Original Article

Distribution of antenatal alloimmunization in the southern districts of West Bengal and its significant associated factor

Archana Naik, Prasun Bhattacharya, Palash Das[1], Krishnendu Mukherjee, Partha Mukhopadhyay[2]

Abstract:

OBJECTIVES: Detection of maternal irregular antibodies against red blood cell antigen is vital in the management of hemolytic disease of fetus and newborn. There are no uniform guidelines related to antenatal antibody screening and identification in the developing Country like India. This study was aimed to identify such alloimmunization and its associations.

MATERIALS AND METHODS: This prospective study was conducted on antenatal mothers at a tertiary care center. The mothers having a history of anti-D administration, blood transfusion, and autoimmune disorders were excluded from the study. Initial indirect antiglobulin test (IAT) was performed in all blood samples by conventional tube technique (CTT) to identify alloimmunization. IAT-positive samples were screened for irregular antibody by column agglutination technology (CAT). Antibody screen-positive samples were further analyzed in 11-cell panel by CAT. Antibody strength was measured by serial double dilution by CTT. The source of isoimmunization was identified by extended Rh phenotype of women, husband, and newborn.

RESULTS: A total of 12 (2.3%) women out of 530 were positive for IAT and antibody screen. Antibody could be identified in 11 women, of which anti-D (5) was the most common, followed by anti-C + anti-D (4), anti-C + anti-E (1), and anti-C (1). All four cases of anti-D + anti-C were distinguished from anti-G by differential adsorption and elution. There was a significant association with alloimmunization versus increased gravid status, antepartum hemorrhage, and past history of newborns with neonatal jaundice.

CONCLUSION: All pregnant women with history of antepartum haemorrhage, newborn with neonatal jundice should be screened for alloantibody for early detection and better management of HDFN.

Keywords:

Alloimmunization, antibody screening, column agglutination technology, indirect antiglobulin test

Department of Immunohaematology and Blood Transfusion, Kolkata Medical College, [1]Department of Community Medicine, College of Medicine and Sagore Dutta Hospital, [2]Department of Obstetrics and Gynaecology, Medical College, Kolkata, West Bengal, India

Address for correspondence:
Dr. Prasun Bhattacharya, Department of Immunohaematology and Blood Transfusion, Kolkata Medical College, 88 College Street, Kolkata - 700 073, West Bengal, India.
E-mail: pbhattach@gmail.com

Introduction

There are more than 50 red blood cell (RBC) alloantibodies causing hemolytic disease of the fetus and newborn (HDFN).[1] Although Rh immunoglobulin prophylaxis has significantly reduced the incidence of pregnancies complicated by anti-D, the need to detect and monitor maternal alloantibodies capable of causing HDFN is still a concern.[1]

After ABO, Rh is the most immunogenic blood group system. It is the most complex of the 36 human blood group systems for its polymorphism. It comprises 54 antigens numbered RH1–RH61, with seven numbers obsolete.[2] Rh antigens are

encoded by two homologous, closely linked genes on the short arm of chromosome 1: RHD, producing the D antigen, and RHCE, producing the C, c, E, and e antithetical antigens.[2]

Isoimmunization in pregnant women has been extensively studied in different area of world, with the frequency being found to range from 0.4% to 2.7% worldwide.[3] Most of the developed countries have guidelines for screening all pregnant women for irregular erythrocyte antibodies. According to the guidelines of the British Committee for Standards in Haematology, all pregnant women should be ABO and D antigen typed and screened for the presence of red cell antibodies early in pregnancy and at 28th weeks of gestation.[4] However, no screening guidelines are followed in developing countries such as India.[3] Further, there is scarcity of data on the prevalence of pregnancy-induced isoimmunization in Eastern India. Detection of maternal irregular antibodies against RBC antigen is vital in the management of HDFN. All other antibodies other than ABO system detected against RBC antigen are considered irregular or unexpected antibodies.[5]

Here, we assessed the overall spectrum and profile of pregnancy-induced isoimmunization in developing country. It was aimed to increase the awareness related to antenatal antibody screening and their regular follow-up. Identification of associated cofactors for the development of alloantibody(s), viz., age, gravid status, gestational weeks, past history of neonatal hyperbilirubinemia, previous pregnancy loss, and antepartum hemorrhage (APH), was also analyzed.

