A case report of multiple Rh alloantibodies in a pregnant female: Resolution by sequential adsorption

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Abstract:
Anti-G antibody mimics the reactivity pattern of coexistent anti-D and anti-C. Differentiating between the two is significant in antenatal females where the decision to administer RhD prophylaxis is based on the presence or absence of anti-D antibody. The aim of reporting this serological challenge is to emphasize the need for phenotyping red cells for sourcing appropriate in house red cell reagents and to help transfusion services sharpen problem-solving skills. A 26-year-old pregnant female with a complicated obstetric history and a positive indirect antiglobulin test presented to the hospital for antenatal assessment at 24 weeks. A positive antibody screen warranted identification of the implicating antibodies. Since identification was suggestive of multiple alloantibodies whose specificities could not be confirmed, step-wise sequential adsorption and elution was required. Anti-D, anti-C, and anti-E antibodies were identified in patient plasma with titers of 1024, 4, and 32, respectively. The absence of anti-G was also confirmed. Multiple alloantibodies can pose a challenge to transfusion services. However, with the help of select cells, phenotyping, adsorption elution studies, and phenotyped donor units; solving complex serological cases can be accomplished.

Keywords: Antenatal, anti-G, elution, multiple alloantibodies, rare donor, sequential adsorption

Introduction
Maternal Rh immunization following pregnancy with a fetus whose red cells possess an Rh antigen which the mother’s red cells lack can lead to the development of hemolytic disease of fetus and newborn (HDFN) in subsequent pregnancies. The risk of HDFN plays an important role in outcome of the subsequent pregnancies. India still has a high number of pregnancies affected by this complication due to lack of awareness and meager policies to reduce Rh isoimmunization. When such a patient approaches the hospital, role of the department of transfusion medicine is to identify specificity of the antibody/antibodies and provide appropriate units for transfusion.

Case Report
A 26-year-old, G4P2L1A1 female presented at 24 weeks’ gestation with a positive indirect antiglobulin test reported from an outside laboratory. The patient’s transfusion history revealed a red blood cell transfusion during her first pregnancy. The husband’s and first child’s blood group was B Positive. Patient’s blood group showed an ABO discrepancy. Forward group was B Negative while reverse group showed unexpected reaction with reagent pooled B cells and pooled O cells. The antibody screen showed a positive reaction with Cell 1 (R1R1) and Cell 2 (R2R2) and a negative reaction with Cell 3 (rr) of antibody screening panel (Surgiscreen, Ortho Clinical Diagnostics, Raritan, New Jersey). This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

How to cite this article: Setya D, Tiwari AK, Arora D, Mehta SP, Aggarwal G. A case report of multiple Rh alloantibodies in a pregnant female: Resolution by sequential adsorption. Asian J Transfus Sci 2021;15:109-12.
NJ, USA). Antibody identification panel (Resolve Panel A, Ortho Clinical Diagnostics, Raritan, NJ, USA) gave a pattern which aroused high suspicion for multiple alloantibodies.

For ruling out/ruling in antibodies, select cells from a second panel (Resolve Panel B, Ortho Clinical Diagnostics, Raritan, NJ, USA) were identified. Three alloantibodies, namely anti-D, anti-C, and Anti-E were suspected. While the patient tested negative for all three antigens, father and first child tested positive for only two; D and C, questioning the source of immunization of E antigen. Anti-E could have been formed after the transfusion episode during her first pregnancy but this could not be established as extended phenotype of the transfused unit was not available at the time of work-up. Since the presence of anti-E in the patient’s plasma was not supported by the father’s or child’s phenotype and the possibility of anti-G could not be excluded, differential adsorption was planned.

The department was conducting a study to gain knowledge about the prevalence of 18 clinically significant blood groups antigens. Coincidently, at the time this patient presented, the department had recently been visited by a very rare donor with D⁻ phenotype. With the help of the pilot samples, sequential adsorption and elution studies were proposed [Figure 1]. Figure 2 shows sequential adsorption and elution studies to check for the presence of anti-G along with other Rh alloantibodies. An alternative approach to identify specificities of these antibodies without use of D⁻ red cells is suggested in Figure 3. The presence of anti-G was ruled out and presence of anti-D, anti-C, and anti-E were confirmed. Serial doubling dilution titration by conventional tube technique was performed with r'r (dCce) adsorbed patient plasma and R1R1 red cells for anti-D, R2R2 (DeE) adsorbed plasma and R1R1 (Dce) red cells for anti-C and R1R1 adsorbed plasma from and R2R2 red cells for anti-E. Titration revealed anti-D titer to be 1024, anti-C to be 4, and anti-E to be 32. In view of multiple antibodies, Doppler ultrasound was done which revealed middle cerebral artery peak systolic velocity >41.9 cm/s, i.e., >1.5 standard deviation above the normal mean for gestation suggesting fetal anemia. A request for 30 ml red cells was received. Group “O” RhD negative, fresh red cells (within 5 days of collection), negative for C, E, and K antigens, hematocrit 75%–85%, leukoreduced, crossmatch compatible with maternal plasma, sickle negative, and irradiated were transfused.

A total of eight intrauterine transfusions were performed till induction of labor. Each time a request was received, antibody screening and antibody titration were performed and an aliquot of patient’s plasma was preserved. No RhD prophylaxis was given to the mother. The neonate developed pathological jaundice after birth which required phototherapy mainly and exchange transfusion once. The unit prepared for exchange transfusion involved reconstituting red cells without additive solution and fresh frozen plasma to a hematocrit of 45%–55%. The red cell unit selected was group “O” RhD negative, fresh, negative for C, E, and K antigens, anti-human globulin phase crossmatch compatible with both the neonate and the patient, sickle negative, leukoreduced, and irradiated. The neonate was able to recover from hyperbilirubinemia and was discharged from the hospital at day 42 of birth. The infant continues to do well.

Discussion

The Rh blood group system is the most complex system. Among the many Rh antigens, five principal Rh antigens D, C, c, E, and e are responsible for majority of

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**Figure 1:** Adsorption studies for identifying the specificity of multiple Rh alloantibodies (anti-D, anti-C, anti-E)
immunization. Rh antigens have high immunogenicity. The administration of IgG anti-D prophylaxis has been effective in prevention of HDFN. Although this discovery declined the number of pregnancies with HDFN significantly in developed countries, India still has not been able to bring about the desired decline primarily because only a small number show up for antenatal check-ups. Second, RhD prophylaxis prevents only RhD alloimmunization; other Rh antigens can still immunize the pregnant female. Hence, antibody screening should be an integral part of antenatal assessment.

Pahuja et al. screened 3577 multigravida women for the presence of unexpected antibodies and reported an incidence of 1.25%. The most common coexistent antibodies were anti-D and anti-C. Makroo et al. reported a case of multiple Rh alloantibodies, namely, anti-G, anti-C, and anti-D. However, to the best of our knowledge, a combination of anti-D, anti-C and anti-E has not been reported in Indian literature. Anti-G is of consideration in such a sample because all cells carrying D or C antigen test positive for G. Hence, ruling out anti-G in the presence of Rh antibodies other than anti-D and anti-C has not been discussed. Although establishing the presence of anti-G in the absence of anti-D is of clinical significance because this patient category requires RhD prophylaxis but this testing is not routinely performed and anti-G remains underdiagnosed due to paucity of knowledge and resources. Hence, steps to educate transfusion services about the need and serological procedures involved to differentiate between anti-D+anti-C and anti-G should be actively taken.

Conclusion

With the help of select cells, phenotyping, adsorption elution studies, and extended phenotype red cells; solving complex serological cases can become facile.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will
be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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