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Original research article

Comparative study of the use of column agglutination technology for detection of weak D compared to conventional tube technique

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Abstract

Background: Rh is one of the most polymorphic and immunogenic blood group system with more than 50 antigens, of which Rh D is the most immunogenic. The red cells giving negative reactions in anti-D test and positive in anti-human globulin phase are considered as weak D. This weak D test isdone by Conventional tube technique. However, Column agglutination technology is considered to be more sensitive than conventional tube technique. This study intends to identify if Column agglutination technology identifies more weak D than conventional tube technique.

Methods: This study was conducted in a tertiary care hospital in Hyderabad for a period of six months. The patients who were typed Rh D negative were further tested for weak D by both CAT and CTT with IgM, IgM + IgG anti-D sera and the results were compared.

Results: Of the 3132 blood samples tested 153 were Rh D-negative (4.8%) of which 78 (51.4%) were O negative, 48(31.4%) were B negative, 21(13.7%) A negative and 6 (3.9%) AB negative. Among these samples5 were positive for weak D (3.3%), of which 3 were belonging to O and one each belonging to B and AB blood groups. The grade of the reaction for the weak D samples was 2-3+ in AHG phase with IgG alone &IgG + IgM (blend) anti-D in 4 of 5 samples tested by CTT

Conclusion: Weak D test by CTT may miss a few weak D when compared to CAT due to lower sensitivity. However further studies regarding this phenomenon and clinical significance for the weak D needs to be evaluated.

Keywords: Weak D, allo-immunization, haemolytic reaction, haemolytic disease of foetus and new born

Introduction

The discovery of Rhesus (Rh) blood group system, which is one of the most clinically important protein-based blood group system [1] was a major breakthrough in the field of transfusion medicine. It is one of the most polymorphic and immunogenic with more than 50 antigens, of which D being the most immunogenic [2].

The two genesRhD and RhCE genes are located on chromosome 1 encode the erythrocyte Rh proteins, RhD and RhCE in which one carries Rh D gene and the other carries Rh CE gene [3]. Mutations in the RhD gene results in amino acid alteration in the Rh D protein which results in a phenotype termed as D variant. Serological studies separate D variant antigen into: weak D, partial D, weak partial D, DEL.

Human red blood cells are classified as Rh (D) positive or Rh (D) negative depending upon the presence or absence of Rh (D) antigen on them. Most Rh (D) positive red cells show clear positive results with Anti-D reagent. The red cells giving negative or weaker reactions in direct Anti-D test and positive by Indirect AntiglobinTest after incubation are considered as weak D. A weakly reacting D antigen was described by Stratton in 1946 as Du variant and later named as weak D [4].

Weak D expression results from single point mutations in RHD leading to amino acid changes in the intracellular and transmembrane regions of RHD resulting in lesser number of D antigen on RBC surface [5]. Though patients with weak D are considered as RhD negative as recipient of transfusion, however,

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transfusion of those cells from a weak D donor to a person who is RhD-negative recipient may result in alloimmunization; and subsequent exposure to such red cells may result in fatal hemolytic reaction. Weak D testing is generally done by CTT using IgG Anti D or IgG + IgM (blend) of Anti D. However CAT is more sensitive to CTT in identification of sensitised RBC. Therefore this exploratory study aims to identify if there any difference in weak D results by testing with CTT and CAT.

Materials and Methods

The prospective exploratory observational study was conducted at the Department of Transfusion Medicine at Kamineni hospitals Hyderabad from September 2022 to February 2023. During this period a total of 3132 samples were included. As a part of routine protocol ABO grouping and Rh typing was done by Conventional tube technique (CTT)/Column agglutination technology (CAT) (Ortho clinical diagnostics). Those who tested negative for D antigen, were further subjected to weak D testing in Conventional tube technique and Column agglutination technology. 100µl of anti-D antisera (IgG, IgG+ IgM) was added respectively in two test tubes followed by the addition of 50µl of patient red cell suspension. The IgG & IgG + IgM blend test tubes were incubated for 45minutes at 37ºCfollowed bycell wash for 3 times and addition of 50µl of AHG and the results were read after centrifugation for1minute at 1000rpm. The IgM anti-D test tube was read after 5 minutes incubation at room temperature followed by centrifugation at 1000rpm for 1minute.

For Column agglutination technology by using Matrix gel cards (Tulip diagnostics India)50µl of 0.8% patient red cell suspension was added to each microwell, followed by addition of 25µl of anti-D IgG& 25µl of IgG+IgM (blend) Anti D. The gel cards were incubated for 15minutes at 37 ºC, followed by centrifugation at 1500rpm for 10 minutes. Theresults were graded based on the level where agglutinates appeared. The results were graded as:

1. **0:** agglutination is not observed with the sample. Passage of red cells across the gelmedia will be exhibited
2. **1+:** agglutinates of red cells observed in the lower half of gel column
3. **2+:** distribution of red blood cell agglutinates throughout the column
4. **3+:** majority of agglutinates of red blood cells amassing in the microtube top area
5. **4+:** solid band of agglutinated red blood cells observed at the gel column top area

Results

Out of a total 3132 samples, 2979 were Rh D positive and153 samples were Rh D antigen negative. The negative samples were further subjected to weak D testing as we have discussed above. The frequency of ABO blood group in the Rh negative sample population is as shown in table-1, with ORhD negative group frequency of (51%)

Table 1: different blood groups& Rh status

Blood groups	Blood grouping, typing& du test		
	Frequency (n=153)	Percentage (%)	Number of Du positive
A RhD negative	21	13.7%	-
B RhD negative	48	31.4%	01
AB RhD negative	06	3.9%	01
O RhD negative	78	51%	03
Total	153	100%	05

Out of 153 RhD negative samples 5 samples were weak D positive, of which3 were blood group O and one each were B and AB blood groups as depicted.

