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Prevalence of Weak D among Blood Donors in a Tertiary Care Hospital, in South India

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ABSTRACT
In the realm of transfusion medicine, after ABO antigens, the Rh D antigen holds significant importance. The prevalence of weak D phenotypes varies significantly among different ethnic populations. Weak D refers to a reduced expression of the D antigen on red blood cells, making it necessary to conduct an extended testing with the Indirect Antiglobulin Test to detect it. The clinical significance of weak D becomes apparent when labeling donors and patients, as donors with weak D are labeled as D positive and recipients as D negative. The aim of this study was to determine the prevalence of weak D among D negative blood donors in our population. The research involved a retrospective analysis of data from the Department of Immuno Haematology and Blood Transfusion at Government Rajaji Hospital in Madurai over a two-year period from January 2021 to December 2022. At our center, all healthy blood donor samples were tested for ABO and Rh D typing using routine tube methods with two different anti-D reagents: Anti-D (IgM) monoclonal and a combination of anti-D (IgM and IgG). Blood samples that did not show agglutination in the immediate spin method were further subjected to weak D testing using IgG anti-D in the IAT phase. Results: Out of the 48,878 donor blood samples analyzed for ABO and Rh blood grouping, 93.47% were Rh-D positive and 6.52% were Rh-D negative. All Rh-D negative samples underwent weak D testing and 2.29% (73 samples) were found to be weak D positive. This study highlights the prevalence of weak D antigen among blood donors in our representative population from Madurai district. Testing for weak D in blood donors using the tube method can sometimes be challenging due to the weak expression of the D antigen. This may lead to false-negative results and misclassification of Rh-negative individuals as weak D individuals. Therefore, it is crucial to employ advanced techniques in weak D testing among blood donors to ensure the safety and effectiveness of blood transfusions.

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Key Words
Weak D antigen, blood donation, conventional tube technique, Rh-D negative, weak-D positive, indirect antiglobulin test

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INTRODUCTION

A weak D phenotype is characterized by a lower expression of the D antigen, a significant determinant in the Rh blood group system, on an individual's red blood cells. This condition is found in a minority of individuals who are categorized as Rh positive but display a diminished D antigen expression on their red blood cells.

Following ABO antigens, the Rh D antigen holds the second-highest importance in the field of transfusion medicine. The prevalence of the weak D phenotype differs notably among various ethnic groups. Weak D denotes a diminished expression of the D antigen on red blood cells, necessitating extended testing with IAT (Indirect Antiglobulin Test) for detection. The clinical significance of weak D becomes apparent during the labeling process of both the donor and the patient[1].

In the past, individuals with a Weak D phenotype were typically labeled as Rh positive. However, there is a growing awareness that they might be more prone to developing antibodies against the D antigen, which could result in transfusion reactions in specific circumstances. This has important consequences for blood transfusion procedures, particularly when weak D individuals act as blood donors. Consequently, comprehending the implications of the weak D phenotype in blood donors becomes essential to guarantee the safety and effectiveness of blood transfusions[2].

Aim and objectives: The objective of this study is to prevalence the frequency of the weak D phenotype among blood donors who are classified as D negative in our population.

MATERIALS AND METHODS

The retrospective research was carried out at the Department of Immunohematology and Blood Transfusion in Madurai Medical College, Tamilnadu, spanning a duration of 2 years from January 2021 to December 2022. At our center, all blood samples from healthy voluntary blood donors were subjected to ABO and Rh D typing using the routine tube method (conventional tube technique)[3].

In some cases, the conventional tube method may not show immediate agglutination. To address this, supplementary techniques like the indirect antiglobulin test (IAT) are employed alongside the tube method, using two anti-D reagents:

- Anti-D (IgG) polyclonal
- Blend of anti-D (IgM and IgG) monoclonal

Blood samples that did not show agglutination during the immediate spin method were subsequently subjected to weak D testing using IgG anti-D in the Indirect Antiglobulin phase.

Procedure for determination of weak D status should be performed on all donor samples giving a negative reaction for Rh (D) antigen on forward grouping:

- Label one test tube with the donor number and name
- To the test tube, add one drop of a 5% suspension of the patient's red blood cells
- Add 2 drops of preferably polyclonal anti-D (IgG), in the absence of which a blend of monoclonal IgG and IgM can also be used to the test tube.
- Incubate at 37°C for at least 30 min
- Wash the red cells 3 times using normal saline and decant the last wash completely
- Add 1 drop of Poly-specific AHG to the washed red cell button
- Centrifuge at 1000 rpm for 1 min
- Re-suspend the red cell button by gentle agitation and examine macroscopically for agglutination
- Add 1 drop of check cells to the tube, giving no agglutination after adding AHG
- Record the results and interpretation

RESULTS AND DISCUSSION

Out of the total 48,878 donor blood samples analyzed for ABO and Rh blood grouping, 45,691 samples (93.47%) were found to be Rh-D positive and 3,187 samples (6.52%) were Rh-D negative. Subsequently, all the Rh-D negative samples underwent weak D testing and out of these, 73 samples (2.29%) were identified as weak D positive (Table 1 and 2).

