What is it really? Anti-G or Anti-D plus Anti-C: Clinical Significance in Antenatal Mothers

Soumya Das¹ · Shamee Shastry¹ · M. Murugesan^{1,2} · Poornima Baliga B¹ · Shamee Shastry¹

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Abstract G antigen of Rh blood group system is present either along with D and/or C positive red cells. Hence, [serologically anti-G presents with the similar picture as that of multiple antibodies (anti-D + anti-C). Differentiating them is important as anti-D + anti-C causes severe hemolytic disease of the fetus and newborn than anti-G. In pregnancies with anti-G alone, alloimmunization due to D antigen could be prevented by prophylactic administration of RhIg. Differentiating between anti-D + C from anti-G in alloimmunized pregnant mothers becomes essential. Sera from antenatal mothers, whose antibody identification by 11-cell panel gave a pattern for anti-D and anti-C were selected. Extended phenotyping for Rh system was performed for these antenatal cases. Differential adsorption and elution testing using R₂R₂ cells initially and r'r cells subsequently were performed to distinguish anti-G from anti-D + anti-C. Antibody titers of these antibodies were determined and their clinical outcome in the newborn was followed. A pattern suggestive of anti D and anti C on

antibody identification were observed in six antenatal cases. On further workup 50 % of them confirmed to have anti G. Antibody titers of anti-G and anti-C were lower than that of Anti-D. All newborns were sensitized in vivo and the antibody specificity in them were confirmed with elution studies. The mothers who had only anti-G were subsequently administered with an appropriate dose of RhIg. Differential adsorption and elution studies help in identifying anti-G and distinguishing it from anti-D plus anti-C, thus helping in better patient management.

Keywords Anti-G · Differential adsorption and elution · Antenatal

Introduction

The Rh system is considered the most important blood group system after ABO. Antibodies against Rh antigens are known to cause hemolytic disease of the fetus and newborn (HDFN). Anti C and anti D can cause HDFN however the severity is less in cases with anti-C antibodies [1].

G antigen was first described by Allen and Tippett [2]. The basis of reactivity for G-antigen is Ser103 which is encoded by RHD and by the C allele of RHCE genes [3]. Hence, anti-G appears serologically similar to anti-C plus anti-D. In exceptional cases G antigen can also be found on D-C- RBCs. There are 3 potential antibodies involved here, anti-D, anti-C and anti-G. The pattern of anti-G or anti-D plus anti-C actually have 5 possibilities to unravel: [1] anti-G + anti-D + anti-C, [2] anti-G + anti-D, [3] anti-G + anti-C, [4] anti-D + anti-C, or [5] anti-G alone [4]. And their differentiation can be a challenge for immunohematologists.

✉ Shamee Shastry
shameegirish@gmail.com

Soumya Das
sdas@gmail.com

Shamee Shastry
shameegirish@gmail.com

M. Murugesan
mohandossmurugesan@gmail.com

Poornima Baliga B
baliga777kmc@gmail.com

¹ Department of Immunohematology and Blood Transfusion, KMC, Manipal University, Manipal 576104, India

² Present Address: Malabar Cancer Centre, Thalassery, Kerala, India

For transfusion support, it is not necessary to differentiate these components as such patients will receive D and C antigen negative PRBCs, regardless of the antibodies present. However, this differentiation is important in pregnancy as the presence or absence of anti-D warrants administration of RhIg. D-negative mothers with anti-G are potential candidate for Rh prophylaxis in order to prevent possible formation of anti-D. The RhIg prophylaxis can be avoided in mothers who have already developed anti-D. Hence the accurate identification of these antibodies is essential for preventing medical and legal complications [4].

The purpose of our study was to differentiate anti-G from anti-D and anti-C which can aid in the administration of RhIg prophylaxis to antenatal mothers.

Materials and Methods

Study Participants

This was a cross-sectional study including antenatal mothers in whom antibody screening was requested from October, 2013 to May, 2015. Preset proforma was used to record the obstetric and transfusion details. The study was approved by institutional research and ethics committee.

Laboratory Investigations

Antibody screening was performed using 3 reagent red cell panel (BioRad ID) using column agglutination technique. In positive cases, antibody identification was performed using 11 reagent red cell panels (BioRad ID). The extended phenotyping for Rh antigens was performed in these cases specific, commercially available antisera.

