Vol 4 (2), July 2013; 16-19

Detection of Du Antigen in Rh Negative Blood Group Individuals

Naheed Afshan[1], Sarah Tariq[1]*

[1]Department of Microbiology, Jinnah University for Women, Karachi -74600, Pakistan

ABSTRACT

Du is the weak expression of D antigen. The cells which are not immediately agglutinated by Anti-D sera cannot be easily classified as D negative because some of these agglutinate after addition of antiglobulin sera. This weak reactivity is termed as Du. Du positive cells are likely to elicit an immune response in D negative individuals and the Du cells could be destroyed if the recepient is already immunized. Therefore, Du positive donor is treated as D positive and recipient is treated as negative. This report is based on Du antigen and the testing of Du antigen. In this report we discussed about the detection of Du Antigen using 2 different protocols that show how to test the presence and absence of Du antigen in Rh negative blood group individuals. In this study we included 100 blood group D-negative individuals .The result showed there were 3% that have Du antigen in their blood.

Keywords: Agglutination, Blood group, Du antigen, Rh antigen, Serum

INTRODUCTION

The Rh system includes many antigens but the major one is D, alternatively reffered as Rho. The term Rh positive is used to denote red cells that carry the D (Rh) antigen or its variant Du. Red cells that have neither D nor Du on their membranes are termed Rh negative. With the exception of A and B, the most important of all blood group antigens is undoubtedly D. This is because the consequence of the presence can be severe and Rh haemolytic disease of the newborn can be tragic: transfusion reaction due to Rh antibodies can be heartbreaking experience. However, unlike the situation in the ABO system, an Rh negative person does not usually have anti-D in his or her serum. Rh antigens are confined to red cells and are not found in body fluids or natural substances; therefore, exposure to red cells is the only way a person can become immunised to Rh. Also contributing to the importance of the Rh system is the fact that the D antigen is one of the most effective blood group immunogens. As stated above, no natural substances chemically similar to the D antigen have been found; therefore when an Rh negative person is found to have anti-D, that individual has invariably been exposed to Rh positive cells. The two most likely ways for Rh positive red cells to reach the circulation of an Rh negative individual are: (1) Transfusion of red cells from an Rh positive donor to an Rh negative recipient. Except in rare circumstances, this is contrary to good transfusion practice; therefore it is usually the result of clerical or technical error, (2) Passage of red cells from an Rh positive foetus through the placenta to the Rh negative mother. This almost always occurs to some extent at delivery and occasionally late in pregnancy.

Du Antigen: In transfusion medicine, after the ABO blood groups, the D antigen is the most significant. A high pro¬portion of people whose red blood cells (RBCs) lack D will make anti-D if exposed to the D antigen by pregnancy or transfusion. Accordingly, all D– patients, especially girls and women who may become pregnant, should be transfused with D– RBCs. The D antigen is in the Rh blood group sys¬tem, which with 49 distinct antigens is the most polymorphic blood group system. This document reviews fundamental in¬formation for the D antigen.

*Corresponding author: naheedafshan07@hotmail.com

Du is the phenotypic term used to denote a weakened expression of the D antigen. Du originally defined as those red cells reacting with anti-D only when a more sensitive indirect antiglobulin test was used. Du phenotype can arise from three different genetic situations. a) A person may inherit a gene coding for weakened quantitative expression of D antigen. b) One gene may interact with another to modify and weaken the expression of the D antigen. c) A gene may not code for the total material that makes up the antigen.The frequency of Du antigen is relatively low less than 1%. Du is a poor immunogen, however, accelerated destruction of Du red cells can result if transfused to a person already making anti-D. Hence Du donor units are currently labelled as Rah positive. Du recipients are labelled as Rah negative. Newborn of Rah negative mother are tested for D & Du and Rh Ig is recommended for mothers of D positive or Du positive infants in order to prevent potential immunisation. The terms Du variant or partial Du are recommended when there is both a qualitative and quantitative difference noted in the D antigen.

Du Testing: Not all red cells can be classified as Rh positive or negative by direct agglutination tests. The cells of a few persons react weakly with anti-D or requires a longer reaction time than most Rh positive cells. An even smaller number of persons have red cells that are not agglutinated by Not Not all red cells can be classified as Rh positive or negative by direct agglutination tests. The cells of a few persons react weakly with anti-D or requires a longer reaction time than most Rh positive cells. antiglobulin serum. An even smaller number of persons have red cells that are not agglutinated by antiglobulin serum. These cells are called Du. Cells of the Du phenotype may fall anywhere within this spectrum of reactivity with anti-D.

