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A CASE REPORT: ANTI-G DETECTION AND MANAGEMENT IN PREGNANCY

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Abstract

Introduction

Red blood cells (RBCs) that carry RhD &/or RhC antigens have the G antigen (Rh12). Since anti-G antibodies show up on antibody screening as anti-D along with anti-C patterns, they were frequently overlooked. To prevent hemolytic disease of the fetus as well as newborn (HDFN) to guide optimal care, it is imperative to detect Rh-negative pregnant mothers.

Case Report

A Rh-negative pregnant woman(32yr/old) presented for antenatal checkup at 10 weeks of gestation. Initial screening revealed a positive indirect antihuman globulin test, with antibody screening patterns evocative of anti-D as well as anti-C. Advanced immunohematological techniques, which included adsorption-elution studies, confirmed the existence of D, C antigen, as well as anti-G antibodies. Antibody titers rose to critical levels of 32 by 33 weeks of gestation, accompanied by ultrasound findings suggestive of fetal anemia based on elevated middle cerebral artery peak systolic volume (>1.5MoM). The patient was managed with steroids for fetal lung maturity, and at 34 weeks C-section is performed. Postnatally, the neonate exhibited signs of HDFN with elevated bilirubin levels, which were successfully managed with phototherapy.

Discussion

This case underscores the clinical significance of distinguishing anti-G from anti-D as well as anti-C antibodies in Rh-negative pregnancies. Early diagnosis facilitated appropriate fetal monitoring and timely interventions, preventing severe complications.

Conclusion

Identifying anti-G antibodies through advanced immunohematological methods is vital for the management of Rh alloimmunization and optimizing maternal and neonatal outcomes. This case emphasizes the need for regular monitoring, multidisciplinary caste, and the critical role of precise antibody characterization in preventing complications in future pregnancies.

INTRODUCTION

The Rh blood group comprises 5 significant antigens (C,c,D,E, along with e) that form among essential human blood group systems. A total of five main antigens form Rh blood group system comprises of D,C,c,E as well as e. Immunogenic D antigen shows strong antigenic properties together with other antigens in this blood group. The production of antibodies occurs in people without the antigen when they encounter either pregnancy-related or transfusion situations. Medical research demonstrates that developed antibodies lead to HDFN and hemolytic transfusion responses (1). The occurrence of HDFN mostly links to antibodies against anti-D as well as anti-C but the severity of its effects tends to be weaker when the antibody is anti-C.

Research by Allen and Tippet established the discovery of Rhesus G antigen (Rh12) in 1958 (2). The cells carry the D or C antigens when they possess Rh12 but this antigen appears absent in antigen-free cells (3). The G-negative blood type appears only when RBCs do not carry either C or D antigens according to current scientific records (4). Studies have confirmed that antihemolytic disease of newborns develops because of anti-G antibodies.

The existence can occur in five ways based on anti-G status combined with anti-D as well as anti-C presence. This antibody exists in isolated form only exceptionally because it coexists with anti-D along with anti-C(5). Distinction between these three antibodies can become troublesome when tested during a prenatal examination. Among female patients of childbearing age it is vital to distinguish between anti-G, anti-C as well as anti-D antibodies. Pregnant women who lack anti-D antibody need to take Rh immune globulin prophylaxis to stop RhD isoimmunization. A person with anti-D antibodies will suffer from a more serious type of HDFN because of their antibodies.

CASE REPORT

A pregnant woman(32yrs/old), with obstetric score $G^2P^1L^1$ (gravida 2, para 1), having O Rh(D) negative blood group, presented to our hospital's Obstetrics department at 10 weeks of gestation for a regular antenatal checkup. Her last childbirth was 4 years ago, a full-term normal vaginal delivery, and the child was having ORh(D) positive blood group. She had no recollection of receiving anti-D at the time of her last childbirth. She gives a history of receiving blood transfusions during her pregnancy because of anemia. The patient's sample was sent to our laboratory in the Department of Transfusion Medicine and Immunohematology for routine investigations, including typing & blood grouping, along with Indirect antiglobulin test (IAT). Her IAT came as positive, which indicated the presence of an alloantibody, following which antibody titration was done, and the titer was found to be too low to be detected. The antibody titer test was done with in-house prepared O-pooled cells. Since IAT was positive, antibody screening was performed with patients's serum, using a 3-cell antibody identification panel (BioRad ID), followed by an 11-cell antibody identification panel (BioRad ID), using column agglutination method. 3-cell panel was done with the automated machine, while the 11-cell panel was done manually. The findings of the antibody identification panel are shown in **Figure 1**. There was a pan-positive reaction of 3+ in Bio-Rad 3 cell panel, while in Bio-Rad 11 cell panel, the 2+ reactivity was observed in cell lines 1, 2, 3, 4, and 8. Extended phenotyping of the patient was also done, which revealed C-c+E-e+K- **(Figure 2)**. The panel of screening of antibodies in patient revealed a pattern that was implying of anti-D with anti-C allo antibodies in red cell panel antigram; the possibility of anti-G antibody was suspected because of the above results **(Figure 3)**. The obstetrics department was informed of the above significant findings. The husband's blood grouping was done; he is BRh-positive with prolonged phenotyping showing C+c+E-e+K- **(Figure 4)**. This was to identify the possible paternal antigens the mother may have been exposed to during the pregnancy.

