# A Study on Weak D Prevalence among Blood Donors and Patients

**Moxda S. Patel*, Sejal R. Kakadiya**, Jayshree M. Shah*****

*Assistant Professor, **2nd Year Resident, *** Professor and Head of the Department, Pathology Department, AMC METMedical College & L.G. Hospital, Ahmedabad-380008, Gujarat, India.

**Abstract:** Background and Objectives: Our study is designed to determine the frequency of weak D antigen in donors and patients coming to our tertiary care hospital which is important when labelling the donor and patient as the donor is labelled D positive, patient as D negative. Methodology: In our centre all blood donors and patients samples are tested for ABO and RhD using anti-D IgM monoclonal and a blend anti-D IgM and IgG reagents. 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 LISS/Coomb's gel card. Results: A total 26020 blood samples were tested(4491 donors, 21529 patients) during the period September 2015 to September 2016.Among these 24489 were Rh D positive(94.11%) and remaining 1531(5.89%) were Rh D negative. A total of 05 were weakD positive constituting 0.326% of Rh D negative and 0.019 % of total individual screened. Conclusion: This study shows the prevalence of weakD antigen in our population and to inform them about their status as donor and recipient of blood to prevent the hazards of blood transfusion. [Moxda P NJIRM 2017; 8(2):35-38]

**Key Words**: Coomb's gel card,Donor,Patient,Weak D antigen

Author for correspondence: Moxda S Patel, Assistant Professor, Department of Pathology, AMC MET Medical college, Ahmedabad-380008. E-Mail: pmoxda70@yahoo.com ,sejalkakadiya1@gmail.com

**Introduction:** The Rh blood group system is one of the most polymorphic and immunogenic blood group systems in humans, among that Rh-D antigen is the most important antigen because of its immunogenicity. At present more than 50 antigens are there in Rh system but only D,C,c,E and e are commonly identified and clinically significant antigens with respect to blood transfusion. D antigen has more than30distinct epitopes,more than 100 known haplotypes with similar phenotypes of different alleles [1,2]. D is labelled as the Rh antigen and the terms Rh positive and Rh negative refer to presence or absence of D antigen respectively. 85% ofthe Caucasian population is Rh-D positive while in India incidence of Rh positivity is 95%.The incidence of Rh negativity worldwide varies between 3-25%[3].

It is easy to detect D antigen in most of the cases. Sometimes, variable expression of Rh-D antigen leads to presence of weak forms. WeakD phenotype, formerly known as Du is a quantitatively weakened form of the D antigen. The most important risk with this phenotype is alloimmunization among the recipients and subsequent exposure to such red blood cells can lead to fatal haemolytic reaction or haemolyticdisease of newborn in a sensitized pregnant female. As D antigen is highly immunogenic, individual 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 positive[4].Thus our study emphasize the importance of weak D serology as a routine procedure.

**Methods:** The study was carried out from September, 2015 to September, 2016 in Department of Pathology (Blood Bank) at L.G. General Hospital in AMC MET Medical college, Ahmedabad which includes patients and donors. Our study had been approved by Ethical committee(IRB).

Two ml blood samples were collected in EDTA vacutainers and tested for ABO forward and reverse grouping by conventional slide, tube and gel card methods.

Samples were tested for RhD typing using two different classes of anti-D reagents by immediate spin tube technique using reagents; monoclonal anti-D IgM (Tulip diagnostics) and a blend anti-D IgM and IgG (Span diagnostics).In tube method, the red blood cells were washed several times to remove any unbound anti-D. A 5% suspension of washed red cells was prepared. Equal volumes each of anti-D serum (IgM + IgG) and 5% red cell suspension were taken in a glass tube, mixed, and incubated at 37°C for 45 minutes and then centrifuged at 1000 rpm× 1 minute. The tube was re-suspended gently and agglutination in the form of cell button observed grossly, which was then confirmed by microscopic examination. When patient's red blood cells were agglutinated with anti-D, that patient was labelled Rh-D positive. When no agglutination was present, then the patient was considered Rh-D negative.

