Original Research Article

The changing trend of alloimmunization in antenatal females: experience from a tertiary care centre in north-western India

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Received: 26 September 2021
Revised: 31 October 2021
Accepted: 01 November 2021

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ABSTRACT

Background: Haemolytic disease of the foetus and new-born (HDFN) is a major concern during the antenatal period, especially in countries with low human development index (HDI). The guidelines for antenatal screening and management significantly vary from one geographical region to another. Since the introduction of RhIG immunoprophylaxis, the incidence of HDFN caused by alloimmunization to D antigen has markedly reduced, while that caused by other minor blood group antigens has not been addressed significantly and needs to be given due consideration.

Methods: The study was carried out to evaluate the incidence of alloimmunization and analyse various factors associated with HDFN in north-western India. A total of 1700 antenatal cases were evaluated over a period of 20 months, antibody screening and identification was performed on their samples and results were analysed.

Results: Out of the 1700 cases, 21 were detected to have the presence of an alloantibody with a prevalence of 1.24%. Out of these, 11 were Rh (D) negative while the remaining 10 were Rh (D) positive. The rate for alloimmunization was higher in females who had a history of blood transfusion (1.24%), bad obstetric history (1.24%), and multigravida status (1.24%).

Conclusions: Screening all pregnant females for alloimmunization to RBC antigens, irrespective of their Rh status will help in minimizing the incidence of the HDFN. The practice of providing partial phenotype matched blood to the females of the childbearing age group should be encouraged to reduce the overall incidence of alloimmunization and HDFN.

Keywords: Alloimmunization, Antenatal screening, HDFN, Rh-negative pregnancy

INTRODUCTION

The guidelines for antenatal screening for the detection of alloimmunization to RBC antigens markedly varies from one geographical region to another and generally determined as per local policies.1 Haemolytic disease of the foetus and newborn (HDFN) is a major preventable cause of mortality and morbidity, especially in countries with a low human development index (HDI).2 Most guidelines recommend screening pregnant women for irregular RBC antibodies early in the pregnancy and around the 28th week of gestation to rule out antibodies that may cause HDFN, but the clinical practice may differ from one centre to another.3,4

HDFN may be caused by naturally occurring ABO blood group antibodies as well as alloantibodies directed against rhesus (Rh) antigens and other minor blood group antigens. The Rh blood group system has been most commonly implicated in the aetiology of HDFN.5 Since the introduction of RhIG immunoprophylaxis, the incidence of HDFN due to alloimmunization to D antigen has significantly reduced.6 Other red cell antigens, namely C, c, E, e, Kell, MNS, Duffy (Fy), Kidd (Jk) have
emerged as an important cause of red cell alloimmunization leading to HDFN. Apart from anti-D; anti-K, anti-e, anti-Fy^a^ and anti-E have been implicated with severe HDFN. These irregular antibodies continue to be a major causative factor as there is no prophylactic immunoglobulin for these antigens. Most of the detected cases of HDFN can be treated with intrauterine transfusions (IUTs) during the antenatal period and with the exchange transfusion after delivery. Hence, if all pregnant women are screened for irregular IgG antibodies during the antenatal period, then it could help in early detection and if needed, appropriate clinical intervention can be done to save the affected foetus. Screening for these antibodies is a regular part of antenatal workup in most of the developed countries, but many countries still don’t have such protocols. Today, cases still occur because of failures in the health care system to ensure that RhIG is appropriately given, and because maternal alloimmunization against blood group antigens other than Rh (D) can cause HDFN. At the authors’ institute, routine antenatal screening is done for all booked cases. As there is limited literature regarding this topic, this study was carried out to find the incidence and identify the antibodies most commonly involved in causing HDFN in antenatal females in north-western India.

This study aimed to evaluate the incidence of alloimmunization and identify the most commonly implicated alloantibodies causing HDFN in antenatal females in north-western India. The secondary objective of the study was to evaluate the association of alloimmunization with other maternal factors like gravida status and bad obstetric history (preterm, previous IUD, hypertension and diabetes mellitus).

**METHODS**

**Study population**

All booked antenatal females undergoing treatment and evaluation at the Department of Obstetrics at the Santokha Durlabhjee Memorial Hospital and Research Institute, Jaipur (Rajasthan).

**Study duration**

The study was carried out over a period of 20 months, from April 2018 to November 2019.

**Inclusion criteria**

All eligible consecutive pregnant women who had not received anti-D and registered at the department of obstetrics at the institute.

**Exclusion criteria**

Antenatal females who had either received anti-D prophylaxis in their current pregnancy or with any other diagnosed cause of haemolysis (for example AIHA) were excluded.