Figure 1: Reactivity pattern of maternal serum with the Bio-Rad ID-Dia 3-cell & 11-cell

Figure 2: Patient Blood group and extended phenotyping

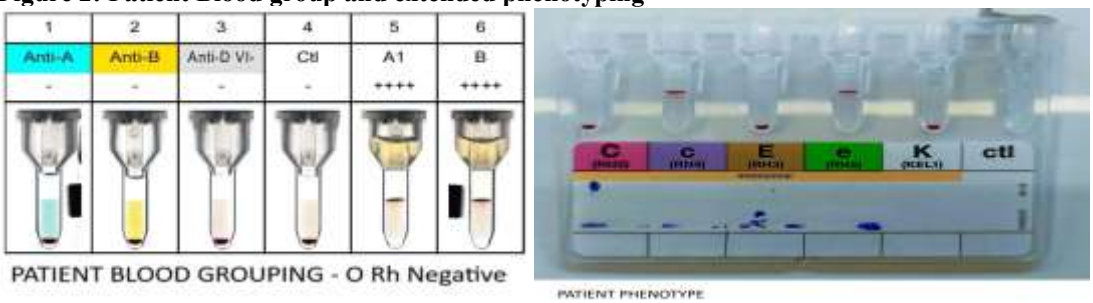


Figure 3: Reactivity pattern of maternal serum with the Bio-Rad ID-Dia 3-cell & 11-cell antibody identification panel