Materials and Methods

The prospective study was conducted in the Department of Immunohematology and Blood Transfusion (IHBT) in collaboration with Department of Obstetrics and Gynaecology (G&O) of a Government Medical College and Hospital, at Kolkata, for a period of 1½ years (from January 2015 to June 2016). The study population consisted of antenatal mothers, irrespective of their gestational weeks who attended G&O outpatient department (OPD) and were referred to the Department of IHBT for ABO and Rh blood group and antibody screening. There were 5625 antenatal mothers who attended the OPD and 1512 deliveries conducted during the study period. A total of 530 antenatal women were evaluated, and informed consent was taken before blood sampling. The study was approved by the Institutional Ethical Committee (IEC).

Patient selection criteria
Antenatal women of both primigravida and multigravida were randomly chosen. The antenatal mothers (a) who had received anti-D prophylaxis within the last 3 months, (b)

who had a past history of blood transfusion, and (c) any pregnant women with a positive history of autoimmune or other immunological disorders were excluded from the study to rule out other causes of sensitization.[6,7]

Medical and obstetrical history documentation
A medical and obstetrical history and follow-up records were reviewed after counseling the antenatal mother and her spouse as per the investigation pro forma. The family history of consanguinity (if any) was also documented.

Blood sample collection and blood grouping of antenatal women, spouse, and their new born
A volume of 3 ml ethyldiaminetetra acetic acid (EDTA) and 3 ml clotted blood sample were collected from the antecubital vein of the antenatal women and their spouse during their first visit under strict aseptic condition. These EDTA and clotted samples were centrifuged at 3000 rpm × 3 min; then, plasma and serum were separated, respectively.[8] The cells from EDTA sample was processed for forward ABO blood grouping and extended Rh typing by conventional tube technique (CTT).[8] Reverse blood group was done using in-house freshly prepared reagent pooled A, B, and O cells.[8] The blood samples of newborn were sent from the ward in EDTA vials for ABO, extended Rh, and Kell phenotype for forward grouping and direct antiglobulin test (DAT).[8] Serum from clotted sample was used for antibody screening and identification. DAT of the antenatal mother was not performed.

Irregular antibody screening and identification
An initial antibody screening was done by indirect antiglobulin test (IAT) using pooled reagent O cell and mother's serum from clotted sample by CTT using poly-specific Coomb's sera (Tulip Diagnostics Pvt. Ltd).[8] Then, both IAT-positive and -negative sera were further used for the antibody screening and identification in column agglutination technology (CAT) using commercially available three cells (R_1R_1, R_2R_2, and rr) and 11 cells, respectively (Dia Panel, Bio-Rad, Switzerland).[9,10] The strength of agglutination was graded (1+ to 4+) in CAT. A flowchart of the initial workup procedure is given in Figure 1.

