Anti-G antibody in alloimmunized pregnant women: Report of two cases

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Abstract:

Anti-G has been reported as a possible cause of hemolytic disease of the fetus and newborn (HDFN), either independently or in association with anti-D, anti-C or both. The antibody mimics the pattern of anti-C and anti-D reactivity in the identification panel and is often present along with either or both of these antibodies. The differentiation of anti-D, -C and-G in routine pretransfusion workup is particularly essential in antenatal cases. We report two antenatal cases where anti-G was identified on advanced immunohematological workup, in addition to other alloantibodies.

Key words:

Alloimmunization, anti-G, Rhesus G antigen

Access this article online

Website: www.ajts.org

DOI: 10.4103/0973-6247.162724

Quick Response Code:

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Introduction

The "Rhesus G" (Rh) antigen was first described by Allen and Tippet in 1958[1] and anti-G has been reported as a possible cause of hemolytic disease of fetus and newborn (HDFN), either independently or in association with anti-D, anti-C or both.[2,3] The antibody mimics the pattern of anti-C and anti-D reactivity in the identification panel and is often present along with either or both of these antibodies.[1] The differentiation of anti-D, -C and G in routine pretransfusion workup is particularly essential in antenatal cases. In the alloimmunized pregnant women showing a reactivity pattern of anti-C plus anti-D, there might be an underlying anti-G causing HDFN either alone or in addition to these antibodies.[4-7] This complex serological condition often accounts for medical dilemmas like anti-D like picture in the maternal serum in spite of Rh D prophylaxis at the time of previous pregnancy or HDFN in a child due to anti-D when the father is D negative. The antibodies in such cases could actually be anti-G or anti-G+C. The former can often be mistaken as failure of Rh D prophylaxis. However the woman still stands a chance of getting immunized against anti-D and should, therefore, receive Rh immunoglobulin (RhIG).[2,4-7] We report here two such cases of HDFN due to multiple alloantibodies including anti-G.

Case Reports

Case 1

A 32-year-old G4P2A1L1 was referred to the Department of Fetal Medicine at our hospital at 24 weeks of gestation with a positive indirect coombs test (ICT) from an outside laboratory. She suffered one early neonatal death due to severe neonatal jaundice and one miscarriage at 12 weeks. Her blood sample was sent to the Department of Transfusion Medicine for blood grouping, typing and antibody screening. Her blood group was A Rh (D) negative, and her antibody screen was positive. Further identification of antibody revealed a multiple alloantibody pattern of anti-D and anti-C specificity. Her extended Rh phenotype was ccee and was Kell negative (K-). Blood grouping and phenotyping of her husband showed O Rh (D) positive blood group with CCee, K-.

The patient had reportedly received RhIG prophylaxis after all her obstetric events. The last dose of anti-D was given 3 years ago. There was no history of blood transfusions. The fetus was monitored with twice weekly middle cerebral artery peak systolic velocity (MCA-PSV) values. The Fetus developed signs of fetal anemia (MCA-PSV >1.5 multiples of median [MOM]) at 29 weeks of gestation and 1st intrauterine transfusion (IUT) was done using Group O Rh (D) negative (ccee K-) leukoreduced, irradiated packed red cells (PRC) with a hematocrit of 80%. Before starting the IUT, a fetal blood sample was sent for blood grouping, phenotyping, and direct antiglobulin test (DAT) and complete blood count. Cell group of the fetus was O Rh (D) Positive and phenotype was Ccee K-. DAT was strongly positive. IUT was repeated at 32 weeks of gestation. In view of falling of hematocrit of 1% per day elective caesarean section was done at 35 weeks. The neonate had a body weight of 2.9 kg with an APGAR scoring of 7 and 8 at 1 and 5 min, respectively. On day 1 the hemoglobin (Hb) was 10.5 g/dL and total bilirubin was 4.7 mg/dL with direct bilirubin being 0.7 mg/dL. Cord blood

obtained also revealed a positive DAT. The neonate received one exchange transfusion with Group O Rh (D) negative blood (ccee K-) and was kept on phototherapy. The direct bilirubin level was all through maintained between 0.2 and 0.7 mg/dL supporting absence of obstructive jaundice. With phototherapy, the total bilirubin was maintained at <6 mg/dL and eventually tapered, and the baby was discharged on day 26 in healthy condition.

Case 2

A 23-year-old female G4P3A1L2 was referred to the Department of Fetal Medicine at our hospital at 29 weeks of gestation with a positive ICT and fetal hydrops on ultrasound. Her sample was sent for blood grouping, typing and antibody screening. Blood group was A Rh (D) negative, ccee K- and antibody screen was positive. Further workup revealed anti-C, anti-D and anti-E. There was no history of any RhIG prophylaxis. Fetal ultrasound confirmed severe fetal hydrops and fetal anemia (MCA-PSV >1.5 MOM). First IUT was given with O Rh (D) negative leukoreduced, irradiated PRC of 80% hematocrit (ccee, K-). Fetal blood sampling prior to transfusion showed a Hb level of 0.7 g/dL, Group A Rh (D) positive with ccEe K-and strong DAT positivity. Blood grouping and phenotyping of father revealed Group O Rh (D) positive, CcEe K-. Second IUT was done a week later. The patient went into preterm labor, and a caesarean section was performed at 30 week's gestation. The neonate, however, did not cry immediately after birth. Attempts to resuscitate the neonate failed, and the neonate died in an hour. Cord blood was not available for any further testing in this case.