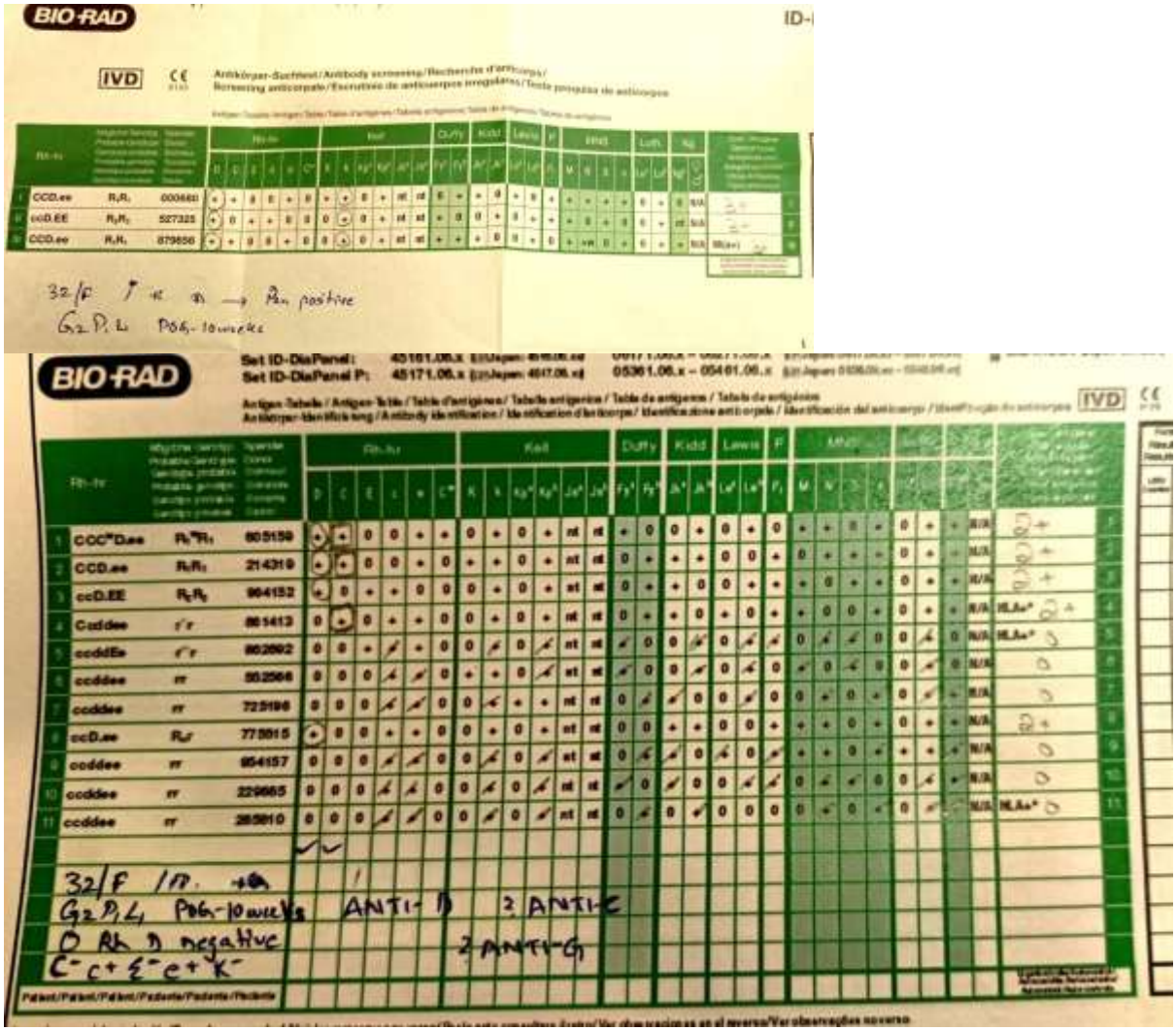
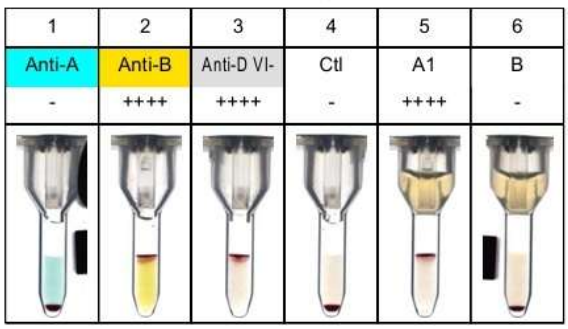
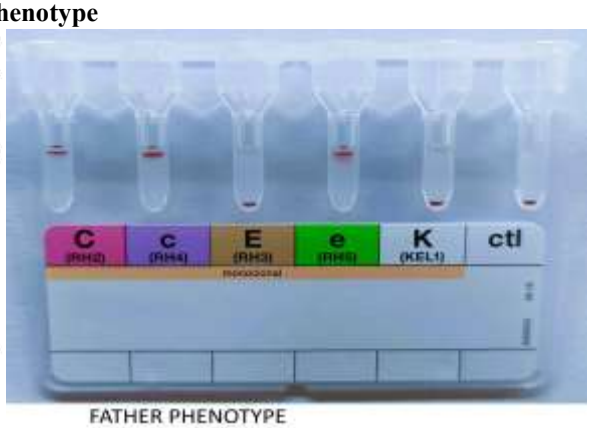


