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Prevalence of unexpected red blood cell antibodies in pregnant women and follow-up of pregnancy outcome in pregnant women treated with intra-uterine transfusion

Sunil Golia, Aseem Kumar Tiwari, Geet Aggarwal, Anil Khetrapal[1], Sandeep Kumar Tyagi[1], Chetna Jain[2], Shubham Gupta, Samruddhi Pawar

Abstract:

BACKGROUND: For the management of hemolytic disease of the fetus and newborn (HDFN), it is important to detect unexpected red cell antibody in pregnant women. We assessed the prevalence of unexpected red cell antibodies in consecutive pregnant women attending antenatal clinic (ANC). More importantly, cases with unexpected antibody causing severe anemia were followed-up for intervention (Intra-uterine transfusion {IUT}) and outcome of pregnancy (still-birth/live-healthy).

AIMS AND OBJECTIVES: The study was conducted with an objective to find the prevalence of unexpected RBC antibodies in pregnant women, their specificity and to do the follow-up for IUT and outcome of pregnancy (still-birth, live-birth) in antibody positive women.

MATERIALS AND METHODS: This was a prospective study from January 2021 to May 2022 at two tertiary care centres. All antenatal samples received by the laboratory were screened for unexpected red cell antibody. Whenever antibody screen was positive, antibody identification was performed. Patients, positive for unexpected antibody and anemia were followed up for any transfusion-based intervention and outcome of pregnancy.

RESULTS: A total of 539 consecutive samples were worked up and among these, 10 samples (1.85%) were found to be antibody positive. The antibodies identified were Anti-D (*n*=6), anti-Le^b (*n*=1), anti-M (*n*=1), anti-C (*n*=1) and anti-E (*n*=1).The prevalence of unexpected antibodies in Rh positive and Rh negative pregnant women was 0.83% and 10.9% respectively. Follow-up was done for all 10 cases with unexpected antibody and anemia was monitored by MCA PSV (middle cerebral artery peak systolic velocity).Two women developed severe anemia thus requiring single intrauterine transfusion (at 26 weeks and 28 weeks respectively) each, for correction of anemia. In both these cases, healthy male child was delivered. At 3-month follow-up both children were alive and healthy.

CONCLUSION: The study found prevalence of unexpected RBC antibodies in pregnant women as 1.85%. The study also underlined importance of transfusion-based interventions contributing to successful outcome in couple of cases with severe anemia.

Keywords:

Alloimmunization, antenatal, antibody screening, pregnant women, prevalence, unexpected antibody intra-uterine transfusion

Department of Transfusion Medicine, Medanta – The Medicity, [1]Department of Transfusion Medicine, Artemis Hospital, [2]Department of Obstetrics and Gynecology, Cloud Nine Hospital, Gurugram, Haryana, India

Address for correspondence:
Dr. Aseem Kumar Tiwari, Department of Transfusion Medicine, Medanta – The Medicity, Sector-38, Gurgaon - 122 001, Haryana, India.
E-mail: draseemtiwari@gmail.com

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Introduction

Unexpected red blood cell (RBC) antibodies may develop in an individual after pregnancy, transfusion, or transplant.[1] The unexpected antibody in pregnancy is against the antigen of paternal origin which presents in the fetus.[2] Mother is sensitized due to fetomaternal bleed which occurs usually at the time of delivery.[2] It, therefore, usually does not affect the first pregnancy; subsequent pregnancy may be affected.[2]

There are few studies on the prevalence of unexpected antibodies, in India.[3-5] However, there is sparse literature on therapeutic intervention in pregnant women with unexpected antibodies and at times, anemia, which is a consequence of maternal-origin unexpected antibody destroying fetal RBCs. We, therefore, undertook a prospective study to find the prevalence of unexpected antibody in pregnant women, therapeutic interventions such as intra-uterine transfusion (IUT), or exchange transfusion (ET) in the baby after birth. The outcome of pregnancy (live birth/stillbirth) and follow-up of mother and child were performed for 3 months, thereafter.

