

Prevalence of Weak D among Blood Donors at a Tertiary Care Hospital in Srinagar, Kashmir

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Abstract: ***Background:** After ABO antigens Rh D antigen is the next most important in the field of transfusion medicine. Among different ethnic populations the prevalence of weak D phenotypes varies significantly. Weak D refers to reduced expression of D antigen on the red blood cells that require an extended testing with indirect antiglobulin test (IAT) to get detected. Clinical importance of weak D arises when labeling the donor and the patient, as the donor is labeled as D positive and the patient as D negative. **Aim:** The study was conducted to determine prevalence of weak D among D negative blood donors in our population. **Material and Methods:** The study was conducted in the Department of Immunohematology and Transfusion Medicine GMC, Srinagar over a period of 18 months from October 2015 to March 2017. It is a hospital based cross-sectional study. In our center all healthy blood donor samples were tested for ABO and Rh D typing by routine serologic methods (conventional tube technique, 'immediate spin method') using two anti D reagents; anti-D (IgM) monoclonal and a blend of anti- D IgM and 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 15680 donor blood samples were analysed for ABO & Rh blood grouping. Among the total 15680 samples 94.6 % (n =14833) were Rh-D positive & 5.4 % (n = 847) were Rh-D negative. All the Rh-D negative (847) samples were subjected to weak D testing. Of the Rh-D negative samples 0.2 % (2/847) were weak D positive and of all the test samples 0.01 % (2/15680) turned out to be weak D positive. **Conclusion:** This study shows the prevalence of weak D antigen in our donor population who are representatives of Srinagar region of J & K. It also stresses the need to identify individuals with variant D (rather than weak D or partial D) and to inform them about their status as donors and recipients of blood/ or organs.*

Keywords: Rh D positive, Rh D negative, Weak D.

1. Introduction

Rhesus (Rh) blood group system is one of the most important as well as highly immunogenic and complex with numerous polymorphisms.¹ It is involved in the incompatible RBC transfusion conflicts and in the hemolytic disease of newborn due to the maternal-fetal blood group incompatibility.² The term "Rh" refers not only to a specific red cell antigen i.e., Rh D but also to a complex blood group system¹. By the year 2015, 58 Rh antigens have been identified. The most common and immunogenic are D, C, E, c and e.³ However, the major antigen of Rh blood group system is the Rh D antigen.⁴ The D antigen is encoded by the RH D gene while RhC, RhE, Rhc and Rhe antigens are encoded by RHCE gene.^{5,6} The RHD gene polymorphism leads to phenotypic polymorphism of D variants including weak D, Del and partial D.⁷

The weak D phenotype corresponds to a decrease in the expression of D antigen.⁸ As a result weak or no agglutination reaction is demonstrated by these RBCs with the anti D reagent at the immediate spin phase.⁹ About 0.1 to 2 % of white Caucasians have this Rh phenotype.¹⁰ Missense mutations observed in the alleles of all weak D types have been demonstrated to be the probable cause or the reduced D antigen expression in these cases.¹¹ Partial D phenotype corresponds to quality changes in D antigen consistent with the absence of some specific epitopes.¹² Although the numbers of Rh D antigens on the RBC surface are normal, alloantibodies and autoantibodies can be formed against the missing epitope. The "DEL" phenotype

(common in Asians) is a very weak form of D expression (D_{el}), which can't be detected by routine serology methods but can be demonstrated by adsorption elution with anti D. Despite extraordinarily low number of D antigens, Del phenotype can cause primary¹³ and secondary¹⁴ immune responses against the D antigen in D negative recipients.

The clinical significance of detecting weak D and other D variants of the Rh (D) system lies in the fact that of all protein antigens, D antigen is the most 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 can't be safely transfused with D positive red cells later.^{1, 15} Even 0.5 ml of Rh D antigen exposure in Rh negative individual can induce antibody response.⁹

The weak D phenotype, formerly known as Du is a quantitatively weakened form of the normal D antigen. The most important risk with this phenotype is alloimmunization among the recipients. As D antigen is highly immunogenic, individuals with weak D phenotype are typed depending upon whether the person is donor or the recipient; the recipients with weak D are considered D negative and must be transfused with D negative blood and the donors are considered as D positive. Mothers with weak D fetus must receive Rh immunoprophylaxis as passage of weak D red cells from fetus to mother may result in sensitization.^{1,16}

The present study will help in estimating the frequency of 'weak D' in healthy blood donors which should be

considered as Rh D positive donors, as weak D blood can provoke synthesis of anti-D in Rh D negative recipients.

