Original Article

DOI: 10.7860/JCDR/2016/16278.7972

Pathology Section

Enigmatic Weak D antigen: An Experience in a Tertiary Care Hospital of East Delhi

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ABSTRACT

Introduction: The Rh blood group system is one of the most polymorphic and immunogenic blood group systems in humans. The expression of Rh blood group antigen is complex, among that Rh-D antigen is the most important antigen because of its immunogenicity. 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. Weak D reacts variably with anti D sera and poses a problem in blood banking. Molecular genetics of Rh-D revealed that weak D antigen is a Rh-D phenotype that possesses less numbers of complete D antigens on the surface of red blood cells.

Aim: Present study was carried out to study weak D positivity in a tertiary neuropsychiatry hospital of East Delhi for compatibility testing in blood transfusion, to assess the implications and need of weak D testing and for population genetics study. This study tried to observe pattern of weak D antigen in four broadly classified religious communities also (Hindus, Muslims, Sikhs and Christians).

Materials and Methods: This was a two years prospective hospital based study including patients as well as donors. All patients were tested for Rh-D factor by commercially available monoclonal anti-D sera. The individuals who were found negative with anti-D were further investigated for weak D antigen by using indirect antiglobulin test (IAT) by tube as well as gel card technique.

Results: The results were compiled by using SPSS software version 21.0 and Microsoft excel. Among 3619 cases, 3502 (96.7%) were Rh-D factor positive while 117(3.2%) were Rh D factor negative. Among these 117 Rh-D negative cases, 9 (7.6% out of total Rh-D negatives and 0.25% out of total samples) were weak D positive and 108(2.98%) were actually D negative individuals after IAT. Weak D positivity showed a slight predominance in females (55.5%). As per broad religious communities, weak D antigen was found in Hindus only and not observed in Muslims, Sikhs and Christians. In weak D positive individuals, B phenotype (0.43%) was found to be most common followed by A (0.26%) and O (0.2%).

Conclusion: Considerably high frequency of weak D antigen was noticed in study samples of this hospital. With this data based information, it is felt worthwhile to perform weak D testing routinely of those individuals who are negative with saline anti-D to prevent possibility of haemolysis and for efficient blood transfusion practices by making compatible blood available.

Keywords: Antiglobulin test, Antigen, Indirect, Neuropsychiatry

INTRODUCTION

In transfusion medicine, Rh system is a complex blood group system having 49 different antigens. Out of all these, D antigen is the most significant. D antigen has more than 30 distinct epitopes, more than 100 known haplotypes with similar phenotypes of different alleles [1,2]. D is often called the Rh antigen and the terms Rh positive and Rh negative refer to presence or absence of D antigen respectively. Eighty five percent of the Caucasian population is Rh-D positive while in India incidence of Rh positivity is 95% [3]. The incidence of Rh negativity worldwide varies between 3-25%. Weaker variants of D, previously known as Du, are defined as quantitative variations with presence of fewer than normal D antigen per red cell but have all the epitopes. Weak D is weakly immunogenic and requires detection by antihuman globulin. Incidence of weak D antigen ranges from 0.2-1% [4,5]. The incidence of weak D varies in different populations and in different geographic locales [5-7]. Partial D antigen lack one or more epitopes on red blood cells [8]. Thus RBCs having partial D antigen are agglutinated distinctively by some but not all monoclonal anti D reagents.

The main concern about weak D phenotype arises due to the risk of alloimmunization among the recipients and subsequent exposure to such red blood cells can lead to fatal haemolytic reaction or haemolytic disease of newborn in a sensitized pregnant female. Individuals whose RBCs carries a partial D phenotype could be sensitized to D epitopes lacking in their RBCs and are at risk during blood transfusion. Therefore, it is necessary to distinguish weak D from partial D [1].

AIM

The current study was designed to determine the frequency of weak D antigen in patients coming to this tertiary hospital so that recommendations can be formulated for considering weak D serology as a routine procedure to avoid transfusion related complications and misdiagnosis.