Figure 4: Father's Blood grouping and extended phenotype



FATHER BLOOD GROUP

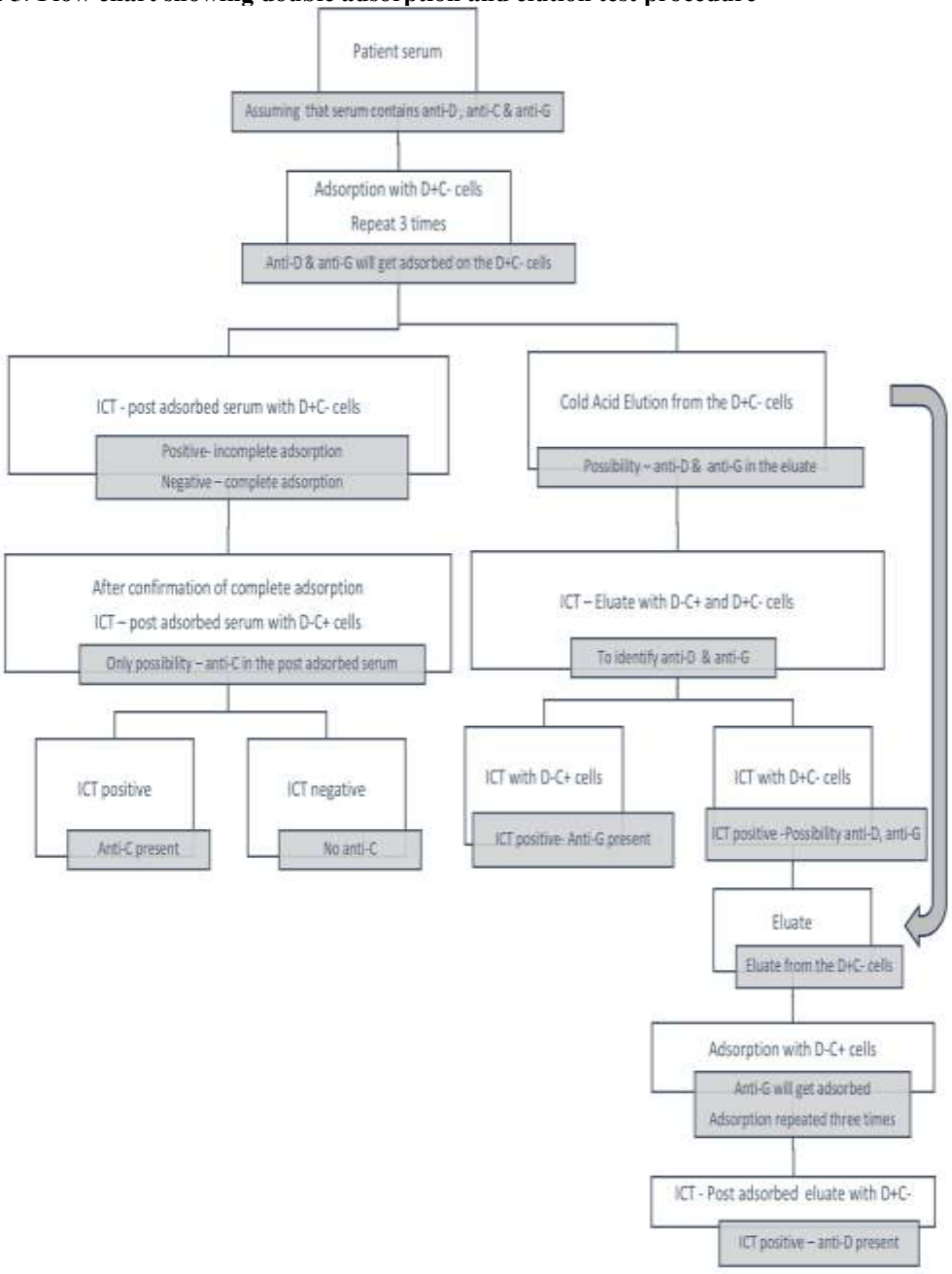


FATHER PHENOTYPE

Since the titer was too low to be detected at 10 weeks of gestation, the patient was advised to repeat the titers every 4 weeks. At 22 weeks of gestation, the titer had increased to 16, following which double adsorption as well

as elution have been planned with patient's plasma using tube method to identify the antibodies present. A flowchart was prepared for the double adsorption and elution **(Figure 5)**.

