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A STUDY ON WEAK D PREVALENCE AMONG BLOOD DONORS AND PATIENTS AT TERTIARY CARE REFERRAL HOSPITAL IN BHAVNAGAR, GUJARAT

Pathology

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ABSTRACT

Background: Rh blood group system is one of the most important as well as highly immunogenic and complex with numerous polymorphisms. 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 and patient as D negative. **Material and method:** All blood donors and patient's samples were 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. **Result:** A total 31778 blood samples were tested (17634 donors, 14144 patients) during the period January 2021 to December 2021.Among these 29755 were Rh D positive (93.63%) and remaining 2023(6.36%) were Rh D negative. A total of 09 were weak D positive constituting 0.44% of Rh D negative and 0.028 % of total samples received. **Conclusion:** It is mandatory to perform weak D testing routinely in donors as well as patients who are negative with saline anti-D, to prevent alloimmunization and for labelling of donors and patients.

KEYWORDS

Donor, Patient, Rhd Antigen, Weak D Antigen

INTRODUCTION

Discovery of ABO blood grouping system was by Landsteiner which became the major tool for testing compatibility for transfusion. In 1940, Landsteiner and Weiner enhanced this by discovering Rh antigen, obtained from Rhesus monkey which was similar to the antibody described by Levine and Stenson in 1939 in the serum of a group 'O' mother (that was responsible for haemolytic disease of new born).[1,2,3] Subsequent to conflicting results in Rh grouping, a weakly reacting D antigen was described by Stratton in 1946.[4,5]

At present more than 50 antigens are there in Rh system but only D, C, c, E and e are important clinically significant antigens with respect to blood transfusion.[1,2] Weak D phenotype, formerly known as Du is a quantitatively weakened form of the D antigen. Weak D red cells have antigen, but have fewer D antigen sites per red cell than normal Rh positive cells. As D antigen is highly immunogenic, individuals with weak D phenotype are considered as Rh D positive when they are blood donors and Rh D negative when they are recipient of blood. [5,6,10] The most important risk with this phenotype is alloimmunization among the recipients. 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, may result in Haemolytic disease of newborn in subsequent pregnancy with Rh positive foetus.[13,11] Sensitization occur in any Rh negative individual if transfused Rh-positive blood, leading to Haemolytic Transfusion Reaction on subsequent transfusion of Rh positive blood.[11]

MATERIAL AND METHODS

The study was carried out from January 2021 to December 2021 in Department of Pathology (blood bank) at Government Medical College, Sir T hospital Bhavnagar which includes patients and donor. Two ml of blood samples collected in EDTA and Plain vacutainers were tested for ABO forward and reverse grouping and RhD typing using two different classes of anti-D reagents by immediate spin tube technique using reagents; monoclonal anti-D IgM and a blend anti-D IgM and IgG. The blood samples which were negative for agglutination by immediate spin method for RhD were further tested for weak D using IgG anti-D in the indirect antiglobulin test (IAT) with low ionic strength solution (LISS)/coomb's gel card.

OBSERVATION AND RESULTS

Table-1: Frequency of Rh-D antigen among Donors and Patients and total study population

RH STATUS	DONORS	PATIENTS	TOTAL
Rh D POSITIVE	16498(93.55%)	13257(93.72%)	29755
Rh D NEGATIVE	1136 (6.44%)	882(6.23%)	2023
WEAK D POSITIVE	05(0.030%) (0.44%)	04 (0.028%) (0.45%)	9 (0.028%) (0.44%)
TOTAL	17634	14139	31778

During the period of study, a total of 31778 blood samples were analysed. Out of 31778 samples 93.63 % (n=29755) were Rh-D positive and 6.36% (n=2023) were Rh-D negative. Among these 2023 Rh-D negative cases, 0.444 %(n=9) were weak D positive which is 0.028% of total samples. Out of total 17634 donors, 0.030 %(n=05) were turned out to be weak-D positive. Among 1136 Rh-D negatives donors 0.44% (n=05) were turned out to be weak-D positive. Out of total 14139 patients, 0.028%(n=04) were turned out to be weak-D positive. Among 882 Rh-D negatives patients 0.45% (n=04) turned out to be weak-D positive. [Table-1]

In our study prevalence of weak D is 0.19%, 0.58%, 0.52%, 210.46% and 0.46% among A, B, AB, and O negative individuals respectively. According to data of our study prevalence of weak D antigen is highest among B negative individuals. [Table 2]

