Original Article

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Pathology Section

Prevalence of Weak D Antigen in Rh Negative Blood Group: An Experience at a Tertiary Blood Centre in Bengaluru, Southern India

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ABSTRACT

Introduction: The Rh blood group system, consisting of more than 50 different antigens, is the most polymorphic among human blood types. Among them, D is the most important antigen. The incidence of weak (Du) antigens ranges from 0.2 to 1%. Routine screening for RhD does not cause agglutination with anti-RhD serum; therefore, it must be detected using anti-human globulin serum. Due to the potential immunological reaction of Du-positive cells in RhD-negative individuals, it is crucial to emphasise the therapeutic implications of this finding and its relation to the risk of alloimmunisation.

Aim: To determine the frequency of the Du antigen among RhD-negative individuals.

Materials and Methods: This five-year hospital-based cross-sectional study was conducted from January 2017 to January 2022 in the Department of Transfusion Medicine at ESIC MC and PGIMSR, Bengaluru, Karnataka, India. ABO and Rh blood group samples were collected in EDTA vacutainers from the blood collection centre and sent to the Department of Transfusion Medicine. Data analysis was performed using Microsoft Excel. Results were presented as numbers and percentages. ABO and Rh blood group typing were carried out using the tube method (immediate spin tube technique), and negative samples were further tested for weak D (Du) using the Gel card system. The Rh-negative cases with Du testing were recorded, and the frequency of the Du antigen in Rh-negative blood group types was determined.

Results: A total of 69,282 blood samples were analysed, of which 66,442 (95.90%) were Rh positive, and 2,840 (4.10%) were Rh negative. The male-to-female ratio was 1:1.79. Out of the total 2,840 Rh-D negative samples, 31 samples were weak D positive. Among them, 06 (0.85%) belonged to the A blood group, 12 (1.63%) to the B blood group, 12 (1.01%) to the O blood group, and 1 (0.47%) to the AB blood group. The Du positivity noted among the Rh-negative cases was 1.09%. The overall Du positivity noted among all the samples was 0.045%.

Conclusion: To prevent RhD mismatches, which can lead to life-threatening complications, it is advised to retype all Rh-negative blood samples using a Du test during routine grouping. This is because weak D antigen is immunogenic and capable of producing alloimmunisation if transfused to RhD-negative individuals.

Keywords: Alloimmunisation, Agglutination, Du antigen, Gel card system

INTRODUCTION

After Landsteiner's discovery of the ABO blood group systems in 1901, Levine and Stetson made the discovery of the Rh antigen in 1939 [1]. The Rh blood group system, which has more than 50 distinct antigens and is the most polymorphic of the human blood types, is second only to ABO in terms of clinical significance in transfusion medicine. Five Rh system components-D, C, c, E, and e-are crucial for producing clinical problems among all the produced antigens, with D being the most significant one expressed by the Rh D protein [2-4]. 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% [5]. A variant of the RhD antigen is the weak RhD phenotype. When conducting conventional RhD typing, it does not agglutinate with potent monoclonal anti-RhD serum; rather, it needs to be combined with antiglobulin serum to show the presence of the D antigen [6]. This weak version of the RhD antigen was first described by Wiener in 1944 and was formally known as the Du antigen [7,8]. Stratton described this type of D as a mild expression of the RhD antigen in 1946 [9]. If the recipient is already immunised, Du cells can be eliminated. Du positive cells are likely to trigger an immunological response in D-negative individuals. Weak Du-positive donors are, therefore, considered D-positive, and weak Du-negative recipients are treated as negative [4]. Therefore, it is crucial to emphasise its clinical implications regarding the possibility of alloimmunisation in RhD-negative individuals. Although the frequency of the weak D antigen is low and its immunogenicity is high, there is a possibility of alloimmunisation if weak D antigen-positive blood is transfused into an individual who is Rh D-negative.

The aim of the current study was to ascertain how frequently the Du antigen is present in Rh D-negative individuals.

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Department of Transfusion Medicine at ESICMC&PGIMSR Bengaluru, Karnataka, India for a period of five years, from January 2017 to January 2022. All the blood samples received from the blood collection centre for blood group confirmation were included. The study was approved by Institutional Ethical Committee (IEC) No 532/L/11/12/Ethics/ESICMC&PGIMSR/Estt.Vol.IV). To determine the age and gender of the patients, the results were analysed using data from the blood group register and the Du register.

Inclusion criteria: The study included all blood grouping performed at the department for a period of five years, from January 2017 to January 2022.

Exclusion criteria: All samples that tested positive for the direct Coombs test and were Rh-negative were excluded from the study.

A total of 69,282 EDTA blood samples were analysed. All samples underwent ABO and Rh blood group testing using the tube method with two types of antisera. Both a polyclonal IgM+IgG anti-D blend and a monoclonal IgM anti-D were used.