Because Du is a form of D, red cells of the Du phenotype can stimulate the production of anti-D in Rh negative recipients and, more importantly, react with anti-D in vivo. It is for these reasons that donor blood must be shown to be negative not only in the test for D but also in the test for Du. In general, testing the red cells of recipients for Du is considered unnecessary. The recipient's welfare is not compromised if he or she is of the Du phenotype but is typed as D negative and receives Rh negative red cells. In such circumstances Rh negative donor blood may be used unnecessarily.

It is important that the Du status of the D negative pregnant woman be established early in pregnancy. If the mother is found to be Rh positive, Du varient, she is not a canditate for Rh immunoglobulin prophylaxis- either antepartum or postpartum- whereas the Rh negative (D and Du negative) mother is a canditate. The reason for performing the Du test early in pregnancy is to avoid mis-interpreting the cause of a positive fetal cell screening test at the time of delivery.

In addition to prenatal patients, newborn babies are also tested for Du if they type as D negative. Again, this relates to the need for Rh immune globulin: the D negative, Du negative baby cannot immunize its mother; for this reason she does not need Rh immune globulin protection. However, the mother should receive Rh immune globulin if the baby is of the Du phenotype.

Du red cells fall into a wide spectrum of reactivity when tested with anti-D reagents. How each cell is detected depends on the type of anti-D that is used and the kind of test that is performed. To test for Du, red cells are incubated at 37^{o}C with an IgG anti-D and an antiglobulin test is performed. If serum suspended cells are used, some blood samples at the upper end of the Du spectrum will be agglutinated weakly by most anti-D reagents prior to the antiglobulin test, either at room temperature or at 37^{o}C. When the same red cells are suspended in saline, direct agglutination may not be observed, or it may be seen with one reagent and not another. Regardless of whether the red cells are agglutinated directly by anti-D or they absorb anti-D and it is detected in the antiglobulin phase of the test, they are Rh positive, provided both controls for D typing and the Du test are negative.

MATERIALS & METHODS

Blood Sample: Any blood group sample with EDTA which is Rh-negative.

Reagents: Anti-D antisera, 3-5% Red cell suspension, Normal saline, Coombs reagent, 37 C incubator, Albumin.

Procedures:

i) Without Albumin: Prepare a washed, 3-5% suspension of RBCs. Add 50ul Anti D in a tube containing 50ul 3-5% Red cell suspension. Incubate the tube at 37 C for 40-45 minutes. After 40-45 min, suspend the tube & examine agglutination, if agglutination occurs it means Rh is positive & if no agglutination present it confirmed that Rh is negative. Centrifuge the suspension at 3500 rpm for 15 seconds. After centrifugation, washed the suspension 3 times with normal saline. After 3rd time washing, tapping should be done so that all the remaining saline should be removed from the cell suspension. Add 1-2 drops of coombs reagent in the tube containing washed red cells. After addition of coombs reagent, centrifuge the tube at 3500 rpm for 15 seconds. Immediately resuspend the tube and examine for agglutination using Electron microscope. Confirm all negative results by adding one drop coombs control cells to all tubes showing no agglutination and centrifuge15-30 seconds at 3500 rpm. Gently resuspend & examine for agglutination. Agglutination should be present in this step or the test is invalid.

ii) With Albumin: Prepare a washed, 3-5% suspension of RBCs. Add 50ul Anti D & 1-2 drops Albumin in a tube containing 50ul 3-5% Red cell suspension. Incubate the tube at 370C for 40-45 minutes. After 40-45 min, suspend the tube & examine agglutination, if agglutination occurs it means Rh is positive & if no agglutination present it confirmed that Rh is negative. Centrifuge the suspension at 3500 rpm for 15 seconds. After centrifugation, washed the suspension 3 times with normal saline. After 3rd time washing, tapping should be done so that all the remaining saline should be removed from the cell suspension. Add 1-2 drops of coombs reagent in the tube containing washed red cells. After addition of coombs reagent, centrifuge the tube at 3500 rpm for 15 seconds. Immediately resuspend the tube and examine for agglutination using Electron microscope. Confirm all negative results by adding one drop coombs control cells to all tubes showing no agglutination and centrifuge15-30 seconds at 3500 rpm. Gently resuspend & examine for agglutination. Agglutination should be present in this step or the test is invalid.