**Sample size**

The sample size was calculated at a 95% confidence interval assuming a 1.1% prevalence of alloantibodies in pregnant females as the reference study. At the absolute allowable error (precision) of 0.5%, 1671 pregnant women were required as sample size. We included 1700 pregnant women as the final sample size for the present study considering attrition.

\[
n = \frac{(Z\alpha)^2pq}{L^2}
\]

n= sample size
p= prevalence
q=1-p
L= acceptable error

n was calculated to be 1671

**Study design**

It was a prospective observational study.

**Consent and ethical clearance**

Ethical clearance was obtained from the institutional ethical committee. Informed consent was taken from each participant after clearly explaining the aim and the nature of the study. Full confidentiality of the patient data was maintained.

**Sample collection**

Maternal blood samples were collected using aseptic precautions during their first antenatal visit at the antenatal clinic in 3.0 ml EDTA tubes. Following tests were then performed on the sample: a) The sample was tested for ABO and Rh typing in Ortho Biovue System (Ortho Clinical Diagnostics, USA) using column agglutination technique as per the manufacturer’s recommendations. b) Direct and Indirect antiglobulin test using the gel technique in LISS-Coombs’ AHG gel Card containing IgG and c3d (Biorad, Morat, Switzerland). c) Antibody screening was done using the commercial 3-cell panel Surgi-screen (OrthoClinical Diagnostics, USA) with the Column Agglutination Technique (LISS-Coombs’AHG gel Card, Biovue, Ortho Clinical Diagnostics, USA). d) The samples that were detected to be positive for antibody screening were further evaluated for antibody identification using the commercial 11-cell panel (Resolve Panel A, Ortho Clinical Diagnostics, USA). e) Antibody titres were done using the standard tube technique as described in the AABB Technical manual. f) The husband’s blood
sample (3.0 ml EDTA) was obtained when feasible. It was tested for ABO and Rh including Rh extended antigen profile in ortho biovue system.

Statistical analysis

Statistical analysis was carried out using Medcalc 16.4 software. Continuous variables were summarized as Mean and S.D. while nominal/categorical variables were summarized as proportions. Unpaired t-test and other appropriate parametric tests were used for the analysis of continuous variables, whereas the chi-square test/Fischer-exact test was used for nominal/categorical variables. A p value <0.05 was considered to be significant.

RESULTS

Over the study period of 19 months, a total of 1828 females were enrolled, out of which 128 did not meet the inclusion criteria and 1700 were finally included in the study. The study population belonging to the childbearing age group ranged from 18 to 45 years of age. The mean age was 26.89 years with an SD of 4.45 years.

Table 1: Incidence of alloimmunization and gravida status of females.

| Gravida status | Alloimmunization | Total |
|----------------|------------------|-------|
|                | Absent | Present | No. % | No. % |
| G1             | 660    | 99.40   | 4     | 0.60  | 664 | 39.06 |
| G2             | 507    | 97.88   | 11    | 2.12  | 518 | 30.47 |
| G3             | 305    | 99.03   | 3     | 0.97  | 308 | 18.12 |
| G4             | 145    | 100.00  | 0     | 0.00  | 145 | 8.53 |
| G5             | 42     | 95.45   | 2     | 4.55  | 44  | 2.59 |
| G6             | 14     | 93.33   | 1     | 6.67  | 15  | 0.88 |
| G7             | 4      | 100.00  | 0     | 0.00  | 4   | 0.24 |
| G8             | 2      | 100.00  | 0     | 0.00  | 2   | 0.12 |
| Total          | 1679   | 98.76   | 21    | 1.24  | 1700| 100.00 |

Out of the 1700 antenatal cases, 21 were detected to have alloantibody during the screening and the overall prevalence of alloimmunization was 1.24%. Out of these, 4 were primigravida and 17 were multigravida. All these cases were diagnosed to have HDFN and managed accordingly. Out of the 17 multigravida females; 11 were G2 (2.12%), 3 were G3 (0.97%), 2 of them were G5 (4.55%) and only one was G6 (6.67%). The distribution has been described in Table 1. The p value was significant (p =0.034) with Chi-square=15.169 and 7 degrees of freedom.

Out of 1679 remaining females, 66 were below 20 years of age, 664 were in the age group of 21-25 years, 636 were in 26-30 years age group, 244 were in the 31-35 years age group, 60 were in 36-40 years age group and 9 were above 40 years.