Each negative Rh-D typing result was confirmed with a weak D test before being reported, because red blood cells expressing weak D antigen can also give a negative reaction in routine Rh-D typing. Weak D antigen testing was done by indirect antiglobulin test by test tube and gel card methods using a commercial polyspecific Antihuman Globulin (AHG) reagent containing anti IgG and C3d. Red blood cells were again washed twice with large volumes of normal saline. After this, the saline was decanted and two drops of antihuman globulin serum was added and the tube centrifuged at 1000 rpm for 1minute. This anti IgG will react with any anti-D IgG that became bound to the red blood cells during the initial typing test. Re-suspension of cell button done and examined macroscopically for agglutination and then result was confirmed by microscopic examination. Simultaneously positive and negative controls were put up. Those samples that showed agglutination with addition of AHG serum were labelled as weak D positive. Only blood sample that was negative macroscopically and microscopically in the weak D test was labelled as D negative.

Gel card system used was Diamed ID Microtyping System containing polyspecific AHG. A 1% red cell suspension of blood sample was prepared in Low Ionic Strength Solution (LISS). Fifty micro litre of 1% Red cell suspension was taken in micro tube of IgG gel card followed by the addition of 50 µL of monoclonal anti IgG (ID Diaclon Anti-D). This was followed by incubation at 37°C for 15 min and fixed centrifugation for 10 minutes[5].

**Results:** A total 26020 blood samples were tested (4491 donors, 21529 patients) .Out of them 94.11 % (n=24489) were Rh-D positive and 5.89% (n=1531) were Rh-D negative. Among these 1531 Rh-D negative cases, 0.326 %(n=5) were weak D positive and 0.019% of total samples.[TABLE-1]

**Table-1: Distribution of Rh-D antigen in total study samples**

| Rh Status | No. Of Cases | Percentage |
|---|---|---|
| Rh D Positive | 24489 | 94.11% |
| Rh D Negative | 1531 | 5.89% |
| Weak D Positive | 05 | 0.019% out of total cases<br>0.326% out of D negative |
| Total | 26020 | |

Out of total 4491 donors, 0.02 %(n=1) were turned out to be weak-D positive. Among 286 Rh-D negatives donors 0.34% (n=1) were turned out to be weak-D positive. Out of total 21529 patients, 0.018% were turned out to be weak-D positive. Among 1245 Rh-D negatives patients0.32% (n=4) were turned out to be weak-D positive. [TABLE-2]

**Table-2: Prevalence Of Weak D Among Donors And Patients**

| Rh Status | Donors | Patients | Total |
|---|---|---|---|
| RhD Positive | 4205(93.63%) | 20284(94.21%) | 24489 |
| RhD Negative | 286(6.37%) | 1245(5.79%) | 1531 |
| Weak D Positive | 01(0.02%) (0.34%) | 04(0.018%) (0.32%) | 05 (0.019%) (0.326%) |
| Total | 4491 | 21529 | 26020 |

**Table-3: Distribution of Rh-D antigen in males and females**

| Rh Status | No. of Male | No. of Female |
|---|---|---|
| Rh D Positive | 9740(94.32%) | 14749(93.97%) |
| Rh D Negative | 586(5.68%) | 945(6.03%) |
| Weak D | 3(0.029%) (0.51%) | 2(0.012%) (0.21%) |
| Total | 10326 | 15694 |

Out of total 10326 male samples, 0.029% were turned out to be weak-D positive. Among 586 Rh D negatives 0.51% (n=3) were turned out to be weak D positive. Out of total 15694 female samples, 0.012% were turned out to be weak-D positive. Among 945 Rh-D negatives 0.21% (n=2) were turned out to be weak D positive. [TABLE-3]

**Discussion:** Following discovery of the ABO blood group system, the greatest breakthrough in transfusion medicine was the discovery of Rh antigen by Landsteiner and Wiener in 1940 by immunizing rabbits and guinea pig with red cells of rhesus monkey[6,7,8].In 1939,Levine and Stenson describe an antibody in the serum of a group 'O' mother that was responsible for haemolytic disease of new born.This antibody was subsequently re-examined and found identical in activity as the anti-Rh antibody found by Landsteiner and Wiener[6,7].