Materials and Methods, Results

Blood grouping, phenotyping, antibody screen (4 cell screening panel; capture ready screen) and antibody identification (Solid Phase Red Cell Adherence [SPRCA], 16 cell Panel), were all performed on the fully automated Immunohematology analyzer (Galileo, Immucor, US). Fetal samples' DAT was performed both by SPRCA and tube techniques.

Double adsorption technique was employed to include/exclude the presence of anti-G alone or in conjunction with anti-D, anti-C or both. Differential adsorption and elution by tube technique were performed using r'r' (O Negative, CCee) and R^0r (O positive, ccee) cells in both the cases.[5]

In both the cases, the patients' plasma was adsorbed onto ficin treated r'r' cells five times to adsorb anti-C and/or anti-G to exhaustion. The adsorbed plasma was then tested against r'r' and R^0r cells showing negative reaction and strength of +4 respectively, confirming complete adsorption of anti-C and/or anti-G and presence of anti-D. Eluate was prepared using Gamma ELU-KIT™ II, Immucor, from the first adsorbing aliquot of r'r' cells and tested with R^0r and r'r' (red blood cells) RBCs which gave +4 and +3 reaction strengths respectively, confirming presence of anti-G. The eluate was then adsorbed on to ficin treated R^0r cells five times to adsorb out anti-G. This adsorbed plasma was again tested with R^0r cells which gave a negative reaction confirming complete adsorption of anti-G. This adsorbed plasma was again tested with R^0r cells, which gave a negative reaction confirming complete adsorption of anti-G. This was then tested with r'r' cells for presence of anti-C which gave +4 reaction in case 1 and negative reaction in case 2 confirming presence of anti-C in case 1 and its absence in case 2. From the first aliquot of R^0r cells elution was done and checked with r'r' RBCs which gave +4 reaction strength re-confirming the presence of anti-G in both the cases.

Discussion

Rhesus (D) alloimmunization was largely recognized as the cause of immune HDFN until 1960s. Antenatal interventions like routine prophylactic RhIG given to D negative women has reduced alloimmunization rate by nearly 90%. However, cases of HDFN resulting from alloimmunization are still reported.[8] The most frequent alloantibodies besides anti-D that are identified in the sera of these patients are directed against: Rh antigens (anti-E, anti-C, anti-c), Kell antigens (anti-K), Duffy antigens (anti-Fy^a), Kidd antigens (anti-Jk^a), and MNSs antigens.[8]

This, to the best of our knowledge is the first report of anti-G antibody from India. Both the cases reported here showed clinical features of severe HDFN. Since anti-D has been independently identified in both the patients, the HDFN can be largely attributed to its presence. However, advanced investigations have revealed the presence of anti-G in both the cases, which could be contributing to the severity of HDFN. In the second case reported, no anti-C antibody was finally identified and the anti-C like picture observed in the identification panel was actually due to the presence of anti-G, which was mimicking the anti D + C picture.

The report, therefore, highlights the importance of performing advanced investigations in order to accurately characterize the immunizing antibodies especially in pregnant women, where it is of paramount importance to confirm the presence or absence of anti-D. The presence of anti-D excludes the need for the administration of prophylactic anti-D immunoglobulin. In addition, the exclusion of the presence of anti-D in samples from D-negative women with D-negative partners or from D-negative recipients of D-negative blood components can avoid potential social or medico-legal complications.[2] In the absence of advanced testing it is likely that anti-G antibodies are being under diagnosed in our country.

Note

Due to nonavailability of the rare r^Gr cells, a direct evidence of anti-G could not be established. However, double adsorption technique has been widely used and reported in literature as highly supportive of the presence of anti-G.[9]

References

1. Allen FH Jr, Tippett PA. A new Rh blood type which reveals the Rh antigen G. Vox Sang 1958;3:321-30.
2. Palfi M, Gunnarsson C. The frequency of anti-C + anti-G in the absence of anti-D in alloimmunized pregnancies. Transfus Med 2001;11:207-10.
3. Huber AR, Leonard GT, Driggers RW, Learn SB, Gilstad CW. Case report: Moderate hemolytic disease of the newborn due to anti-G. Immunohematology 2006;22:166-70.
4. Schulze TJ, Goebel M, Scharberg EA, Bugert P, Janetzko K. Development of anti-G, anti-C and anti-Jk(b) in a 22-year-old mother during her fourth pregnancy. Transfus Med Hemother 2013;40:207-9.

5. Shirey RS, Mirabella DC, Lumadue JA, Ness PM. Differentiation of anti-D, -C, and -G: cDlinical relevance in alloimmunized pregnancies. Transfusion 1997;37:493-6.
6. Hadley AG, Poole GD, Poole J, Anderson NA, Robson M. Haemolytic disease of the newborn due to anti-G. Vox Sang 1996;71:108-12.
7. Yesus YW, Akhter JE. Hemolytic disease of the newborn due to anti-C and anti-G masquerading as anti-D. Am J Clin Pathol 1985;84:769-72.
8. Solheim B, Gronn M. Hemolytic disease of the fetus and newborn. In: Simon TL, Snyder EL, Solheim BG, Stowell CP, Strauss RG, Petrides M, editors. Rossi's Principles of Transfusion Medicine. Bethesda: AABB Press; 2009. p. 418-25.
9. Vos GH. The evaluation of specific anti-G (CD) eluate obtained by a double absorption and elution procedure. Vox Sang 1960;5:472-8.

Cite this article as: Makroo RN, Kaul A, Bhatia A, Agrawal S, Singh C, Karna P. Anti-G antibody in alloimmunized pregnant women: Report of two cases. Asian J Transfus Sci 2015;9:210-2.

Source of Support: Nil, **Conflicting Interest:** None declared.