Prevalence, Specificity and Titration of Red Cell Alloantibodies in Multiparous Antenatal Females at a Tertiary Care Centre from North India

Meena Sidhu^{1,2} · Renu Bala¹ · Naveen Akhtar¹ · Vijay Sawhney¹

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Abstract Screening and detection of clinically significant antibodies among antenatal women plays an important role in transfusion safety and preventing hemolytic disease of fetus and newborn. Routine screening of antenatal women for antibodies is not done in all blood centres of our country and so immunization rates are not known in pregnant women. We studied the prevalence of alloantibodies and titration of Anti D among antenatal multiparous women in Jammu region. In present prospective study, 750 antenatal multiparous women attending antenatal clinics were typed for ABO and D antigens. Alloantibody screening was done, if positive, specificity of alloantibody was ascertained by using commercially available red cell panel by tube method. Rate of alloimmunization was correlated with Rh D status, gravida, previous transfusion history and bad obstetric history. Titration of alloantibody D was done in first and third trimester of pregnancy. In present study most common blood group detected was B positive (38.4 %). Rh D negative cases constituted 7.6 % of total cases. Rate of alloimmunization was 2 %. A significant correlation was seen between Rh D-negative and alloimmunization (21 % in D-negative and 0.45 % in D-positive). There is significant increasing degree of alloimmunization with increase in Gravida. Alloimmunization in females with bad obstetric history was high (4.41 %) as compared to females with no bad obstetric history showing only 1.76 %. Alloantibodies detected were

Anti-D, Anti-E, Anti-C and Anti-K. Anti-D constituted 80 % of all alloantibodies detected. Six women in their third trimester had raised titers of anti-D. Most common alloantibody detected was anti-D (80 %). Alloantibodies to other Rh antigens and Kell blood group systems were also identified. To minimize alloimmunization in Rh D negative women, proper Anti D immunoprophylaxis should be implemented.

Keywords Alloimmunization · Alloantibodies · Multiparous · Rh D · HDFN · Antenatal

Introduction

Antibody detection plays a critical role in transfusion medicine. It is a key process in pretransfusion testing. Antibody screening in antenatal women aids in the detection and monitoring of patients who are at the risk of delivering infants with Hemolytic disease of foetus and Newborn (HDFN). Investigations for presence of irregular antibodies and its titers during pregnancy are important to calculate the risk of developing HDFN [1]. Most developed countries have guidelines for screening all pregnant women for irregular red cell antibodies. According to the guidelines of British Committee for standards in Hematology, all pregnant women should be ABO and D antigen typed and screened for presence of alloantibodies early in pregnancy and at the 28th week of gestation [2]. As per guidelines of The Netherlands, it has been mandatory to screen all pregnant females for presence of alloantibodies [3].

Even though, in India, DGHS Technical manual recommends screening for alloantibodies during pregnancy in India, however these guidelines are not being followed strictly [4]. Limited data is available on presence of

✉ Meena Sidhu
minapthapa@gmail.com

¹ Department of Transfusion Medicine, Government Medical College, Jammu, Jammu and Kashmir, India

² F-234, Raipur Satwari, Jammu Cantt, Jammu and Kashmir, India

alloantibodies in Northern part of India. The present study was undertaken to look for prevalence of alloantibodies, their specificity and titers of alloantibody D in 1st and 3rd trimester of pregnancy in multiparous antenatal women.

Materials and Methods

The present prospective study was carried out in multiparous antenatal women attending antenatal clinics in Shri Maharaja Gulab Singh Hospital, Government Medical College, Jammu from November 2012 to October 2013. Antenatal females on their booking visit get ABO and Rh D grouping from Blood bank. During the study period Blood Bank performed additional screening for irregular antibodies in these cases. An informed consent was taken from the pregnant women/their husbands before screening them for the presence of clinically significant antibodies. Titration of Rh D alloantibody was also done.

For each pregnant woman, name, age, obstetrical history (including stillbirths, abortions, medical termination of pregnancy) history of blood transfusion, history of having received Anti-D were recorded prior to taking samples from them.

The blood samples, 2 ml collected in EDTA tube and 5 ml in plain glass test tube, centrifuged to separate plasma/serum from red cells for performing ABO (forward and reverse) and Rh D grouping, Direct Antiglobulin test, Indirect Antiglobulin tests and antibody screening as per departmental Standard operating procedures.

Antibody Screening was done using serum from the blood samples, performed by using commercial three red cells panel (Immucor, Inc. Norcross, USA) in IAT and Enzyme phase at 37 °C. Samples which were screened positive for alloantibodies were stored at –80 °C for

further alloantibody identification. For identification samples were thawed at room temperature before performing the test.