Rh blood group system is one of the most important system as well as it is highly immunogenic and complex with numerous polymorphism comprising

more than 50 antigens of which five are clinically significant. These antigens are C,c,D(d),E, and e (five major Rh system antigens). 'd' simply represents the absence of D antigen. Genes for the five Rh antigens are encoded by two autosomal dominant genes RHD and RHCE on chromosome1[6,9].Anindividual phenotype is thus reported as DCE[10]. D antigen is a mosaic of epitopes (antigenic determinants). When Rhpositive RBC samples are typed for the D antigen they are expected to react strongly (macroscopically) with anti-D sera. However with certain RBCs, the testing must be carried through the antiglobulin phase to demonstrate the presence of the D antigen. These weaker D antigens have been referred to as the Du type described by Stratton in 1946 and are considered Rh positive[10]. Race et al., and Renton and Stratton observed that Du red cells were not agglutinated directly by anti Rh D serum but required subsequent addition of antiglobulin to detect D antigen [11,12]. This Du term was replaced by more appropriate term- the weak D antigen in 1984 [13].Weak D expression results from single point mutations in RHD leading to amino acid changes in intracellular or in the transmembraneregions of RHD resulting in lesser number of D antigen[14].

**There are three genetic mechanisms postulated for weak expression of the D antigen. These are:**

1. Individuals inherit the RHD gene which encode for a weakly expressed D antigen;
2. D antigen is weakly expressed due to presence of C antigen in the transposition on the opposite chromosomes such as Dce/dCe genotype. This is seen more commonly in blacks;
3. When one or more epitopes of the D antigen are missing, a weak D phenotype may be expressed and these individuals may be alloimmunized if transfused with D positive blood possessing the missing epitope[15].

Red blood cells with weak D antigen do not show agglutination with routine anti D reagents unless the indirect anti globulin test is performed. The incidence of weak D antigen ranges from 0.01%- 0.4% among Rh negative individual in different regions of India[3,14] . [TABLE-4]

**Table-4: Comparison With Other Study**

| No | Year | Author | Region | Weak D In Rh D Negative | Weak D In Total Population | Rh Negative | Rh Positive |
|---|---|---|---|---|---|---|---|
| 1 | 2016 | Our Study | Gujarat | 0.326% | 0.019% | 5.89% | 94.11% |
| 2 | 2014 | Kotwal U[16] | Jammu | 0.14 % | 0.0075 % | 5.48 % | 94.5 % |
| 3 | 2014 | Pahuja S[17] | Delhi | 0.2 % | 0.009 % | 5.4% | 94.6 % |
| 4 | 2013 | Das S[2] | Kolar and South Karnataka | 0.15 % | ---- | 12.76% | 88.8 % |
| 5 | 2013 | Agrawal[18] | Northern hilly areas of Uttarakhand | 0.09 % | 0.005 % | 5.2% | 94.8 % |
| 6 | 2010 | MakrooRN[19] | Delhi | 0.01 % | 0.0086 % | 7.19 % | % |

In our study incidence of weak D antigen was 0.019% out of total individual taken and 0.326 % out ofRh D negative individuals which is comparable with the study of other authors.

It is therefore hoped that the simple data generated of Rh-D negative and weak D positive patients in this study would be helpful in many ways: firstly the patients who have weak expression of D antigen with lesser number of D antigens on red cells surface, can

be transfused with Rh-D positive donor blood with no prior sensitization. Secondly, Rh-D negative blood would be conserved for genuinely Rh-D negative patients making optimal usage of scarce Rh-D negative blood. Thirdly, weak D positive females do not require anti-D immunoglobulins in case of Rh-D positive babies. So this would assist in the planning and establishment of a more efficient blood transfusion services that would meet the ever increasing demand for safe blood and blood products.