Measurement of antibody strength/titer in case of single antibody
Antibody strength was determined by the titration method in CTT using corresponding antigen-positive cells in doubling dilution with normal saline.[11] Any antibody(s) strength ≥16 was consider to be significant for Rh antibody(s), and titer ≥8 was significant for Kell antibody(s).[11] After initial titration, the serum/plasma sample was aliquot and preserved in −40°C for comparison of titer during follow-up.
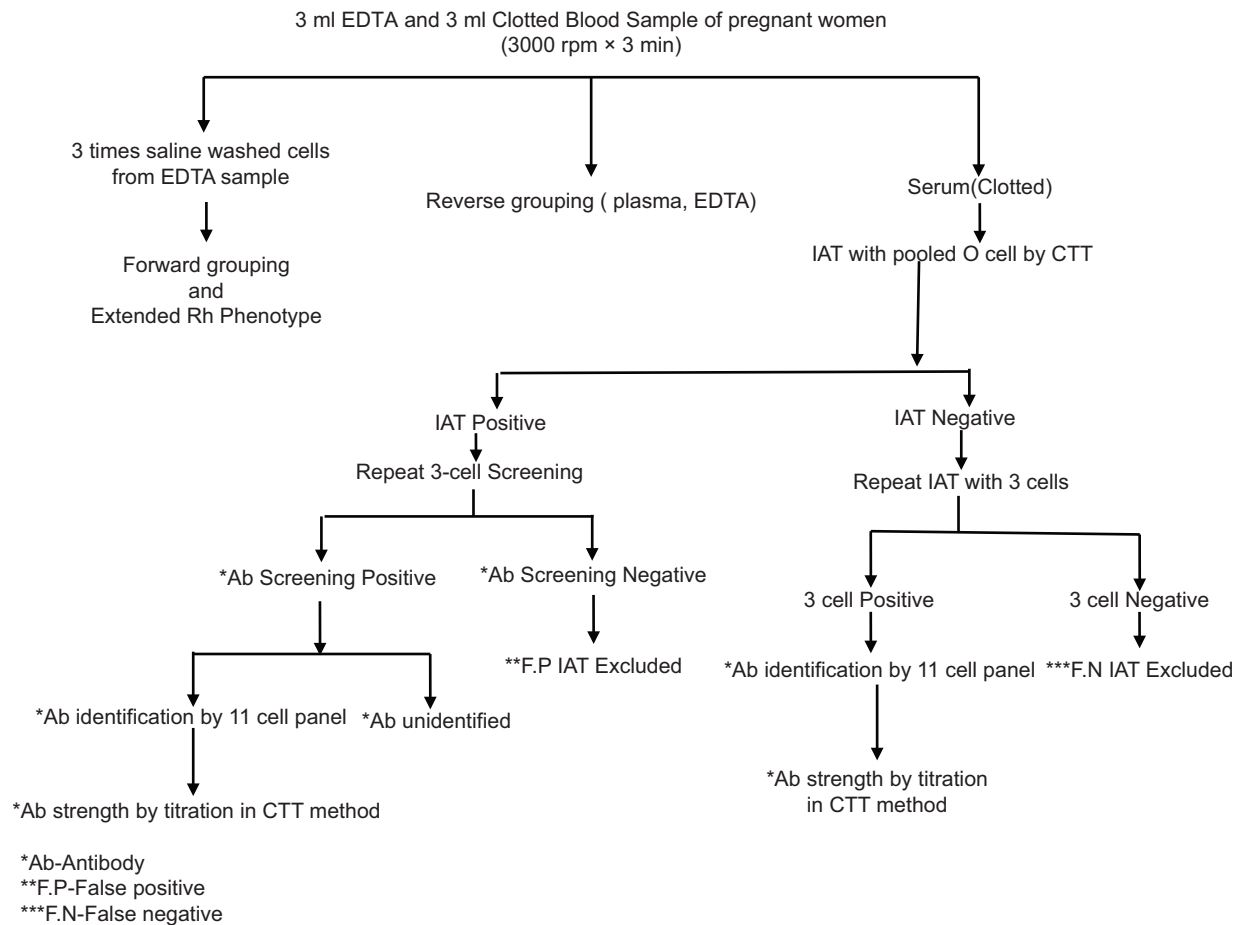
3 ml EDTA and 3 ml Clotted Blood Sample of pregnant women
(3000 rpm × 3 min)

3 times saline washed cells
from EDTA sample

Reverse grouping (plasma, EDTA)

Serum(Clotted)

Forward grouping
and
Extended Rh Phenotype

IAT with pooled O cell by CTT

IAT Positive

IAT Negative

Repeat 3-cell Screening

Repeat IAT with 3 cells

*Ab Screening Positive

*Ab Screening Negative

3 cell Positive

3 cell Negative

**F.P IAT Excluded

*Ab identification by 11 cell panel   ***F.N IAT Excluded

*Ab identification by 11 cell panel   *Ab unidentified

*Ab strength by titration
in CTT method

*Ab strength by titration in CTT method

*Ab-Antibody
**F.P-False positive
***F.N-False negative

Figure 1: The flowchart of initial workup of the study population

Analysis of the antibody specificity and strength in case of multiple antibodies

In case of antenatal mothers who were having multiple antibodies, the individual antibody and their strength were determined using differential adsorption methods (i.e., corresponding single antigen-positive and other antigen-negative in-house freshly prepared individual blood group O donor cells).[4,5,12]

Statistical analysis of data

Categorical variables are expressed as number of patients and percentage of patients and compared across the groups using Pearson's Chi-square test for the independence of attributes. The Statistical Software SPSS Version 20 (IBM Corp, Armonk, NY, USA) has been used for the analysis. An alpha level of 5% has been taken, i.e., if any $P < 0.05$, it has been considered statistically significant.

Results

Profile and distribution of study population

A total of 530 antenatal women were randomly selected and followed up during their antenatal period. Among them, 153 were primigravida and the rest 377 were multigravida (G2–G7). The age group of these women was 18–40 years. The spouses of 343 antenatal women were available for analysis of their ABO and Rh phenotype. The blood group and extended Rh phenotype of only 27 newborns delivered by these mothers were available for analysis.