Patient Selection

Serum of antenatal mothers, whose antibody identification gave a pattern for anti-D and anti-C were selected and included for further analysis. Testing was repeated on previously frozen aliquots of serum from the patients during their antenatal checkup.

Differential Adsorption and Elution

Differential adsorption and elution studies using R₂R₂ cells initially and r'r cells subsequently were performed to exclude the presence of anti-D (Fig. 1). The first adsorption was performed by incubating equal volumes of enzyme treated R₂R₂ cells and serum at 37 °C for 60 min. The adsorption was performed 2–3 times, in order to obtain a serum that is non-reactive with those RBCs. An

eluate was prepared from these adsorbed cells using cold-acid elution technique. The eluate was then tested against a panel of RBC which are D+C- and D-C+ in antiglobulin phase.

The eluate was then adsorbed with enzyme treated r'r cells, and a second elution was performed on these adsorbed cells using the cold-acid elution technique. The second eluate was again tested against D+C- and D-C+ in antiglobulin phase for confirmation. The techniques of differential adsorption and elution studies to identify anti-C plus -D, and -G were performed as described by Issitt et al. [5].

Antibody screening was performed on the adsorbed serum after each step using D+C- and D-C+ cells. All reagents and cells used for the differential adsorption and elution were prepared in house.

Antibody Titration

Titration was performed for anti-G, anti-D and anti-C using O-pooled cells, R₂R₂ cells and r'r cells respectively in saline phase without any enhancement medium. After incubation for 60 min incubation at 37 °C, the test was repeated in antiglobulin phase. The end point of titration was reported as the reciprocal of the highest dilution of serum at which 1+ agglutination was observed.

Outcome in Newborn

These mothers and their newborns were followed up to note the clinical outcome. The newborn blood group and a direct antiglobulin test were performed to confirm in vivo sensitization. The antibody specificity in the DAT positive cells were confirmed by performing heat elution and testing the eluate with D+C- and D-C+ cells. The newborn's hemoglobin, bilirubin and any treatment received were recorded. All the immunohematological tests were carried out as per AABB [6].

Results

During the study period, 2336 antenatal cases were screened for red cell antibodies, among them 510 were RhD negative. Among the Rh negative pregnancies, six patients (1.2 %) gave a pattern of anti-D + anti-C on identification panel. Anti-G was confirmed in three cases, while other three had anti D + C using double adsorption and elution technique (Table 1). Rh phenotype of all mothers was rr (dce).

In the cases with anti-D + C, maternal anti-D titers ranged from 16 to 128, while for anti-C, it was between 2 and 4. For anti-G the titers was between 16 and 128.

Fig. 1 Flowchart of the differential adsorption and elution technique **Table 1** Results obtained in the study of 6 serum samples with presumed anti-D and anti-C specificity

Case	DAT after adsorption R_2R_2 cells	Eluate of 1st Adsorption with R_2R_2 cells		IAT adsorbed serum with cells		DAT after 2nd adsorption r'r cells and 1st eluate	2nd eluate of 2nd adsorption with r'r cells		Probable antibody
		D+C-	D-C+	D+C-	D-C+		D+C-	D-C+	
1	+	+	0	0	+	0	0	0	D+C
2	+	+	0	0	+	0	0	0	D+C
3	+	+	+	0	0	+	0*	0*	G
4	+	+	+	0	0	+	0*	0*	G
5	+	+	+	0	0	+	0*	0*	G
6	+	+	0	0	+	0	0	0	D+C

* Elution couldn't be performed due to weak DAT strength

Details of pregnancies and their outcome are depicted in Table 2. Signs of fetal anaemia were noted in one case by Doppler ultrasound. This particular newborn showed features of severe HDFN. In spite of preterm delivery and aggressive invasive treatment with

exchange transfusion and top-up transfusion supports, baby succumbed.