The Agglutination/Gel Card Method (Diamed ID Microtyping System) was employed to test samples that were negative by

the tube method (immediate spin tube technique) for weak D. A mixture of anti-D serum (IgM+IgG) and 2-5% red cell solution was combined in equal quantities, incubated at 37°C for 45 minutes, and then centrifuged at 1000 RPM in a clean glass tube. The cell button was gently resuspended in the tube, and agglutination was microscopically confirmed.

The tube test result was reported as D antigen positive if the test red cells agglutinated. If the test cells did not agglutinate (D antigen negative), they were washed three to four times with normal saline. Two drops of anti-human globulin serum were added after the last wash, and the tubes were centrifuged at 1000 rpm for one minute. The cell button was resuspended and examined macroscopically and microscopically for evidence of agglutination. Samples that showed agglutination after the addition of anti-human globulin serum were considered weak D positive. Further testing was conducted using the Gel Card method. A 50 µL 1% red cell suspension of the sample to be tested for weak D was added to one microtube of Anti-IgG cards. A 50 µL of anti-D blend was added, incubated for 15 minutes at 37°C, and then centrifuged for 10 minutes in an Bio-Rad ID centrifuge.

The immediate spin tube technique was used for routine Rh typing. Blood samples that showed no agglutination were further tested. Samples that exhibited agglutination after incubation or the addition of AHG serum were considered weak D. Appropriate controls were utilised, including positive control (check cells, i.e., washed O positive cells with diluted anti-D) and negative control (washed O positive cells). All negative results were observed after the addition of the control sample. All Rh-negative cases with Du testing were recorded, and the data was tabulated in Microsoft Excel. The frequency of different ABO blood groups was calculated, and the frequency of the Du antigen in Rh-negative blood group types was determined.

RESULTS

In this study, a total of 69,282 blood samples were analysed. Among these, 66,442 (95.90%) were Rh D positive, and 2,840 (4.10%) were Rh D negative [Table/Fig-1]. Out of the Rh D-negative samples, 1,018 (35.84%) were males, and 1,822 (64.15%) were females, resulting in a male-to-female ratio of 1:1.79 [Table/Fig-2].

Blood samples	Frequency	Percentage
Rh Positive blood samples	66442	95.90
Rh Negative blood samples	2840	4.10
Total blood samples	69282	100

[Table/Fig-1]: Distribution of Rh positive and Rh negative blood samples.

	Male	Female
Overall	1018	1822
Du Positive	9	22

[Table/Fig-2]: Gender distribution in Rh negative and Du positive donors.

Among the Du-positive cases, nine were males, and 22 were females. Among the 31 Du-positive cases, distribution according to age is given in [Table/Fig-3].

Age (years)	Du Positive
<18	01
18-45	17
46-65	11
>66	02

[Table/Fig-3]: Age distribution of Du positive cases.

Out of the total 2,840 samples, 704 (24.79%) were Group A negative, 737 (25.95%) were Group B negative, 1185 (41.72%) were Group O negative, and 214 (7.54%) were Group AB negative [Table/Fig-4].

All Rh D-negative samples were tested for weak D. Out of the total 2,840 Rh D-negative samples, 31 samples were weak D positive. Among these, 6 (0.85%) belonged to Group A, 12 (1.63%) belonged to Group B, 12 (1.01%) belonged to Group O, and 1 (0.47%) belonged to Group AB. The frequency of Du positivity among the Rh D-negative cases was 1.09% [Table/Fig-4]. The overall frequency of Du positivity among all the samples was 0.045%.

ABO blood group	Total Rh negative blood groups	Percentage	Du negative	Du positive	% Du positive
A Negative	704	24.79	698	06	0.85
B Negative	737	25.95	725	12	1.63
AB Negative	214	7.54	213	01	0.47
O Negative	1185	41.72	1173	12	1.01
Total	2840		2809	31	1.09

[Table/Fig-4]: Frequency of different Rh negative blood groups and percentage of Du positivity among Rh negative blood groups.

DISCUSSION

The immediate spin tube method is commonly used to detect the D antigen. Weak expression of the D antigen was first described by Stratton in 1946, and it is now referred to as the weak D antigen. Routine monoclonal anti-D sera are not sufficient to detect weak Rh antigens on the red cell surface, which is why the use of anti-human globulin is necessary [3,10]. The genes RHD and RHCE, located on chromosome 1, encode the five Rh antigens. The D antigen, being the most immunogenic, is of great importance in immunohaematology and blood banking [11,12]. Weak D is a phenotype that results in a diminished expression of the D antigen by altering either the quality or quantity of the RhD protein. The prevalence of Rh D-negative individuals varies from 3 to 25% depending on the ethnic group [13]. In India, the prevalence of weak D is estimated to affect between 0.0075% and 0.2% of the donor population, depending on geographic distribution [14]. Determining the weak D antigen (and other D variants) is extremely important in blood banking to prevent the clinical implications of alloimmunisation due to weak D antigen positivity.