MATERIALS AND METHODS

This two years hospital based cross-sectional study was carried out at licensed blood storage centre under Department of Pathology from January, 2012 to December, 2014 in a 350 bedded neurosciences Institute catering to patients from Delhi, Ghaziabad, Bulandshahar, Baghpat, Muradnagar, Moradabad and Meerut districts of Uttar Pradesh and Karnal, Jind, Jhajjar and Sonipat districts of Haryana. This blood storage centre is attached to Regional blood transfusion centre, Guru Teg Bahadur (GTB) Hospital (mother blood bank) to meet its demand of whole blood and blood components. The sample study included patients, both admitted as well as attending outpatient departments, and donors. Informed consents of all the subjects were taken. 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. Rh-D forward typing was done using commercially available monoclonal anti D sera having blend of IgM and IgG (Tulip

diagnostics) by slide, conventional tube and gel card methods. 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 labeled 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 C_3d. 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 labeled as weak D positive. Only blood sample that was negative macroscopically and microscopically in the weak D test was labeled 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 microlitre of 1% RBC suspension was taken in microtube 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 [9].

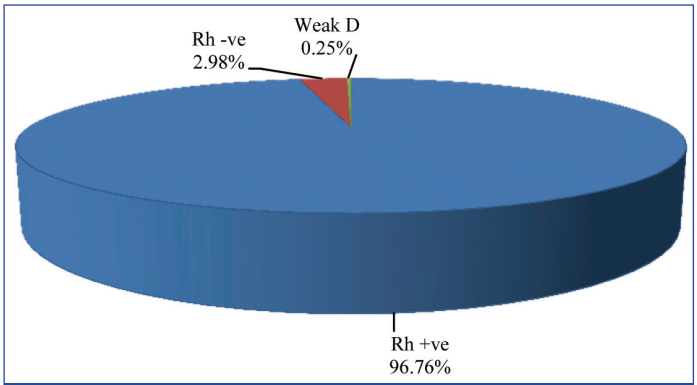
RESULTS

The results were prepared using statistical package for social sciences (SPSS) version number 21.0 and microsoft excel. Of the 3619 samples evaluated,117(3.2%)were found to be negative by tube technique. These samples were further evaluated for weak D positivity using antihuman globulin serum by tube as well as gel card technique. A total of 9 (0.25%) cases were found weak D positive with polyspecific AHG (anti IgG and C3d) and 108(2.98%) were actually D negative individuals after IAT as shown in [Table/Fig-1,2].

Rh-D status	No. of cases (n)	%age
D positive	3502	96.76
D negative	108	2.98
Weak D positive	9	0.25 (out of total cases) 7.69 (out of D negative)
Total	3619	

[Table/Fig-1]: Distribution of Rh-D antigen in total study samples.

[Table/Fig-3] showed RH-D status in both males and females. In total 1885 males, Rh-D antigen was found to be positive in 97.4%(1836), weak D in 0.2%(4) and negative in 2.3%(45). Similarily, out of 1734 females, D antigen was found to be positive in 96.0%(1666), weakly positive in 3.7%(63) and negative in 0.3%(5).Thereby, Rh-D negative and weak D indicated a slight predominant pattern in females.

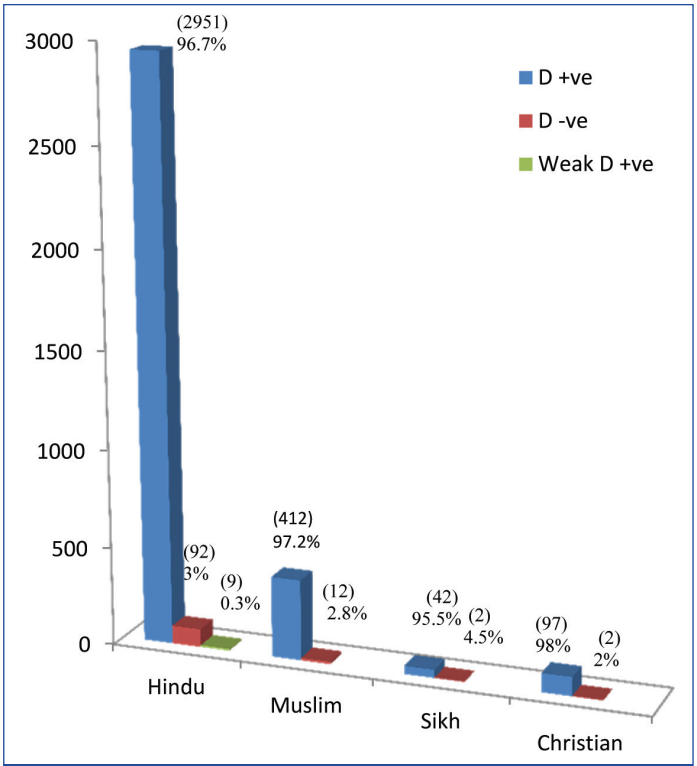


[Table/Fig-2]: Distribution of Rh-D antigen in total samples.

