Original Article

Evaluation of Prevalence of Weak D Antigen among Rhesus-Negative Patients in Tertiary Care Hospitals in Bangladesh: A Multicenter Study

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ABSTRACT

Background and Objectives: In 1939, D antigen was discovered which is believed to be the most immunogenic antigen in Rhesus (Rh) blood group system. There are some D variants such as weak D, partial D, and DEL due to gene polymorphism. These variants can cause RhD-positive person to behave like RhD negative which could result in alloimmunization. Clinically weak D antigen is very important due to its strong immunogenicity in spite of its low frequency. Hence, we need to know the prevalence of weak D variants in the community. The purpose of this study is to find out weak D prevalence among the Bangladeshi population. **Methods:** It is a retrospective study done over the last 5 years, from January 2015 to December 2019, at the department of transfusion medicine of three tertiary care hospitals in Bangladesh. A total of 177,702 patients were enrolled in the study. Blood samples that were negative for RhD were tested for weak D by indirect antiglobulin test according to institutional protocol. **Results:** Out of 177,702 patients, 7359 (4.1%) were found to be RhD negative and among those, 14 (0.19%) were weak D antigen positive. **Conclusion:** Weak D antigen is prevalent in Bangladesh and every RhD-negative individual should be checked for the presence of weak D to prevent RhD alloimmunization.

Keywords: *Bangladesh, D antigen, Rh negative, weak D antigen*

Introduction

After discovery of ABO grouping, the significant breakthrough in transfusion medicine was the discovery of rhesus (Rh) (International Society of Blood Transfusion no 004) blood group system. It is one of the most polymorphic and immunogenic blood group systems which consists of more than 50 antigens and among them, D is the most immunogenic. Molecular genetics has showed Rh system comprised 2 genes RHD and RHCE on chromosome 1. Each gene consists of 10 exons that encode 417 amino acid polypeptides.[1] Amino acid alterations in the RhD protein may result from mutations in RHD gene and the resulting phenotype is called D variant.[2] Serological studies separate D variant antigens into three broad categories, namely weak D, partial D, and DEL.

A weakly reacting D antigen was described by Stratton in 1946 as Du variant and later on renamed weak D.[3] Weak D is considered a quantitative alteration expressing the entire D antigen complex in reduced quantities. Flow cytometry shows that individuals with weak D had at least 10 times lower expression than RhD-positive individuals.[4] In 1999, Wagner *et al*. indicated that an amino acid change in the transmembrane and intracellular region of D antigen occurs due to point mutation in RHD gene affecting its insertion and hence density on the surface.[5] Other studies revealed that a weak expression of normal polypeptide can occur in a normal RHD gene with severely reduced messenger RNA transcript.[6] Three different genetic mechanisms are believed to be responsible for weak D expression:

1. A person may be inheriting the RHD gene which codes for weakly expressed D antigen

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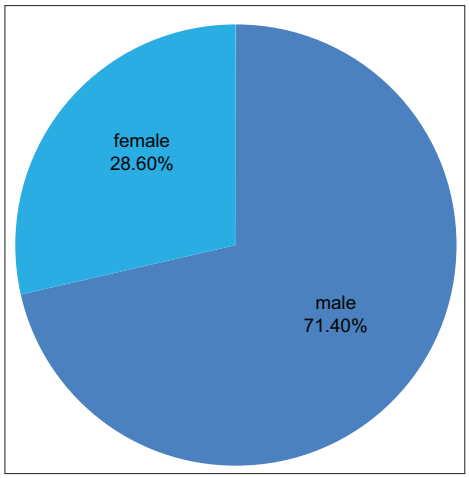

Chart 1: Distribution of weak D antigen-positive patients by gender

2. Presence of C antigen in the transposition on the conflicting chromosomes may cause weak expression of D antigens such as Dce/dCe genotype
3. Partial "D" antigen when one or more epitopes of the D antigen are missing, a weak D phenotype may be seen.[7]

Testing for D antigen is included in routine blood typing. Most serological weak D phenotypes are detected when RhD typing gives a weaker reaction (≤2+). Problems in immunohematological testing occur when blood donors are wrongly typed as RhD negative in spite of having a trace amount of RhD antigen which can cause alloimmunization. In many blood transfusion centers of developing countries like Bangladesh, test for weak D antigen is not carried out in RhD-negative individuals. Hence, we need to know the prevalence of weak D variants in the community.

Aims and objectives

The purpose of this study is to find out weak D prevalence among the Bangladeshi population.

Materials and Methods

This retrospective study was done at the department of transfusion medicine from three different tertiary care hospitals of Bangladesh. A total of 177,702 subjects were enrolled in the study from a period of January 2015 to December 2019. All data were obtained from patients' hospital records by electronic and laboratory systems. In the study, all the RhD-negative cases have been included for the analysis.

