Prevalence of Weak D Antigen In Western Indian Population

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ABSTRACT

Introduction: Discovery of Rh antigens in 1939 by Landsteiner and Weiner was the revolutionary stage in blood banking. Of these antigens, D, which decides Rh positivity or negativity, is the most antigenic. A problem is encountered when an individual has a weakened expression of D (Du), i.e., fewer numbers of D antigens on red cell membrane. **Aims and Objectives:** To know the prevalence of weak D in Indian population because incidence varies in different population. To determine the risk of alloimmunization among Rh D negative patients who receives the blood of weak D positive donors. **Material and Methods:** Rh grouping of 38,962 donors who came to The Department of Immunohematology and Blood Transfusion of Civil Hospital, Ahmedabad from 1st January 2013 to 30th September 2014 was done using the DIAGAST (Automated Grouping). The samples that tested negative for D antigen were further analysed for weak D (Du) by indirect antiglobulin test using blend of Ig G and Ig M Anti D. This was done using Column agglutination method in ID card (gel card). **Results:** The total number of donors studied was 38,962. Out of these 3360(8.6%) were tested Rh D negative. All Rh D negative donors were tested for weak D (Du). 22 (0.056% of total donors and 0.65% of Rh negative donors) turned out to be weak D (Du) positive. **Conclusion:** The prevalence of weak D (Du) in Western Indian population is 0.056 %, So the risk of alloimmunization in our setting due to weak D (Du) antigen is marginal. But, testing of weak D antigen is necessary in blood bank because weak D antigen is immunogenic and can produce alloimmunization if transfused to Rh D negative subjects.

Keywords: Immunogenic, Weak D antigen, Western Indian population.

Introduction

In transfusion medicine, the discovery of the blood group antigen is the revolutionary step in the history of blood transfusions. Following the discovery of ABO antigens by Landsteiner in 1901,[1] the next most important discovery was that of Rh antigens in 1939 by Landsteiner and Weiner further leading to description of haemolytic disease of new born by Levine and

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Genetically Rh system is one of the most complex blood group systems in humans. The Rh gene lies on chromosome number 1 and is carried in group of three [3]. The Rh locus is composed of two highly homologous genes: the RHD gene, which encodes the D protein, and RHCE gene, which encodes the c, C, e and E proteins[4]. 6 alleles have been identified (c, C, e, E, d, D) as against only 5 antigens (c, C, e, E, D), d being an amorph gene[5]. Among these, D is the most immunogenic. Consequently, D often is called the Rh antigen, and the individuals who possess Rh D antigens are known as Rh +ve and those who lack Rh D antigens are typed as Rh-ve.

Rh D antigen consists of mosaic of at least 9 epitopes (epD1-epD9)[6]. Recent work has suggested the presence of a minimum of 30 different epitopes distributed along the extracellular portions of Rh D protein[7]. Thus a change in amino acid sequence of Rh D may not ablate the entire D antigen but can cause epitope loss, giving rise to variant forms of D antigen. Most common of them are Weak D, Partial D and DEL phenotype.

"Weak D" RBCs demonstrate reduced quantities of D antigen. As a result, weak or no agglutination reaction is demonstrated by these RBCs with anti D reagents at the immediate spin phase. About 0.1 to 2 percent of white Caucasians have this Rh phenotype[8]. Missense mutations observed in the alleles of all weak D type have been demonstrated to be the probable cause for the reduced antigen D expression in these cases[9].

Materials and Methods

This study was conducted in our Tertiary Care Teaching Centre, Ahmedabad from 1st January 2013 to 30th September 2014. During this period, a total of 38,962 donors came to our blood bank. As a routine protocol, Rh typing was done for all these donors. Those who tested negative for Rh D antigen were further subjected to weak D testing.

Routine Rh typing was done in DIAGAST using principle of Erythrocyte Magnetized Technology. Groupa 2 Lys kit was used in DIAGAST. Groupa2lys plate contains wells which are coated with anti A, anti B, anti D which is Ig M monoclonal. Antisera used for Rh typing were antiD1 (IgM) and antiD2 (blend of IgM and IgG).

Weak D testing of all negative donors was done in ID card (gel card) using indirect antiglobulin test. 50ul of 1% suspension of donors' red cells was added to microtube of an ID card labelled with donor unit number. To this microtube 25ul of D2 (blend of IgG and IgM) was added. Then ID card was incubated in dry incubator at 37 C for 15 minutes and centrifuged for 10 minutes in ID centrifuge.