Rh-D status	Male n %		Female n %	
D positive	1836	97.4	1666	96.0
D negative	45	2.3	63	3.7
Weak D positive	4	0.2	5	0.3
Total	1885		1734	

[Table/Fig-3]: Distribution of Rh-D antigen in males and females.

Rh-D antigen was also studied in relation to various religious communities which are broadly classified into Hindus, Muslims, Sikhs and Christians. All 9 cases of weak D positivity were observed in Hindus only and none was reported in other religious groups as depicted in [Table/Fig-4].



[Table/Fig-4]: Frequency distribution of Rh-D antigen in various religious communities.

In [Table/Fig-5], ABO blood group phenotype was studied along with Rh-D antigen status. It was noticed that weak D positivity is found in maximum number (0.4%) of B phenotype individuals followed by A(0.26%) and O(0.2%) phenotypes.

DISCUSSION

Rh antigen was discovered by Levine and Stenson in 1939 which was the greatest breakthrough in transfusion medicine after the discovery of blood group system [10]. D antigen is the most

immunogenic antigen in the complex Rh system, discovered in 1939 by Fischer and Race. They postulated that three closely linked sets of alleles produced antigens of the Rh system, with each gene producing a particular product/antigen on the RBC surface. Antigens of the system were named as D (d), C, c, E, e (five major Rh system antigens). 'd' simply represents the absence of D antigen. Rh genes are co-dominant, each expressing its corresponding antigen on the RBC. An individual phenotype is thus reported as DCE [11].

D antigen is a mosaic of epitopes (antigenic determinants). 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 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 [11]. 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 [12,13]. This Du term was replaced by more appropriate term- the weak D antigen in 1984 [14].

Two genes– RHD and RHCE on chromosome 1, encode antigens of the Rh blood group. There are three genetic mechanism 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 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. This is termed as partial D antigen and these individuals may be alloimmunized if transfused with D positive blood possessing the missing epitope [12,14,15].

Blood Groups	Rh-D positive		Rh-D Negative		Weak D		Total
A	730	96.4%	25	3.3%	02/757	0.26%	757
B	1126	96.7%	33	2.84%	05/1164	0.43%	1164
O	1085	96.8%	34	3.0%	02/1121	0.2%	1121
AB	561	97.2%	16	2.8%	-		577
Total	3502	96.8%	108	2.99%	09/3619	0.24%	3619

[Table/Fig-5]: ABO phenotype with Rh-D antigen.

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. While some partial D, similar to weak D, results from point mutations in RHD that cause single amino acid changes in the extracellular regions and alter or create new epitopes [16-18]. The anti D produced by individuals expressing partial D can cause Haemolytic Disease of Newborn (HDN)/ transfusion reaction/ both.

Incidence of Rh negativity is 3-25% worldwide depending upon the ethnic group. Approximately 5% of Indian population is negative for the D antigen, though the incidence varies from community to community with 15-17% Rh negativity in Parsis, Chitrapur saraswat and Goans [14,15,19]. Our study of 3619 cases revealed 2.98 % of Rh negative subjects.

Incidence of weak D antigen ranges from 0.2-1% worldwide [14]. The incidence of weak D antigen is reported 0.23% to 0.5% in Europe and 3% in USA [20-22]. Studies conducted in India showed an incidence of 0.189% and 0.15% [5,14,22]. In our study, incidence of weak D antigen was 0.25% out of total individuals taken and 7.6% out of Rh-D negative individuals. In our study, potent monoclonal antisera were used with a high antibody titre. These antisera detected Rh-D positive cells that would be difficult to detect with less sensitive polyclonal reagents. Gel card system was also used for weak D detection which was more sensitive than tube method.

The incidence of Rh-D negative and weak D antigen is variably reported in different ethnic groups that have been summarized in [Table/Fig-6].