Antibody identification was performed by using a commercial RBC panel (Panoscreen-10, a 12 vial set including a buffered preservative diluents, Immucor, Inc. Norcross, USA) with known antigens against patient's serum as per manufacturer's instruction. Titration of Rh D alloantibody was also done by serial dilutions of patients till the agglutination is negative.

Statistical Analysis

The Data was analyzed with the helper computer software Epi-info version(CDC Atlanta) Statistical significance of the results were evaluated by using χ^2 /Fisher's Exact test. A p value of <0.05 was considered significant.

Results

The study included 750 multiparous antenatal females in the age group of 19 to 38 years with majority of cases between 23 and 26 years (39.33 %), mean age was 26.5 years.

Gravida II constituted 55.6 %, gravida III 30.94 %, gravida IV 11.06 % and gravida V 2.4 % of the total cases.

Adverse obstetric history was present in 9.06 % (68/750) of antenatal females. Prior history of transfusion was present in 8.67 % (65/750) females. Most common blood group detected in study population was B positive 38.4 %. Rh D negative cases constituted 7.6 % of total cases as shown in Table 1. Direct antiglobulin test was non-reactive in all the cases.

Indirect Antiglobulin test (IAT) was reactive in 2.67 % (20/750) of the cases and 80 % of these reactive cases were Rh D negative which was highly significant ($p < 0.05$). IAT was reactive in 6.15 % patients with history of prior blood transfusion as shown in Table 2.

On alloantibody screening 2 % (15/750) antenatal females were found to be alloimmunized. It was observed that 21.06 % (12/57) Rh D negative females were alloimmunized, whereas only 0.44 % (3/693) Rh D positive were alloimmunized. A significant correlation was seen between alloimmunization and D negative status, $p < 0.01$ (Table 3).

Table 1 Distribution of ABO and Rh(D) blood group system in study population

ABO	Rh (D positive) N = 693 (92.4 %)	Rh (D negative) N = 57 (7.6 %)
A	160 (21.33 %)	12 (1.6 %)
B	265 (35.3 %)	23 (3.07 %)
O	206 (27.5 %)	19 (2.5 %)
AB	62 (8.3 %)	3 (0.4 %)

Table 2 Distribution of cases according to IAT reactivity

IAT	Rh (D)positive (693)	Rh (D negative) (57)	Transfusion history present (65)	Transfusion history absent (685)
Reactive (n = 20)	4 (0.5%)	16 (28.0%)	4 (6.1%)	16 (2.3%)
Non reactive (n = 730)	689 (99.4%)	41 (70.17%)	61 (93.8%)	669 (97.6%)

Table 3 Association of Rh (D) antigen and bad obstetrical history with alloimmunization

	Rh (D)positive No = 695	Rh (D)negative No = 57	Bad obstetrical history present No = 68	Bad obstetrical history (Absent) No = 682
Antibodies detected	3 (0.44 %)	12 (21.06 %)	3 (4.41 %)	12 (1.76 %)
Antibodies not detected	690 (99.56 %)	45 (78.44 %)	65 (95.59 %)	670 (98.24 %)

Table 4 Alloimmunization in relation to gravida status

Gravida status	II No = 417	III No = 232	IV No = 83	V No = 18
Antibodies detected	7 (1.68 %)	3 (1.29 %)	3 (3.61 %)	2 (11.11 %)

Alloimmunization in females with bad obstetric history was 4.41 % (3/68) as compared to females with no bad obstetric history showing only 1.76 % (12/682). There was significant increase in degree of alloimmunization with increase in Gravida status, $p < 0.029$ (Table 4).

On identification of alloantibodies, 93.3 % belonged to Rh blood group system. Of these 80 % (12/15) were Anti D, 6.7 % (1/15) were Anti C and 6.7 % (1/16) were Anti E and there was one case (6.7 %) having Alloanti K belonging to Kell blood group system. In Rh D negative females, alloantibody D and C were detected. Anti D was the only antibody detected in all the alloimmunized cases having bad obstetric history (4.41 %). Females with prior history of transfusions were alloimmunized with Anti D and Anti K (Table 5).

Only 7/12 females with Alloanti D could be followed in both 1st and 3rd trimester of their pregnancy. Titration of the Anti D was done in these cases. Two females, case 1 and case 7 with bad obstetric history had more than two fold increase and high titers (from 1:16 and 1:32 to 1:128 and 1:256) of Anti D in third trimester (Table 6).

Discussion

Many countries have specific guidelines for antenatal screening for irregular red cell antibodies as routine procedure. Drug Controller General of India recommends

Table 6 Titration of alloanti D in 1st and 3rd trimester

Case no	Gravida	Ist trimester	3rd trimester
1	V	1:32	1:128
2	IV	1:8	1:16
3	III	1:8	1:32
4	III	1:16	1:64
5	II	1:4	1:4
6	III	1:16	1:64
7	V	1:16	1:256

screening for alloantibodies [5] but hardly followed, may be due to lack of resources available in many blood transfusion services. Mostly indirect antiglobulin test (IAT) is done in Rh D negative females for screening and titration of antibodies in this part of the country. In this study IAT was reactive in 2.67 % of the cases. The frequency of alloantibodies in multiparous antenatal females was 2 % slightly higher than other studies from other parts of India. In a study from Delhi prevalence of alloimmunization among multigravida was 1.25 % [6].