Materials and Methods

Settings

The study was a prospective, observational analysis conducted in the Department of Transfusion Medicine of a Tertiary Care Multispecialty Hospital in North India from January 2021 to May 2022.

Patient inclusion

All consecutive pregnant women attending the antenatal clinic were offered informed consent for tests for unexpected antibodies and follow-up. Those who consented were included in the study.

Tests performed

Blood samples were collected in a 3-ml ethylenediaminetetraacetic acid tube, BD Vacutainer (Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, NJ). Immunohematology testing on this sample included blood group (BG) and antibody screen (AS). BG and AS were done using an automated machine, Vision (Ortho Clinical Diagnostics, Raritan, NJ, USA). Forward ABO and RhD grouping was done on ABD cards. Reverse grouping was done using in-house pooled A-cell, B-cell, and O-cell on Reverse Diluent cards. Antibody screening of all patients was done using a three-cell antibody screen panel (Reagent RBCs Surgiscreen) on AHG (Anti-Human Globulin) cards. If an antibody screen was positive, alloantibody was identified using 11-cell ID Panel A (Reagent RBCs, Resolve Panel A) or/and Panel B (Reagent RBCs, Resolve Panel B). These pregnant women were followed up for the outcome of the pregnancy and 3-month follow-up postdelivery for the well-being of the baby, telephonically. All reagent cassettes and panels were from Ortho Clinical Diagnostics, Raritan, NJ, USA.

Data collection

Data collection included age, BG, name of the clinician, gravida, para, abortus, living, previous pregnancy outcome, history of sensitization (previous pregnancy, previous abortion, and history of transfusion), duration of pregnancy (weeks), anti-D immunization dose (number), antibody screen, antibody identification, and antibody titer. Antibody-positive cases were followed up till delivery for any intervention required (IUT done on the basis of middle cerebral artery [MCA] peak systolic velocity [PSV] [middle cerebral artery peak systolic velocity] >1.5 MoM [multiples of median]) and outcome of pregnancy.

Statistics

Data were entered in Microsoft excel 2007 version. Categorical data were presented in absolute numbers and percentages.

Table 1: Demographics of pregnant women

Parameters	*n* (%)
Age distribution (years)	
Below 25	36 (6.67)
25–35	429 (79.59)
Above 35	74 (13.72)
Gravida status (*n*=539)	
Multigravida	351 (65.12)
Primigravida	188 (34.88)
Duration of pregnancy (weeks)	
Mean±SD	35.27±6.20
Median (range)	37.30 (5.60–40.50)
Previous pregnancy outcome (*n*=539)	
Live birth	217 (40.25)
Abortion	127 (23.56)
NA	194 (35.99)
Transfusion history (*n*=539)	
No	533 (98.88)
Yes	6 (1.11)
RhIg immunization (*n*=539)	
2 dose	21 (3.89)

SD=Standard deviation, NA=Not available, RhIg=Rho (D) immune globulin

Table 2: ABO and RhD blood group distribution of pregnant women

ABO BG	*n* (%)	Rh BG	*n* (%)
B	177 (32.83)	D positive	484 (89.79)
O	167 (30.98)	D negative	55 (10.20)
A	144 (26.71)		
AB	51 (9.46)		
Total	539	Total	539

BG=Blood group

Ethical clearance

The study was approved by the institutional ethics committee, "Medanta Institutional Ethics Committee."

Results

Demographics

As shown in Table 1, most of the 539 pregnant women, i.e., 429 (79.59%) were aged between 25 and 35 years. Three hundred and fifty one (65.12%) were multigravida and 188 (34.88%) were primigravida. Among 351 multigravida women, the previous pregnancy outcome of 224 (41.55%) was reported as live birth and in 127 (23.56%) pregnancies, it was abortion. Of 539 pregnant women, six (1.11%) also reported a history of previous transfusion. As shown in Table 2, the most common BG reported was the 'B' BG followed by "O," "A," and "AB."