The tube method is a widely used approach for conducting weak D testing in blood donors. In this method, the donor's red blood cells are mixed with anti-D antibodies in a test tube and the presence of agglutination indicates the presence of the D antigen on the red blood cells. However, because weak D individuals have a less pronounced expression of the D antigen, there are situations where agglutination is not immediately visible using this method. Consequently, this can lead to false-negative results, misclassifying weak D individuals as Rh negative[4].

To overcome this challenge, the tube method can be complemented with additional techniques like the indirect antiglobulin test (IAT). By incorporating the IAT, anti-human globulin reagent is introduced to

Table 1: Blodd samples

Initial Rh (D) test	Weak D test	Check cells	Interpretation
+	NA	NA	+
-	+	NA	+
-	-	+-	
-	-	RT	

+: Positive, -: Negative, NA: Not applicable and RT: Repeat test

Table 2: Total donor samples 2021-2022

Years	Total donor samples	Total Du done	Du positive
2021	21633	1333	33
2022	27245	1854	40

enhance the detection of weak D antigens. This approach enhances the sensitivity of weak D testing, reducing the likelihood of false-negative results and ensuring more accurate identification of weak D individuals[5].

In the domain of blood group systems, the Rh blood group system stands out as a matter of great significance, ranking second only to the ABO system among the 36 identified blood group systems. Among the 54 blood group antigens within the Rh system, the RhD antigen is of exceptional importance in clinical practice due to its highly immunogenic nature[6,7].

Indeed, for RhD-negative women, the development of anti-D antibodies can have grave implications during subsequent pregnancies when the fetus is RhD-positive. This circumstance places the pregnancy at risk for complications linked to Rh hemolytic disease of the fetus and newborn, which could result in significant morbidity and even mortality for the baby. It underscores the importance of proper RhD typing and vigilant monitoring during pregnancy to manage and prevent potential complications associated with Rh incompatibility[8,9].

Similarly, for any RhD-negative individual who forms anti-D antibodies, the option of emergency transfusion with D+ (RhD-positive) red blood cells is no longer feasible. Consequently, there becomes an absolute lifetime necessity to receive only D- (RhD-negative) red blood cell transfusions. This underscores the importance of proper Rh typing and blood matching to avoid these potentially serious consequences in clinical settings[10,11].

In 2015, the AABB (formerly known as the American Association of Blood Banks) and the College of American Pathologists (CAP) collaborated to form a Work Group on RHD Genotyping, aiming to address clinical concerns regarding RhD typing[12,13]. The Work Group released its recommendations, introducing the term "Serologic weak D phenotype" to distinguish the results of serological weak D testing performed in clinical laboratories using anti-human globulin from the outcomes of RHD genotyping for weak D types, which are determined using molecular methods[14].

This differentiation was implemented to enhance clarity and facilitate a better understanding of the testing methodologies and their implications for clinical practices. By providing clear definitions, these recommendations aimed to promote accurate and effective RhD typing in medical settings[15].

Furthermore, with technological advancements, molecular methods like polymerase chain reaction (PCR) have emerged, enabling precise identification of the presence or absence of weak D alleles. These molecular techniques offer enhanced clarity and accuracy in identifying weak D individuals, ensuring proper blood compatibility and significantly reducing the risk of transfusion reactions. By utilizing these sophisticated methods, healthcare professionals can confidently match blood donors and recipients, leading to safer and more effective blood transfusions[16].

CONCLUSION

Indeed, weak D testing using the tube method in blood donors can be problematic due to the limited expression of the D antigen. This can result in false-negative outcomes, leading to the incorrect categorization of Rh-negative individuals as weak D individuals. To address these challenges effectively, it becomes essential to complement the tube method with additional techniques such as the indirect antiglobulin test (IAT) or molecular methods like PCR. By doing so, accurate identification of weak D individuals can be achieved, ensuring appropriate blood compatibility and minimizing the risk of transfusion reactions. The implementation of these advanced techniques in weak D testing among blood donors plays a critical role in upholding the safety and effectiveness of blood transfusions. It empowers healthcare professionals to make well-informed decisions in matching blood donors with recipients, ultimately promoting the success of transfusion practices and safeguarding patient health.

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