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ANTI-D IN RHESUS D POSITIVE PREGNANCT MOTHER: A CASE REPORT.

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ABSTRACT

An accurate ABO and Rhesus (Rh) blood group identification, especially D antigen, is essential for pre-transfusion evaluation in pregnancy. This procedure will prevent allo-immunisation or haemolytic disease of foetus and newborn (HDFN). A 32-year-old pregnant lady with an underlying autoimmune disease was noted to have positive antibody screening with the possibility of auto anti-D or anti-LW. Even though the latter antibody is not clinically significant, these two antibodies need to be differentiated. Anti-D may develop in partial D or variant D individual who was mistakenly labeled as Rh (D) positive previously. Unfortunately, it is difficult to establish the blood grouping of patients with an underlying autoimmune process. Therefore, in this case, a molecular study for blood group D was required.

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ABSTRACT

An accurate ABO and Rhesus (Rh) blood group identification, especially D antigen, is essential for pre-transfusion evaluation in pregnancy. This procedure will prevent allo-immunisation or haemolytic disease of foetus and newborn (HDFN). A 32-year-old pregnant lady with an underlying autoimmune disease was noted to have positive antibody screening with the possibility of auto anti-D or anti-LW. Even though the latter antibody is not clinically significant, these two antibodies need to be differentiated. Anti-D may develop in partial D or variant D individual who was mistakenly labeled as Rh (D) positive previously. Unfortunately, it is difficult to establish the blood grouping of patients with an underlying autoimmune process. Therefore, in this case, a molecular study for blood group D was required.

Keywords: Anti-D, Anti-LW, Haemolytic disease of foetus, Molecular, Pregnancy, Rhesus.

INTRODUCTION

Rhesus (Rh) blood group system contains the D antigen, which is immunogenic. The D antigen differs from the other Rh antigens (C/c and E/e) by 31 to 35 amino acids, making the antigens potent at stimulating an immune response.[1] The Rh locus is located on the long arm of chromosome 1 and contains the *RHD* and *RHCE* genes.[2] However, more than 100 different *RHD* alleles encode a protein with different types of amino acids, causes different D variants (partial D and weak D).[3] Partial D red cells are the red blood cells of individuals that lack some part of D antigen. Their D epitopes are altered due to amino acid changes, or portions of *RhD* joined to portions of *RhCE* producing hybrid proteins and generated new antigens. Therefore, they can produce antibodies to the missing portion of *RHD*.[4]

The Landsteiner-Wiener (LW) system encoded by a single gene located on chromosome 19, and the gene is independent of Rh genes.[5] However, the LW glycoprotein expression requires interaction with Rh proteins.[3] Therefore, LW antigen is expressed more strongly on Rh(D) positive than Rh(D) negative red blood cells, and more strongly on umbilical cord red blood cells than adult red cells.[3]

Most of the Rh antibodies are IgG subtype, which is considered a potential cause of

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severe haemolytic disease of foetus and newborn (HDFN) and haemolytic transfusion reaction (HTR). Individuals with partial-D have altered RhD proteins, and are at risk of producing alloantibody (anti-D) or mistakenly described as auto anti-D in Rh(D) positive individuals. However, anti-LW, which is directed against the LW antigen, can be wrongly identified as anti-D. Hence it is essential to differentiate anti-D and anti-LW in pregnancy using molecular testing such as array-based systems (bloodChip microarray, multiplex ligation-dependant probe amplification) and polymerase chain reaction with sequence-specific primers (PCR-SSP) designed to detect more than 70 RhD variant genes.[6,7] We report here a case of a 32-year-old pregnant woman with an underlying autoimmune disease noted to have positive antibody screening with the possibility of auto anti-D or anti-LW who required molecular typing to confirm and identify the specific genotype in RhD variants for better mother and newborn management.

CASE REPORT

A 32 year-old-Malay lady Gravida 2 Para 1, who was diagnosed with systemic lupus erythematosus (SLE) with Evan Syndrome in 2002, was referred from Health Clinic for further management of small for gestational age (SGA) at 38 weeks of gestation to Obstetrics and Gynaecology (O&G) clinic. Ultrasound showed the foetus correspond to 36 weeks of gestation with a normal amniotic fluid index. Routine group, screen, and hold (GSH) testing was positive; therefore, further testing to look for auto/alloantibody towards red blood cell antigen was done. She was currently on azathioprine 50mg daily for her SLE.