Out of the 5 samples that were tested with Anti D IgG+IgM (blend) using CTT and CAT, 4 samples showed same results while one sample had weakly positive reaction in CTT but showed 3+ positivity in CAT IgG+IgM (blend). The reaction of each sample with IgG, IgM and IgG+IgM (blend) by CTT and IgG anti D and IgG+IgM (blend) by CAT are mentioned table 2

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Fig 1: Reactions of the discrepant sample

Table 2: Reaction of each sample

Samples	Anti-D (IgG)	Anti-D (IgM)	Anti-D(IgG+IgM)	CAT (IgG)	CAT (IgG+IgM)
Sample 1	3+	0	3+	3+	3+
Sample 2	3+	0	3+	4+	4+
Sample 3	3+	0	2+	4+	4+
Sample 4	2+	weak	2+	3+	3+
Sample 5	weak	0	weak	3+	3+

Discussion

The Rh system is the most important blood group system after ABO.It is highly polymorphic, complex and immunogenic with more than 50 antigens, of which Rh D is the most immunogenic [2]. Red blood cells are classified as Rh positive and Rh negative based on the presence or absence of D antigen. Majority of the world population is Rh D positive. The incidence of RhD negativity worldwide varies between 3%-25% and the incidence weak D antigen varies from 0.2% to 1% [6]. In our study we found 153 samples out of 3132(4.88%) of our population was Rh D negative of which 5 samples (3.3%) were weak D, majority of these belonged to O group (51%) followed by B (31.4%), A(13.7%) and AB blood group(3.9%). In the study done by Amit Agarwal *et al* [7] the predominant blood group was O in the southern part of India(40.05%) followed by B(27.39) unlike the northern part of India where B is the most common blood group(37.50%) followed by O(32.50%)

The major concern during clinical transfusion practice is to prevent D alloimmunization among the Rh D- negative individuals. Approximately 80% of them will develop anti-D after the first exposure to Rh D- positive blood and only 7-8% will remain nonresponsive [8]. In 1946 Stratton described D variant as weak D or Du for those red blood cells that reacted with a variable intensity with the anti-D sera.

The incident rates of Rh D negative and weak D which have been reported around the world are different due to genetic diversity among different study populations.

The discrepancies in Rh D typing may due to various reasons such as [9]:

1. Testing methods(tube, microplate, column agglutination technology)
2. Saline or Coombs phase of testing
3. Specificities and avidity of anti-D sera.

The number of D antigen sites on the Rh (D) - positive red cells is normally in the range of 9900 to 33000. The weak D phenotype appears to be a quantitative variation in the number of D antigen sites on the red blood cells with 110 to 9000 per red blood cell [10].

There are three genetic mechanism postulated for the weak expression of the D antigen these are:

1. Individuals who inherit the RHD gene which encode for a weakly expressed D antigen.
2. D antigen is weakly expressed due to the presence of C antigen in the trans position on the opposite chromosome 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 [11].

Weak D expression results from single point mutations in RHD leading to amino acid changes in the intracellular and transmembrane regions of RHD resulting in lesser number of D antigen on RBC surface [5]. Though patients with weak D are considered as RhD negative as recipient of transfusion, however, transfusion of those red cells from a weak D donor to a person who is RhD-negative recipient may result in alloimmunization; and subsequent exposure to such red cells may result in fatal hemolytic reaction. Mothers with weak D foetus should also receive Rh monoprophylaxis as passage of weak D cells from the foetus to the mother can cause sensitization and may result in haemolytic disease of new born in subsequent pregnancies

In our study, after typing for Rh D antigen and these samples were further tested for weak D. Out of 153 RhD negative samples which were collected 5 of them were tested positive for weak D- (3.3%).

In our study majority of Rh D negative belonged to O (51%) followed by B (31.4%), A (13.7%) & AB (3.9%).

The prevalence of weak-D in our study is (3.3%), compared to0.01%-0.06% [12, 13] in Indian population, 0.19% [2] among Bangladeshi population, 1% [14]. In the Pakistani population, 0.14% in the Albanian population [15], 0.5% in Europe, 3% in USA and 0.8% in Brazil [14, 16, 17]. The molecular basis for the higher weak D prevalence in our population needs to be explored.

The higher prevalence rate of (3.3%) could be due to use of more sensitive technique of CAT, where Number of IgG molecules/ red cells is (120-180) can be detected compared to CTT which has sensitivity of 300-500 [18] Number of IgG molecules/ red cells. In our study 1sample of the total 5 samples reported a weaker reaction with the CTT. Further testing using molecular genotyping to identify this phenomenon and testing its ability to sensitize RhD negative individuals on transfusion may help in determining the

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clinical significance.

Conclusion

A number of weak D phenotypes may be missed on conventional tube technique as the number of sensitized Rh D antigens on the surface of RBCmight be lesser than the threshold of detection by CTT compared to CAT. Further testing may be required to establish the clinical significance of this phenomenon.

Conflict of Interest: None

Funding Support: Nil

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