OBSERVATION

Table I. How to read the result.

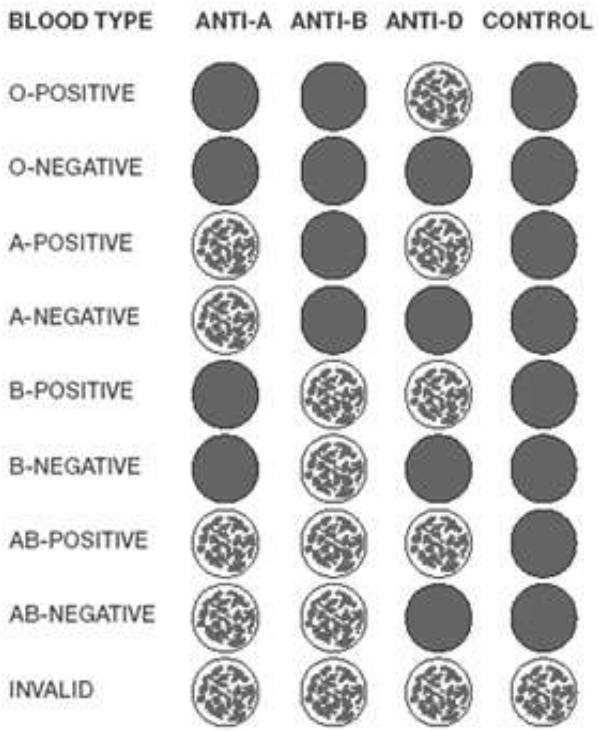

Table II. Results showing presence and absence of Du antigen.

Blood Groups	% of Patients	Result		
		Positive	Negative	Invalid
A	22.64%	1.89%	16.98%	3.77%
B	31.13%	0%	24.53%	6.60%
AB	11.32%	0%	10.38%	0.94%
O	34.91%	0.94%	31.13%	3.77%

RESULTS & DISCUSSION

100 blood samples taken from different individuals having different blood groups but all were Rh negative individuals. From 100 samples, 3 were Du positive.

As described earlier, people whose RBCs have a weak D phenotype (quantitative D variant) do not make anti-D, whereas people whose RBCs have a partial D phenotype (qualitative D variant with or without weakening of the D antigen) can make alloanti-D. This presents a different problem depending on whether the person is a donor or a patient. For donors, detection of weak and partial D antigens would eliminate the possibility of immunization should such blood be transfused to a true D-negative patient. However, historical data show that weakly expressed D antigens are most unlikely to be immunogenic.

Clinical complications result from RBC destruction due to the interaction of an alloantibody with RBCs carrying the corresponding antigen. The D antigen is highly immunogenic and induces an immune response in 80% of D-negative persons when transfused with 200 mL of D-positive blood. For this reason, in most countries D typing is performed routinely on every blood donor and transfusion recipient so that D-negative patients receive D-negative RBC products. Consequently, clinical complications due to mismatched transfusions are infrequent. In contrast, despite the use of immunosuppressive therapy with anti-D immunoglobulin prophylaxis, D alloimmunization in pregnancy still occurs.

Patients with acute or chronic myeloid leukemia, myeloid metaplasia, polycythemia, or myelofibrosis occasionally have 2 populations of RBCs of different Rh type. In some cases, a loss of Rh antigens is associated with chromosome aberrations.

CONCLUSION

From the result, it is concluded that there were rare cases in peoples that have Du antigen in their blood. We cannot avoid to detect Du antigen before transfusion because if Donor is Rh negative and Recipient is also Rh negative but Du antigen is present in the donor blood so if we can't test Du antigen mismatched in transfusion occur which will results in mild to life threatening complications or death.

REFERENCES

Reid ME and Lomas-Francis C. 2004. Blood Group Antigen FactsBook. 2nd ed. San Diego: Academic Press.

Reid ME and Mohandas N. 2004. Red blood cell blood group antigens: Structure and function. Semin Hematol, 41: 93-117.

Reid ME and Yazdanbakhsh K. 1998. Molecular insights into blood groups and impli¬cations for blood transfusions. Curr Opin Hematol, 5:93-102.

Tayyab M, Malik AR and Khan AS. 2000. Du phenotype - a review. JAMC,12(3): 41-44.

Westhoff CM. 2007. The structure and function of the Rh antigen complex. Semin Hematol, 44:42-50.