The patient blood group was A, Rh (D) positive. Initial antibody screening in 2017 during her first pregnancy showed pan agglutination. Antibody identification showed an anti-D pattern at low ionic strength saline (LISS) phase and pan agglutination at the enzyme phase. Subsequently, direct Coomb's test (DCT) was positive for IgG, and eluate from the cells demonstrated anti-D specificity. Auto adsorption of the serum was done, and repeat antibody identification later showed no reaction, indicating no alloantibody. The extended rhesus phenotype by serology suggested that the patient was RhD+, C+/c-, and E-e+ (likely R1R1).

During this current admission, her haemoglobin was 10.8 g/dL, and platelet was 212 x 10^9/L. Antibody screening was positive for all screening panels with 2+ reactivity. A further test for antibody identification at LISS and enzyme phase showed no specificity with auto control 1+. The DCT was positive for IgG. To differentiate the possibility of anti-D with anti-LW, red blood cells were treated with dithiothreitol (DTT), and it showed 1+ reaction with patient serum. There was no reaction with cord blood of a similar blood group. Blood crossmatch was done, and it was compatible with A, Rh (D) negative blood, but incompatible with A, Rh (D) positive blood. Table I showed a summary of immunohaematological results for this patient.

The patient went on to deliver a healthy baby boy one week later via vaginal delivery spontaneously. Her delivery was uneventful, and she required no blood transfusion. She was discharged well. Her baby was not jaundice, and no other sign of haemolysis was detected.

DISCUSSION

An accurate Rhesus blood group identification, especially D antigen, is essential for pre-transfusion evaluation in pregnancy to prevent alloimmunisation or HDFN. It is known that RhD is the most important antigen in the Rh system due to its immunogenicity. Our immunohaematological results showed this patient has autoantibody towards D antigen,

Table I: Immunohematology results at first (2017) and current Pregnancy.

Immunohematology Tests	2017 (1st pregnancy) Results	2019 (Current pregnancy) Results
Blood grouping	A RhD+	A RhD+
Rh phenotyping	R1R1 (DCe/DCe)	R1R1 (DCe/DCe)
Antibody screening	Positive (2+) at cell I, II and III	Positive (2+) at cell I, II and III
Antibody identification		
• LISS*	Anti-D	No specificity
• Enzyme	Pan-agglutination (4+)	Pan-agglutination (4+)
Autocontrol	Positive (1+)	Positive (1+)
Auto adsorption	Repeat antibody identification post autoadsorption showed no reaction.	Repeat antibody identification post autoadsorption showed no reaction.
Direct Coombs test	Positive (IgG 1+)	Positive (IgG 1+)
DTT treated RBC	Not done	Post DTT treatment, the patient's serum reacted with the patient's RBC

*LISS: Low ionic strength salt solution

which was diagnosed during the first pregnancy and persistently present during the current pregnancy. Similar cases have been reported in a para one lady who had been typed as RhD positive previously.[8] Repeated immunohaematological test showed agglutination pattern with monoclonal anti-D reagents, and she was typed as DCcee. However, antibody identification showed the presence of anti-D. A molecular study using PCR-SSP confirmed D^{DBT}Ccee phenotype. Another study in Croatia involving 102,982 women found anti-D immunization occur in three pregnant women who were initially serologically typed as RhD positive, later identified as D variant carrier.[9]

SLE is an autoimmune disease, and patients with SLE can produce warm-reactive autoantibodies towards a wide diversity of cellular antigens, including red blood cell antigen. Therefore, autoantibodies with specificity towards D antigen is highly suspicious in this case. Haemolytic workup has been suggested to exclude active auto immune haemolytic anaemia (AIHA). In a transfusion, red blood cell (RBC) autoantibody may interfere with the serological testing especially warm-reactive autoantibodies. These autoantibodies present in the serum may react with all cells in the antibody panels resulting in panagglutination reaction. In this condition, detection of alloantibody, which is more significant, is difficult. Furthermore, in AIHA patients, one-third to one-half of patients were reported to have underlying alloantibodies.[10] Therefore, to exclude the presence of alloantibody in this patient is crucial.