**Conclusion**: Our study concluded that the incidence of Rh negative blood group was 6.37 % in donors and 5.79 % in patients in our tertiary care hospital. We found a very low weak D positivity, possibly due to routine use of two potent monoclonal anti D blood group typing antisera. It is mandatory to perform weak D serology testing routinely in donors as well as patients who arenegative with saline anti-D to prevent

possibility of haemolysis and hazards of blood transfusion and also labeling of donors and patients.

**References:**


1. Srikrishna A, Sitalakshmi S, Devi S, Damodhar P, Jayanti M, Paul R. The Rh enigma-problems encountered. Indian Journal of Hematology and Blood Transfusion. 2001;19(4):102-03.
2. Das S, Kumar H. The Rh enigma prospects and pitfalls International Journal of Basic and Applied Medical sciences. 2013;3(1):210-14.
3. Kumar H., Mishra D.K., Sarkar R.S. and Jaiprakash M. - Difficulties in Immunohaematology: The weak D Antigen. MJAFI. 61:348-350, 2005.
4. Mayne K, Bowell P, Woodward T, Sibley C, Lomas C, Tippett P. Rh immunization by the partial D antigen of category DVa. Br J Haematol 1990;76:537-9.
5. Rafei UM. Standard operating procedures for blood transfusion service. World Health Organization. New Delhi. 2002:1-116.
6. Henry's Clinical Diagnosis and Management by Laboratory methods,1st South Asia edition:680-695,2016
7. WHO Transfusion Medicine,Technical Manual 2nd Edition:75-87,2003
8. Philip; Rufus E. Stetson. - An unusual case of intragroup agglutination. J Am Med Assoc. 113(2):126-127, 1939.
9. Wagner F.F., GassnerC., Muller T.H., Schorritzer D., Schunter F., Flegel W.A. - Molecular basis of weak D phenotypes. Blood. 93:385-393, 1999
10. Merilyn W. The Rh blood group system. In: Modern blood banking and transfusion practices. Harmening DM (ed), Philadelphia FA Davis Co.2008: pp134-47.
11. Stratton F. A new Rh allelomorph.Nature. 1946;158:25-26.
12. Race RR, Mourant EE, Lawler SD, Sanger R. The Rh chromosome frequencies in England. Blood. 1948;3:689-95.
13. Acharya S, Kumar R, Acharya R, Kudesia S, Kishore S. Weak D antigen – Revisited. Indian Medical Gazette. 2011:342-45
14. Bhatia H.M. - Mumbai: Blood Group Reference Centre, Indian Council of Medical Research : 44, 1977.
15. Tippet P. - Subdivisions of the Rh antigen D, review. Med Lab Sci. 45:888-891, 1988.
16. Kotwal U, Raina TR, Sidhu M, Dogra M. Distribution of ABO &Rh(D) blood groups among blood donors of Jammu region with respect to various ethnic groups. Journal of Medical Thesis. 2014;2(1):31-34.
17. Pahuja S, Pujani M, Sethi N, Kushwaha S, Jain M, Kumari R. Frequency of variant D in Delhi,India. Asian J Transfus Sci. 2014;8(2):142-43.
18. Agarwal N, Chandola I, Agarwal A. Prevalence of weak D in northern hilly areas of Uttarakhand, India. Asian J Transfus Sci. 2013;7(1):90-91.
19. Makroo RN, Raina V, Chowdhry M, Bhatia A, Gupta R, Rosamma NL. Weak D prevalence among indian blood donors. Asian J Transfus Sci. 2010;4(2): 137-39.



| |
|---|
| Conflict of interest: None |
| Funding: None |
| Cite this Article as: Moxda P, Sejal K, Jayashree S. A Study On Weak D Prevalence Among Blood Donors And Patients. Natl J Integr Res Med 2017; 8(2):35-38 |