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Dr. Shruti Bharatbhai Patel
Resident Doctor, Department of Pathology, Narendra Modi Medical College & Sheth L.G. General Hospital, Maninagar, Ahmedabad, Gujarat, India

Dr. Ankita Dipakkumar Solanki
Resident Doctor, Department of Pathology, Narendra Modi Medical College & Sheth L.G. General Hospital, Maninagar, Ahmedabad, Gujarat, India

Dr. Pooja Pravinsinh Padheria
Resident Doctor, Department of Pathology, Narendra Modi Medical College & Sheth L.G. General Hospital, Maninagar, Ahmedabad, Gujarat, India

Dr. Moxda Suresh Patel
Associate Professor, Department of Pathology, Narendra Modi Medical College & Sheth L.G. General Hospital, Maninagar, Ahmedabad, Gujarat, India

Corresponding Author:
Dr. Shruti Bharatbhai Patel
Resident Doctor, Department of Pathology, Narendra Modi Medical College & Sheth L.G. General Hospital, Maninagar, Ahmedabad, Gujarat, India

Investigation of weak d phenotype frequency in blood donors and patients: A tertiary care hospital study

Dr. Shruti Bharatbhai Patel, Dr. Ankita Dipakkumar Solanki, Dr. Pooja Pravinsinh Padheria and Dr. Moxda Suresh Patel

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Abstract
Background and objectives: RhD is the most important and complex blood group system after ABO bloodgroup system for transfusion purpose. Weak D refers to reduced expression of D antigen on red blood cells. Our study is designed to determine the frequency of weak D antigen among healthy donors and patients coming to our tertiary care hospital which is important while labelling the donor and patient; as the donor is labelled D positive, patient as D negative also to highlight its clinical implications related to risk of alloimmunization in blood recipients having RhD negative blood group. The probable cause for reduced antigen D expression could be missense mutations seen in alleles of all weak D type.
Material and Methods: 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 54222 blood samples were tested(4977 donors, 49245 patients) during the period of January 2023 to December 2023.Among these 51242 were Rh D positive(94.50%) and remaining 2180 (4.02%) were Rh D negative. A total of 80 were weak D positive constituting 3.66% of Rh D negative and 0.147% of total individual screened.
Conclusion: This study shows the prevalence of weak D antigen in our population and to inform them about their status as donor and recipient of blood to prevent the hazards of blood transfusion.

Keywords: Alloimmunization, D antigen, Weak D, Rh blood group

Introduction
The Rh blood group system is one of the complex and most polymorphic blood group systems in humans, among that Rh-D antigen is the most important antigen because of its immunogenicity. Following discovery of ABO blood group system the greatest breakthrough in transfusion medicine was the discovery of Rh antigen by Landsteiner and Weiner in 1940 by immunizing rabbits and guinea pig with red cells of rhesus monkey "Macacus Rhesus" species [1-3]. The term "Rh positive" and "Rh negative" refer to the presence or absence of D antigen [4]. Molecular genetics is controlled by two Rh genes, one encoding RHD (D) and the other encoding the RHCE (Cc and Ee) antigens. These can be found on 1p34-p36 of chromosome one [5]. The incident of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%- 1% [6]. 85% of the Caucasian population is Rh-D positive while in India incidence of Rh positivity is 95%.
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. Rh negative mothers with weak D foetus must receive Rh immunoprophylaxis as passage of weak D red cells from foetus to mother may result in sensitization. There are 51 antigens in the Rh system and more than 200 alleles for the RHD gene [7]. The objectives were to find the distribution of weak D antigen from the total study samples. Our study emphasize over distribution of weak D serology among the total study sample as a routine procedure.

Materials and Methods

The study was carried out from January 2023 to December 2023 in Department of Pathology (Blood Bank) at Narendra Modi Medical college and hospital, Sheth L.G. General Hospital, Ahmedabad which includes patients and donors. 2ml 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 anti globulin test by test tube and gel card methods using a commercial poly specific 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. Resuspension 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. If the test result was recorded as positive (3+/4+), then a grade was assigned to it. If the test result was doubtful (1+/2+) or negative, a confirmation of weak D Antigen was done. The results were always compared with negative controls. 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 confirmed 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). 50 µL 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 [8].

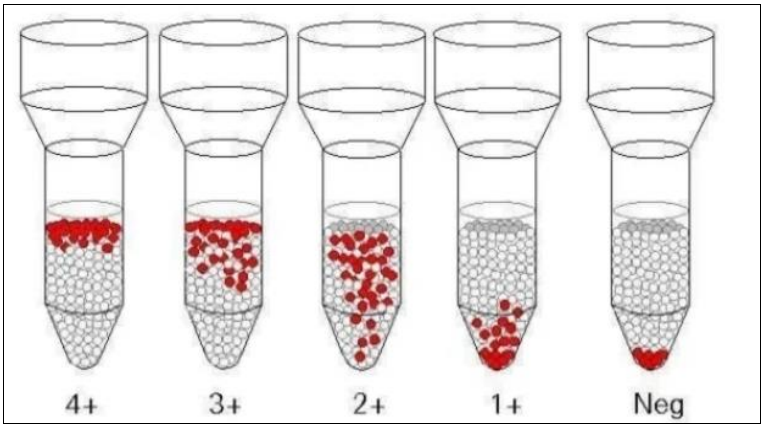

Fig 1: The collected data were recorded in Microsoft Excel and data analysis was done in same.