Rest of the newborns showed moderate features of HDFN and were improved clinically with phototherapy. Details of the pregnancies and their outcome are depicted

Table 2 Details of the Pregnancy and its outcome

Case	Antibody	Titre			Gestational History	Delivery	Newborn DAT	Mother Blood Group	Newborn blood group	Newborn treatment	Status
		D	C	G							
1	D+C	128	2	–	G4P3L2IUD1	Preterm	+	A Neg	A Pos	ET	Not alive
2	D+C	16	4	–	G2P1L1	Term	+	A Neg	A Pos	PT	Improved
3	D+C	32	4	–	G2P1L1	Term	+	B Neg	B Pos	PT	Improved
4	G	–	–	128	G2P1L1	Term	+	O Neg	O Pos	ET + PT	Improved
5	G	–	–	16	G2P1L1	Term	+	AB Neg	B Pos	PT	Improved
6	G	–	–	16	Primi*	NA	NA	AB Neg	NA	NA	NA

History of previous transfusion is present

in Table 2. In case number 6, the patient had received transfusion in childhood during cardiac surgery done for correcting the congenital heart defect. And that could be the sensitizing event. The mothers who had only anti-G were subsequently administered with the appropriate dose of Rh Immunoglobulin (RhIg).

Discussion

The G antigen is present on almost all D-positive or C-positive red cells. The differentiation of anti-D+ C and anti-G specificities are rarely necessary for routine pre-transfusion testing because the transfusion support will be with rr (dce/dce) phenotyped RBC, the most common genotype among all the RhD Negative donors. However, characterizing these antibodies is necessary for an alloimmunized antenatal mother. The clinical outcome in the fetus as well as RhIg prophylaxis is based on the exact identification of these antibodies [7]. The identification of anti-G is a tedious and relatively complex procedure, an area of interest for inquisitive immunohematologist involving challenging workups of double adsorption and elution techniques [8]. In our study, we confirmed three cases of anti-G and another three with anti-D plus anti-C by performing differential adsorption and elution technique. The mean time taken for the full work-up is approximately 6 h. Fatima et al. in their study reported the use of a similar technique for distinguishing anti-D + C from anti-G [9]. The technique of differential adsorption and elution was initially described by Issitt [5]. Palfi et al. and Shirey et al. had followed the technique using R0 and r' cells while in our study R2R2 and r'r cells were used [4, 8]. We have modified the techniques with respect to the types of cells used and number of adsorption required in order to obtain an IAT negative serum to those RBCs. Our technique is simple and less time consuming. The mean time taken for the full work-up is approximately 6 h.

In the present study, the prevalence of Rh D negative cases was high in comparison to the other studies and the

prevalence in the general population of our region [10, 11]. The reason for the above difference could be that the study is conducted in the tertiary care set-up and the majority of the Rh D negative cases from peripheral hospitals are referred to our center for further management. Higher prevalence of Rh D negative cases (14 %) was also noted in similar study by Al-Ibrahim et al. (14 %) in Saudi Arabia [12].

From a clinical point of view, identification of anti-C is useful only in giving transfusion support with C antigen negative blood. The clinical significance of anti-G alone still remains controversial [13]. However, the identification of anti-G helped in providing appropriate transfusion support and to prevent Rh D alloimmunization by administering immunoprophylaxis in such cases. We observed that one newborn required both exchange transfusion and phototherapy while the second one could be managed with phototherapy alone.

In our study, mothers, with dual antibody specificity i.e. anti-D + anti-C showed features of moderate to severe HDFN. Alloimmunization with a combination of three or four antibodies is very rare. A study by Nordvall et al. showed 27 % of cases with multiple antibodies, among which majority (67 %) had anti-D as one among the many antibodies [14]. It's relevant to assess whether only anti-D is present or second antibody are also present with it. A similar study by Howard et al. showed anti-D in combination with another RBC antibody results in a more serious form of HDFN, especially with anti-C [14, 15]. Our observations confirmed that anti-D with anti-C resulted in significant morbidity in newborns.

Conclusion

Antibody identification with extended cell panel is recommended even if the pattern on antibody screen is that of the anti-D and this step will help us to identify the mimicking antibodies like anti-G. The testing method

mentioned in the present study helps us to delineate anti-G from anti-D plus anti-C, and thus the better patient care.

Author Contribution Dr. Soumya Das: Planned and performed the immunohematology workup, Dr. Shamee Shastry: Planned, guided the study and corrected the manuscript, Dr. Mohandoss Murugesan: Guided and corrected the manuscript, Dr. Poornima Baliga: Corrected the manuscript.

Compliance with Ethical Standards

Conflict of Interest No conflict of interest among the authors.

Ethical Standards The study protocol was approved by the Institutional Ethics Committee prior to its commencement. The Institutional Ethics Committee clearance number was IEC 353/2013.

Informed Consent A written informed consent was taken from the antenatal patients before taking part in the study.

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