



**PREVALENCE OF WEAK D AMONG BLOOD DONORS AT A TERTIARY CARE HOSPITAL IN TAMAKA, KOLAR, KARNATAKA**

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**ABSTRACT**

**Introduction:** The study “Prevalence of Weak D among Blood Donors at a Tertiary Care Hospital in Tamaka, Kolar, Karnataka” was conducted in the Department of pathology Sri Devaraj Urs Medical College, over a period of 6 years from January 2012 to December 2017. **Method:** Blood samples that were negative for RhD by immediate spin tube method were tested for weak D by indirect antiglobulin test. **Observation and results:** Among 45272 healthy blood donors, 42853(94.65%) were RhD factor positive while, 2419(5.33%) were RhD negative. Among these, 2419 RhD factor negative individuals 5 (0.20%) were weak D positive. **Conclusion:** Numerous studies conclude that weak D antigen is immunogenic and is capable of producing alloimmunisation if transfused to RhD negative subjects. Ergo, it is pertinent to also detect the Weak D or partial D status of those individuals who are negative with saline anti-D.

**KEYWORDS:** Weak D antigen, Rh blood group.

**INTRODUCTION**

In transfusion medicine, determination of weak D (and other D variants) is important to ensure blood safety. The term Du was coined by Stratton.<sup>1</sup> Later, Race et al<sup>14</sup> and Stratton et al<sup>17</sup> studied this antigen further and showed that it was an inherited characteristic. The currently preferred term for Du is weak D.<sup>[1]</sup>

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.<sup>[2]</sup> 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.<sup>[3]</sup> Mothers with weak D fetus must receive Rh immunoprophylaxis as passage of weak D red cells from fetus to mother may result in sensitization.<sup>[4]</sup>

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.<sup>1, 15</sup> Even 0.5 ml of Rh D antigen exposure in Rh negative individual can induce antibody response.<sup>[5]</sup>

**MATERIAL AND METHODS**

The study “Prevalence of Weak D among Blood Donors at a Tertiary Care Hospital in Tamaka, Kolar, Karnataka” was conducted in the Department of pathology Sri Devaraj Urs Medical College, over a period of 6 years from January 2012 to December 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 37o 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  $1000 \times g$  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  $\mu$ L of 1% RBC suspension was dispensed in microtube of IgG card followed by the addition of 50  $\mu$ L 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.

## RESULTS

The present study is a hospital based cross-sectional study. During this 18 month study period, a total of 45272 donor blood samples were analyzed for ABO & Rh blood grouping. Among the total 15272 samples **23.25%** (10529) were of **group A**, **30.61%** (30.61) of **group B**, **40.94%** (18530) of **group O** & **5.2%** (2355) were of **AB group**. **94.6%** (n =42853) were Rh-D positive & **5.4%** (n = 2419) were Rh-D negative. Table 1. All the Rh-D negative samples were subjected to weak D testing. Of the Rh-D negative samples **0.20%** (5/2419) was weak D positive and of all test samples **0.01%** (7/45272) turned out to be weak D positive. Table 2.

**Table 1: Showing blood group distribution and weak D positivity among blood donors for the year 2012-2017.**

Blood group	Rh positive	Rh Negative	Total	Weak D positive
A group	9996 (22.07%)	533 (1.17%)	10529 (23.25)	3
B group	13184 (29.12%)	674 (1.48%)	13858 (30.61)	2
O group	17503 (38.66%)	1027 (2.26%)	18530 (40.94)	1
AB group	3170 (7.00%)	185 (0.4%)	2355 (5.2%)	1
Total	42853 (94.65%)	2419 (5.3%)	45272 (100 %)	7 (0.01%)

**Table 2: Showing frequently of weak D positivity among Rh negative blood donors for the year 2012-2017.**

Blood group	Number	Weak D positives	%
A negative	533	2	0.08%
B negative	674	1	0.04%
O negative	1027	1	0.04%
AB negative	185	1	0.04%
Total	2419	5	0.20%

## DISCUSSION

The Rh blood group system is one of the most complex blood systems, including 49 different antigens. The D antigen itself consists of more than 30 distinct epitopes, more than 100 known haplotypes and similar phenotypes of different alleles.<sup>[6]</sup> Initially weak D formerly, called Du was characterized by a reduction of antigen D expression on red cells. However, known variants of the D antigen, also-called partial D phenotypes, can have a weak D expression, such as the Dva and Dvi phenotypes. Individuals whose RBCs carried a partial D phenotype could be immunized to D epitopes lacking in their RBCs and were at risk. Therefore, it is necessary to distinguish weak D from partial D.<sup>[7]</sup> Individuals with weak D phenotypes do not produce antibodies in response to pregnancy or transfusion, however, these RBCs can provoke anti D immunization in D negative recipients.<sup>[8]</sup>

The D antigen is unique among blood groups because it expresses 30 epitopes distributed along the extracellular portions of the RhD protein. Changes, in the amino acid sequence of RhD may not ablate the entire D antigen but can cause epitopes loss, giving rise to variant forms known as partial D.<sup>[9]</sup> New discoveries relating to the RhD gene, and an appreciation of its variant phenotypes such as weak D and partial D, have challenged the way

that D status is assigned to both blood donors and blood product recipients.<sup>[10]</sup> The Rh antigen D positive is highly immunogenic and clinically significant in transfusion and pregnancy. As many as 80% of D negative recipients of a unit of D positive red cells will form anti-D. Ideally D negative patients should be transfused with D negative components.<sup>[11]</sup>

Weak D is phenotype with either qualitative or quantitative difference in the Rh Du moiety resulting in a weakened expression of the antigen. The problems that arise from weak Du antigen are due to its low immunogenicity which gives rise to conflicting laboratory reports, as to whether an individual is Rh D positive or negative.<sup>[12]</sup> Weak Du demonstrates reduced quantities of the D antigen because of mutations in the protein's transmembrane domains. As the name implies, these RBCs tend to demonstrate either weak or no hemagglutination at IS phase.<sup>[13]</sup>

Weak D Rh antigen presents a peculiar clinical situation. The subject may face problems in Rh blood group typing and prevent a potential risk of alloantibody formation when transfused with Rh positive blood.<sup>[14]</sup> Allo-immunisation of females with weak-D while in the child-bearing age is disastrous and results in haemolytic

disease of the newborn. It would be prudent to consider individuals with a weak D antigen as Rh D positive when presenting as a donor and Rh D negative when confronted as a recipient.<sup>[15]</sup>

### 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.<sup>[16]</sup>

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.<sup>[17]</sup>

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