DETERMINATION OF WEAK DU ANTIGEN AMONG RHEUSUS NEGATIVE BLOOD DONORS

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Abstract

Introduction: Rh antigen is major blood group of Rh blood group system. The ''Weak-D'' antigen indicates the presence of aberrant expression of Rh -D protein on red cells. In vitro, Rh antigen does not react routinely by agglutination with potent monoclonal anti – D antisera, but it requires adding up of antiglobulin serum to detect the presence of antigen. Expression of weak D antigen is necessary in certain cases where risk of sensitization and allo-immunization is present.

Objective: The main objective of this study is to detect the presence of ''Weak D'' antigen in Rh-Negative blood donors.

Methodology: This study was conducted at Department of Pathology, Indus Medical College, Tando Muhammad Khan during the period of 6 months (November 2017 to May 2018). A total of 2281 participants were included in this study. 3mL blood was collected from each participant for detection of ABO and Rh blood typing. Rh -Negative individuals were further evaluated for the presence of ''Weak-D antigen'' with the addition of anti-human antiglobulin. Data was analyzed using SPSS 21.0. P – value of <0.05 was considered as statistically significant.

Results: Out of 2281 participants, 2122 (93.02%) were Rh-Positive while 159 (6.97%) were Rh-Negative. Rh-Negative individuals were evaluated for the presence of weak D antigen. Out of 159 Rh-negative individuals, 8 (5.03%) were positive for weak D antigen with P – value of <0.001.

Conclusion: Overall incidence of weak D antigen in our study was 0.3%. Detection of weak D antigen is not routinely performed in laboratories, but their presence may be important factor in certain cases where risk of sensitization and allo -immunization is high.

Keywords: Weak – D antigen, Rheusus, Blood Group, ABO, Sensitization, Allo - immunization

INTRODUCTION:

After discovery of ABO blood grouping system, the greatest evolution was the discovery of Rh antigen in 1939. However, contradictory reporting of Rh blood grouping, a week D antigen was found in 1946. The incidence rate of Rh negativity globally is approximately

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3-25% and weak D antigen is present in 0.2-1% population. (1,2) The greatest evolution in the field of transfusion medicine RhD antigen contains a variant weak RhD phenotype that does not react by agglutination with routine potent monoclonal serum (anti-D)', though requiring addition of antiglobulin serum to evaluate the presence of D antigen. (3)

Weak D phenotype of Rh antigen is shown to be come up from three mechanisms. One mechanism is gene interaction; in which there is C gene is dormant when in trans to D gene (i.e. Dee/Ce). Second mechanism causes absence of fraction of D antigen (partial D). Third mechanism is occurrence of atypical form of D antigen which causes fragile expression of phenotype. Individual will be referred to as ''Weak D'', indicating aberrant RhD protein on red cells and express reduced D antigen membrane surface. Partial/weak D antigen sometimes shows positive phenotypic reactions in serological procedures. (4–6) When tested with anti – D reagents, Du red cells show various reactivity. (7) This study was conducted to determine the presence of Du antigen among individuals with RhD negative phenotype.

METHODOLOGY:

This was a prospective study, conducted at Department of Pathology, Indus Medical College Tando Muhammad Khan. This study was performed between November 2017 to May 2018. A total of 2281 participants were included in this study through probability consecutive sampling technique. From each blood donor, 3mL blood was collected by sterile and standard venipuncture technique in EDTA tube.

All blood samples were processed for blood group typing (ABO and Rh). ABO blood group typing was determined by making suspension of 5% red blood cells and were mixed with anti-A and anti-B antisera in test tube at standard room temperature. Samples were then centrifuged and examined for presence or absence of agglutination both macroscopically and microscopically. Rh blood group typing was determined by immediate spin tube method. Suspension of 5% red blood cell was mixed with anti-D antisera in test tube. Samples were centrifuged and examined both macroscopically and microscopically. Rh – positive reaction was labelled as proper agglutination.

All samples labelled as ''Rh-Negative'' were further processed for the presence or absence of ''Weak D antigen'' by Du testing. Balanced amount of 5% washed red blood cells and anti – D reagent were mixed, followed by incubation at 37OC for 30 minutes. After centrifugation cells were re-suspended and observed for agglutination both macroscopically and microscopically. Samples which showed agglutination were labelled as ''Rh-positive''. In samples with no agglutination, the mixtures were washed with normal saline three times. After washing, saline was poured off and mixed with two drops of Anti – Human Globulin (AHG). After centrifugation, samples were examined both macroscopically and microscopically for the presence or absence of agglutination. Agglutination at this stage was labelled as ''Weak – D positive reaction''. The positive control was made up of check cells, consisting of washed ''O'' positive cells with anti – D. The negative control consisted of washed ''O'' positive cells with normal saline (0.9%).

Data was analyzed using SPSS 21.0. P – value of <0.05 was considered as statistically significant.

RESULTS

A total of 2281 participants were included in this study. All participants were categorized according to ABO and Rh blood group systems. Most common blood group found was ''O-Positive'', followed by ''A-Positive''. Least common group found was ''B-Negative'' (Figure 1 and 2). Weak D antigen was also determined from all Rh – negative individuals. 8 out of 159 Rh-negative individuals were turned out to be weak D positive. The frequency of weak D positivity was 5.03% out of all Rh -negative individuals and 0.3% from total participants (Table 1 and 2).

