Prevalence of 'Serologic Weak D' among blood donors at a tertiary care hospital: An Observational study from North India.

Rubiya Ryhan , Hilal Ahmad Tali, Saima Muzaffer , Rumana H Makhdoomi, Kaneez Fatima, Shagufta Roohi

Abstract

Background: Weak D refers to reduced expression of D antigen on the red blood cells that require an extended testing to get detected. The most important risk with this phenotype is alloimmunization among the recipients.

Materials and Methods: It is a hospital based observational study conducted over a period of 3 years. During this period all blood donor samples were tested for ABO and Rh D typing by routine serologic methods. Samples which were initially negative were further tested for weak D antigen using IgG Anti-D in the indirect antiglobulin test phase.

Results: A total of 37334 blood donor samples were analysed for ABO & Rh grouping. Out of the total samples 34037 (91.2 %) were Rh-D positive & 3297 (8.8 %) were Rh-D negative. Of all the test samples, 09/37334 (0.03 %) turned out to be weak D-antigen positive. And of all the Rh-D negative samples 09 /3297 (0.3 %) were weak D- antigen positive.

Conclusion: This study stresses the need to identify individuals with weak D and other D variants and to inform them about their status as donors and/ or recipients of blood or blood products

JK-Practitioner2026;31(2):41-44.

Introduction

Rh system is the 2nd most immunogenic and complex blood group system after ABO with more than 58 antigens included in it. [1] Rh D antigen is considered the major antigen of this blood group system. [2] The Rh D antigen is encoded by the RH D gene which is highly polymorphic and any mutation in this gene may lead to different phenotypic expressions of the D antigen known as D-Variants (including Weak D, Partial D, and Del, etc). [3,4]

The term "Weak D" Phenotype was 1st described by Stratton in 1946 as "Du". [5,6] It corresponds to a quantitative decrease in the expression of normal D antigens on RBCs. [7] As a consequence of decreased number of D antigens, weak or no agglutination reaction is demonstrated by these RBCs with the anti D reagent by conventional tube technique, [8,9] and require an extended testing with indirect antiglobulin test or molecular tests to get detected. So any test sample which may be labeled as Rh D negative by routine serologic methods may be found Rh D positive on further confirmation.

The clinical significance of detecting weak D and other D variants of the Rh (D) system lies in the fact that even lessthan 0.5 ml of Rh D antigen exposure in Rh negative recipient can induce antibody response. [8] So The most important risk with this phenotype is alloimmunization among the recipients. 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]

This study aims to estimate prevalence of weak D antigen among D-negative blood donors and to provide data for improving blood transfusion protocols, especially for recipients prone to alloimmunization.

Materials and Methods

Study Design: This is an observational, hospital-based study

Study Population: The study was conducted in the Department of Blood Transfusion and Immunohematology, SKIMS Soura, Srinagar,

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Indexed

EMBASE, SCOPUS, IndMED , ESBCO, Google Scholar besides other national and international databases.

Cite this article as

Ryhan R , Tali HA , Muzaffer S, Makhdoomi RH, Fatima K, Roohi S. Prevalence of 'Serologic Weak D' among blood donors at a tertiary care hospital: An Observational study from North India.JK Pract2026;31(2):41-44.

Full length article available at **jkpractitioner.com** one month after publication

Keywords

Rh D-positive, Rh D- negative, Weak D antigen.

over a three-year period from March 2021 to April 2024. Blood donors who met the criteria for donation and were deemed fit during this period were included in the study. Donors who were deferred or found unfit for donation were excluded.

Sampling Procedure: The study encompassed all blood donor samples collected during the specified period, with no additional sampling procedure required.

Sample Size: The sample size was determined by the total number of donors (n = 37,334) who donated blood during this specified period.

Human Subjects Protection: Informed consent was obtained from all blood donors prior to blood donation and sample collection, and proper ethical approval for the study was secured from the institutional ethics committee.

Blood Group Typing: A total of 37334 blood donor samples were tested routinely for ABO and Rh blood groups. Rh blood group typing was done by conventional 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 for Rh D by conventional tube technique (CCT) were further tested by Indirect Antiglobulin Test (IAT) and Gel Card System (GCS) (ID Diaclon Anti-D, ID Microtyping System) for weak D antigen.

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 RT for 45- 60 minutes (as advised by the manufacturer)

(Sedimentation method) or the tube was incubated at 37^{O}C for 10-15 minutes and centrifuged at 1000× g for one minute (spin tube method). The tube was gently resuspended and the cell button was observed for agglutination. If the test red cells were agglutinated, the conventional tube test (CCT) 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 Antihuman globulin (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^{o}C for 15 minutes and centrifugation. All the results were read and interpreted by two observers independently

Statistical Analysis; Descriptive statistics was performed for categorical variables in the form of numbers (n) and percentages (%), using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 29.