For the interpretation of result, if red cells settle to the bottom of microtube then it is weak D negative. In weak D positive sample, red cell agglutinates are trapped in gel matrix.

Results

A total of 38,962 donors were studied. Among these 3360 (8.6%) were tested to be Rh negative. All the Rh negative samples were subjected to weak D testing. Of the 3360 samples that tested to be Rh D negative, 22 (0.056% of total donors and 0.65% of Rh negative donors) turned out to be weak D (Du) positive.

Image -1 Chart showing Prevalence of Rh negative donors

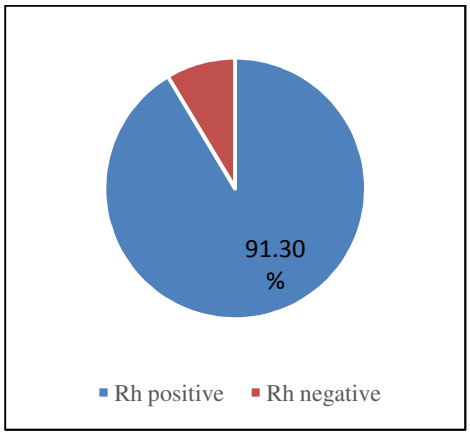
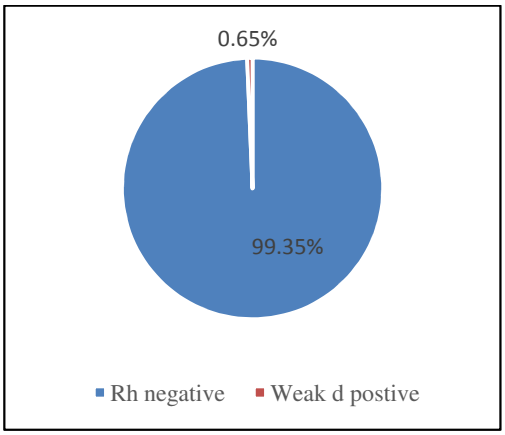


Image-2 Chart showing Prevalence of weak D positive donors among Rh negative



Discussion

In our study weak D variant was detected in about 0.056% of total donors. Our hospital, being a major tertiary care centre of the country, caters to a population covering large geographical areas and therefore our results can be considered as representative of the western Indian Population.

Table 1: prevalence of weak D antigen in different study

	Present study	**Study done by R. N. Makroo and Vimarsh Raina.[10]**
Prevalence of Rh negative donors	8.6%	7.2%
Prevalence of weak D among total donors	0.056%	0.01%

Prevalence of weak D is slightly higher in our study compared to study done by R. N. Makro and Vimarsh Raina[10]. showing 0.01% of total donors, probably because of use of column agglutination technology which is more sensitive than tube technique.

Rh system is the most complex of all blood group systems. New discoveries relating to RHD gene and its variant phenotype have challenged the way that D status is assigned to both blood donors and recipients. The weak D has been the subject of many studies ever since it was identified. RBCs that react with anti D only after extended testing in the AHG phase are called weak D.

The number of samples classified as weak D depends on the characteristics of the typing reagent[11]. The improved sensitivities of anti D sera have decreased the prevalence of weak D phenotypes. The prevalence also varies from region to region. We reported weak D in 0.056% of our donor population. Slightly lower values have been reported in Korean study and higher values have been reported in Caucasians[12, 13].

The main concern about this Rh phenotype arises due to the risk of alloimmunization among the recipients. Since, the “D” antigen is highly immunogenic; donors with the weak D

phenotype are designated Rh positive. The patients with weak D phenotype are considered Rh negative.

Rh negative mother with weak D foetus must receive Rh immunoprophylaxis[14] as passage of weak D red cells from foetus to mother may cause sensitization.

Conclusions

The prevalence of Rh D negativity in our setting is estimated to be about 8.6% and that of weak D antigen is 0.056 % that is very small. Several research studies proved that weak D antigen is immunogenic and can produce alloimmunization if transfused to Rh D negative subjects.

So however, the risk of alloimmunization in our setting due to weak D (Du) antigen is marginal study of Rh D negative patients with weak D alleles who have been exposed to Rh D positive RBCs is needed to quantify the absolute risk of sensitization.

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