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Anti-G with concomitant anti-C and anti-D: A case report in a pregnant woman

Rabeya Yousuf, Ahmad Nasirudin Mustafa, Siew-Ling Ho, Yee-Loong Tang, Chooi-Fun Leong

Abstract:

The G antigen of Rh blood group system is present in almost all D-positive or C-positive red cells but absent from red cells lacking D and C antigens. The differentiation of anti-D and anti-C from anti-G is not necessary for routine transfusion; however, during pregnancy, it is important because anti-G can masquerade as anti-D and anti-C with initial antibody testing. The false presence of anti-D will exclude the patient from receiving anti-D immunoglobulin (RhIG) when the patient actually is a candidate for RhIG prophylaxis. Moreover, patients with positive anti-D or anti-G are at risk of developing hemolytic disease of the fetus and newborn and need close monitoring. Thus, proper identification allows the clinicians to manage patients properly. This case report highlights a rare case of anti-G together with anti-D and anti-C in a pregnant woman. This report disseminates knowledge on identification of anti-G and its importance in pregnant women.

Key words:

Antenatal antibody, anti-C, anti-D immunoglobulin, anti-G, hemolytic disease of the fetus and newborn

The G antigen belongs to the Rh blood group system and is produced by RhD gene and RHCE gene and is present in almost all D-positive or/and C-positive red blood cells (RBCs) while absent from those of D-negative and C-negative red cells. This unique phenotype was first described by Allen and Tippet in 1958.[1] Its apparent co-distribution with either the C or D antigen causes anti-G to appear serologically as anti-C plus anti-D activity.[2]

The presence of anti-G antibody together with anti-D and anti-C is important in women with rr phenotype during pregnancy and can lead to hemolytic disease of fetus and newborn (HDFN).[3] The differentiation of anti-D, anti-C, and anti-G specificities for routine blood transfusion in rr patients is not necessary as these patients will receive RhD and C antigen negative blood that would also be G antigen negative.[4] However, distinguishing these antibodies during pregnancy is very important for proper antenatal management of the patient.[5] Issitt and Tessel first described the method for separation of these antibodies by serial double-elution procedure using $D^+ C^-$ RBCs followed by $D^- C^+$ RBCs to identify the presence of anti-G.[6] The correct identification of the antibody specificity, particularly anti-D is very important as the absence of anti-D requires the need for administration of prophylactic anti-D immunoglobulin (RhIG) to prevent HDFN due to anti-D during pregnancy.[7] Therefore, the false presence of anti-D antibody together with anti-C due to anti-G antibody will exclude the patient from receiving RhIG when the patient actually is a candidate for RhIG prophylaxis.[8] In a previous study, it was shown that anti-D was absent in two out of seven (30%) alloimmunized pregnant women with apparent anti-D plus anti-C and anti-D was not administered where actually the patients were the candidate for RhIG.[5] Therefore, appropriate identification of anti-D antibody in pregnant women is very essential for proper management. It has been reported that anti-G antibody has displayed mild, moderate, or even severe forms of HDFN.[8] Thus, proper antibody identification allows the clinicians to take necessary steps in patient management. We report here a rare case of anti-G in combination with anti-D and anti-C in a pregnant woman, identified during the antenatal visit. This report helps to disseminate knowledge on identification of anti-G and its importance in pregnant women.

Department of Pathology, Blood Bank Unit, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur, Malaysia

Address for correspondence:
Dr. Leong Chooi Fun, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
E-mail: cfleong@ppukm.ukm.edu.my

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Case Report

A 32-year-old, gravida 4, para 3 woman at 28 weeks of pregnancy was monitored during her antenatal visit. The patient had no history of blood transfusion and no RHIG administration at that point of time. She had a previous history of neonatal jaundice in her third baby. Her husband's blood group was A with R1r (DCe/dce) phenotype.

She was grouped as A with rr (dce/dce) phenotype. Direct Coomb's test was performed using a gel technique (ID-Card "LISS/Coombs") and revealed as negative. Antibody screening (3-cell panels, Bio-Rad ID-DiaCell I-II-III Asia) was positive (2+) by gel technique (manual method). Antibody identification (11 cell panel, Bio-Rad ID DiaPanel, ID DiaPanel-P, gel technique) revealed the presence of anti-C and anti-D specificities. In view of the presence of combined anti-C and anti-D, the presence of anti-G was suspected and further testing by double adsorption and elution using R_2R_2 (DcE/DcE) and rr' (dce/dCe) cells was performed by the tube technique.

