Difficulties in Immunohaematology : The Weak D Antigen

Lt Col H Kumar*, Lt Col DK Mishra⁺, Lt Col RS Sarkar[#], Col M Jaiprakash**

Abstract

Background: The Rh blood system is one of the most polymorphic and immunogenic systems known to humans. The expression of Rh blood group antigen is complex. The Rh D antigen is the most important of the antigens that constitute the Rh antigen system. In most cases, D antigen can easily be detected. However, due to variability of expression, weak forms antigen are encountered. The reactivity of weak D with antisera is variable and presents as a problem in blood banking.

Methods: A retrospective analysis for a five-year period was done. Blood samples that were negative for Rh D by immediate spin tube method were tested for weak D antigen by additional lab tests.

Result: Of 34932 serial Rh grouping tests done in our Blood Bank, the incidence of weak D Rh antigen was 0.189%. All these were confirmed by the antiglobulin test.

Conclusion: These patients present as a problem for the blood banker and a curiosity to the clinician. Although uncommon, all health care workers should be aware of this entity to avoid anti D alloimmunisation.

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Key Words: Weak D; Rh Blood Group

Introduction

The discovery of Rh antigen began with the detection of antibody to Rh antigen by Levine and Stetson in 1939. This was the second major discovery in immunohaematology after the discovery of the ABO blood groups by Kari Landsteiner in 1900. The human race was divided into, those who possessed the Rh antigen (Rh positive) and those who did not (Rh negative). Subsequently, Fisher and Race published their work on Rh antigens, wherein the CDE nomenclature of Rh blood group system was proposed and accepted. The main antigen in the Rh blood group is the 'D' antigen expressed by the Rh D protein. Depending upon the ethnic group about 3% to 25% of the human population lacks Rh D antigen. As anti-D immunization can occur in D negative recipients, antigen D is of critical importance for blood transfusion. Around the same time weak-D antigen was also detected and described. It was then called the D^u antigen. The difference between D and D^u antigen is that the latter is weakly immunogenic and difficult to detect. About 0.2% to 1% of white individuals carry weak D antigen [1].

Material and Methods

Rh blood group typing of all donors and patients in our blood bank was analysed for a five-year period from 01 January 1998 to 31 December 2002. There were a total of 34942 subjects. Routine Rh typing was done using the

immediate spin tube technique. Blood samples, which were negative for agglutination by immediate spin tube method were further tested. Samples showing agglutination after incubation or after addition of AHG serum were considered to be weak D. Appropriate controls were used. Equal volumes each of anti D serum and 2-5% washed red cell suspension were placed in a clean glass test tube. They were mixed and incubated at 37°C in a water bath for 45-60 minutes (as advised by the manufacturer). Alternatively, in an emergency situation we incubated for 10 minutes and then centrifuged at 1000 rpm for one minute to obtain a rapid result. However, standard AHG technique was always performed for confirmation. The tube was gently re-suspended and the cell button observed for agglutination. If the test red cells were agglutinated (but not in the negative control tube) the test was recorded as positive and there was need to proceed to the antiglobulin phase of the test. If the test cells were not agglutinated or the results were doubtful, the cells were washed three to four times with large volumes of normal saline. After the final wash, the saline was decanted and one to two drops of antiglobulin serum was added according to manufacturers instructions. Following this, the contents of the test tube were mixed and the tube was centrifuged at 1000 rpm for 30 seconds. The cell button was then gently re-suspended and examined for agglutination. All negative results were confirmed under the microscope.

Results

The results are depicted in Table 1. All samples that were negative for Rh antigen by the immediate spin tube method

*Associate Professor, **Professor and Head, Department of Transfusion Medicine, AFMC, Pune. ⁺Classified Specialist (Pathology and Haematology), AHDC(R&R), Delhi Cantt. [#]Cl Spl (Path & Haem) MH Roorkee.

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Table 1

Total samples tested	Rh D+		Rh D-		Rh D ^a	
	n	%	n	%	n	%
34932	32731	93.7	2201	6.3	66	0.189

were subjected to further tests. Appropriate controls were used. Of a total of 34932 blood samples we found 32731(93.7%) to be Rh D positive. Of 2201 (6.3%) were Rh D negative even after antiglobulin phase of testing. There were 6(0.189%) with D antigen. All cases of D antigen presented as Rh D negative with immediate spin tube method.

Discussion

Weak D is a phenotype with either a qualitative or quantitative difference in the Rh 'D' moiety resulting in a weakened expressed of the antigen. Stratton first described the weak D or D antigen in 1946. Unlike in the Caucasian population where the incidence of Rh D antigen positivity is 85%, the incidence of Rh D positivity in India is around 95% [2,3]. The incidence of weak D is 0.23% and ranges from 0.2% to 1% in the general population [1]. The problems that arise from weak D antigen are due to its low immunogenicity which gives rise to conflicting laboratory reports, as to whether an individual is Rh D positive or negative.