Sensitization and its relationship to the prevalence of unexpected antibodies

As depicted in Figure 1, previous pregnancy, previous abortion, and history of transfusion were found in 351, 127, and 6 women, respectively. One hundred and twenty seven, four, and three had an overlapping history of two events of sensitization, while three had all three events of sensitization. Unexpected antibodies were present in four patients out of 127 cases which were multigravida and history of abortion both and in all three patients with all three events of sensitization.

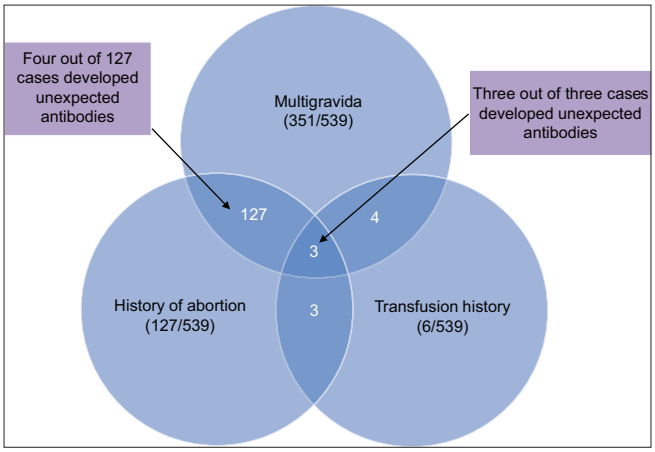

Figure 1: Sensitizing events and unexpected antibodies

Table 3: Prevalence, specificity and titer of unexpected RBC antibodies in pregnant women

Prevalence of unexpected RBC antibody (*n*=539)		Specificity	Number (*n*) of cases Total *n*=10	Titer
Antibody screen	*n* (%)			
Positive	10 (1.85%)	Anti-D	6	8,8,16,16,128,128
Negative	529 (98.15%)	Anti-C	1	4
		Anti-E	1	64
		Anti-Le(b)	1	8
		Anti-M	1	16

Rho (D) immune globulin prophylaxis

The present study showed that only 38.2% (*n* = 21) had received prophylactic Rho (D) immune globulin (RhIg) immunization out of a total of 55 Rh-negative cases.

Prevalence of unexpected red blood cell antibodies

Out of the 539 pregnant women that were included in the study, unexpected RBC antibodies were detected in 10 women. The prevalence of unexpected antibodies was found to be 1.85%. If we look at unexpected antibodies, only in the subgroup of Rh-negative pregnant women, the prevalence was much higher at 10.9%. In the subgroup of Rh-positive pregnant women, the prevalence was only 0.83%.

Unexpected antibody specificity and titer

As shown in Table 3, out of the 10 unexpected antibodies, the most common antibody was found to be anti-D (*n* = 6) followed by anti-C (*n* = 1), anti-E (*n* = 1), anti-Le (b) (*n* = 1), and anti-M (*n* = 1). Anti-Le (b) and anti-M are usually not clinically significant and not known to cause HDFN. In our cohort, although these were detected at 37°C at the antihuman globulin phase, they did not cause significant anemia in the fetus. The titer of these ten antibodies varied between 4 and 128 with a median of 16.

Intervention and outcome of pregnancy

Out of the 10 pregnant women having unexpected antibodies, two pregnant women developed anemia, which was diagnosed with MCA PSV >1.5 MoM (multiples of median) in both cases. The MCA PSV of >1.5 MoM was defined as a diagnostic of anemia by Mari *et al*.[6] A study by Alessandra Cacciatore[7] defines titer ≥16 as clinically significant. As depicted in Table 4, one IUT, each, was performed in both cases without any complications. In both these pregnant women, a live male child was delivered at 30 and 31 weeks of pregnancy, respectively, following lower-segment cesarean section. Both the male children were healthy when followed up, telephonically, again 3-month postdelivery.