FIGURE 5: **Flow chart showing double adsorption and elution test procedure**

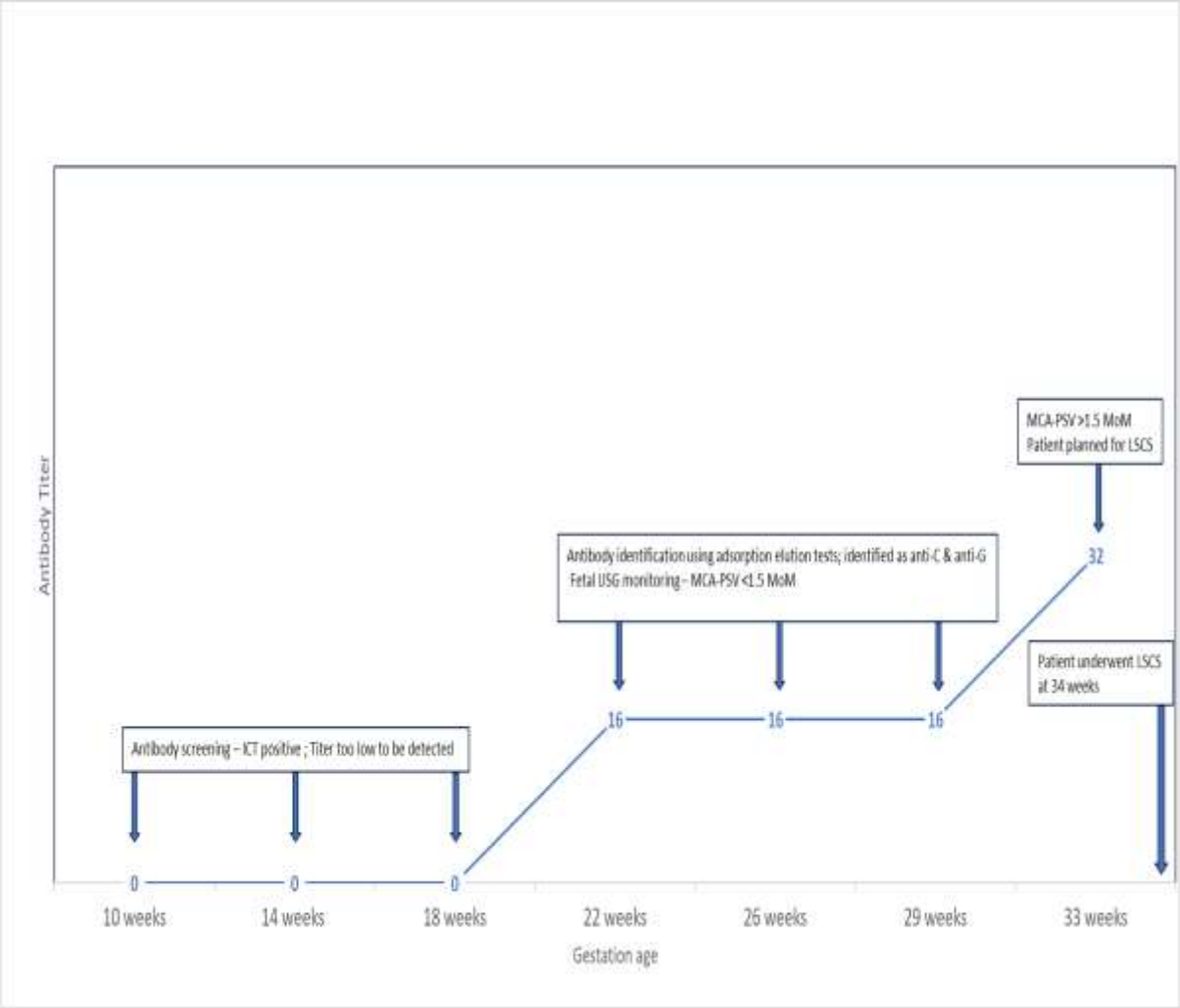


Double adsorption as well as elution have been done using O positive D+C-(R2R2) as well as O negative D-C+ (rr'). Patient's serum was assumed to have all 3 "antibodies—anti-D, anti-C, plus anti-G. D+C- is used for the first adsorption, which would absorb both anti-D even anti-G antibodies (as G antigen is seen on all D+ RBCs)". The adsorption was done by incubating the D+C- cells with the patient's serum at 37°C for 45min. Adsorption was repeated three more times with different sets of O Rh-positive D+C- cells, which will leave only the unbound antibodies (i.e., anti-C) to remain in the adsorbed plasma. With the post-adsorbed serum, IAT was done with O cells D+C- cells, to confirm complete adsorption (IAT should be negative). After confirmation of the complete adsorption, IAT was then done with the post-adsorped serum using O cells D-C+, for which we got an IAT-positive result. This confirmed that anti-C have been in the serum of patient.

Elution (cold acid elution) was done on the cells (D+C-) which was used for adsorption at the first step. With the eluate, "IAT was done with D+C- cells (to confirm anti-G & D) as well as D-C+ cells (to confirm anti-G). IAT was positive with both D+C- and D-C+ cells, which meant patient had anti-G antibodies. To determine whether anti-D is present, we did adsorption of the eluate with D-C+ cells, the adsorption was repeated 5 times (serial adsorptions will remove the anti-G from the eluate). Post-adsorption, with the eluate, ICT was done on D+C- cells, which came as positive. This meant that the patient's serum had anti-D antibodies also. Hence, we found that the patient had all 3 anti-D, anti-C, anti-G antibodies". After identifying antibodies, the patient antibody titers were checked with D+C- as well as D-C+ O cells, both titers came to 16. The above results were informed to the obstetrics department.