Anti-LW, which is directed against the LW antigen, can be mistakenly identified as anti-D. LW antigens have intramolecular disulfide bonds that are sensitive to DTT treatment. The DTT treatment is used to differentiate between anti-LW and anti-D because the D antigen is resistant to DTT.[3] In this case, anti-LW has been excluded from the serological test result. Another possibility is partial D, in which the patient may produce alloantibody when exposed to RhD+ red cell. Partial D antigen lack one or more of D epitopes. There are amino acid changes outside the membrane in the RhD proteins. This is difficult to detect serologically as a particular commercial monoclonal antibody may not identify it, or it causes weak expression of the epitope by a specific method. Furthermore, the reactivity of different partial D with anti-D reagents is variable. DIII can produce similar reactions to RhD+ cells, and DIVa can produce stronger reaction.[11] Recently, monoclonal antibodies for the characterisation of the partial-D phenotype have shown that D variants are more common than previously reported.[12]

In pregnancy, the determination of accurate RhD phenotype is critical in the patients' management. Our patient is para 2, which molecular testing for RhD will benefit her future pregnancy and transfusion decision. Some RhD variants prone to make anti D, therefore, should be typed as RhD negative and given RhD negative blood. Those pregnancies should be given Rh immunoglobulin (RhIg) prophylaxis during pregnancy and after delivery to a RhD positive baby.[13] However, certain weak D especially type 1,2, and 3, rarely cause anti-D allo-immunisation. Therefore, identifying an accurate RhD phenotype will prevent multiple RhIg exposures as prophylaxis and avoid allo-immunisation in the other group. Furthermore, pregnant women and transfusion recipients in this type of weak D may be safely transfused with RhD positive blood, conserving limited storage of RhD negative blood.

CONCLUSION

This case probably represents another example of an auto anti D after anti-LW has been excluded. However, alloantibody with variant D phenotypes needs to be excluded. This is important, especially for future management during pregnancy. Therefore, in this case, a molecular test for the D blood group is required.

CONFLICTING INTEREST

We declare that we have no conflict of interest.

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INFORMED CONSENT

Verbal informed consent (verbal) was obtained from the participant.

REFERENCES

1: Westhoff CM. The Rh blood group system in review: A new face for the next decade. Transfusion. 2004;44(11):1663–1673.
2: Franz Wagner and Willy A Flegel. RHD gene deletion occurred in the Rhesus box. Blood. 2000;95:3662-3668. [Accessed on 6 April 2021.]
3: Fung MK, Grossman BJ, Hillyer CD, Westoff CM (Editors). AABB.Technical Manual.2014. 18th Edition. [Accessed on 6 April 2021.]
4: Westhoff CM, Siegel DL. (2016) Chapter 14: Rh and LW blood group antigens. Simon TL., et al (Ed). Rossi's principles of Transfusion Medicine. John Wiley & sons, Ltd. 2016:176–184.
5: Bailly P, Tontti E, Hermand P Cartron JP, Gahmberg CG. The red cell LW blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leukocyte integrins. Eur J Immuno. 1995;25(12):3316–3320.
6: Ouchari M, Srivastava K, Romdhane H, Jemni Yacoub S, Flegel WA. Transfusion strategy for weak D Type 4.0 based on RHD alleles and RH haplotypes in Tunisia. Transfusion. 2018;58 (2):306–12. [Accessed on 6 April 2021.]
7: Haer-Wigman L, Veldhuisen B, Jonkers R, Loden M, Madgett TE, Avent ND, de Haas M and van der Schoot CE. RHD and RHCE variant and zygosity genotyping via multiplex ligation-dependent probe amplification. Transfusion. 2013;53(7):1559-74.
8: Stefania Vaglio, Maria Paola Perrone, Maria Cristina Arista, Luca Laurenti, and Gabriella Girelli. Anti-D in a D-positive patient: autoantibody or alloantibody? Blood Transfus. 2007;5 (1):44. [Accessed on 6 April 2021.]
9: Jelena Lukacevic Krstic, Slavica Dajak, Jasna Bingulac-Popovic, Vesna Dogic, Jela Mratinovic -Mikulandra. Anti-D Antibodies in Pregnant D Variant Antigen Carriers Initially Typed as RhD+. Transfus Med Hemother 2016;43:419–424. [Accessed on 6 April 2021.]
10: Branch DR, Petz LD. Detecting alloantibodies in patients with autoantibodies. Transfusion. 1999;39:6–10.
11: Daniels G. Human Blood Groups. 3rd edition. Wiley Blackwell. 2013. ISBN: 978-1-444-33324-4
12: Denomme GA., et al. Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients: implications for anti-D alloimmunization and prevention. Transfusion. 2005;45 (10):1554–1560.
13: Geoff Daniels. Variants of RhD-current testing and clinical consequence. British Journal of Haematology. 2013;161:461-470. [Accessed on 6 April 2021.]