Original Article:

A study on Rh incompatibility and frequency of weak D among blood donors and patients at a tertiary care referral teaching hospital in Tirupati, Andhra Pradesh

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ABSTRACT

Background: Rh D antigen is the next most important after ABO antigens in the field of transfusion medicine. Weak D refers to reduced expression of D antigen on the red blood cell that requires an extended testing with indirect antiglobulin test (IAT) to get detected. Clinical importance of weak D arises when labelling the donor and patient, as the donor is labelled as D positive, patient as D negative.

Methods: In our center all blood donor and patient samples are tested for ABO and Rh D by conventional tube technique using two anti-D reagents; anti-D immunoglobulin M (IgM) monoclonal and a blend of anti-D IgM and immunoglobulin G (IgG). The blood samples which were negative for agglutination by immediate spin method were further tested for weak-D using IgG anti-D in the IAT phase with low ionic strength solution (LISS)/Coombs'gel card.

Results: A total of 46,654 blood samples were tested (22,326 donors and 24,328 patients) during the period January 2012 to August 2014. Among these 43,771 (93.82%) were Rh D positive and remaining 2,883 (6.18%) were Rh-D negative. A total of 30 individuals (16 donors and 14 patients) were weak D positive constituting 1.04% of Rh-D negatives and 0.06% of total individuals screened.

Conclusions: This study shows the prevalence of weak D antigen in our population who are representative of Rayalaseema region of Andhra Pradesh. It also stresses the need to identify individuals with variant D (rather than weak or partial D) and to inform them about their status as donor and recipient of blood/organ.

K**ey words:** *Rho D antigen, Coombs test*

Deepthi Krishna G, Sreedhar Babu KV, Arun R, Jothibai DS. A study on Rh incompatibility and frequency of weak D among blood donors and patients at a tertiary care referral teaching hospital in Tirupati, Andhra Pradesh. J Clin Sci Res 2015;4:281-4. DOI: http://dx.doi.org/10.15380/2277-5706.JCSR.14.053

INTRODUCTION

Rhesus (Rh) blood group system is one of the most important as well as highly immunogenic and complex with numerous polymorphisms. The term "Rh" refers not only to a specific red cell antigen i.e., Rh D but also to complex blood group system. At present 58 antigens are there in the Rh system[1] but only D, C, c, E and e are the commonly identified and the important clinically significant antigens with respect to blood transfusion. Clinical significance of Rh antigens stems from the fact that the antigen D of the Rh system is highly immunogenic; if a unit of D-positive blood is transfused to a D-negative recipient, approximately 90% of recipients result in the formation of anti-D which cannot be safely transfused with D-positive red cells later.[2] In the routine blood banking protocol of grouping only Rh D antigen is tested and individual's Rh is reported as Rh positive or negative. The Rh D antigen can vary in both the quantity of antigen expressed and the qualitative nature of the antigen, so RhD antigen has multiple antigenicity and has variants like incomplete D, partial D, D mosaic;

Received: September 16, 2014; Revised manuscript received: May 17, 2015; Accepted: June 01, 2015.

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Online access
http://svimstpt.ap.nic.in/jcsr/Oct-dec15_files/4oa15.pdf
DOI: http://dx.doi.org/10.15380/2277-5706.JCSR.14.053

weak D etc.[3,4] The weak D phenotype , formerly known as D^u is a quantitatively weakened form of the D antigen. Weak D red cells have the D antigen, but have fewer D antigen sites per red cell than normal Rh positive cells. The currently preferred term for D^u is "weak D." The most important risk with this phenotype is allo immunization among the recipients. As D antigen is highly immunogenic, individuals with weak D phenotype are typed depending on whether the person is donor or the recipient; so recipients with weak D are considered D negative and must be transfused with D negative blood and donors are considered as D positive. Mothers with weak D foetus must receive Rh immunoprophylaxis[5] as passage of weak D red cells from foetus to mother may result in sensitization. In the present study we tried to estimate the prevalence of weak D at our blood bank based on the serological techniques.

MATERIAL AND METHODS

During the study period January 2012 to August 2014, at the Department of Immuno Haemotology and Blood Transfusion all blood donor as well as patient samples were tested for ABO grouping and RhD typing using two different classes of anti-D reagents by immediate spin tube technique using monoclonal anti-D immunoglobulin M (IgM) (Tulip diagnostics Pvt. Ltd, Verna, Goa) and blend of IgM and IgG anti-D immunoglobulin (Span diagnostics Ltd, Surat, India). All the blood samples which were negative for agglutination by immediate spin method for Rh D were further tested for weak-D using IgG anti-D (Tulip diagnostics Pvt. Ltd, Verna, Goa) in the indirect antrglobulin test (IAT) phase with low ionic strength solution (LISS)/Coombs' gel card. For this 1000 µL of LISS (DiaMed AG, Morat, Switzerland) was taken in a test tube. To that, 10 µL of donor/patient packed cells or 20 µL of donor/patient whole blood was added. The sample was mixed properly in LISS to make a 1% red cell suspension. Gel card (DiaMed AG, Morat, Switzerland) was taken and 50 µL of 1% red cell suspension that was already prepared was added along the sides of the reaction chamber. Twenty five µL of the anti-D IgG (Tulip Diagnostics Pvt. Ltd, Verna, Goa) was added directly into the reaction chamber and the column was incubated at 37 ^oC for 15 minutes. Later centrifugation of the gel card was done for 10 minutes. and the results were tabulated.

