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Original Research Article

PREVALENCE OF WEAK D (Du) ANTIGEN IN Rh NEGATIVE BLOOD GROUP INDIVIDUALS – AN EXPERIENCE AT A TERTIARY BLOOD CENTRE

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ABSTRACT

Background: The Rh blood group system has over 50 antigens, with the D antigen being crucial for determining Rh-positive or negative status. Weak D (Du) refers to reduced expression of the D antigen, often missed by routine anti-D serum testing and requiring anti-human globulin (AHG) for detection. Identifying weak D individuals is vital to ensure safe and appropriate transfusion practices. **Aim:** To determine the prevalence of Du antigen among the blood donors and recipients who were found to be Rh - negative in conventional grouping.

Materials and Methods: This prospective study, conducted from May 2024 to April 2025 at a tertiary blood centre, tested Rh-negative donor and patient samples for the Du antigen. ABO and Rh typing were performed using the tube method, and Rh-negative samples were further tested for weak D using the gel card system, with results recorded as numbers and percentages.

Results: A total of 25,512 blood samples were analysed with 25,073 Rh D positive and 439 Rh D negative cases. Among the Rh D-negative, 97 (22.1%) were Group A, 124 (28.2%) Group B, 43 (9.8%) Group AB, and 175 (39.8%) Group O. Of the 439 Rh D-negative samples, 48 (10.93%) were weak D positive: 8 (8.24%) Group A, 14 (11.29%) Group B, 4 (9.30%) Group AB, and 22 (12.57%) Group O.

Conclusion: Weak D prevalence was 10.93% among Rh-negative individuals. Routine weak D testing is essential for accurate typing; donors considered Rh-positive and recipients Rh-negative to prevent alloimmunization and ensure transfusion safety.

Keywords: Antigens, Blood Group, Blood Transfusion, Hemagglutination Tests, Rh-Hr Blood-Group System.

INTRODUCTION

The ABO blood group system was discovered by Karl Landsteiner in 1901, who identified that blood could be classified into four groups: A, B, AB, and O, based on the presence of specific antigens (A and B) on the surface of red blood cells. Then, in 1939, Landsteiner and Alexander Wiener discovered the Rh antigen. It also played a key role in understanding hemolytic disease of the newborn (HDN), a condition first described by Levine and Stetson in 1939.[1] Five Rh system components-D, C, c, E, and e-are crucial in Rh systems with D being the most significant. Weak D refers to a situation where the D antigen is expressed on red blood cells, but at a reduced or weak level. Standard testing with monoclonal anti-D sera (which is typically used to detect the presence of the D antigen) may not detect this weak expression. In these cases, the Indirect Antiglobulin Test (IAT) is required.[2] Worldwide, the prevalence of the Rh-negative blood group ranges from 3 to 25%, while that of the weak D antigen is between 0.2% and 1%.[3-5] The weak Rh D phenotype was first identified by

Wiener in 1944 and was referred to as the Du antigen. Later, in 1946, Stratton described this as a milder form of Rh D antigen expression.[6,7,8] Weak D positive cells are likely to trigger an immunological response in D-negative individuals. Weak Du-positive donors are, therefore, considered D-positive. Though the number of weak D is less but its detection helps in safe blood transfusion.[4,5] However, the prevalence of weak D antigen varies across different populations and geographical regions. Limited data are available regarding its frequency among Rh-negative individuals in tertiary care settings. O: 10539 (42.03) to determine the prevalence of weak D antigen among individuals identified as Rh-negative by conventional grouping methods.

MATERIALS AND METHODS

This was a prospective study conducted in the tertiary blood centre for a period of one year from May 2024 to April 2025. All the blood samples received from the blood collection centre for blood group confirmation were included. The study was approved by Institutional Ethical Committee. A written informed consent was taken from all the subjects. The results were analysed using data from the donor group register and the patient sample register.

Inclusion and Exclusion criteria

The study included all donors and recipients who attended the blood centre over a one-year period from May 2024 to April 2025. Donors deferred from blood grouping and recipients with unavailability of blood components were excluded from the study.

