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Case report

Cases of RhD variants RhD*DAU2/DAU6 and RhD*weak D type 4.1 in pregnant women in Saudi Arabia

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Abstract. The D antigen is one of the most immunogenic and clinically significant antigens of the Rh blood group system due to its various genotypes that encode for more than 450 different variants. Accurate RhD typing and D variant identification is crucial specially in prenatal screening during pregnancy. Women with RhD -ve phenotype are eligible to Rh immune globulin (RhIG) prophylaxis for the prevention of anti-D alloimmunization and hemolytic disease of the fetus and newborn (HDFN). However, there are some women who possess RhD variant alleles, who are mistakenly grouped as RhD positive and considered not eligible for RhIG prophylaxis, putting them at risk of anti-D alloimmunization and consequently leading to HDFN during subsequent pregnancies. Here, we describe two cases of RhD variants DAU2/DAU6 and Weak D type 4.1 in obstetric patients who were grouped as RhD +ve with negative antibody screening during routine serologic testing. Weak/Partial D molecular analysis using genomic DNA Red Cell Genotyping (RCG) revealed that both patients had RhD variants, one of which DAU2/DAU6 allele associated with anti-D alloimmunization. According to routine testing neither patients received RhIG or transfusion. In this case report we document to our knowledge the first reported cases of RhD variants among pregnant women in Saudi Arabia. (www.actabiomedica.it)

Key words: RhD Variants, RhD alloimmunization, HDFN, Prenatal testing, RhIG prophylaxis

Introduction

The Rh blood group system is the most clinically significant blood group system after the ABO blood group system due to its ability to cause hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN) (1). It is also the most complex and polymorphic blood group system consisting of 56 antigens, encoded by two homologous closely linked genes *RHD* and *RHCE* that run in opposite directions (2). The close proximity of these genes are responsible for the rise of a plethora of alleles (2,3). The D antigen is the most immunogenic antigen of the Rh blood group system as 80% of RhD negative individuals are capable of producing anti-D when exposed to RhD positive red cells (4). RhD phenotypes is classified as RhD positive, RhD negative, or RhD variants. Identification of RhD phenotype can be challenging and uncertain in some cases with RhD variant, due to the discrepancy in the reactivity of D antigen with different anti-D reagents and testing policies .

RhD incompatibility between a mother and her fetus is responsible for severe cases of hemolytic disease of the fetus and newborn (HDFN). Although the majority of RhD HDFN cases are caused by RhD negative mothers carrying RhD-positive fetuses, some cases are caused by RhD positive mothers carrying RhD positive fetuses. These cases have been associated with RhD variants that are mistakenly grouped as RhD +ve (5,6)

The RhD variants are a result of an alteration in the expression of RHD protein leading to a reduction in the quantity of RhD epitopes (Weak D variant) on the red cell membrane or for the absence of parts of the RhD epitope or its complete absence (Partial D variant) on the red cell membrane (7,8). Therefore, to prevent anti-D alloimmunization accurate identification of RhD phenotypes is essential specially in obstetric patients.

RhD variants are suspected when the grade of RBC agglutination with different anti-D reagents is weak (≤+2). However, molecular analysis is required to confirm the RhD phenotype and to identify the type and subtype of RhD variants (9). Therefore, when individuals with some types of D variants are exposed to RhD positive RBCs through pregnancy or transfusion, they are capable of producing anti-D. Although anti-D alloimmunization cases are rare among pregnant women with D variants, cases with specific types of D variants have been reported, such as weak type 4.2, 15, and 21 (7,10,11). Up to 78% of pregnant women with discrepant RhD serological typing results are at risk of RhD alloimmunization and should receive anti-D prophylaxis (12). Recent cases of alloimmunization among pregnant women were found to be from RhD variants such as DVI, DIIIa, Del, type 42, and DNB, causing neonatal jaundice, severe anemia, and hydrops fetalis (2,13,14).

In this case report, we describe two obstetric patients with RhD variant phenotype in King Fahd Hospital of the University were classified as RhD+ during standard prenatal serologic testing resulting in considering these patients as not eligible for RhIG prophylaxis.