Results

A total 54222 blood samples were tested (4977donors, 49245patients). Out of them 94.5% (n=51242) were Rh-D positive and 4.02% (n=2180) were Rh-D negative. Among these 2180 Rh-D negative cases, 3.66% (n=80) were weak D positive and 0.147% 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	51242	94.5%
Rh D Negative	2180	4.02%
Weak D Positive	80	0.147% out of total cases 3.66% out of D negative
Total	54222	

Out of total 4977 donors, 0.02% (n=1) were turned out to be weak-D positive. Among 513 Rh-D negatives donors 0.19% (n=1) were turned out to be weak-D positive. Out of total 49245 patients, 0.16% were turned out to be weak-D positive. Among 1667 Rh-D negatives patients 4.73% (n=79) 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	4463(89.67%)	47499(94.21%)	51962
RhD Negative	513(10.30%)	1667(5.79%)	2180
Weak D Positive	01(0.19%)	79(3.62%)	80(3.66%)
	(0.02%)	(0.16%)	(0.147%)
Total	4977	49245	54222

Discussion

In 1939, Levine and Stenson discovered the Rh antigen by detection of antibody from 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. The frequency of weak D among Blacks is higher than in Whites.

Partial D is a rare variant in which part of D antigen is missing. Partial D RBCs react with some monoclonal anti-D antibodies and not each one. Rh-null is in which there is absence of expression of all Rh blood group system. It causes membrane abnormality which shortens RBC lifetime, although normal Rh genes are present.

There are more than 50 types of weak D antigens and majority (90%) of individuals with weak D phenotype is Type 1, 2 or 3 and these expresses normal. Among them 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 non-glycated palmitoylated proteins encoded by two homologous genes RHD and RHCE located on Chromosome 1p34-p36. D antigen is a mosaic of epitopes (antigenic determinant). When Rh positive 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 anti globulin phase to demonstrate the presence of the D antigen. Red blood cell antigens that react with anti-D only after extended testing with the indirect antiglobulin test are called weak D. Weak D expression results from single point mutations in RHD leading to amino acid changes in intracellular or in the transmembrane regions of RHD resulting in lesser number of D antigen. During arise of any confusion additional steps which may be prolonged incubation with the anti D reagent or addition of ant globulin serum after incubation with anti D would be required. Reduced expression of D antigen occurs in an estimated 0.2%–1% of Caucasians.

Patients with weak D phenotype are designed as D negative and should get D negative blood products. Sensitization may occur as a result of passing weak D cells from fetus to pregnant mother. Thus for the safety measures, women having weak D fetus should get Rh Immune-prophylaxis to avoid Rh alloimmunization.

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 or duplication 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.

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. [TABLE-3].

Table 3: Comparison with other study

No	Year	Author	Region	Weak D In Rh D Negative	Weak D In Total Population	Rh Negative	Rh Positive
1	2023	Our Study	Gujarat	3.66%	0.15%	4.02%	95.83%
2	2017	Desai *et al.* [9]	Mumbai	0.8%	0.39%	5.36%	94.63%
3	2015	Krishna *et al.* [10]	Andhra Pradesh	1.04%	0.06%	6.18%	93.82%
3	2014	Kotwal U [11]	Jammu	0.14%	0.0075%	5.48%	94.5%
4	2014	Pahuja S [12]	Delhi	0.2%	0.009%	5.4%	94.6%
5	2013	Das S [13]	Kolar and South Karnataka	0.15%	----	12.76%	88.8%
6	2013	Agrawal [14]	Northern hilly areas of Uttarakhand	0.09%	0.005%	5.2%	94.8%
7	2010	MakrooRN [15]	Delhi	0.01%	0.0086%	7.19%	-----

In our study incidence of weak D antigen was 0.15% out of total individual taken and 3.66% out of Rh D negative individuals which is comparable to studies 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 to minimize misdiagnosis and transfusion related problem [16].

In chronically transfusing conditions such as sickle cell disease, thalassemia major or chronic renal failure requires Weak D antigen testing and detection. Suppose, Rh negative people on further testing tests positive for weak D antigen, they should be transfused with Rh-negative blood of the same ABO group. This decrease the risk for alloantibody formation against weak D antigen which could be missed on routine method of blood grouping.

Conclusion

Our study concluded that the incidence of Rh negative blood group was 10.30% in donors and 5.79% in patients in our tertiary care hospital. We found low weak D positivity, possibly due to routine use of two potent monoclonal anti D blood group typing anti sera. It is mandatory to perform weak D serology testing routinely in donors as well as patients who are negative with saline anti-D to prevent possibility of haemolysis and hazards of blood transfusion and also labelling of donors and patients.

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