Of them, 496 (93.58%) women were Rh (D) positive and 34 (6.42%) were Rh D negative. A total of 12 (2.3%) women were IAT positive with both pooled O cell and 3-cell panel. Samples that were positive in CAT were also positive in CTT. Results in both the techniques were same.

Among these 343 couples with known blood groups, 32 women had Rh incompatibility with their spouses (32 couples had Rh (D)-negative women having Rh (D)-positive spouses). In these 32 Rh D-incompatible couples, 10 (31.25%) women developed alloantibody, whereas only 2 (0.64%) women were alloimmunized among the rest 311 Rh-compatible couples ($P < 0.0001$). In the other 187 couple, spouse's blood group could not be done. IAT positivity was observed in nine women out of 377 multigravida and three out of 153 primigravida.

Frequency and distribution of alloantibodies (*n* = 12)

In these 12 alloimmunized women, five developed single alloantibody against D antigen (41.7%) followed by anti-C (1 woman). In the rest six mothers who developed multiple alloantibodies, anti-D + anti-C combination was seen in 4 (33.3%) and the other combination was anti-C + anti-E, who was also an Rh-negative primigravida. Antibody could not be identified in one woman.

All of the anti-D + anti-C combination of alloantibodies was distinguished from anti-G (D + C antibody) by differential adsorption and elution method as anti-G has a specificity for both D and C antigens at the same time. Figure 2 shows the distribution of the identified alloantibodies. The profile and spectrum of alloantibodies in these 12 antenatal women in their course of gestational journey are summarized in Table 1. The critical titer of ≥16 in Rh antibody was observed in eight women, and the titer ranges from 16 to 2048.

Extended Rh profile of isoimmunized women (*n* = 12), their spouses, and new born

To identify the cause of alloimmunization other than alloanti-D, an extended Rh phenotype was performed in the women, their spouses, and the implicated newborns [Table 2]. The underlined italicized antigen(s) was inherited from the father to the newborn.

Strength of alloantibody versus its outcome during the course of gestation

In 12 alloimmunized women, eight were having antibody above the critical titer (i.e., ≥16, ranged from 16 to 2048) during their gestational period, seven women were during the first trimester, and one woman during the mid-trimester reached critical label of titer [Table 1].
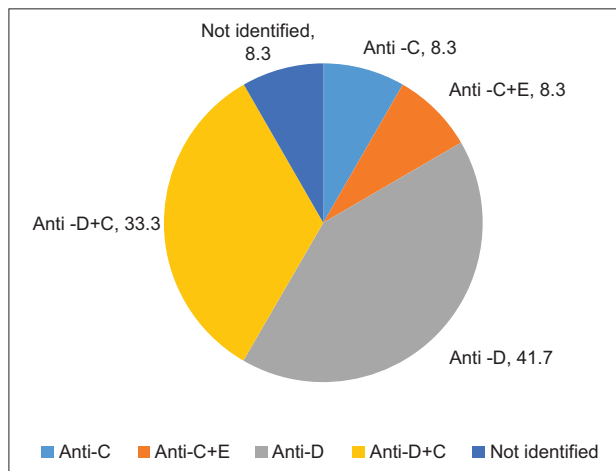

Figure 2: Distribution of alloantibody specificity in pregnant women

The course of gestation was uneventful in the rest 4 women, who had antibody titre of below critical label. In eight antenatal mothers whose titer was above the critical level, four of them had an uncomplicated gestational journey with delivery of healthy newborn. In the rest four women (whose antibody titer was ≥16), one of them had a premature delivery at 30 weeks, one had a severe hydrops at 28 weeks, and another two women delivered at 36 weeks of gestation with severe jaundice requiring exchange transfusion. An overall poor outcome of 50% (4/8) was seen in mothers having antibody titer well above 16.

Factors associated with development of antenatal alloimmunization

There was a significant increase in alloimmunization (*P* < 0.001) in the third gravida (G3) onward [Table 3].

In 521 antenatal women who were without any history of APH, among them, 10 (1.92%) were IAT positive. In the rest nine women who had a history of APH, 2 (22.22%) of them were IAT positive, which was statistically significant (*P* < 0.001) [Table 4].