According to our study, weak D antigen was seen to be positive in Hindus only and not reported in other communities whereas Rh-D negative was reported in both Hindus and Muslims. This was an observation as bulk size of study group comprised of Hindus who had mainly migrated from different parts of country to Delhi.

Females had more weak D positivity than males in our study. Out of ABO blood group system, B phenotype individuals (0.4%) showed maximum number of weak D positivity followed by A(0.26%) and O(0.2%) phenotypes.

However, even after so many years of weak D antigen discovery, its clinical significance, immunogenicity and guidelines are controversial [14,15]. If weak D antigen is transfused to Rh D sensitized subject, it can result in haemolytic transfusion reaction. If weak D red cells are transfused to an Rh negative subject, it may lead to alloimmunization and recipients may develop anti D antibodies

Sl. No.	Year	Authors	Ethnic Group Studied	Region	Weak D(%) in D-ve in Total Population		Rh-ve (%)	Rh+ve (%)
1	2015	Our study	Indians-Hindus, Muslim, Sikh and Christians	East Delhi	7.6	0.25	2.98	96.7
1	2014	Kabiri Z [8]	Morocco	Moroccons	5	0.05	11	88.9
2	2014	Pahuja S [23]	Indians	Delhi	0.2	0.009	5.4	94.6
3	2014	Kotwal U[24]	Indians – *Hindus* (Dogras, Kashmiri pandits, Sikhs, Brahmins, Rajputs, Thakurs, Jats) & *Muslims* (Gujjars & non Gujjars)	Jammu	0.14	0.0075	5.48	94.5
4	2013	Usman M [20]	Pakistanis	Pakistan	0.8	0.05	6.9	93
5	2013	Sharma DC [25]	Indians	Greater Gwalior		0.036	8.9	91.1
6	2013	Agarwal N [26]	Indians	Northern hilly areas of Uttarkhand	0.09	0.005	5.2	94.8
7	2013	Das S [2]	Indians	Kolar and South Karnataka	0.15		12.76	88.8
8	2011	Acharya S [14]	Indians	Garhwal region of Uttrakhand	0.135	0.017	12.62	87.3
9	2010	Makroo RN [27]	Indians	Delhi	0.01	0.0086	7.19	92.81
10	2005	Kumar H [3]	Indians		0.189	0.18	6.3	93.7

[Table/Fig-6]: Percentage of weak D positivity in different ethnic groups by various studies.

[28]. But this is debatable because there are not enough cases to substantiate this contention. Only two case reports have reported alloimmunization of D negative patient following transfusion with weak D blood [3,14,15]. Another important fact is that if sensitized Rh-D negative women conceives Rh-D positive fetus, the passage of anti D antibodies across the placenta to the fetus results in HDN. It is mandatory to detect the weak D/partial D status of the donor but the recipient can be safely considered as Rh-D negative [29]. Therefore, most of the blood banks evaluate all D negative subjects for weak D antigen by AHG though the cost effectiveness of the same has never been studied [14,15].

Although it is difficult to differentiate between partial D and weak D, molecular analysis of RHD and RHCE genes can identify the majority of D variants in our population and also resolve serological discrepancies but that requires expertise in interpreting genes's many alleles.

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.

LIMITATIONS

The main limitation of our study is small sample size. Also, it is an observational study so causative association between weak D expression and various religious communities could not be established. Further, elaborative study is required involving larger sample size from different communities and using many antibodies panel to differentiate partial D antigen from weak D.

The main problem that arises from presence of weak D antigen is of conflicting laboratory reports, as to whether an individual is Rh-D positive or negative. Thus, there should be a standard protocol for investigating every case of Rh-D negative samples for weak D by IAT or more sensitive gel card system to prevent transfusion related complications and to avoid wrong diagnosis.

CONCLUSION

As D antigen is immunogenic and can produce alloimmunization if transfused to Rh-D negative subjects and with high frequency of weak D reported in our hospital, serological analysis at first glance seemed to be sufficient to make testing of weak D antigen mandatory in those individuals who are negative with saline anti-D although cost effectiveness, time and effort needed to be further evaluated along with clinical justification in future study.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 17, 2015**
Date of Peer Review: **Oct 21, 2015**
Date of Acceptance: **Apr 25, 2016**
Date of Publishing: **Jun 01, 2016**