Routine ABO and RhD typing was done by column agglutination technique using Ortho BioVue ABD forward and reverse cassettes (Ortho Clinical Diagnostics, USA). Samples which were negative for agglutination with anti-D were further evaluated for weak D testing with Ortho BioVue®AHG polyspecific cassettes where 10 ml 3%–5% cell suspension, 40 ml anti-D (Monoclonal, IgM + IgG, Tulip Diagnostic P, LTD, India), and 50 ml Ortho BLISS solution were kept in Ortho BioVue Heat Block at 37°C for 10 min. Then, it was centrifuged for 5 min and the results were noted and compared with positive and negative controls. Weak D testing was also repeated through the conventional tube method. For the tube method, 3%–5% washed red cells and anti-D reagent (Tulip Diagnostic P, Ltd, India) were mixed and incubated at 37°C for 1 h. After centrifugation, the cell button was resuspended, and agglutination was read both macroscopically and microscopically. Reactions showing agglutination at this stage were reported as RhD positive. Then, the mixture was washed three times with normal saline. After the last wash, saline was decanted off and two drops of anti-human globulin (Polyspecific, Biorex Diagnostics Ltd) were added. The contents of the tube were mixed and centrifuged at 1000 rpm for 30 s. Macroscopic and microscopic agglutination was observed and any agglutination at this stage was recorded as serological weak D positive. Washed O-positive red cells with anti-D were used as a positive control and washed O-positive cells with 0.9% normal saline was used as a negative control. Direct agglutination test (DAT) was performed in all cases which tested positive for weak D antigen as false-positive results may occur in cases with positive DAT.

Statistics

Statistical analysis was done by computing percentages and calculating a 95% confidence interval for proportion by using OpenEPI Version 3.01. The pie chart was done by Microsoft Excel.

Ethics

Ethical approval was obtained from Research and Ethics Committee on March 7, 2021.

Results

Among the patients tested, 170,343 (95.9%) were RhD positive and 7,359 (4.1%) were RhD negative. Among the RhD negative, weak D was found to be positive in 14 patients (0.2%) with male predominance (male:female 2.5:1). All 14 samples which were tested for weak D were found to be DAT negative and weak D positive by tube technique also.

Table 1 and Chart 1 show the distribution of RhD among patients and gender-wise weak D positivity and Table 2 shows frequency of ABO group types among RhD negative patients.

Table 1: Distribution of RhD status among patients

	Total sample tested	RhD positive	RhD negative	Weak D percentage among RhD negatives	Weak D percentage of total
n (%)	177,702	170,343 (95.9)	7359 (4.1)	14/7359 (0.19)	14/177,702 (0.008)
95% CI		95.77-95.95	4.05-4.235	0.113-0.319	0.005-0.013

CI: Confidence interval

Table 2: Frequency of ABO group types among RhD-negative patients

Blood groups	*n*	Percentage (95% CI)
A RhD negative	1807	24.6 (23.6-25.6)
B RhD negative	2296	31.2 (30.15-32.27)
AB RhD negative	660	8.96 (8.34-9.64)
O RhD negative	2596	35.28 (34.2-36.4)

CI: Confidence interval

Discussion

The Rh positivity and negativity imply the presence or absence of D antigen on red blood cell (RBC) surface. The purpose of RhD typing is to ensure RhD-negative blood transfusion to negative recipients to avoid alloimmunization. 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.[8] This antibody is involved in erythrocyte immune-mediated destruction and hemolytic disease of fetus and newborn. Hence, appropriate assessment of RhD status is necessary. We investigated the status of patients in the Bangladeshi population.

Majority of the world's population is RhD positive. Different incidence rates of RhD negative and weak D have been reported around the world due to genetic diversity among different study populations. The incidence of Rh negativity worldwide varies between 3% and 25% and that of weak D antigen from 0.2% to 1%.[9] Our study revealed that out of 177,702 patients, 7359 (4.1%) patients were RhD negative and of negative patients, 14 (0.19%) expressed the weak D antigen. Makroo *et al*. found 7.19% RhD negativity in the Indian population and weak D in 0.01%.[10] Another study done by Krishna *et al*. reported the prevalence of weak D antigen to be 0.06% in the Indian population.[11] In the Pakistani population, the prevalence of RhD negative was found to be 13.7% and among them, 1% were weak D antigen-positive in Lahore, Punjab.[12] A study conducted in Africa by Okrah found 7.75% RhD negative and 6.45% weak D positive in blood donors which is slightly high.[13] Xhetani *et al*. reported 10.86% RhD-negative donors out of which 0.14% were weak D positive in the Albanian population.[14] The prevalence of weak D antigen is 0.5% in Europe, 3% in USA, and 0.8% in Brazil.[12,15,16]

Clinically, weak D antigen is very important due to its strong immunogenicity in spite of its low frequency. In a study, it was found that in childbearing women expressing weak D antigen, 90% of institutions transfuse RhD-positive blood components and only 10.2% transfuse RhD-negative components.[17] Hemolytic disease of fetus and newborn can occur in an already sensitized pregnant female with weak D-positive fetus. Hemolytic transfusion reaction may occur in a sensitized RhD-negative individual when transfused with weak D antigen-positive blood. There are several evidences reporting the development of alloimmunization due to weak D or partial D.[3,18-20] However, there are not enough evidence to prove this. In a follow-up study of 45 RhD-negative cases who were transfused with weak D antigen-positive blood, none developed anti-D antibodies even though the RBCs remained in the circulation for 100 days in 34 cases.[21] Hence even after several years of discovery of weak D antigen, its clinical significance, immunogenicity, and guidelines are controversial. However, it is usually recommended to consider recipients with weak D antigen positive as RhD negative and must be given RhD-negative blood and donors are considered as RhD positive.[22]

Limitations of the study were molecular genotyping could not be done and also from these data, we cannot generalize the prevalence of weak D as it was done in a metropolitan area and more rural areas need to be included. Further research can be done to find out the prevalence of weak D among the Bangladeshi population from this study.

Conclusion

The study result shows that the prevalence of weak D antigen is considerable in Bangladesh. Considering the safety issue of blood transfusion, it is worth recommending that in all blood transfusion centers, RhD-negative blood samples should be further tested for weak D antigen.

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Conflicts of interest

There are no conflicts of interest.

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