Among 502 women who did not have a previous history of newborn with neonatal jaundice, 8 (1.59%) had a positive IAT. In the rest 28 women, there was a past history of neonatal jaundice, and out of them, 4 (14.29%) were positive on IAT (*P* < 0.001) [Table 5].

There is no significant correlation between alloimmunization and age, gestational week, and previous pregnancy loss.

Discussion

We observed that all the 16 antibodies in 11 women were against the antigen of Rh system except one which could not be identified. In our study, the patients with a history of blood transfusion, RhD immunoglobulin prophylaxis, and autoimmune disease were excluded by the selection criteria. This was not followed in most of the previous studies.[13-15] The antibody other than Rh system was mostly developed due to previous blood transfusion.[16] Our results showed almost a similar rate of isoimmunization among Rh-negative women in comparison to earlier studies ranging from 0.4% to 2.7% [Table 6].

The present study showed that anti-D was the most common single alloantibody (41.7%), and alloantibody against multiple red cell antigens anti-D + anti-C was the most common (33.3%). This result is similar to the study by Pahuja *et al*.[3] from Northern India. In all of these four women, anti-D + anti-C was distinguished from anti-G. It is

Table 1: Profile and titer of maternal alloantibodies during their gestational course

Gravida and parity	Antibody specificity	Titer at 1st trimester	Titer at 2nd trimester	Titer at 3rd trimester	Range of titer
G6P3+2	Anti-D	16*	32*	32*	16-32
G3P2+1	Anti-D	64*	64*	128*	64-128
	Anti-C	4	4	8	4-8
G4P1+2	Anti-D	512*	512*	2048*	512-2048
	Anti-C	4	4	8	4-8
G2P1+0	Anti-D	Negative	4	8	4-8
G3P2+1	Anti-D	16*	64*	512*	16-512
G6P5+0	Anti-D	16*	32*	256*	16-256
G2P1+0	Anti-D	8	16*	16*	8-16
G2P1+0	Anti-D	512*	512*	1024*	512-1024
	Anti-C	64*	64*	128*	64-128
G1P0+0	Not identified	NA	NA	NA	NA
G1P0+0	Anti-C	1	1	1	1-1
G1P0+0	Anti-C	2	2	2	2-2
	Anti-E	4	4	4	4
G4P1+2	Anti-D	32*	64*	128*	2-4
	Anti-C	2	2	4	32-128

*Significant titer ≥16. NA=Not available

Table 2: An overall distribution of ABO extended Rh phenotype

Alloantibody specificity	Women blood group and extended Rh phenotype	Husbands' blood group and Rh phenotype	New borns' blood group and extended Rh phenotype
Anti-D	B, dccee	B, *DC*cee	B, *DC*cee
Anti-D+C	B, dccee	O, *DC*Cee	B, *DC*cEe
Anti-D+C	B, dccee	B, *DC*cee	B, *DC*cee
Anti-D	O, dccee	O, *DC*cee	Not done
Anti-D	B, dccee	B, DCCee	Not done
Anti-D	A, dccee	AB, *DC*cEe	Not done
Anti-D	A, dccee	A, *DC*Cee	A, *DC*cee
Anti-D+C	A, dccee	A, *DC*Cee	O, *DC*cee
Anti-C+E	O, dccee	O, D*C*Cee	O, D*C*cee
Anti-D+C	B, dccee	B, *DC*Cee	B, *DC*cee

The underlined italic antigens were inherited from the father to new born

Table 3: Correlation between gravid status versus alloimmunization

Gravida	IAT		Total	P	Significance
	Negative	Positive			
G1	150 (98.04)	3 (1.96)	153 (100)	<0.001	Significant
G2	200 (99.01)	2 (0.99)	202 (100)		
G3	117 (97.5)	3 (2.5)	120 (100)		
G4	40 (95.24)	2 (4.76)	42 (100)		
G5	8 (100)	0 (0)	8 (100)		
G6	2 (50)	2 (50)	4 (100)		
G7	1 (100)	0 (0)	1 (100)		
Total	518 (97.74)	12 (2.26)	530 (100)		

IAT=Indirect antiglobulin test, IAT=Indirect antiglobulin test

important to distinguish anti-G from anti-D + anti-C as women with anti-G without anti-D should be eligible for anti-D immunoprophylaxis.[4]

A proportion of antibodies with apparent anti-D + anti-C specificity but with disproportionately high anti-C titers may be demonstrated by advanced serological technique, to be anti-G.[4] However, in our cases, none

of the anti-D + anti-C had a titer of anti-C above anti-D. This rules out the possibility of anti-G.