In earlier years, blood banks used polyclonal antisera and these contained low titres as compared to monoclonal antibodies. The blood group reporting was affected by the titre of anti D antibodies in the test reagent. An individual with weak D antigen was labeled Rh D positive by one laboratory and Rh D negative by another. Such conflicting reports lead to inadvertent transfusion of Rh D positive blood to Rh D negative recipients. High potency monoclonal reagents detect Rh D positive cells that would be difficult to detect with less sensitive polyclonal reagents [4]. The use of modern sensitive gel system for ABO and Rh-D typing has given concordant result when compared with the conventional blood grouping system [5].

The "D" antigen is highly immunogenic, and if a Rh D positive blood is transfused to an Rh D negative recipient, the recipient is likely to develop anti D allo-antibodies and thereafter cannot be transfused with Rh D positive blood. Furthermore, if sensitized Rh negative women, conceived a Rh D positive foetus, the passage of anti D antibodies across the placenta to the unborn baby resulted in hemolytic disease of the newborn. The clinical significance of weak D is that transfusion of such red cells to a Rh D immunized subject can result in a haemolytic transfusion reaction. If Weak-D red cells are transfused to a Rh negative subject it may lead to alloimmunisation to the Rh D antigen. Subsequent transfusion of blood from these donors to a sensitized

individual may result in accelerated destruction of donor RBC. This contention is however debated, as there are not enough cases recorded in literature to give conclusive evidence. There are only two reports of formation of anti Rh D antibodies in Rh D negative subjects after exposure to D positive RBC. In contrast, in a follow up of 45 Rh D negative subjects who had been transfused with weak Rh D positive red cells, none developed anti D. In 34 of these subjects, the Rh D positive red cells could be detected up to 100 days after the transfusion. Therefore, the transfusion of Rh D negative blood to individuals with weak D blood group was considered wasteful by some workers. The current opinion of the majority, albeit debatable is that weak D subjects especially if they happen to be women in the child bearing age group should be treated as Rh D positive when they are donors and D negative when they are recipients of blood transfusion.

Two genes, the RHD and RHCE encode the antigens of Rh blood group. It is generally believed that weak D phenotype could arise from three different genetic mechanisms.

1. A person may inherit a RHD gene that encodes for a weak expression.
2. The 'D' gene may interact with the 'C' gene of the corresponding chromosome in the genotype Cde/Cde or cDe/Cde and for unknown reasons, the expression of the D antigen is depressed.
3. The RHD gene may not encode for all the epitopes that make up the complete D antigen [6].

It will be observed, that although present in reduced amounts, all epitopes of 'D' are present in mechanism 1 and 2. In these situations, weak D antigen is expressed and such individuals do not have the capability to produce allo anti-D antibody. Those cases that manifest due to mechanism 3 do not have all the epitopes and therefore have the capability to produce allo anti-D to the missing epitopes of antigen D. These cases represent partial-D antigen. In recent years, using modern techniques like molecular studies, workers analysed different genomic DNA samples derived from individuals expressing D positive, D negative, weak D and partial D phenotype. It was observed that some serologically Rh D negative individuals possessed an intact RHD gene but had a point mutation which resulted in the expression of Rh D negative phenotype [7]. Individuals with weak D phenotype, may show amino acid substitution in the intra cellular and transmembranous protein segment of the Rh antigen [8]. Analysis of Taiwanese population, using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) and direct sequencing, showed four types of mutations that accounted for the

weak D antigen [9]. Some workers observed that most weak D individuals carry altered antigens due to RHD alleles encoding aberrant Rh D protein [10], while others observed that genes encoding weak D phenotype showed a normal RHD gene sequence but a severely reduced expression of RHD messenger RNA [11]. Recently, UV spectrophotometric approach to typing weak D has been successfully used [12].

The incidence of weak D varies worldwide, and it ranges from 0.2% to 1% in whites [1]. In our study, the incidence of weak-D was 0.189%. Bhatia et al placed the incidence of weak D in India at 0.3-0.5% [2]. A recent study in India noted the incidence to be 0.15% [13]. It is possible that the use of potent monoclonal anti D reagents may account for the slightly higher incidence of weak D in our study.

To conclude, weak D Rh antigen presents a peculiar clinical situation. The subject may face problems in Rh blood group typing and prevent a potential risk of alloantibody formation when transfused with Rh positive blood. Alloimmunisation of females with weak-D while in the child-bearing age, is disastrous and results in haemolytic disease of the newborn. It would be prudent to consider individuals with a weak D antigen as Rh D positive when presenting as a donor and Rh D negative when confronted as a recipient.

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