Results:

A total of 37334 donor blood samples were analysed for ABO & Rh blood grouping. Out of these total 37334 samples 34037 (91.2 %) were Rh-D positive & 3292 (8.8 %) were Rh-D negative. Among the all total 37334 samples 8850 (**23.7 %**) were of group A {8129 (28.1%) A positive and 721 (1.9 %) A negative], 12463 (**33.3 %**) were of group B {11433 (30.6 %) B positive and 1030 (2.7 %) B negative}, 13243 (**35.5 %**) of group O {11866 (31.8 %) O positive and 1377 (3.7 %) O negative} & 2778 (**7.44 %**) were of AB group {2609 (6.9 %) AB positive and 7.7 (0.5 %) AB negative} (Table 1)

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

Blood Group	Rh Positives n (%)	Rh Negatives n (%)	Total (%) n (%)	Weak D positives n (%)
A	8129 (21.8 %)	721 (1.9 %)	8850 (23.7 %)	2 (0.01 %)
B	11433(30.6 %)	1030 (2.7 %)	12463(33.3 %)	3(0.01 %)
O	11866 (31.8 %)	1377 (3.7 %)	13243(35.5%)	4 (0.01%)
AB	2609(6.9%)	169 (0.5%)	2778(7.44%)	00
Total	34037(91.2%)	3297(8.8%)	37334(100 %)	9 (0.03 %)

All the Rh-D negative (3297) samples were subjected to weak D testing. Of all the test samples 09/37334 (0.03 %) turned out to be weak D positive. **Table 1**. And of all the Rh-D negative samples 09 /3297 (0.3 %) were weak D positive.

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

Blood Group	Number	Weak D positives	%
A Negative	721	02	0.3 %
B Negative	1030	03	0.3 %
O Negative	1377	04	0.3 %
AB Negative	169	00	00 %
Total	3297	09	0.3 %

Discussion

For safety of blood transfusion, determination of Serologic Weak D (and other D variants) is of utmost importance. In literature more than 100 variant of D phenotypes have been reported. [3] The incidence of weak D (& other D variants) varies worldwide. Although various authors have given the prevalence of serologic weak D (& other D variants) in their populations, the comparative analysis becomes difficult due to the lack of set standards for performing the tests, type of reagents used (monoclonal / polyclonal, single / blended), objective and subjective variation in interpretation of test results. [10] Further, it has been adequately documented that D epitopes distribution differs with different geographic locales & ethnicities of the populations. [11] Kulkarni et al highlighted this fact by testing 42 confirmed D variants with 7 commercially available anti-D reagents in India. [12]

In our study, the incidence of weak D comprised 0.03 % of all study samples & 0.3 % of all Rh - negative samples. Comparable results were obtained by Makroo RN et al, 2010, [8] Devi G et al 2016 [13] and Srivastava RK et al (2018). [14] Agarwal N et al 2013 [15] 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 Deepthi Krishna G et al 2015 [1] (0.6 % of total individuals and 1.04 % of Rh negative individuals screened), Lamba HS et al 2017, [16] Rehmani MT et al 2017, [17] and Brar RK et al (2020). [18] Table 3. The occurrence of weak D antigen is about 0.23 % to 0.5 % in Europe and 3 % in USA, [19,20] while in India, the frequency of weak D antigen turned out to be 0.3 to 0.5 %. [21,22]

This study highlights the prevalence of serologic weak D in our blood donor population who are prototype representatives of this region. 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 and blood products. The current opinion is that weak D & other D variant subjects should be treated as D positive as donors to prevent alloimmunization if accidently 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. [23] Alloimmunization of D negatives can occur with weak D, while in childbearing 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

Table 3: Comparison of prevalence of Weak-D in blood donors from different regions of the world

Sr. no.	Author	Total Study participants/ donors	Weak D Prevalence	
			% among Rh D negative donors	% among total donors
1.	Makroo RN et al, 2010 [8]	184072	0.12	0.01
2.	Devi G et al, 2016 [13]	7019	0.43	0.01
3.	Srivastava RK et al, 2018 [14]	1,66,338	0.35	0.004
4.	Agarwal N et al, 2013 [15]	58,614	0.09	0.005
5.	Deepthi K G et al, 2015 [1]	46654	1.04	0.0.06
6.	Lamba HS et al, 2017 [16]	13043	0.94	0.06
7.	Rehmani MT et al 2017 [17]	55874	0.98	0.08
8	Brar RK et al 2020 [18]	6415	1.51	0.07
9	Our Study	37334	0.3	0.03

immunoglobulin is recommended for mothers of weak D positive infants in order to prevent immunization. [23] The study, despite successfully highlighting the prevalence of serological weak D in the blood donor's population has certain limitations, as it doesn't distinguish between weak D & other D variants (like partial D & Del types) in our study.

Conclusion

The present study provides us the information regarding prevalence of serologic weak D antigen in our blood donor population. Not testing for the weak D antigen in the blood group may have devastating immunological and medical consequences especially in chronically transfused patients like thalassemia, sickle cell anemia, chronic renal failure and in pregnant women. Alloimmunization (with weak D) in such patients especially women in the child bearing age is catastrophic. It also stresses the need to identify individuals with other D variants (rather than weak D or partial D) and to inform them about their status as donors and recipients of blood and blood products.

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