Double adsorption and elution

Differential adsorption and elution were performed with the patient's plasma by the tube technique using R_2R_2 (O positive, $D^+ C^-$) and rr' (O negative, $D^- C^+$) cells to evaluate the presence or absence of anti-G. R_2R_2 cells were used for the first adsorption, reason being that G antigen is present in almost all D-positive RBCs, and adsorption with R_2R_2 cells are G antigen positive ($D^+ G^+ C^-$) and will adsorb the anti-G and/or anti-D from patient's plasma. The adsorption was done in a LISS environment with polyethylene glycol (RAMPEG CSL), and elution was performed using ELU-KIT™ Plus Kit, Immucor Gamma red cell elution system.

The first adsorption of patient's plasma with R_2R_2 ($D^+ G^+ C^-$) cells was performed three times and allowing the unbound anti-C to remain in the postadsorbed plasma (first postadsorbed plasma). After adsorption, the postadsorbed plasma and postadsorbed cells were separated. The postadsorbed plasma was tested with rr', R_2R_2, and rr cells. Test results showed a positive reaction (1+) with rr' ($C^+ D^- G^+$) and confirmed presence of anti-C antibody while negative on R_2R_2 ($C^- D^+ G^+$) and rr ($C^- D^- G^-$), confirming complete adsorption of anti-D and/or anti-G. On the other side, the eluate was prepared from the postadsorbed R_2R_2 cells (first adsorption) that were suspected to contain anti-D and/or anti-G.

Subsequently, the next step was the second adsorption of the eluate prepared from the above-mentioned step with rr' ($C^+ D^- G^+$) cells for three times with an intention to adsorb the anti-G by the rr' cells if present, leaving behind the unbound anti-D in the postadsorbed eluate. After completion, the postadsorbed eluate and postadsorbed cells were separated. The postadsorbed eluate was tested positive (3+) on R_2R_2 ($D^+ G^+ C^-$) cells, confirmed the presence of anti-D and negative on rr' ($C^+ D^- G^+$). Concomitantly, the final eluate was prepared from this second step of postadsorbed rr' cells by using the same kit as mentioned earlier and tested positive with R_2R_2 ($D^+ G^+ C^-$) cells and rr' ($D^- G^+ C^+$) cells, as well as R_1R_1 ($D^+ G^+ C^+$) cell. The results confirmed the presence of anti-G. Thus, the presence of combined anti-D, -C and -G was confirmed. The steps are shown in Figure 1, and test results are shown in Table 1.

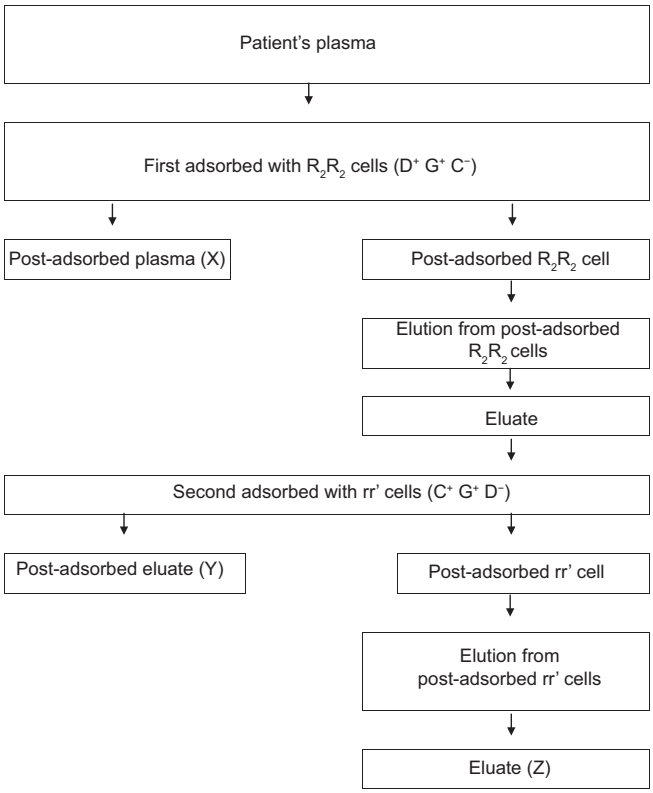

Figure 1: *Flow chart showed the test procedure of double adsorption and elution. X, Y, and Z represent the respective specimens for further testings*