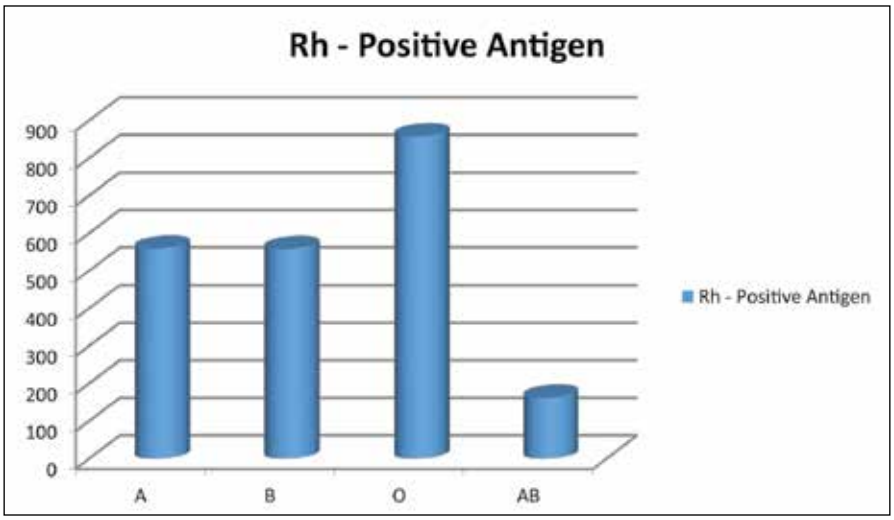

Figure 1 Rh-Positive Antigen in Blood Donors (n=2281)

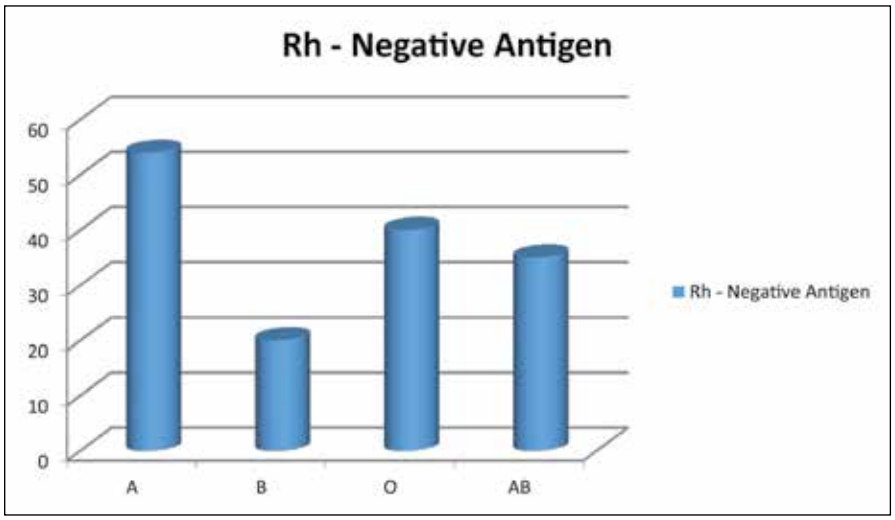

Figure 2 Rh-Negative Antigen in Blood Donors (n=2281)

Table 1: Blood Groups in Blood Donors (n=2281)

Blood Group	Number of Blood Donors	Rh Antigen		P – value
		Positive	Negative	
A	613	559	54	<0.001
B	578	558	20	<0.001
O	898	858	40	<0.001
AB	192	162	30	<0.001
Total	2281	2122	159	<0.001

Table 2: Weak D Antigen in Rh-Negative Blood Donors (n=2281)

Total Rh Negative	Weak D Antigen		P – value
	Positive	Negative	
159	8 (5.03%)	151 (94.96)	<0.001

DISCUSSION

The discovery of Rh antigen made revolutionary success to ensure safe blood transfusion in the field of transfusion medicine. (8) The presence of weak D antigen has not been evaluated to a larger extent. As weak D antigen is usually reported as Rh-negative blood type, so transfusion of Rh-Negative/Positive type does not cause transfusion reaction. Although identification of weak D antigen may be significant in certain cases. Transfusion o red cells containing weak D may have allo -immunization or risk of sensitization in patients with Rh-negative phenotype. Likewise, hemolytic disease of the newborn can occur in pregnancy females with Rh-negative phenotype carrying babies with weak Rh-positive phenotype. (3,9) Red cell surface expresses Rh with the association of RhAg, a glycoprotein. This protein has 36% sequence identity with Rh protein. This protein is located at chromosome 6. The Rh blood groups is expressed by 2 proteins that are non-palmitolyated and non-glycosylated and are encoded by 2 homogenous and identical genes RHD and RHCE that are located on chromosome 1. (10–12) Our study showed 5.03% incidence of weak D antigen from individuals with Rh-negative phenotype and 0.3% from all participated individuals. This incidence is variable among races and geographical distribution. Kumar et al showed the frequency of 0.18% weak RhD antigen from blood donors. (13) Makroo et al showed that weak D antigen was present in 0.01% of blood donors at Delhi. (14) Agarwal et al showed the incidence of weak D antigen as 0.005%. (2) Usman et al showed that weak D antigen was present in 0.8% of blood donors. (15) In contrast, study from Africa by Okrah et al showed incidence of 6.45% weak D positive phenotype which varies from other studies. (16) This indicates variation of weak D antigen due to geographical distribution.

CONCLUSION

In our study, the incidence of weak D antigen was 0.3% from all blood donors and 5.03% from all Rh-negative individuals. This ratio vary in different populations are races. Though not performed routinely, its clinical implementation is important in certain cases to avoid sensitization and allo-immunization.

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