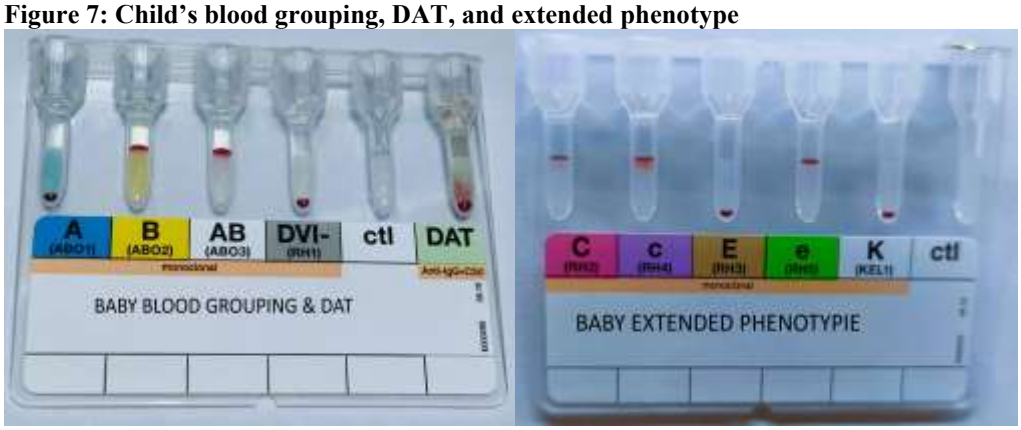
The patient was further advised to review every 4 weeks. Further reviews at 26 weeks and 30 weeks of gestation, the titer remained the same at 16. By 33 weeks (33 weeks + 4 days) of gestation, the titer had risen to 32 **(Figure 6)**. Given the elevated titer values (above the critical titer value of 16), fetal ultrasound was performed on the patient in order to detect fetal anemia. Middle cerebral artery peak systolic velocity (MCA-PSV) was analyzed, and readings came to>1.5multiples of the mean (MoM). This predicted that the fetus was developing anemia. Given the above finding, the patient was started on steroids with a plan to deliver the fetus. The infant had been delivered through elective lower segment C-section (LSCS) at 34 weeks of gestation. For additional care, the infant was brought to the Neonatal ICU.

Figure 6: Graph depicting titer levels and timing of interventions



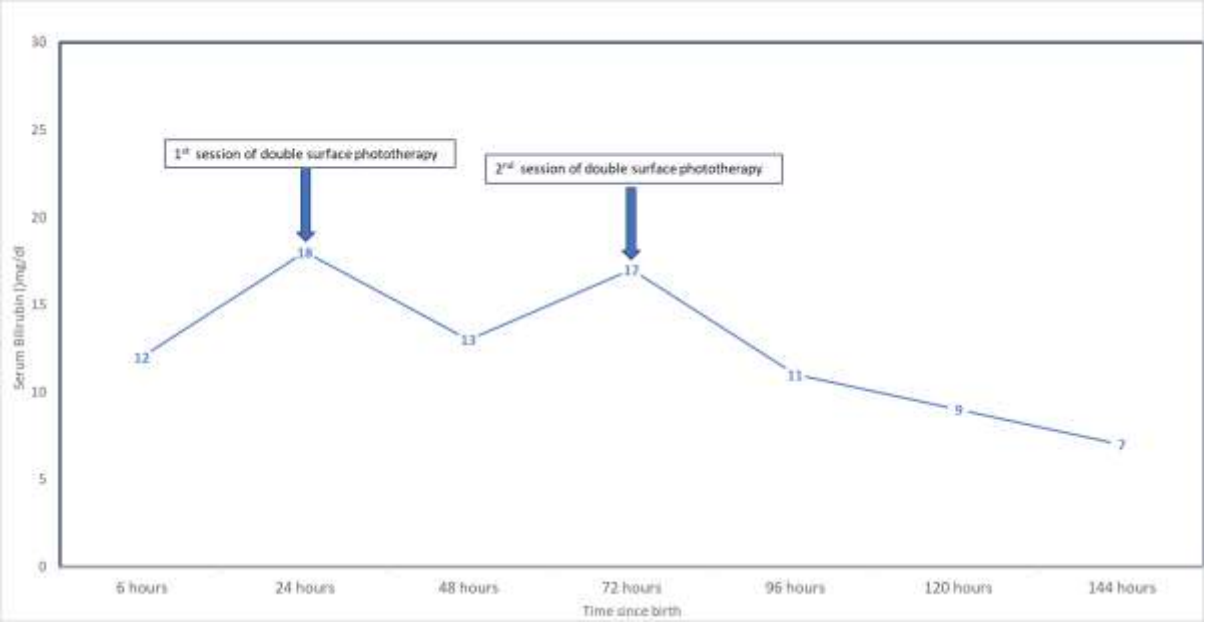
The baby was B Rh negative, with extended phenotyping revealing C+c+E-e+K- and direct antihuman globulin test (DAT) positive **(Figure 7)**.

