

Prevalence of Weak D (Du) in blood donors in a referral teaching hospital

Lamba HS¹, Kaur K², Kaur K³, Vij AS⁴

¹Dr HS Lamba
Assistant Professor

²Dr Kulbir Kaur
Professor

³Dr Kulwant Kaur
Tutor

⁴Dr Amarjit Singh Vij
Professor

Department of Medicine
dr.asvij@gmail.com

^{1,2,3}Department of Pathology

^{1,2,3,4}Punjab Institute of Medical
Sciences, Jalandhar, Punjab, India

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Correspondence to:

Dr HS Lamba
lambahsdr@gmail.com

ABSTRACT

Background: Rh D is the most important Blood Group antigen after ABO Blood group antigen for transfusion purpose. All negative blood units by routine methods must be tested to detect weak D using IAT method. When the test for D and D^u is positive, the label should read Rh(D) Positive. When the test for D and D^u is negative, the label should read Rh(D) Negative.

Objective: To know the prevalence of weak D in the donor population. No study has been done in this part of the country earlier. It will help in the knowledge of weak D, which is very important for better patient care and prevent allo-immunization in blood recipients.

Materials and Methods: Blood samples were tested by ID Gel technique or by tube method with two anti D reagents – anti-D IgM monoclonal and blend of anti-D IgM & IgG. All negative samples were further tested for weak D in IAT phase by LISS/Coombs' gel card.

Results: A total of 13043 samples were tested from January 2011 to December 2013. 12196 were Rh positive and 847 were Rh D negative. Weak D was positive in 8 samples.

Conclusion: The study shows the prevalence of weak D as 0.07% in blood donors who were primarily from in and around Jalandhar in Punjab. These donors may have posed problem to the recipients of blood and blood product and their detection prevented them from alloimmunisation.

Key Words: D^u, Rh-D, Weak D, Rh negative, anti D

Introduction

The Rh blood group is the most important blood group system after ABO blood group system and is more complex. Rh antigens are present only on red blood cells.^[1,2] Its discovery is the most important event in the blood group field since discovery of ABO system. The term "Rh positive" and "Rh negative" refer to the presence or absence of D antigen.^[3] More than 50 Rh antigens have been characterized although the five principal antigens – D, C, c, E & e are responsible for majority of clinically significant antibodies.^[3] It has been well established for decades that no "d" antigen exists. When D antigen is weakly expressed on RBCs, it cannot be detected by routine monoclonal anti-D sera. It requires testing by Indirect Antiglobulin Test (IAT). The RBCs found positive after IAT are referred as "Weak D". Though the number of Weak D positive is less but its detection helps in safe blood transfusion.^[4] The significance of weak D lies in the fact that transfusion of red cells from a 'weak D, person to a 'D Negative' person may result in alloimmunization and subsequent exposure to

such 'D Positive' red cell can lead to fatal hemolytic reaction or hemolytic disease of newborn in a sensitized pregnant female. The present study was done to find out the prevalence of weak D in the Blood Donors in this part of Punjab, India.

Materials and Methods

Rh Blood Group of blood donors (selected as per Drug & Cosmetic Act norms) was analyzed in our blood bank from 2011 to 2013. Routine Rh typing analyzed using the immediate spin tube technique and by LISS/Coombs' gel card. The blood samples which were negative were further tested by blend of anti-D IgM & IgG after addition of AHG serum by tube method or by LISS/Coombs, gel card. The results were always compared with negative controls. The samples found +ve were considered as weak D. The controls were also used. All negative results were confirmed under microscope. IgM anti D monoclonal is highly specific saline reacting working equally well at room temp and at 37°C. (unable to detect weak

D). IgG & IgM blend monoclonal reagents & blend IgM reagents were used for D^u testing.

Weak D testing of all negative donors was done in ID card (gel card) using indirect antiglobulin test. 50 ul of 1% suspension of donors' red cells [1000ul of Diluent -2 +10 ul of Test Packed Red Cells] was added to microtube of an ID card labelled with donor unit number. To this microtube 50ul of blend of IgG and IgM was added. Then ID card was incubated in dry incubator at 37°C for 15 minutes and centrifuged for 10 minutes in ID centrifuge. For the interpretation of result, if red cells settle to the bottom of microtube then it is weak D negative. In weak D positive sample, red cell agglutinates are trapped in gel matrix. Data was analysed in

percentage and prevalence of weak D was calculated.

Results

5267 blood samples were analyzed in 2011, 5138 in 2012, 2638 in 2013. A total of 13043 blood samples were analyzed during the period 2011 to 2013. Out of these 12196 (95.51%) were Rh D positive and 847 (6.49%) were Rh D negative. These negative samples when tested for weak D, 8 (0.06%) samples were found to be weak D or D^u positive. Out of these 12196 (95.51%) were Rh D positive and 847 (6.49%) were Rh D negative. These negative samples when tested for weak D, 8 (0.06%) samples were found to be weak D or D^u positive. (Table: 1)

Table: 1 Prevalence of weak D: Year wise

Year	No. of Samples	Rh D+ve		Rh D -ve		Weak D +ve	
		Numbers	%	Numbers	%	Numbers	%
2011	5267	4915	93.31	352	6.69	0	0.00
2012	5138	4782	93.37	356	6.93	6	0.12
2013	2638	2499	94.73	139	5.27	2	0.08
Total	13043	12196	93.51	847	6.49	8	0.06