Case report

Patient A, a 27-year-old Saudi woman (gravida 2 para 1) was admitted to the OB/GYNE department for delivery. History of the mother shows that her blood group was A RhD positive with a negative antibody screening with no history of previous transfusions. Upon routine testing during admission for ABO/Rh grouping and antibody screening, the patient's blood group was confirmed as A RhD positive. However, this patient showed discrepancy in RhD typing using ABO/RhD ID-card (DiaMed, Cressier, Switzerland) which contains two types of anti-D (polyclonal anti-D and monoclonal anti-D) Figure 1. Further investigation ID-Partial RhD Typing set which consists of 6 panels of monoclonal anti-D [cell lines LHM76/55 (IgG), LHM77/64 (IgG), LHM70/45 (IgG), LHM59/19 (IgG), LHM169/80 (IgG), and LDM 1 (IgM)]. These panels were selected for their ability to detect the partial D categories such as DII, DIII, DIVa, DIVb, DV, DVI, DVII, DFR, DBT, and DHAR. Based on the panel reactivity the patient showed to be DIII variant. To confirm the variant specificity, genomic DNA was purified and sent for RhD genotyping at Versiti Immunohematology Reference Laboratory (IRL) in Milwaukee, Wisconsin, USA. Results of RhD genotyping using polymerase chain reaction with sequence-specific priming (PCR-SSP) for the detection of the most common weak D and partial D type alleles associated with the variable expression of the D antigen confirmed the patient had DAU2/DAU6 allele (Figure 2A). The patient gave birth to a healthy baby girl with O RhD positive blood group and a negative DAT. The patient did not receive RhIG phrophylaxis.

Patient B, a 38-year-old Saudi woman (gravida 4 para 0) was admitted to the OB/GYNE department for delivery. History of the mother shows that her blood group was A RhD positive with a negative antibody screening with no history of previous transfusions. Upon routine testing during admission for ABO/Rh grouping and antibody screening, the patient's blood group was confirmed as A RhD positive. However, this patient showed discrepancy in RhD typing using ABO/RhD ID-card (DiaMed, Cressier, Switzerland) which contains two types of anti-D (polyclonal anti-D and monoclonal anti-D) Figure 2. Further investigation ID-Partial RhD Typing set which consists of 6 panels of monoclonal anti-D [cell lines LHM76/55 (IgG), LHM77/64 (IgG), LHM70/45 (IgG), LHM59/19 (IgG), LHM169/80 (IgG), and LDM 1 (IgM)]. These panels were selected for their ability to detect the partial D categories such as DII, DIII, DIVa, DIVb, DV, DVI, DVII, DFR, DBT, and DHAR. Based on the panel reactivity the patient showed to be DIII variant. To confirm the variant specificity, genomic DNA was purified and sent for RhD genotyping at Versiti Immunohematology

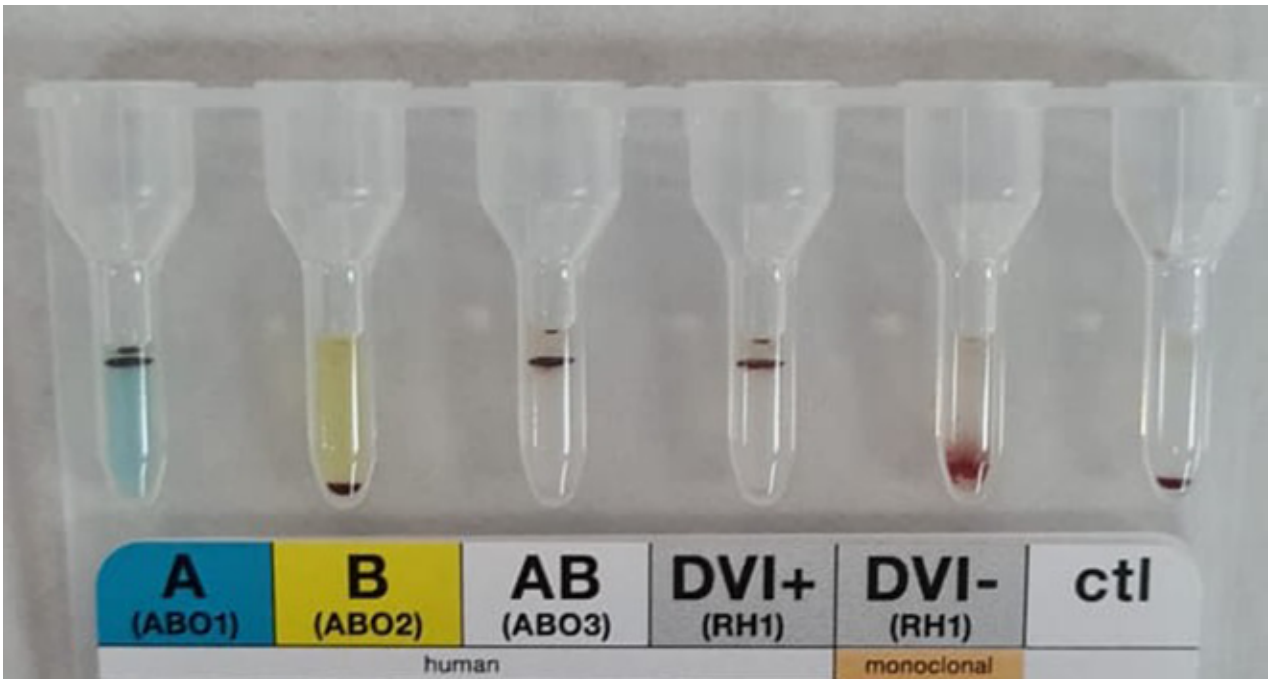

Figure 1. D variant reactivity using serological methods showing +4 agglutination with DVI+ polyclonal anti-D antibody and +1 agglutination with DVI- monoclonal anti-D antibody.