2. Materials and Methods

The study “Prevalence of Weak D among Blood Donors at a Tertiary Care Hospital in Srinagar, Kashmir” was conducted in the Department of Immunohematology and Transfusion Medicine GMC, Srinagar over a period of 18 months from October 2015 to March 2017. It is a hospital based cross-sectional study.

During this period a total of 15680 healthy blood donors were tested routinely for ABO and Rh blood groups. Rh blood group typing was done by immediate spin tube method using two anti D reagents; **monoclonal** IgM anti-D (Tulip Diagnostics Private Limited, Verna, Goa, India) and **polyclonal** IgM+IgG anti-D blend (Tulip Diagnostics Private Limited, Verna, Goa, India). Blood samples that were negative by immediate spin tube technique were further tested by Indirect Antiglobulin Test (IAT) and Gel Card System (GCS) (ID Diaclon Anti-D, ID Microtyping System) for weak D.

A 5% suspension of the cells to be tested was made. Equal volumes (2 drops) of each of anti-D serum and 5% red cell suspension were placed in a clean glass tube, mixed well, and incubated at 37° C for 60 minutes. The tube was gently resuspended and the cell button was observed for agglutination. If the test red cells were agglutinated, the immediate spin tube test result was recorded as D antigen positive. If the test red cells were not agglutinated, the test was recorded as D antigen negative. Further, the D negative cells were washed 3-4 times with large volumes of normal saline. After the final wash, the saline was decanted and two drops of antihuman globulin serum was added and the tube centrifuged at 1000× g for 1 minute. The cell button was resuspended and examined for agglutination. All negative results were confirmed by microscope. The samples showing agglutination after addition of AHG serum (J. Mitra & Co. Pvt. Ltd) were considered weak D positive. Parallel positive and negative controls were set up to rule out any DAT-Positive sample.

For testing of weak D by gel card method, 1% red cell suspension was prepared in LISS. 50 µL of 1% RBC suspension was dispensed in microtube of IgG card followed by the addition of 50 µL of monoclonal anti-D IgG (Diamed ID Microtyping System). This was followed by incubation at 37°C for 15 minutes and centrifugation. All the results were read and interpreted by two observers independently

3. Results

The present study is a hospital based cross-sectional study. During this 18 month study period, a total of 15680 donor blood samples were analysed for ABO & Rh blood grouping. Among the total 15680 samples **25 %** (3920) were of **group A**, **34 %** (5332) of **group B**, **31 %** (4860) of **group O** & **10%** (1568) were of **AB group**. **94.6 %** (n =14833) were Rh-D positive & **5.4 %** (n = 847) were Rh-D negative. Table 1. All the Rh-D negative samples were subjected to weak D testing. Of the Rh-D negative samples

0.2 % (2/847) were weak D positive and of all test samples **0.01 %** (2/15680) turned out to be weak D positive. Table 2.

Table 1: Showing Blood Group Distribution and Weak D Positivity Among Blood Donors

Blood Group	Rh Positives	Rh Negatives	Total (%)	Weak D positives
A Group	3732 (23.8 %)	188 (1.2 %)	3920 (25 %)	1
B Group	4987 (31.8 %)	345 (2.2 %)	5332 (34 %)	1
O Group	4640 (29.6 %)	220 (1.4 %)	4860 (31 %)	0
AB Group	1474 (9.4 %)	94 (0.6 %)	1568 (10 %)	0
Total	14833 (94.6 %)	847 (5.4 %)	15680 (100 %)	2 (0.01 %)