In Rh-negative women, nine out of 16 antibodies (56.25%) were anti-D (alone or in combination with C), six were anti-C (37.5%) (in combination with anti-D or alone), and one was anti-E (6.25%) in combination with anti-C [Figure 2].

Antibodies other than anti-D were inherited from the spouse's phenotype are shown in the Table 2. One women who developed a combination of anti-C + anti-E was found to be mismatch phenotype with her spouse for C, but the development of anti-E could not be explained. To explain this phenomenon of the development of anti-E, molecular genetic analysis could have been helpful. She had a history of APH during the second trimester.

We found a statistically significant correlation between the development of antibody versus gravid status of pregnant women, APH, and past history of neonatal jaundice. Similar results were shown by Pahuja *et al.*,[3] Al-Joudi *et al.*,[22] and Sidhu *et al.*,[23] which shows

Table 4: Correlation between bleeding pervagina and alloimmunization

History of bleeding PV	IAT		Total	P	Significance
	Negative	Positive			
No	511 (98.08)	10 (1.92)	521 (100)	<0.001	Significant
Yes	7 (77.78)	2 (22.22)	9 (100)		
Total	518 (97.74)	12 (2.26)	530 (100)		

PV=Pervagina, IAT=Indirect antiglobulin test

Table 5: Correlation between history of neonatal jaundice and alloimmunization

History of NNJ	IAT		Total	P	Significance
	Negative	Positive			
No	494 (98.41)	8 (1.59)	502 (100)	<0.001	Significant
Yes	24 (85.71)	4 (14.29)	28 (100)		
Total	518 (97.74)	12 (2.26)	530 (100)		

NNJ=Neonatal jaundice, IAT=Indirect antiglobulin test

Table 6: Prevalence of alloimmunization among pregnant women in different areas of the world

Authors of study (place)	Year	Total number of women screened	Number of patients with antibodies	Number of antibodies identified	Overall prevalence	Type of antibodies	Special comments
Pahuja *et al.*[3] (Delhi, India)	2008-2009	3577	45	51	1.25%	Rh D contributed to 78.4% of all the antibodies formed	
Koelewijn *et al.*[15] (The Netherlands)	2008	305,000	1002		1.232% of all, 0.328% of non-RHD patients		First-trimester screening enables timely treatment of HDFN caused by antibodies other than Anti-D
Gottvall *et al.*[17] (Sweden)	2008	78,145	316	376	0.4%	0.16% symptomatic Anti-D 60%, Fya 10%, c 7%, K 4%	
Al-Ibrahim *et al.*[18] (Saudi Arabia)	2008	1195	42		1.92%	52.38% Rh group, 2.38% Kell, 2.38% Kidd, 2.38% Lewis, 2.38% Duffy, 4.76% nonspecific, 33.33% autoantibodies	
Lee *et al.*[19] (China)	2003	28,303	213	230	0.79%	Clinically significant 0.27%, Anti- Mi 57.6%, Anti-E 19.7%, Anti-S 10.6%, Anti-c 7.6%	Routine antenatal antibody screening for Chinese women may not be worthwhile
Chandrasekar *et al.*[20] (Ireland)	2001	34,913	186			85 Antibody other than Anti-D	
Howard *et al.*[21] (Liverpool, UK)	1998	22,264	244	244	1%	100 anti-D, 144 non-Rh D	
Present study (West Bengal, India)	2015-2016	530	12	16	2.3%	Rh D contributes to 56.25% of all antibodies formed	

increase in alloimmunization with increasing gravida status.

Conclusion

Alloimmunization due to Rh system antigen was the most common. Anti-D immunoprophylaxis may prevent alloanti-D-induced HDFN, but it is ineffective against other antibodies of the polymorphic Rh system-like C and E. Antibody screening should be incorporated in antenatal checkup of all pregnant women with history of antepartum hemorrhage and newborn with neonatal jaundice.

Conflicts of interest
There are no conflicts of interest.

References

1. Smith HM, Shirey RS, Thoman SK, Jackson JB. Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary-care facility. Immunohematology

2013;29:127-30.

2. Daniels G. Rh and RHAG blood Group Systems. Human Blood Groups. 3rd ed. F.A. Davis Company; 2013. p. 182-258.

3. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus 2011;9:388-93.