Table 2: Distribution of pregnant females according to history of blood transfusion.

| History of previous transfusion | Alloimmunization | Total |
|---------------------------------|------------------|-------|
|                                 | Absent | Present | No. % | No. % | No. % |
| Absent                          | 1342   | 99.11   | 12    | 0.89  | 1354 | 79.65 |
| Present                         | 337    | 97.40   | 9     | 2.60  | 346  | 20.35 |
| Total                           | 1679   | 98.76   | 21    | 1.24  | 1700 | 100.00 |

Chi-square =5.311 with 1 degree of freedom; p=0.021

In females with a previous history of blood transfusion, the incidence of alloimmunization was 2.6%, whereas in females without any history of transfusion, it was 0.89% with a significant p value (0.021), with Chi-square =5.311 and 1 degree of freedom. In non-alloimmunized females, a history of previous blood transfusion was present in 337 (20.07%) cases whereas it was absent in 1342 females (79.92%) as described in Table 2.

In cases with BOH (history of preterm delivery, previous IUD, hypertension, and diabetes mellitus) alloimmunization rate was 6.09%, whereas in patients with no BOH; it was 0.88% with a significant p value, with odds ratio =7.273 (95% confidence interval: 2.875 to 18.397) Chi-square =19.723 and 1 degree of freedom and p<0.001.

Table 3: Distribution of pregnant females according to bad obstetric history and association with alloimmunization.

| Adverse obstetric history | Alloimmunization | Total |
|---------------------------|------------------|-------|
|                           | Absent | Present | No. % | No. % | No. % |
| Present                   | 108    | 93.91   | 7     | 6.09  | 115  | 6.76 |
| Absent                    | 1571   | 99.12   | 14    | 0.88  | 1585 | 93.24 |
| Total                     | 1679   | 98.76   | 21    | 1.24  | 1700 | 100.00 |

Odds ratio =7.273 (95% confidence interval: 2.875 to 18.397); Chi-square =19.723 with 1 degree of freedom; p<0.001.

In non-alloimmunized women, BOH was observed in 108 (6.43%) cases whereas it was absent in 1571 (93.56%) females (Table 3).

Table 4: Distribution of pregnant females according to Rh and antibody specificity.

| Rh | Alloimmunization | Total |
|----|------------------|-------|
|    | Absent | Present | No. % | No. % | No. % |
| RH-| 127    | 92.03   | 11    | 7.97  | 138  | 8.12 |
| RH+| 1552   | 99.36   | 10    | 0.64  | 1562 | 91.88 |
| Total | 1679 | 98.76   | 21    | 1.24  | 1700| 100.00 |

Chi-square =50.005 with 1 degree of freedom; p<0.001

The frequency of alloimmunization according to the different ABO blood groups were in the decreasing order of B (1.61%) > O (1.50%) > AB (1.17%) > A (0.51%).
Alloimmunization rates were higher in Rh negative pregnancies (7.97%) as compared with Rh-positive ones (6.64%), Chi-square = 50.005 with 1 degree of freedom and p<0.001 (Table 4).

**Alloimmunization and Rh status**

Out of the 21 females, who were detected to have alloantibodies, 11 were Rh (D) negative. Out of the 11 cases, 9 were detected to have anti-D and 2 of them were detected to have anti-C+D. The presence of anti-G was ruled out in the latter 2 cases using differential adsorption technique. 10 of the remaining detected cases of alloimmunization were found in Rh (D) positive pregnancies and this included anti-c (3), anti-c+E (1), anti-E (3), anti-K (1), anti-e (1) and anti-S (1) with a significant p value. The prevalence of detected alloantibodies was: anti-D (42.86%), anti-C+D (9.52%) anti-c (14.29%), anti-c+E (4.76%), anti-E (14.29%), anti-K (4.76%), anti-e (4.76%) and anti-S (4.76%) (Table 5).

### Table 5: Comparison of different studies from India and antibody specificities.

| Study Name | Year | Anti-D (%) | Anti-C (%) | Anti-E (%) | Anti-c (%) | Anti-e (%) | Anti-K (%) | Anti-S (%) | Other (%) | Anti-c+E (%) | Anti-D+C (%) |
|------------|------|------------|------------|------------|------------|------------|------------|------------|-----------|-------------|-------------|
| Pahuja et al16 | 2011 (New Delhi) | 78.43 | 85.7 | 1.96 | - | - | 1.92 | 3.92 | - | - |
| Sankaralingam et al14 | 2016 (Punjab) | - | - | 71.4 | - | - | 14.3 | 14.3 | - | - |
| Sidhu et al14 | 2013 (Jammu) | 80 | 6.7 | 6.7 | - | 6.7 | - | - | - | - |
| Present study | 2020 | 42.86 | 12.5 | 14.29 | 14.29 | 4.76 | 4.76 | 4.76 | 4.76 | 9.52 |

### DISCUSSION

The health care service in India is decentralized and though there are guiding documents for antenatal screening issued by central regulatory agencies like the Director General of Health Services (DGHS), the practices significantly differ across the centres. Most of the guidelines and literature address the issue of alloimmunization in Rh (D) Negative antenatal females and the data from Rh D positive females is limited.13 There is limited literature available on this topic from India and no data pertaining to the antenatal cases in north-west India.