RESULTS

During the period of study a total of 46,654 blood samples were analyzed. Out of them 22,326 were of donors' and 24,328 were of patients (Table 1). Among the total 46,654 individuals 94 % (n = 43,771) were Rh-D positive and 6% (n = 2,883) were Rh-D negative. A total of 22,326 donor samples were analyzed and found to be positive for Rh-D in 93% (n = 20,820); negative for Rh-D in 7% (n = 1506). Out of Rh-D negatives 1.06% (n = 16) were turned out to be weak-D positive (Table 2). Among the 24,328 patients screened 22,951 (94%) were Rh-D positive and 1377 (6%) were Rh-D negative. Of the Rh-D negative persons 14 (0.037%) turned out to be weak-D positive (Table 3).

Table 1: Prevalence of weak D

Variable	Donors	Patients	Total
Rh positive (No.)	20,820	22,951	43,771
Rh negative [No. (%)]	1,506 (6.7)	1,377 (5.6)	2883 (6.2)
Weak D positive [No. (%)]	16 (0.03)	14 (0.03)	30 (0.06)
Total	22,326	24,328	46,654

DISCUSSION

Weak D is a phenotype with either a qualitative or quantitative difference in the Rh 'D' moiety resulting in a weakened expression of the D antigen. Red blood cells (RBCs) which react with anti-D only after testing in the Coombs' phase are called weak D. The term D^u was coined by Stratton.[6] Later, Race et al[7] and Stratton et al[6] studied this antigen further and showed that it was an inherited characteristic. Unlike the incidence of Rh D antigen positivity in the Caucasian population which is 85%,[8,9] it is around 95% in India. Prevalence of weak D in our population is 0.06%.[10]

Point mutation in the *RHD* gene results in an amino acid change in the transmembrane and intracellular regions of the D antigen affecting its insertion and hence density on the surface.[11] Other entity called high grade weak D is a term used for only a minor reduction of Rh D antigen expression. It is due to suppressive effects of Cde haplotypes in trans position.[12] They possess normal *RHD* allele, because the carriers' parents and children often express a normal Rh D antigen density and they are typed as normal RhD, due to increased sensitivity to anti-D monoclonal antisera.

Using flow cytometry it was established that the weak D subjects had at least 10 times lower expression of the antigen as compared to D positive individuals.[13] There are three genetic mechanisms proposed for weak expression of D antigen. These include a point mutation in the *RHD* gene that codes for a weak expression of D antigen proposed, presence of C antigen in trans position on the opposite chromosome as in *Dce/dCe* genotype which was most common in African-Americans. Further, a gene may not code for the total material that makes up the D antigen resulting in missing of one or more epitopes (termed as partial D antigen) which may also result in weak expression of D antigen. These individuals can be allo immunized if transfused with D positive blood bearing that missing epitope.[14] Partial D antigen present as normal D type and they may be detected when they form anti-D even though transfused with D positive blood.

The low immunogenicity of weak-D antigen result in problems, such as, conflicting laboratory reports as to whether an individual tests Rh D positive or negative. However, the number classified as weak D depends on the characteristics of the typing antisera. Earlier, blood banks were using polyclonal antisera containing low titers of antibodies as compared to monoclonal antisera. The blood group reporting was dependent on the titer of anti D antibodies in the test reagent. This led to conflicting reports of Rh D positive and negative by different labs leading to inadvertent transfusion of Rh D positive blood to Rh D negative recipients. Monoclonal reagents having high potency detect Rh D positive cells that would be difficult to detect with less sensitive polyclonal reagents.[15]

The use of modern sensitive gel system for ABO and Rh D typing has given concordant result when compared with the conventional blood grouping system.[16] This improved sensitivity of the anti-D antisera is also responsible for the decreased frequency of the weak D phenotypes. Prevalence also varies with region to region.

The incidence of weak D varies worldwide, and it ranges from 0.2% to 1% in Caucasians.[10] In the present study, we observed weak D in 0.06% subjects. In another study[8] the incidence of weak D in India was found to be 0.3% to 0.5%. A recent study[17] in India reported the incidence to be 0.15%.[17] It can be explained by the fact that the use of monoclonal anti-D reagents of different potency may account for the difference in the incidence of weak D antigen.

When weak D red cells are transfused to an Rh negative recipient it may lead to alloimmuni-

zation to the Rh D antigen. Subsequent transfusion of blood from these donors to a sensitized individual may result in accelerated destruction or hemolysis of donor RBC. There are studies showing more than 90% of Europeans with most common weak D types (1, 2, 3, 4.0, and 4.1), which do not appear to be susceptible to immunization to the D antigen.[18] These individuals could safely receive D positive blood and do not require Rh immune globulin prophylaxis during pregnancy. However, serological tests cannot discriminate between these weak D types and those susceptible to alloimmunization; except by a molecular analysis of the *RHD* gene.

The current opinion is that weak D subjects especially if they happen to be women in the child bearing age group should be treated as Rh D positive when they are donors and D negative when they are recipients of blood transfusion. There is a potential risk of alloantibody formation when transfused with Rh positive blood. Allo-immunization of females with weak D while in the child-bearing age is disastrous and results in hemolytic disease of the newborn. It would be prudent to consider individuals with weak D antigen as Rh D positive when they are donors and Rh D negative when they are recipients. Though in our hospital, we evaluate all D negative patients and donors for weak D antigen, studies with molecular analysis should be conducted to formulate a cost-effective policy. Knowledge of blood group phenotype distribution is very important for blood banks and transfusion service policies.

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