Discussion

Demographics

The present study included pregnant women in the age group 19–46 years with a majority of cases between 25 and 35 years (79.59%), with the average age being 30.79 years. This was very similar to other Indian studies, i.e., Sidhu *et al*. (19–38 years),[3] B Suresh *et al*. (18–39 years),[8] and Soumya Das *et al*. (18–42 years).[4] The

Table 4: Intervention, outcome, and follow-up of antibody screen-positive cases

Titer	GPAL	First IUT		Presence of anemia	IUT approach	Number of IUTs	Outcome	Follow-up
		Gestational age in weeks	Hb (g/dL)					
Anti-D (128)	G3A2	27 weeks 3 days	5.1	Yes	Transplacental intravascular	1	Male child delivered at 30 weeks	Healthy at 3-month postdelivery
Anti-D (128)	G2P1L1	28 weeks 1 day	4.5	Yes	Transplacental intravascular	1	Male child delivered at 31 weeks	Healthy at 3-month postdelivery

GPAL=Gravida, para, abortus, living, IUTs=Intrauterine transfusions, Hb=Hemoglobin

Table 5: Comparison of demographics between present study and other published studies in India

Study	Age group (years)	Gravida status (%)	Previous pregnancy outcome (%)	Transfusion history (%)	RhIg immunization	BG (%)
Sangeeta Pahuja *et al*.[9] (2008–09)	-	Multigravida 100	-	1.4	RhIg immunized pregnant women were excluded	B (37.18) O (27.45) A (25.49) AB (9.86) Rh positive 88.98 Rh negative (11.02)
Meena Sidhu *et al*.[3] (2012–13)	19–38 (majority in 23–26-year age group)	Multigravida 100	-	8.67	-	B (38.37) O (30) A (22.93) AB (8.7) Rh positive 92.4 Rh negative 7.6
B. Suresh *et al*.[8] (2012–13)	18–39 (96.5% in 18–30 age group)	Multigravida 100	-	0.7	-	O (41.9) B (31.8) A (20.5) AB (5.8)
Soumya Das *et al*.[4] (2013–15)	18–42 (72% in 21–30-year age group)	Multigravida 56, primigravida 44	History of abortion 27.5	2.6	13% of Rh-negative pregnancy	O (40.5) B (28) A (26.6) AB (4.7) Bombay (0.2) Rh positive 78.2 Rh negative 21.8
Present study	19–46 (79.59% in 25–35-year age group)	Multigravida 65.12 Primigravida – 34.88	History of abortion (36.18)	1.11	2 doses in 38.18% of Rh-negative pregnancy	B (32.83) O (30.98) A (26.71) AB (9.5) Rh positive (89.81) Rh negative (10.19)

BG=Blood group, RhIg=Rho (D) immune globulin

present study also showed that the most common BG among pregnant women was "B" (32.82%) followed by "O" (30.97%), "A" (26.71%), and "AB" (9.5%). Overall, 89.81% of pregnant women were Rh positive and the rest 10.19% were Rh negative. This was similar to other Indian studies as shown in Table 5.

Sensitization and its relationship to the prevalence of unexpected antibodies

As depicted in Table 5, 351 (65.12%) cases were multigravida and 127 (23.56%) had a previous history of abortion which is similar to studies done previously (multigravida [56%–100%],[3,4,8,9] history of abortion [Soumya Das *et al*. (27.5%)]). History of transfusion was also present in 1.11% (similar to previous studies [0.7%–8.67%]).

