Weak D antigen - Revisited

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Abstract

D antigen is the most immunogenic antigen in the complex Rh blood group system discovered in the year 1939. There is a lot of polymorphism in its phenotype due to genetic heterogeneity. Certain mutations and/or deletions lead to a weak phenotype defined by decreased density of antigen sites which require the use of anti human globulin for detection. The need for detection of the weak D antigen was to prevent alloimmunization by this blood if transfused to a D negative patient especially to women in child bearing age group. This contention is however, controversial and not proven beyond doubt. Moreover, the use of potent monoclonal D typing antisera detects low density of weak D antigens thus obviating the use of anti human globulin.

We have assessed the incidence of Rh negative and weak D blood groups in the Garhwal region of Uttarakhand and reviewed the literature regarding the controversies in the clinical significance of weak D antigen.

Keywords

incidence, weak D antigen, anti human globulin, alloimmunization, immunoprophylaxis

Introduction

Following the discovery of the ABO blood group system, the greatest breakthrough in transfusion medicine was the discovery of the Rh antigen by Levine and Stetson in 1939. Its role in hemolytic disease of the newborn cannot be overemphasized. Subsequent to conflicting results in Rh grouping, a weakly reacting D antigen was described by Stratton in 1946.

Weak D represents a D phenotype where due to decreased antigen sites the antigen is not detected by routine grouping (using immediate spin tube methodology). Demonstration of this weakly expressed antigen requires evaluation by prolonged incubation and use of antihuman globulin.

The incidence of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%-1%.

Fifty years after the description of weak D antigen much debate over its clinical significance and reagents used for detection continue. In weak D positive pregnant women guidelines regarding Rh group to be transfused and postpartum immunoprophylaxis are not established.

In the present study we have sought the incidence of Rh negative blood group and that of weak D antigen in the Garhwal region of Uttarakhand state and have reviewed its mode of inheritance and clinical relevance of additional testing for weak D antigen.

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Aims and Objectives

This study was conducted with the aim of seeking the incidence of Rh negativity and weak D antigen in Garhwal region of Uttarakhand and to review the literature regarding mode of inheritance of weak D antigen. The objective being, a review of the clinical significance of weak D antigen and the justification of testing for it.

Materials and Methods

Rh blood group and typing of all donors and patients in blood transfusion centre under the Department of Pathology, SGRIMS, Dehradun Uttarakhand, was analyzed for a period of four and a half years from 01 Jan 2006 to 15 June 2010.

There were a total of 5855 subjects of which 565 were antenatal cases registered with the Obstetric & Gynaecology department of our hospital. Routine Rh typing was done using the immediate spin tube technique (using monoclonal anti D antisera, IgG & IgM from two different companies, Diamed and Tulip).

Samples which were negative for agglutination by both the antisera were further evaluated. Equal volume of 2-3% of washed cells and antiD sera were mixed and incubated at 37°C for 45minutes. The cell button was resuspended and agglutination was looked for. In presence of macroscopic or microscopic agglutination the sample was recorded as Rh positive. In case there was no agglutination the mixture was washed 4 times with normal saline. After the last wash, saline was decanted and 2 drops of monoclonal, polyvalent anti human globulin was added. The contents of the tube were mixed and centrifuged at 1000 rpm for 30 seconds. Macroscopic and microscopic agglutination was looked for and any agglutination at this stage was recorded as weak D antigen. Positive control (check cells i.e washed O positive cells with diluted antiD) and negative control( washed O positive cells) were always put. All negative results were observed for agglutination after addition of check cells.

Results

Rh grouping was done in a total of 5855 samples of which 739 samples (12.62%) were negative by immediate spin tube method or on prolonged incubation.

Of the 739 Rh negative samples, 738 samples were negative even after AHG (anti human globulin) phase of testing. There was a single case which was positive for weak D antigen (0.135% of Rh negative samples).

Discussion

Rh blood group system is a complex system comprising more than forty antigens of which five are clinically significant. These antigens are C,c,D,E, and e. Genes for the five Rh antigens are encoded by two autosomal dominant genes RHD and RHCE on chromosome 1.

The D antigen is most immunogenic and plays an important role in immunohaematology and blood banking. Consequently Rh positivity and negativity imply presence or absence of the D antigen on the surface of red blood cell. There is a lot of polymorphism in the D antigen phenotype because of variations due to deletions and missense mutations. There is an evidence to indicate that the D antigen is a mosaic of many epitopes.

Detection of the D antigen is done routinely by immediate spin tube method. A weak expression of the D antigen was described by Stratton in 1946 and was called the Du antigen. This term was abandoned in 1984 and replaced by more appropriate terminology, the weak D antigen. This is detected by use of anti human globulin.

Wagner et al in 1999 indicated that point mutation in the RHD gene results in an amino acid change in the transmembrane and intracellular regions of the D antigen affecting its insertion and hence density on the surface. Using flow cytometry it was established that the weak D subjects had at least ten times lower expression of the antigen as compared to D positive individuals.