Discussion

The Rh blood group system is highly immunogenic, complex and polymorphic.^[5] The discovery of Rh antigen began with detection of antibody to Rh antigen by Levine & Stetson in 1939 in the serum of a recently delivered woman whose fetus died in-utero. Rhesus group was discovered by Landsteiner & Weiner in 1940. They immunized rabbits and guinea pigs with blood of monkey "Macacus rhesus" and made the surprising discovery that the resulting antibodies agglutinated not only monkey red cells but also red cells of 85% white people in New York. The 85% whose red cells were agglutinated by rabbit anti-rhesus serum were called Rh positive and remaining 15% Rh negative.^[6] Rh D negativity is highest in Basque population.^[7]

Rh expression at red cell surface requires presence of Rh associated glycol protein RhAg which exhibits 36% sequence identity with Rh

protein and is encoded by a gene located at chromosome 6. The Rh blood group is carried by two nonglycosylated palmitoylated proteins encoded by two homologous genes RHD and RHCE located on chromosome 1. RHCE encodes CcEe set of antigens and RHD encodes D antigen.^[5] There are approximately 10,000 to 30,000 copies of RHD & RHCE proteins and 100,000 to 200,000 copies of Rh antigen per cell.^[5] The number of D antigen sites on the Rh(D)-positive red blood cell is normally in the range of 9900 to 33000. There are many variants of RhD antigen and the important variants of D antigen are weak D, Partial D, Rh null. The current preferred term for D^u is "weak D." Weak D red cells have fewer D antigens per cell than normal Rh positive cells. (110 to 9000 per red blood cell). In Weak D one or more amino acid substitutes are found in region that are presumed to be in or below the

membrane and may interfere with assembly of Rh complexes.^[8] They react with anti-D only after extended testing with the indirect anti-globulin test. It is customary to regard Weak D subjects as Rh -negative when they are recipients of transfusion and Rh-positive when they are blood donors.^[9] The frequency varies with the method used, the reagent used, and the race. The frequency of weak D among Blacks is higher than in Whites. The expression of D antigen occurs in an estimated 0.2%–1% of Caucasians. [approximately 0.3 percent (3 in 1000)]. Partial D, a rare variant in which part of D antigen is missing. Normally it is not possible to detect by routine D^u testing.^[2] Partial D RBCs react with some monoclonal anti-D antibodies and not with others.^[8] Rh null, in which there is absence of all Rh blood group system antigens. It causes membrane abnormality which shortens RBC survival.^[4] though normal Rh genes are present.

Table: 2 Comparison of weak D in different studies

Author	Description	Percentage
H Kumar ^[10]	Blood Donors	0.189
Makroo RN et al ^[11]	Blood Donors Delhi	0.01
Nitin Agarwal et al ^[12]	Uttarakhand	0.005
S Das & H Kumar ^[13]	Kolar	0.15
Kotwal ^[14]	Jammu	0.0075
Sadaria ^[15]	Ahmedabad	0.056
Deepthi Krishna et al ^[16]	Tirupati Blood Donors	0.07
Deepthi Krishna et al ^[16]	Tirupati Patients	0.06
Present study	Jalandhar	0.06

The clinical importance of the detection of weak D is consequence of the fact that Rh -ve individuals are easily stimulated to form Rh antibodies if transfused with Rh +ve blood or in pregnant woman if exposed to fetal Rh +ve cells of the fetus which leaked through placenta into maternal circulation. In our study weak D variant

was detected in about 0.06% of total 13043 blood donors. Our Institution is the only Medical College Hospital in the Doaba region of Punjab and caters to the need of Jalandhar, Kapurthala & Hoshiarpur Districts of Punjab, besides part of adjoining Himachal Pradesh. The prevalence of weak D varied in the study by different persons, which is given in Table 2. Our study result of 0.06 % weak D incidence is nearly same as that of Deepthi Krishna et al in blood donors 0.07%^[16], Deepthi Krishna et al in patients 0.06%^[16] and Sadaria et al 0.056%^[15] but higher than that of Makroo et al 0.01^[11], Nitin Agarwal et al 0.005%^[14] and Kotwal et al 0.0075.^[18] The other studies by Kumar H et al 0.189%^[10] and Das et al as 0.15%.^[13] This variation may be due variation in incidence of Rh negativity in different races and regions. The variation can also be due to characteristic of typing reagents^[4] used. The detection by gel technique is more sensitive but still use of other sensitive methods and techniques, and standardisation of kits & techniques may result in better and uniform results. The main concern of study was to create awareness and stop alloimmunisation of recipients of blood and blood products from weak D because D is highly immunogenic followed by c>E>C>e.

The prevalence of Rh D negativity in our setting is estimated to be about 6.49%, and that of weak D antigen is 0.06 % - which is very small. Several research studies proved that weak D antigen is immunogenic and can produce alloimmunization if transfused to Rh D negative subjects. The risk of alloimmunization in our setting due to weak D (D^u) antigen is very low. However the study of Rh D negative patients with weak D alleles who have been exposed to Rh D positive RBCs is needed to quantify the absolute risk of sensitization

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