Figure 7: Child's blood grouping, DAT, and extended phenotype



A DAT test showed positivity in the newborn patient. The patient received a serum bilirubin test which revealed 12.2 mg/dl at the 6-hour mark following birth before the levels increased to 18 mg/dl by the 24-hour period. Given the rising bilirubin levels, the baby underwent double-surface phototherapy. After the first session of phototherapy, bilirubin levels decreased to 13.3 mg/dl. But by the third day, the bilirubin levels had risen to 17.4 mg/dl; consequently, a second session of double surface phototherapy was done. By 96 hours, serum bilirubin levels decreased to 11 mg/dl **(Figure 8)**. On further monitoring of the baby, the bilirubin levels had reached within the safe limits, with no evidence of bilirubin-induced neurological dysfunction (BIND). The parents of the baby were advised to regularly follow up with the pediatrics department to ensure a thorough evaluation and assessment of the baby's growth and development.

Figure 8: Graph depicting serum bilirubin levels and timing of double surface phototherapy intervention



DISCUSSION

HDFN is caused by immune hemolysis of fetal and neonatal RBCs due to transplacental passage (IgG antibodies) of the maternal antibodies. The antibodies involved in HDFN usually arise following a sensitizing event, which usually can be due to blood transfusions or previous pregnancy. Because of the hemolytic process, either fetal/neonatal anemia, hyperbilirubinemia, maybe both, that can result in mortality and morbidity (6).

The Rhesus system is known to be highly polymorphic and immunogenic blood group systems known to humans (3). It comprises 61 different antigen specificities (1). Rh antibodies arise following sensitization events, unlike in ABO blood group systems, where antibodies arise naturally (7). Rhesus D alloimminization is well known to cause HDFN. This was before the anti-D immunoglobulin prophylaxis practice. Antenatal intervention for Rh D-

negative mothers with prophylactic anti-D immunoglobulin has been shown to reduce alloimmunization by nearly 90% (8). The other antibodies identified to cause HDFN are "in **Table 1**.

Table 1: **Antibodies identified to cause HDFN in prenatal specimens** (1)

Common	Rare
Anti-D	Anti-Fy[a]
Anti-D + C	Anti-s
Anti-D + E	Anti-M
Anti-C	Anti-N
Anti-E	Anti-S
Antic	Anti-Jk[a]
Anti-e	
Anti-K	

RhD" and "RhC antigens on RBCs contain G antigen (Rh12). G reactivity is thought to be mostly dependent on the amino acid serine located at position 103 on the RhD, RhCe, as well as RhCE proteins (1) (9). When M. Palfi et al. examined 27 women with alloimmunization, they discovered that G antigen is highly immunogenic. Of them, 11.1% (anti-D with anti-C), 14.8%(anti-G with anti-C), 25.9%(anti-D with anti-G), as well as 48.1% (anti-D, anti-C, as well as anti-G) (10). Of the 27 women who participated in the study, 24 (88.9%) had anti-G in addition to anti-D and/or anti-C. However, according to different study by P. D. Issitt et al., G antigen is less immunogenic in D-C-individuals instead of C antigen" (11). "About 30% of pregnant women with an apparent anti-C, as well as anti-D pattern, showed detectable anti-D, according to another study (12); Issit et al. previously reported this same finding (11). People with normal D antigen but lacking G antigen have been reported (13)". "When D-negative people are exposed to G antigen, either through pregnancy or transfusion, they will exhibit anti-G. Originally discovered in Rh D-negative (dce/dce) persons, anti-G is typically produced in conjunction with anti-D & anti-C antibodies (14). Anti-G is frequently misidentified because a thorough immunohematology workup is not performed. Anti-G is difficult to identify since it mimics the anti-D and anti-C patterns on antibody screening. Adsorption elution methods can be utilized for identification of anti-G antibodies". (13).

