frequency of Rh alloimmunization and adverse obstetric history (odd ratio = 13.38, $\chi^2 \approx 234$, $P < 0.001$) which means that the occurrence of an antibody-positive women having an adverse obstetric history was higher than women who were antibody

**Table 3: Distribution of alloantibodies detected**

| | | | Distribution | | |
|---|---|---|---|---|---|
| Antibodies ($n$=126) | Number of alloantibodies detected in patients | Percentage of alloantibodies | D-antigen-positive women | D-antigen-negative women | Adverse obstetric history OR=13.38, $\chi^2 \approx 234$, $P$<0.001 |
| Anti-D | 78 | 61.91 | 0 | 69 | 58 patients |
| Anti-C and Anti-e | 9 | 7.14 | 0 | 9 | 6 patients |
| Anti-E | 13 | 7.14 | 10 | 3 | 12 patients |
| Anti-M | 5 | 10.32 | 5 | 0 | 5 patients |
| Anti-c | 9 | 0.79 | 8 | 1 | 8 patients |
| Anti-S | 8 | 3.96 | 7 | 1 | 0 |
| Anti-Fya | 3 | 6.35 | 2 | 1 | 0 |
| Anti-Cw | 1 | 2.39 | 1 | 0 | 0 |
| Total | 126 | 100 | 33 | 84 | 89 |

OR = Odds ratio

**Table 4: Antibody formation in relation to gravida status**

| Gravida status | II | III | IV | V | Total |
|---|---|---|---|---|---|
| Total cases | 5890 | 2426 | 580 | 24 | 8920 |
| Antibody positive | 58 | 30 | 28 | 1 | 117 |
| Percentage of antibody to total cases of respected gravid | 0.98 | 1.24 | 4.83 | 4.17 | |

$\chi^2 \approx 27.86$, $P$<0.001

**Table 5: Comparison between various studies regarding rate of alloimmunization**

| Various studies | Rate of alloimmunization of all antibodies (%) | Rate of alloimmunization in clinically significant antibodies (%) |
|---|---|---|
| Gottvall and Filbey | 0.4 | 0.16 |
| Koelewijn *et al*. | 1.2 | 4 |
| Al-Ibrahim *et al*. | 1.92 | 1.0 |
| de Vrijer *et al*. | 2.71 | - |
| Pahuja *et al*. | 1.2 | - |
| Varghese *et al*. | 1.48 | - |
| Present study | 1.31 | 3.4 |

negative. The gravida status of women showed a statistically significant ($\chi^2 \approx 27.86$, $P < 0.001$) positive correlation with alloantibody formation. Koelewijn *et al*.[20] found that the prevalence of alloantibodies other than anti-D is 0.38% whereas that with another study carried out from North India was about 0.45%;[26] another study from South India showed prevalence of about 1.48.[27] Lurie *et al*.[14] have suggested that antibody screening is not warranted from a cost-clinical benefit perspective. Lee *et al*.[25] supported the view that routine antenatal antibody screening for Chinese women may not be worthwhile.

Based on the fact that anti-D accounted for 64.10% of all alloantibodies, we need to focus more on anti-D immunoprophylaxis. In the present study, there was a glaring, statistically significant difference between alloimmunization rates in RhD-negative versus Rh D-positive group comparable with another study from North India showing statistical significance[26] (odds ratio = 0.0162, $\chi^2 = 138.47$, $P < 0.001$).

On the basis of the above results and discussion, we conclude that prevalence of anti-D alloimmunization among antenatal women with D-antigen negative group was maximum as compared to other studies of western region.

Hence, anti-D immunoprophylaxis measures should be taken with great efficiency for antenatal pregnant women. Apart from anti-D, there are other clinically significant antigens responsible for alloimmunization as mentioned above; hence, there should be one universal protocol framed for the screening of all antenatal women. Moreover, with respect to adverse obstetric history and gravida status, there was a significant correlation established between the adverse obstetric history and gravida status and the rate of alloimmunization.

Hence, by the above study, we can conclude that there is yet to go a long way in obtaining best antenatal care practices as far as developing country like India is concerned.

## Limitation

It is possible that some antibodies in the present study were missed by the absence of routine third-trimester screening. In addition, the present study included only hospital attendees and do not represent the prevalence of anti-D among a large number of Indian women who do not have access to obstetric care. Any other population specific antigen may account for large proportios of undentified antibodies in the present study, needs further evaluation. However, there still remains rare possibility of other antibodies which remain unreported/unidentified due to limitations in facilities for their identification.

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## Conflicts of interest

There are no conflicts of interest.

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