4. White J, Qureshi H, Massey E, Needs M, Byrne G, Daniels G, *et al*.; BCSH. Guideline for blood grouping and red cell antibody testing in pregnancy. Transfusion Med 2016;26:246-63.

5. Harmening D.M. Modern blood Banking and Transfusion Practice 6th ed. Philadelphia, USA: Bristol, UK, Wiley Blackwell; 2012.

6. Bhattacharya P, Samanta E, Afroza N, Naik A, Biswas R. An approach to incompatible cross-matched red cells: Our experience in a major regional blood transfusion centre at Kolkata, Eastern India. Asian J Trans Sci 2018;12:51-6.

7. Datta SS, Mukherjee S, Talukder B, Bhattacharya P, Mukherjee K. Frequency of Red Cell Alloimmunization and Autoimmunization in Thalassemia Patients: A Report from Eastern India. Adv Hematol. 2015;2015:610931. doi: 10.1155/2015/610931. Epub 2015 Sep 6. PMID: 26425124; PMCID: PMC4575732.

8. Saran RK. Transfusion Medicine Technical Manual. 2nd ed. New Delhi: Ministry of Health and FW; 2003.

9. Brecher ME. Blood group Genetics, Identification of alloantibodies against Red cell antigens & Methods of Red cell typing, Antibody detection & Serologic Compatibility Testing. In: AABB Technical Manual. 15th ed. Bethesda, Maryland: AABB; 2005. p. 226-71, 731-61.

10. Aggarwal G, Tiwari AK, Arora D, Dara RC, Acharya DP, Bhardwaj G, *et al*. Advantages of type and screen policy: Perspective from a developing country. Asian J Trans Sci 2018;12:42-5.

11. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM. AABB Technical Manual. 18th ed. Bethesda: AABB; 2014.

12. Distinguishing Anti-G from Anti-C+D in Prenatal Serological Studies. Available from: http://cbbsweb.org/enf/2003/antibodypreg_g.html. [Last accessed on 2018 Jan 12].

13. Varghese J, Chacko MP, Rajaiah M, Daniel D. Red cell alloimmunization among antenatal women attending a tertiary care hospital in south India. Indian J Med Res 2013;138:68-71.

14. Suresh B, Sreedhar Babu KV, Arun R, Jothibai DS, Bharathi T. Prevalence of 'unexpected antibodies' in antenatal women attending the Government Maternity Hospital, Tirupati. J Clin Sci Res 2015;4:22-30.

15. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: A population study in the Netherlands. Transfusion 2008;48:941-52.

16. Koelewijn JM, Vrijkotte TG, de Haas M, van der Schoot CE, Bonsel GJ. Risk factors for the presence of non-rhesus D red blood cell antibodies in pregnancy. BJOG 2009;116:655-64.

17. Gottvall T, Filbey D. Alloimmunization in pregnancy during the years 1992-2005 in the central west region of Sweden. Acta Obstet Gynecol Scand 2008;87:843-8.

18. Al Ibrahim, Al Saeed . Red blood cell alloimmunisation among Saudi pregnant women in the central province of Saudi Arabia. Kuwait Med J 2008;40: 116 23.

19. Lee CK, Ma ES, Tang M, Lam CC, Lin CK, Chan LC. Prevalence and specificity of clinically significant red cell alloantibodies in Chinese women during pregnancy-a review of cases from 1997 to 2001. Transfus Med 2003;13:227-31.

20. Chandrasekar A, Morris KG, Tubman TR, Tharma S, McClelland WM. The clinical outcome of non-RhD antibody affected pregnancies in Northern Ireland. Ulster Med J 2001;70:89-94.

21. Howard H, Martlew V, McFadyen I, Clarke C, Duguid J, Bromilow I, *et al*. Consequences for fetus and neonate of maternal red cell allo-immunisation. Arch Dis Child Fetal Neonatal Ed 1998;78:F62-6.

22. Al-Joudi FS, Ahmed Al-Salih SA. Incidence of Rhesus isoimmunization in Rhesus-negative mothers in Ramadi, Iraq, in the mid-1990s. East Mediterr Health J 2000;6:1122-5.

23. Sidhu M, Bala R, Akhtar N, Sawhney V. Prevalence, specificity and titration of red cell alloantibodies in multiparous antenatal females at a tertiary care centre from North India. Indian J Hematol Blood Transfus 2016;32:307-11.