Methods

Labelling the tubes with Donor/ Recipients ID and test identification. ABO and Rh blood grouping was done by conventional forward grouping by the known Anti serum antibody. Du testing was done using Standard operating procedure for MatrixTM Gel system. Dispense 1 mL (1000 µL) of Matrix™ Diluent 2 (LISS**)** into a clean, labelled test tube**.** Add 25 µL of packed red cells to the diluent. Mix gently to ensure a uniform 0.8% red cell suspension. Label the Matrix™ Gel Card microtube with the donor's or recipient's identification details. Pipette 50 µL of the 0.8% red cell suspension into the labelled microtube on the card. Add 25 µL of AGTROL or Ercyclone Anti-D IgG to the microtube. Incubate the card at 37°C for 30 minutes using the Matrix™ Card Incubator. After incubation, centrifuge the card using the Matrix™ Card Centrifuge for 1 cycle (10 minutes)**.** Observe the card for agglutination and interpret the result accordingly. Record the results as per the standard format.

RESULTS

A total of 25512 blood samples were analysed during the period May 2024 to April 2025. Out of these 25073 (98.27%) were Rh D -positive and 439 (1.73%) were Rh D - negative as shown in Table 1 and Figure 1. Out of the total 439 samples, 97 (22.11%) were Group A negative, 124 (28.24%) were Group B negative, 43 (9.79%) were Group AB negative, and 175 (39.86%) were Group O negative. All Rh D-negative samples were tested for weak D. Out of the total 439 Rh D-negative samples, 48 samples were weak D positive. Among these, 8 (8.24%) belonged to Group A, 14 (11.29%) belonged to Group B, 4 (9.30%) belonged to Group AB, and 22 (12.57%) belonged to Group O. The frequency of Weak D positivity among the Rh D-negative cases was 10. 93 % (Table 2, Figure 2 and 3).

Table 1: Frequency of Rh antigen status

Blood group	Rh -positive	Rh - negative (%)	Total
A	5213 (20.79)	97 (22.1)	5310
B	7449 (29.71)	124 (28.2)	7573
AB	1872 (7.47)	43 (9.8)	1915
O	10539 ((42.03)	175 (39.9)	10714
Total	25073	439	25512
Percentage	98.27	1.73	

Table 2: Frequency of different Rh -negative blood groups and percentage of Du positivity among Rh -negative blood groups

Blood group	Rh- negative N (%)	Weak D positive	Du negative	Weak D positive in %
A	97 (22.11)	8	89	8.24 %
B	124 (28.24)	14	110	11.29 %
AB	43 (9.79)	4	39	9.30 %
O	175 (39.86)	22	153	12.57 %
Total	439	48	391	10.93%

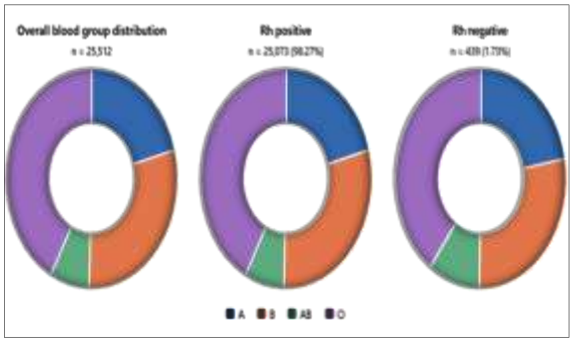

Figure 1: Distribution of ABO blood groups among Rh-positive and Rh- negative donors (n = 25,512)

Figure 2A

Figure 2B

Figure 2: Photograph with Test sample of a B Negative Individual. A) Rh- negative blood sample with weak D (DU) antigen negative B) Rh - negative blood sample with weak D (DU) antigen positive - Test Sample Shows Agglutination

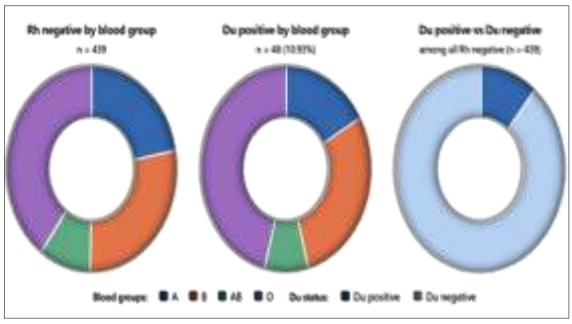

Figure 3: Frequency of Weak D positivity among the Rh D-negative blood samples

DISCUSSION

The Rh (Rhesus) blood group system is a highly complex system, primarily due to its large number of antigens and the diverse genetic variations that occur within it.[9] While the system includes over 40 different antigens, the most clinically significant ones are C, c, D, E, and e. The genes responsible for encoding these antigens, RHD and RHCE, are located on chromosome.[10] The Rh D antigen, in particular, plays a crucial role in blood transfusions and pregnancy, as its presence or absence determines Rh -positivity or negativity. The Rh D antigen is considered to be highly immunogenic, meaning it can trigger an immune response if mismatched between a donor and recipient during blood transfusion.6 There are several variants of the Rh D antigen, which can complicate compatibility testing in transfusion medicine and organ transplantation.[11,12]