Less than optimum rho (D) immune globulin immunization

The frequency of the RhD-negative phenotype is common in individuals of European and North American descent (15%–17%), while it is much lower in India (5.39%). Agrawal *et al*.[10] showed that this is similar to RhD frequency in Africa (3%–8%). Despite this vast difference in the frequency of RhD in Caucasians as compared to Indians, the prevalence of unexpected antibodies is fewer in Western nations because of robust routine antenatal

Table 6: Present study in comparison to other published studies on prevalence and specificity of irregular red blood cell antibodies in India

Study	Number of samples	Overall prevalence (%)	Prevalence in Rh positive (%)	Prevalence in Rh negative (%)	Specificity (%)
Jophy Varghese *et al*.[13] (2008–2009)	5347	1.48	0.65	13.5	-
Sangeeta Pahuja *et al*.[9] (2008–2009)	3577	1.25	0.125	10.4	-
Meena Sidhu *et al*.[3] (2012–2013)	750	2	0.44	21.06	Anti-D 80 Anti-C 6.7 Anti-E 6.7 Anti-K 6.7
B. Suresh *et al*.[8] (2012–2013)	2060	1.1	0.26	12.78	Rh 86.4 Anti-M 4.5 Anti-Le (a) 4.5 Anti-Le (b) 4.5
Soumya Das *et al*.[4] (2013–2015)	2336	2.27	0.8	6.9	Anti-D 49 Anti-D + Anti-C 5 Anti-G 5 Anti-C 5 Anti-E 2 Anti-E 2 Anti-H 7 Anti-M 2 Anti-Le (a) 2 Anti-Le (b) 12
Present study	539	1.85	0.83	10.9	Anti-D 60 Anti-C 10 Anti-E 10 Anti-M 10 Anti-Le (b) 10

anti-D prophylaxis. According to a study done on anti-D prophylaxis by de Haas *et al*. in the British population, the use of anti-D Ig has been shown to reduce RhD immunization from 16% of all RhD-negative women to 0.3% of RhD-negative women using anti-D.[11] In the American population, the postpartum administration of RhD immune globulin reduced the rate of alloimmunization in at-risk pregnancies from approximately 13%–16% to approximately 0.5%–1.8%. The risk was further reduced to 0.14%–0.2% with the addition of routine antepartum administration.[11,12] Unfortunately, in India, the rate of prophylaxis is not optimum. In the present study, only 38.18% of RhD-negative pregnant women received RhIg immunoprophylaxis; it was dismal at 13% in the study done by Soumya Das *et al*.[4] This brings out the fact that the RhIg immunoprophylaxis varies between 13% and 38%, which is much lower than what is desirable, i.e., 100%.

Prevalence of unexpected antibodies

As shown in Table 6, the 1.85% (n = 10) prevalence of unexpected antibodies was in concordance with different studies (1.1%–2.27%) in India. The prevalence in Rh-negative pregnant women was 10.9% and in Rh-positive women was 0.83%. The prevalence reported in various studies [Table 6] ranges from 6.9% to 21.06% (Rh negative) and 0.125% to 0.8% (Rh positive).

Specificity of unexpected antibodies

Out of the 10 unexpected antibodies, the most common antibody was found to be anti-D (60%) followed by anti-C (10%), anti-E (10%), anti-Le (b) (10%), and anti-M (10%). As shown in Table 6, the antibodies belonging to the Rh group were 80% which was in concordance with the studies done previously (68%–93%).

Intervention and outcome of pregnancy

Two out of 10 antibody-positive cases required a single IUT each. Soumya Das *et al*.[4] showed that two out of 36 cases required ET. Zwiers *et al*.[14] showed that the survival of fetuses had increased from 88.6% between 1988 and 2001 to 97% between 2001 and 2015. This would have occurred because of the enhanced safety of IUT/ET, over a period of time. Survival in various other studies[14-17] varied between 93% and 100%. In the present study, the survival at birth was 100%, though the sample size of patients receiving IUT was very small (n = 2). Both the children were alive and healthy at the time of 3-month follow-up, after successful delivery.

Limitations

A sample size of 539 in the present study was small; studies with larger sample sizes may be required to corroborate the finding of the present study.

Conclusion

The study found the prevalence of unexpected RBC antibodies in pregnant women as 1.85%. The study also found that two out of ten pregnancies with unexpected antibodies were affected with fetuses showing anemia. The study also underlined the importance of transfusion-based interventions in such pregnancies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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