Table 1: Reaction of specimen X, Y, Z when tested with R_2R_2, rr', and rr cells

Specimen	R_2R_2 ($D^+ G^+ C^-$)	rr' ($D^- G^+ C^+$)	rr ($D^- G^- C^-$)	Result
X	0	1+	0	Anti-C
Y	3+	0	0	Anti-D
Z	2+	+	0	Anti-G

Additional test of specimen Z with R_1R_1 ($D^+ C^+ G^+$) cell showed a positive result (2+)

The mixed antibody titer (anti-D, -C and -G) was 1:512 and remains steady to this level in her subsequent follow-up till delivery. The patient was most likely sensitized and developed anti-D, C, and G from previous pregnancies. No RhIG was administered to the patient during this current pregnancy as the patient was anti-D positive. The anti-D titer of the patient and the fetus were monitored closely until and after delivery. A baby boy was delivered by cesarean section at 39 weeks of pregnancy with mild HDFN. Baby's blood group was A Rh D positive and his direct coombs test was positive (3+). Total bilirubin was 71.7 umol/L with mild anemia (Hb 12.5) at birth. Full blood picture showed the evidence of hemolysis with normochromic normocytic RBC, presence of spherocytes, and occasional nucleated RBC. The bilirubin was gradually increased to 232.5 umol/L and Hb decreased to 9.7 on day-5. The baby was managed with phototherapy. However, the parents preferred to get the baby discharged and subsequently, there was no follow-up at our hospital.

Discussion

D antigen is highly immunogenic, and the presence of anti-D can cause severe HDFN. Anti-C also has the potential

to cause some degree of HDFN.[5] However, there are controversial reports regarding the potential of anti-G to cause HDFN. It is mentioned that anti-G rarely reaches high titer that can pose a risk to the fetus compared to anti-D and anti-C.[5] However, Hadley *et al*. claimed anti-G could cause HDFN with high titers of anti-G consistent with moderate to severe HDFN.[9] Palfi and Gunnarsson mentioned that the G antigen is highly immunogenic and showed the presence of anti-G in different combination in 24 (88.9%) cases out of 27 cases.[10] Huber *et al*. mentioned isolated anti-G causing moderate HDFN in an infant with hyperbilirubinemia requiring a prolonged stay in the hospital for phototherapy.[2] In another report, moderately severe HDFN was reported in a RhD-negative woman with anti-G.[11] All these cases showed the evidence of HDFN due to anti-G. Thus, it is important to correctly identify the specificity of the antibodies during pregnancy in patients who have initial antibody testing showed concurrent anti-C and anti-D antibodies.

In addition, the differentiation of anti-D from anti-G is also helpful in solving medicolegal aspects such as excluding questions of paternity for RhD-negative couple due to anti-D like picture in the maternal serum in spite of RhD 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, thus the exclusion of the presence of anti-D in samples from D-negative women with D-negative partners can avoid potential social or medicolegal complications. Similarly, differentiation of anti-D also helps to avoid confusion regarding previous transfusion history in D-negative recipients of D-negative blood components as anti-G and anti-C can be found in recipients of RhD-negative products.[2,12]

This report highlights the importance to recognize anti-D, anti-C, and anti-G in a pregnant woman with initial antibody screening positive for anti-D and anti-C to decide the administration of prophylactic RhIG and management of HDFN. A pregnant woman who possesses anti-G without anti-D requires prophylactic RhIG to prevent Rh D HDFN. For transfusion in patients with anti-G, anti-D, and anti-C, D- and C-antigen-negative red cells that are also G antigen-negative should be given.

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Conflicts of interest

There are no conflicts of interest.

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