Table 2: Showing Frequency of Weak D Positivity Among Rh Negative Blood Donors

Blood Group	Number	Weak D positives	%
A Negative	188	1	0.5 %
B Negative	345	1	0.3 %
O Negative	220	0	0.0 %
AB Negative	94	0	0.0 %
Total	847	2	0.2 %

4. Discussion

In transfusion medicine, determination of weak D (and other D variants) is important to ensure blood safety. The term Du was coined by Stratton.¹⁷ Later, Race et al¹⁴ and Stratton et al¹⁷ studied this antigen further and showed that it was an inherited characteristic. The currently preferred term for Du is weak D.¹

The incidence of weak D (& other D variants) varies worldwide. More than 100 variant of D types have been reported in literature.⁷ Although various authors have given the prevalence of weak D (& other D variants) in their populations, the comparative analysis becomes difficult due to the lack of set standards & the type of reagents used (monoclonal / polyclonal, single / blended). Further, it has been adequately documented that D epitopes distribution differs with different geographic locales & ethnicities of the population.¹⁸ It is being felt that the reagents produced in western countries may not be suitable for Indian population as D antigen is genetically controlled & major variations may exist in the D antigen profile of the populations. Kulkarni et al highlighted this fact by testing 42 confirmed D variants with 7 commercially available anti-D reagents in India.¹⁹

In our study, weak D comprised **0.01 %** of all study samples & **0.2 %** of all D antigen- negative samples. The serological method has not distinguished between weak D & other D variants (like partial D & Del types) in our study. This study shows the prevalence of weak D antigen in our blood donor population who are representatives of Srinagar region of J & K State. It also stresses the need to identify individuals with variant D (rather than weak D or partial D) and to inform them about their status as donors and recipients of blood/ or organs. Comparable results were obtained by **Mak KH et al, 1993²⁰** (0.016 % in Chinese donors), **Makroo RN et al,**

2010⁹ (0.01% of total donors and 0.12 % of Rh negative donors), **Manjula Jain et al, 2014**²¹ (0.009 % of total population and 0.2 % of the Rh negative samples), and **Geeta Devi et al, 2016**²² (0.014 % of total donors and 0.43 % of Rh negative donors). **Agarwal N et al, 2013**²³ observed a lower prevalence of weak D in their study(0.005 % of total donors and 0.09 % of Rh negative donors). However, higher results were obtained by **Opoku-Okrah C et al, 2008**²⁴ (0.5 % of total donors and 6.45% of Rh negative African donors) and **Deepthi Krishna G et al, 2015**¹ (0.6 % of total individuals and 1.04 % of Rh negative individuals screened). The occurrence of weak D antigen is about 0.23 % to 0.5 % in Europe and 3% in USA.^{25, 26} while in India, the frequency of weak D antigen turned out to be 0.3 to 0.5 %.^{27, 28}

Since the clinical significance of detecting weak D (& other D variants) lies in the fact that of all protein antigens, D antigen is the most immunogenic. The current opinion is that **weak D & other D variant subjects** should be treated as **D positive as donors** to prevent alloimmunization if accidentally transfused to D negative recipients. **Partial D recipients** should be considered as **D negative**, else they will form antibodies against the missing epitopes of the D antigen when transfused with D positive blood.²¹ There is one misconception that individuals with weak D phenotypes cannot make anti-D in contrast to partial D because they have low levels of complete D antigen.²⁹ Alloimmunization of D negatives can occur with weak D, while in child-bearing age can be disastrous and can lead to hemolytic disease of newborn. Newborns of D negative mother should be tested for weak D and Rh immunoglobulin is recommended for mothers of weak D positive infants in order to prevent immunization.²¹

5. Conclusion

This study shows the prevalence of weak D antigen in our donor population (0.01 %) which is substantial. Not testing for the weak D antigen in the blood group may cause transfusion reactions and allo-immunization. It also stresses the need to identify individuals with variant D (rather than weak D or partial D) and to inform them about their status as donors and recipients of blood/ or organs.

For safe blood transfusion & to prevent transfusion related complications, comprehensive national transfusion guidelines need to be laid down to standardize the protocol for D antigen testing for donors as well as patients.

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