Another study from South India showed it to be 1.48 % [7]. Alloimmunization rates in antenatal females ranged from 0.4 to 2.71 % [8–16].

In our study there was significant difference between the alloimmunization rates in Rh D negative (21 %) versus Rh D positive group similar to other studies [6, 7] at 10 and

Table 5 Antibody specificity in relation to Rh (D) status, bad obstetrical history and transfusion history

Antibody specificity	Total number of Alloimmunized females N = 15	Rh positive women N = 3	Rh negative women N = 12	Bad obstetrical history (present) N = 68	Bad obstetrical history (absent) N = 682	Transfusion history (present) N = 65	Transfusion history (absent) N = 685
Anti-D	12		12	3	9	2	8
Anti-C	1	1		–	1	–	1
Anti-E	1	1		–	1	–	1
Anti-K	1	1		–	1	1	–

13.5 % respectively. A wide variation from 0.9 to 7.1 % can be seen regarding alloimmunization rates in the D antigen-negative group [8, 10, 17]. The rate of alloimmunization in the D antigen-negative group is higher in present study. The reason for higher rate may be that unassisted home deliveries and abortions which are common in many rural areas. These are far flung hilly areas and access to health care facilities is poor due to bad road connectivity and ignorance about the importance of implications of being D negative mother. So many of these D negative women do not receive anti-D immunoprophylaxis during their first deliveries/abortions and came to hospital later with repeated fetal loss or stillbirths.

Frequency of alloimmunization was more in females with bad obstetric history than in females not having bad obstetric history, however the difference was not significant as reported by Pahuja et al. [6]. There was also significant increase in the degree of alloimmunization with increase in the gravida status again comparable to another study [6]. Alloimmunization rate was higher in females with transfusion history as transfusions expose them to multiple allogenic red cell antigens causing alloantibody formation.

Alloanti-D was the most common alloantibody detected, similar to other studies. Pahuja et al. [6] detected in 78.43 %, Gottvall and Filbey [10] found in 60 % of cases. Howard et al. [15] and Filbey et al. [16] found that anti-D was responsible for 41 and 45.6 % and it was the leading offender despite immunoprophylaxis. The alloimmunization rate within the Rh-positive group in our study was comparable to other studies [6, 11].

Apart from anti D, other alloantibodies implicated in HDFN are anti-E, anti-c, anti-C, anti-K and anti-Fy. HDFN caused by anti-K is generally severe [18]. Anti K was found in one of the females who had received transfusions, thus K antigen should also be typed in females of reproductive age group along with ABO and Rh D typing. There is evidence that anti-K can recognize K antigens expressed in the early stage of erythroid development in the fetal liver and can cause anemia by suppressing erythropoiesis [19]. HDFN caused by anti-C is usually mild as the C antigen has weak immunogenicity [20]. In present study Rh system contributed about 93.3 % and Kell system about 6.7 % to red cell alloimmunization in pregnancy. There is limited published data regarding the correlation of alloimmunization in antenatal multiparous women with their transfusion history and bad obstetrical history.

Titration for Anti-D could be performed in 7 out of 12 cases (58.33 %) during their third trimester, due to poor access to obstetric care for the people in rural areas. Except one all the cases had increase titers in third trimester. All four alloimmunized women with bad obstetrical history had raised Anti-D titers at the end of pregnancy. Two of these females with gravida V had multiple stillbirths. Rahman et al. [21] found raised titers which could lead to

HDFN in 42.85 % women which could be followed in their 3rd trimester. In general, a titer of 16–32 and difference of >2 dilutions is considered a significant factor in causing HDFN. However, antibody titers consistently below the laboratory's critical titre throughout the pregnancy predict an unaffected or mild to moderately affected fetus [22].

Thus antenatal antibody screening and identification along with titrations predicts the severity of HDFN and adequate interventions can be done to save the foetus/neonate in utero by intrauterine transfusions and exchange transfusion. At least Rh antigens (D,C,E,c,e) and Kell (K) antigen matched blood should be transfused in females till their child bearing age to avoid sensitization [2].

Conclusion

Alloanti-D is still a common antibody identified as the major cause of alloimmunization in pregnancy. Alloimmunization due to anti-D can be prevented by proper and adequate implementation of anti-D prophylaxis. Routine antenatal alloantibody screening of all the multiparous women is recommended. All the females should be typed and screened for at least Rh blood group system antigens and Kell blood group system along with ABO and D typing in first and third trimester of pregnancy.

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