One of the important outcomes of the study was the prevalence rate of alloimmunization in Rh D positive females, which was 0.64% and represents a significant amount of detectable alloimmunization cases considering that the majority (85%) of the females will belong to this group. Similar studies from India by Sidhu et al had reported the prevalence of Anti-D antibody to be 80% and Pahuja et al had reported it to be around 78%.14,15 While Anti-D was implicated in only 48.8% of cases observed at our Institute. This change in the trend can be attributed to the fact that these studies were conducted few years earlier and the incidence of HDFN due to anti-D antibody has gradually subsided since then. The findings affirm that screening antenatal females with Rh D positive status will help in further reducing the incidence of alloimmunization.

In our study, the mean age group of the antenatal females was 26.89 years which was comparable with the study by Sidhu et al which included 750 females with a mean age of 26.5 years.15 The ABO blood group distribution in 1700 females showed the blood group B with the highest prevalence of 35.35 %, followed by group O (31.29%), A (23.29%), and AB (10.06%), which is in accordance with the blood group distribution in general Indian population.16

The overall prevalence of alloimmunization in antenatal females was 1.24% (21 out of 1700) which is similar to the studies from other parts of India by Pahuja et al (North India) and Verghese et al (South India).15,17 The prevalence rate of alloantibodies across various studies have been summarized in Table 5. In our study, alloimmunization in Rh (D) positive females was 0.64% (10/1562) whereas, in Rh (D) negative females, the frequency was 7.97% (11/138) which was similar to study conducted by Pahuja et al showing alloimmunization rates in Rh (D) negative and Rh (D) positive groups (10.7% versus 0.12% respectively) and Sidhu et al (21% in D-negative and 0.45% in D positive).14,16 There were 7 antibodies found in 21 alloimmunized pregnant females. 3 of them were present in combination with another antibody which included anti-c+E and anti-C+D. Antibody specificities identified were anti-D (42.86%), anti-C+D (9.52%), anti-c (14.29%), anti-c+E (4.76%), anti-E (14.29 %), anti-K (4.76%), anti-e (4.76%) and anti-S (4.76%) in this study which was comparable with other studies conducted in different parts of India.

The prevalence rate for alloimmunization was higher in pregnant females with a history of blood transfusion (1.24%), bad obstetric history (1.24%), and multigravida status (1.24%).17 Out of 21 were multigravida while the remaining 4 alloimmunized cases were primigravida with history of blood transfusion. In total, 9 out of 21 alloimmunized females (42.8%) had a previous history of
blood transfusion which was comparable to the study conducted by Sankarlingam et al.18

The study found that the antibodies implicated in alloimmunization mostly belonged to the Rh and Kell blood group system and more than 40% of alloimmunized females had a history of blood transfusion. In view of these findings, selecting antigen phenotype matched blood for Rh (D, C, E, c, e) and Kell (K) antigens should be done and phenotypically matched blood for transfusion in females of childbearing age will have a significant impact on reducing sensitization and subsequent alloimmunization.

Antigen phenotyping of the father also helped in confirming the cause of alloimmunization in positive cases. It is recommended that this practice should be followed in future for confirming the cause of alloimmunization.33 Establishing advanced immunohematological centres with facilities for free foetal DNA detection and molecular phenotyping can also help in overcoming these issues.

The main limitation of this study is the lack of follow up data of alloimmunized cases and the newborns of these mothers, though the patients with high antibody titres and different paternal antigen profiles were advised for a regular follow up at the department of obstetrics. The observations and recommendations of this study are restricted only to antenatal cases and cannot be generalized for the entire population in this region.

CONCLUSION

Based on the findings of this study, it is advised to screen all pregnant females for alloimmunization, irrespective of their Rh status. It is a simple and cost-effective test that can significantly reduce the adverse consequences of HDFN by early detection and subsequent interventions like IUT. It is also recommended to encourage the practice of providing Rh-Kell phenotype matched blood to the females of the childbearing age group to reduce the overall incidence of alloimmunization and HDFN. Similar studies in future can help in establishing these guidelines at the national and global forum as well.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Yadav A, Joshi CS, Gupta GN, Dubey R. The changing trend of alloimmunization in antenatal females: experience from a tertiary care centre in north-western India. Int J Res Med Sci 2021;9:3637-42.