There are three genetic mechanisms postulated for the acquisition of weak expression of the D antigen. These are:

1) Individuals inherit the RHD gene which codes for a weakly expressed D antigen.
2) D antigen may be weakly expressed due to presence of C antigen in the trans position on the opposite chromosomes such as Dce/dCe genotype. This is seen fairly commonly in blacks.
3) When one or more epitopes of the D antigen are missing a weak D phenotype may be seen. This is
termed as partial D antigen and these individuals may be alloimmunized if transfused with D positive blood bearing the missing epitope.

At times partial D antigens may present as normal D types and may remain undetected unless they form antiD.

Using molecular techniques, DNA genomic samples of D positive, D negative, weak D and partial D individuals have been extensively studied. These studies showed that some serologically Rh negative individuals had an intact RHD gene but for a point mutation which caused the negative phenotype. There is heterogeneity in the inheritance of a weak D phenotype. Some studies revealed that RHD gene alteration leads to amino acid substitution in the cellular and transmembranous part of the D antigen resulting in a weak D phenotype. Other workers observed that a normal RHD gene with a severely reduced messenger RNA transcript can also cause a weak expression of the normal polypeptide.

Incidence of D negative and weak D antigen are variably reported around the globe. Incidence of Rh negativity is 3-25% worldwide depending upon the ethnic group. Approximately 5% of the Indian population is negative for the D antigen, though the incidence varies from community to community with 15-17% Rh negativity in Parsis, Chitrapur Saraswats and Goans. Review of literature showed no study for the incidence of Rh negative blood group in the Garhwal region of Uttarakhand. Our study of 5855 cases revealed that 12.62% subjects were Rh negative.

The incidence of weak D antigen ranges from 0.2%-1%, worldwide. Studies conducted in India showed an incidence of 0.189% and 0.15%.

In our study we found that 0.14% of negative subjects expressed the weak D antigen.

The use of potent monoclonal antisera with a high antibody titre may be responsible for detecting the Rh D positive cells that would be otherwise difficult to detect with less sensitive polyclonal reagents.

The D antigen is highly immunogenic and a significant antibody response is seen when a D negative patient receives D positive blood. Hemolytic disease of the newborn is also caused by an already sensitized pregnant D negative female with a D positive fetus. Thus the explanation behind the mandatory anti D immunoprophylaxis in all D negative females who give birth to D positive babies.

However, even after so many years of the discovery of the weak D antigen, its clinical significance, immunogenecity and guidelines are controversial. Therefore 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.

Theoretically, transfusion of weak D positive blood to a D negative patient may lead to alloimmunization. But this is debated because there are not enough cases to substantiate this contention. In a follow-up of 45 RhD negative cases who received weak D blood, none developed anti D antibodies even though the weak D positive erythrocytes remained in the circulation for hundred days in 34 cases. Only two case reports have reported alloimmunization of D negative patients following transfusion with weak D blood. In a study it was seen that in child bearing women who expressed weak D antigen, 10.2% institutions transfused D negative blood components while approximately 90% transfused D positive components. The study recommended that obstetric patients who test positive clearly for weak D by AHG (2+ macroscopic agglutination) can be safely regarded as D positive and transfused with D positive blood components.

Most of the institutions recommend that a weak D status of gravidae should avoid postpartum or antepartum antiD immunoprophylaxis. This is contradictory to the decision of American Association of Blood Banks in 2003 that it is no longer necessary to test for weak D antigen in obstetric patients. The reason behind this was that the present day blood typing reagents are more potent. They recommend that patients should be typed either as D positive or D negative by immediate spin tube method. The clinical implication of this being that a few women who actually have a weak expression of the D antigen will receive Rh immunoglobulin which has no adverse outcome.

In our blood bank the incidence of weak D is very low, probably due to routine use of two potent monoclonal anti D blood typing antisera. We do test all donors and women of child bearing ages for weak D antigen. Weak D individuals are treated as D positive when they are donors and D negative when recipients of blood transfusion. The drain on effort, time and money though needs to be evaluated.
further and should be clinically justified. Newer techniques like UV spectrophotometric approach to blood group typing and molecular analysis may be more accurate.

Issues related to weak D phenotype should be undertaken in conjunction with molecular studies to formulate beneficial, cost effective standardized guidelines.

**Conclusion**

Our study concluded that the incidence of Rh negative blood group was 12.62% in the Garhwal region of Uttarakhand. There was no similar study from this region for comparison and this study can be used for future reference. We found a very low weak D positivity, possibly due to routine use of two potent monoclonal antiD blood group typing antisera. Clinical relevance of weak D is debatable. Though in our hospital, we evaluate all D negative patients and donors for weak D antigen, studies with molecular analysis should be conducted to formulate a cost effective policy.

**References**