Anti-G antibody consists of IgG isotypes; hence, it can cross the placenta and result in HDFN. Identifying anti-G in the patient's serum that contains anti-D and anti-C is important because D-negative women with no anti-D antibody should receive anti-D immunoglobulin prophylaxis. Failure to identify the above can endanger future pregnancies (14). Severe HDFN is reported to be caused by anti-D antibodies. Women with anti-G+C as well as no anti-D should be identified and have to be given anti-D prophylaxis. While anti-C with anti-G, as per studies done by Palfi et al. and Hadley et al. (10) (15), has been found to have a lower incidence of severe HDFN. Similar findings were shown by Cash et al. as well as Shirley et al. (16,17). This is mainly because anti-G antibodies rarely reach high titers. There have been cases reported of isolated anti-G, which resulted in moderate HDFN in the infant, requiring phototherapy and prolonged hospital care of the baby (18).

In our case, the patient had presented to our hospital for an antenatal checkup at 10 weeks of gestation. She was a Rh-negative individual with a history of childbirth four years ago. She had no recollection of receiving anti-D prophylaxis for her last childbirth. On routine follow-up, she had antibodies in her serum. Antibody screening patterns revealed anti-D and anti-C patterns, which gave suspicion of the possible anti-G antibodies also. Initial titers were too low to be detected; hence, the patient was advised to follow up regularly every 4 weeks. Further follow-up at 22 weeks revealed the titers to have risen to 16. At this antenatal checkup visit, an adsorption elution study was done, which revealed the patient to have anti-D, anti-C, and anti-G antibodies. Also, fetal monitoring with ultrasound was done to detect any signs of hydrops and MCA-PSV assessment. In anemic fetuses, there will be lower viscosity of blood as well as elevated cardiac output, resulting in greater PSV. The threshold value is considered to be around 1.5MoM, which is predictive of moderate to severe fetal anemia (14). Signs of hydrops include soft tissue edema, ascites, scalp hepatomegaly, edema, pleural effusion, cardiomegaly, as well as portal hypertension. Since she had titers at critical values, the patient was advised to follow up regularly till the third

trimester. By 33weeks (33 weeks + 4 days) of gestation, the patient's antibody titers had risen to 32, and MCA-PSV was found to be above 1.5 MoM, which implied that the child may be developing anemia. Because of the above finding, the patient was given steroid cover to ensure lung maturity of the fetus. The patient underwent a C-section at 34 weeks of gestation. Patient was not provided anti-D prophylaxis, as the patient had already been sensitized. The newborn was monitored closely and was given phototherapy. There were no other major complications in parents as well as the child.

This report highlights importance of identifying antibodies detected in pregnant patients. In this case, the antibody screening revealed a picture of anti-D as well as anti-C, with possibility of anti-G. Antibodies were identified performing advanced immunohematological investigations, and they were revealed to be anti-D, anti-C along anti-G. After identifying the antibodies, the patient can decide whether to receive anti-D immunoglobulin to prevent further sensitization, which will complicate further pregnancies. Regular antibody titer and close fetal monitoring were performed as per guidelines to assess the risk of HDFN. Early intervention and multidisciplinary approaches helped to optimize maternal and fetal outcomes.

CONCLUSION

This case report emphasizes the significance of identifying as well as creating different anti-G antibodies from anti-D and anti-C in pregnant women, particularly in those who are Rh-negative. The advanced immunohematological techniques employed in this case, such as double adsorption elution studies, were critical in accurately identifying presence of anti-G, anti-D as well as anti-C antibodies. This finding has significant clinical importance as it influences the decision to administer Rh immunoglobulin prophylaxis and in the management of pregnancies at risk of developing HDFN. Regular antibody titration as well as close fetal monitoring, which included MCA-PSV and USG fetal assessment, were vital in the timely intervention, which ensured optimal maternal and fetal outcomes. This instance emphasizes the necessity of a multidisciplinary approach along with detailed immunohematology workup in managing Rh alloimmunization, emphasizing the role of early diagnosis and targeted interventions in preventing complications and improving perinatal care.

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