Table-2: Distribution of Rh antigen among ABO Phenotype

BLOOD GROUP	Rh D POSITIVE		Rh D NEGATIVE		WEAK D POSITIVE	TOTAL
A	7144	93.53%	507	6.43%	1(0.19%)	7651 (24.07%)
B	10645	94.81%	681	5.19%	4(0.58%)	11326 (35.64%)
AB	2830	95.38	192	4.55%	1(0.52%)	3022 (9.50%)
O	9136	94.69%	643	5.26%	3(0.46%)	9779 (30.77%)
TOTAL	29755	94.57%	2023	5.40%	9(0.44%)	31778

DISCUSSION

The purpose of RhD typing is to avoid alloimmunization in RhD negative individuals and prevent blood transfusion hazards. Among the RhD-negative individuals, approximately 80% will develop anti-D after the first exposure to RhD-positive blood and only 7%–8% will remain nonresponsive.[2,8] Antibody response in Rh Negative persons can elicit even with exposure of 0.1 ml of RhD antigen positive cells. Once initiated Rh antibody production is irreversible & circulating antibodies last for years.[11] Even if the level of circulating antibodies falls below detectable threshold, subsequent exposure to antigen results in rapid secondary response and antibody formation.[2]

Due to genetic diversity among different study populations, different incidence rates of RhD negative and weak D have been reported around the world. The incidence of Rh negativity worldwide varies between 3% and 25% and that of weak D antigen from 0.2% to 1%.[7,8] With different races, prevalence of the different Rh group antigens varies. As example, the prevalence of D antigen in Indians is 93.6% whereas in our neighbouring country China, it was observed to be 99%. India being vast country with diverse population groups, it is required that phenotypic frequencies be studied in different parts of India. So, here we investigate the RhD status of population of Bhavnagar region of Gujarat.

The incidence of weak D antigen ranges from 0.01%- 0.4% among Rh negative individual in different regions of India.[5,7,14]. The results of weak D in Rh -ve and in total population was found in concordance with various other studies as shown in Table no 3. But, in our study a slightly higher incidence of weak D is found. The reason could be higher sensitivity of monoclonal anti-D compared to less sensitive polyclonal anti-D used in other studies.[6]

Table 3: Comparison between various study

Sr. No	Year	Author	Region	Weak D in Rh D-ve	Weak D in Total Population	Rh-ve	Rh +ve
1	2021	Our Study	Gujarat	0.44%	0.028%	6.36 %	93.63 %
2	2020	Afroz T	Bangl adesh	0.19%	0.008%	4.1%	95.9%
3	2018	Brar RK	Andaman Nicobar	1.51%	0.077%	5.14 %	94.86 %
4	2017	Lamba HS	Punjab	0.9%	0.06%	6.49 %	95.51 %
5	2016	Moxda S	Gujarat	0.326%	0.019%	5.89 %	94.11 %
6	2014	Kotwal U	Jammu	0.14%	0.0075%	5.48 %	94.5%
7	2015	Anshu Gupta	East Delhi	0.25%	7.69%	2.98 %	96.7%

It was also noticed that weak D positivity is maximum in B phenotype (0.58%) individuals which is similar to the study conducted by Anshu Gupta (0.4%).

Genes for the five Rh antigens are encoded by two autosomal dominant genes RHD and RHCE on chromosome 1.[1,5,7] An individual phenotype is reported as DCE.[12] Weak D expression results from single point mutations in RHD leading to amino acid changes in intracellular or in the trans membrane regions of RHD resulting in lesser number of D antigen.[5,7,15]

There are three genetic mechanisms postulated for weak expression of the D antigen:
(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 trans position 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.[5,7]

Depending on the variable expression of D antigen weak D and partial D are the two variants of D antigen due to many RhD alleles. Quantitative and qualitative changes in Rh protein result in Weak D and partial D respectively.[6] Rh positive RBC samples should react strongly (macroscopically) with anti-D sera when typed for the D antigen.[5] Weak D antigen is not detected by routine techniques (spin tube method) due to reduced D antigen expression on red cells. However, by prolonged incubation and the use of anti-human globulin this weakly expressed antigen can be demonstrated.

CONCLUSION

Compared to other study, our study has high prevalence of weak D amongst the population in Bhavnagar region, so in case of natural calamities, to tackle emergency situations for antigen negative blood, enlisting such donors and patients with Weak D expression, informing them about their Rh status and setting an alarming system in our software for future reference is beneficial. If the phenotyping of the person is known, suitable blood can be made available in appropriate time, even if he/she travels to other parts of the world. It is mandatory to perform weak D testing routinely in donors as well as patients who are negative with saline anti-D to prevent alloimmunization and for labelling of donors and patients.

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