A

IMMUNOHEMATOLOGY MOLECULAR

WEAK RHD ANALYSIS

COLLECTED DATE/TIME				
[redacted]				
PROCEDURE	**RESULT**			**COMPLETED DATE**
WEAK RHD ANALYSIS	**NONE DETECTED**			[redacted]

PARTIAL RHD ANALYSIS

COLLECTED DATE/TIME				
[redacted]				
PROCEDURE	**RESULT**			**COMPLETED DATE**
PARTIAL RHD ANALYSIS	**DAU-2/DAU-6**			[redacted]

B

IMMUNOHEMATOLOGY MOLECULAR

WEAK RHD ANALYSIS

COLLECTED DATE/TIME				
[redacted]				
PROCEDURE	**RESULT**			**COMPLETED DATE**
WEAK RHD ANALYSIS	**TYPE 4.1**			[redacted]

Figure 2. RhD molecular analysis report. (A) patient A, RhD genotyping report showing the detection of partial D allele DAU2/DAU6. (B) Patient B, RhD genotyping report showing the detection of weak D allele type 4.1.

Reference Laboratory (IRL) in Milwaukee, USA. Results of RhD genotyping using polymerase chain reaction with sequence-specific priming (PCR-SSP) for the detection of the most common weak D and partial D type alleles associated with the variable expression of the D antigen confirmed the patient had Weak D type 4.1 allele (Figure 2B). The patient gave birth to a healthy baby girl with O RhD negative blood group and a negative DAT. The patient did not receive RhIG phrophylaxis.

Discussion

In this report, we have described two cases of RhD variants, one with partial D variant DAU2/DAU6 and another with weak D variant weak D type 4.1. Both cases were typed as RhD positive based on the strong 4+ reactivity using one polyclonal anti-D reagent. Neither woman received RhIG prophylaxis. Fortunately, neither newborns in both cases developed anemia nor required phototherapy or transfusion.

These cases highlight the importance of accurate RhD typing to be able to identify and specify RhD variants, especially among women in child-bearing age and during prenatal follow-up to allow women at risk of anti-D alloimmunization to receive RhIG prophylaxis and to avoid transfusion of RhD positive packed red blood cells (pRBCs) if required. A multiethnic study on the prevalence of D variants among pregnant women in the United States showed that D variants are present in 2.6% of women of African origin, 2.7% of Hispanic-Latino women, and 1% of Caucasian (15). Therefore, it is recommended to use at least two RhD antibodies with different specificities in order to increase the probability of identifying RhD variants and to further confirm those with RhD genotyping if detected (16,17). Up to date there are approximately 450 RhD variant alleles. However, not all are capable of anti-D alloimmunization such as weak D types 1,2,3 and 4.1 which could be managed safely as RhD positive. In these cases the administration of RhIG prophylaxis is unnecessary and it could be reserved for eligible women only (18).

In the first case the patient had DAU2/DAU6 alleles and had experienced one previous abortion, she was grouped as RhD positive therefore, was not eligible for RhIG prophylaxis and her newborn had an RhD-positive blood group. Therefore, this mother should have been considered at high risk of RhD alloimmunization and a candidate for RhIG prophylaxis. The DAU2/DAU6 allele has been previously reported to stimulate the immune response for anti-D production. On the other hand, the second case the patient had weak D type 4.1 allele This type of weak D variant is not associated with anti-D development during pregnancy or blood transfusion. Therefore, this patient will not be a candidate of RhIG prophylaxis nor her infant at risk of HDFN.

In 2015, the American Association of Blood Banks (AABB) and the College of American Pathologists (CAP) working group recommended molecular analysis using RhD genotyping for the identification of D variants. Accordingly, the strategy of transfusion and the administration of anti-D prophylaxis should be determined after properly identifying the D variant type(18). According to the AABB-CAP work group recommendation, the weak D subtype 4.1 should be grouped as RhD positive, and it is not mandatory for this group to receive injection of the anti-D prophylaxis (19).

To our knowledge, these are the first reported cases of RhD variants among obstetric patients in Saudi Arabia.

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Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Consent to Participate: All participants provided written informed consent to participate in this study.

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