These variants include weak D, partial D, and Rh null: Weak D variant individuals have fewer Rh D antigens due to genetic mutations affecting Rh protein structure and assembly. Partial D variant involves altered or missing Rh D antigen components, risking immune response upon exposure to normal Rh D.[13] This is critical in pregnancy, as maternal antibodies can cause hemolytic disease of the newborn (HDN). Rh null individuals lack all Rh antigens (D, C, E), a rare condition. Without Rh proteins, Rh null individuals face increased risk of hemolytic anaemia due to unstable red cell membranes. It reacts with anti-D only after extended testing using the indirect antiglobulin test. It is conventional to consider weak D individuals as Rh-negative when they are recipients and Rh-positive when they are blood donors.[13,14]

In particular, individuals with Du positive (weak D) status require special attention, especially in settings where frequent blood transfusions are necessary, such as for patients with HIV/AIDS, chronic renal failure, advanced malignancies, aplastic anaemia, bone marrow failures, sickle cell anarhemia, and other forms of chronic anaemia.[15]

The immediate spin tube method is commonly used to detect the D antigen, but routine monoclonal anti-D sera cannot detect weak D antigens, requiring anti-human globulin for confirmation. Weak D antigen expression, first described by Stratton in 1946, refers to diminished Rh D presence on red cell surfaces. In the immediate spin phase, diluted red cells and serum or plasma are centrifuged at room temperature and checked visually for agglutination.[16]

To accurately detect weak D antigens, especially in individuals with the weak D phenotype, the indirect antiglobulin test (IAT) is employed. This method enhances the detection of weak D antigens by using anti-human globulin, which can detect antibodies that are not detectable at the immediate spin phase. It's important to note that discrepancies in D antigen phenotyping can occur due to different monoclonal antibody formulations used in reagent manufacture. These variations can affect the ability to detect partial D antigen and weak D phenotypes.[16,17] The present study was conducted to determine the prevalence of weak D antigen among individuals identified as Rh-negative by conventional grouping in a tertiary care setting. In the present study, donor and recipient samples were analysed.

Table 3: Comparison table for Du positivity frequency in several studies

Study	Place	Sample size	Du positivity frequency
Srivastava AR et al., (2021)[18]	Maharastra	1866	0.03%
Afshan N and Tariq S (2013)[19]	Pakistan	100	3%
Gundrajukuppam DK et al., (2016)[20]	Tirupathi	1000	0.76%

Githiomi R and Kuria KM, (2016)[21]	Kenya	384	2.10%
Deepthi Krishna G et al., (2015)[22]	Tirupati	1377	1.04%
Singh A et al., (2022)[23]	Lucknow, Uttar Pradesh	3153	1.11%
Brar RK et al., (2020)[24]	Andaman and Nicobar Islands	330	1.51%
Present study	Anantapuramu	439	10.93%

In present study, a weak D prevalence of 10.93 % over one year was reported among the Rh-negative samples, which was comparatively higher to the findings of other studies conducted by Deepti Krishna et al., (1.04%), Singh et al., (1.11%), and Brar et al., (1.51%) as shown in Table 3.[22-24] The higher prevalence observed in this study may be attributed to the use of more sensitive detection techniques such as the indirect antiglobulin test, differences in study population, and possible regional genetic variations.

This finding indicates that a considerable proportion of individuals initially classified as Rh-negative may actually express weak D antigen, highlighting the importance of additional testing in all Rh-negative samples. These findings have important implications in transfusion practice, as failure to detect weak D antigen may lead to alloimmunization. Therefore, routine screening for weak D in Rh-negative individuals is essential for ensuring safe transfusion practices.

CONCLUSION

Our study found that the prevalence of weak D antigen among Rh-negative individuals was 10.93% in a tertiary blood centre. This indicates that a significant proportion of individuals initially classified as Rh-negative may express weak D antigen. Detection of weak D is essential for appropriate transfusion management, where donors are considered Rh-positive and recipients as Rh-negative to prevent alloimmunization and ensure optimal utilization of Rh-negative blood.

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