In present study, out of a total of 2,840 Rh-negative blood samples analysed, 1,018 (27.5%) were males, and 1,822 (72.5%) were females, with a higher proportion of females likely due to the majority being pregnant women. The majority of Du-positive cases were in the age group of 18-45 years (17 cases), and there was one Du-positive case in a newborn out of the total 31 Du-positive cases. The prevalence of Rh-negative blood group in present setting was 4.09%, which was similar to the prevalence of Rh-negative donors in India.

In present study, a weak D prevalence of 1.09% over five years was reported among the Rh-negative samples, which was similar to the findings of other studies conducted by Deepti Krishna G et al., (1.04%), Singh A et al., (1.11%), and Brar RK et al., (1.51%) [Table/Fig-5] [15-19].

Study	Place	Sample size	Du Positivity frequency
Srivastava AR et al., (2021) [15]	Maharashtra	1866	0.027%
Afshan N and Tariq S (2013) [5]	Pakistan	100	03%
Gundrajukuppam DK et al., (2016) [16]	Tirupati	1000	0.756%
Githiomi R and Kuria KM, (2016) [6]	Kenya	384	2.1%
Brar RK et al., (2020) [2]	Andaman and Nicobar Islands	330	1.51%
Deepthi Krishna G et al., (2015) [17]	Tirupati	1377	1.04%
Kanwar R et al., (2018) [18]	Moradabad, Uttar Pradesh	2052	0.19%
Singh A et al., (2022) [19]	Lucknow, Uttar Pradesh	3153	1.11%
Present study (2023)	Bengaluru	2840	1.09%

[Table/Fig-5]: Comparison table for Du positivity frequency in several studies.

To best of authors knowledge, no studies have been conducted on the distribution of ABO and D antigens in various ethnic groups [20].

Limitation(s)

This study was conducted in a hospital-based setting, and larger community-based studies are needed to determine the true prevalence.

CONCLUSION(S)

The weak D antigen is immunogenic and can lead to alloimmunisation if transfused to RhD-negative individuals, even if its occurrence is low. Patients with chronic conditions such as thalassaemia, sickle cell anaemia, and chronic renal failure also require weak D antigen testing. Additionally, if the recipient is of childbearing age, it can result in haemolytic disease of the newborn in subsequent pregnancies, which is a major concern. It is recommended that all blood samples identified as Rh negative during routine grouping should be retested using the Du test to avoid RhD mismatches, which can result in mild to life-threatening complications or even death. To ensure patient safety and prevent transfusion-related problems, comprehensive national transfusion guidelines should be established, including standardised procedures for D antigen testing in both donors and patients.

REFERENCES

[1] Philip L, Stetson RE. An unusual case of intra group agglutination. J Am Med Assoc. 1939;113(2):126-27.
[2] Brar RK, Shaiji PS, Sehgal S. Testing for weak D antigen: Spectrum and its applied role in rhesus-negative transfusions in Andaman and Nicobar Islands. Tzu Chi Med J. 2020;32(2):167-70.
[3] Ahmad S, Tiwari N, Tiwari K, Anwar M, Farhana, Siddiqui F, et al. Incidence of DU antigen at a blood bank of tertiary care centre: A statistical study. Annals of International Medical and Dental Research. 2017;3(2):10-13.
[4] Avent ND, Reid ME. The Rh blood group system: A review. American society of Hematology. 2000;95(2):375-87.
[5] Afshan N, Tariq S. Detection of Du Antigen in Rh Negative Blood Group Individuals. J of Biological Res App Sci. 2013;4(2):16-19.
[6] Githiomi R, Kuria KM. Prevalence of weak RhD phenotype in the blood donor population of Nairobi Regional Blood Transfusion Centre- Kenya. Africa Sanguine. 2016;18(2):13-15.
[7] Agre PC, Davies DM, Issitt PD, Lamy BM, Schmidt PJ, Treacy M, et al. A proposal to standardize terminology for weak D antigen. Transfusion. 1992;32(1):86-87.
[8] Storry JR, Castilho L, Daniels G, Flegel WA, Garratty G, Francis CL, et al. International Society of Blood Transfusion working party on red cell immunogenetics and blood group terminology: Berlin report. Vox Sang. 2011;101(1):77-82.
[9] Daniels G. The molecular genetics of blood group polymorphisms. Transpl Immunol. 2005;14(03-04):143-53.
[10] Gujar RK, Uniya U, Kirar D. Prevalence of weak D antigen and Rh positive and Rh negative blood groups among blood donors-A Six year experience in a blood bank of tertiary health care centre of central India. Med Pulse International Journal of Pathology. 2018;6(1):20-22.
[11] Hoffbrand AV, Higgs DR, Keeling DM, Mehta AB. Postgraduate hematology. 7th ed. Hoboken, New Jersey: John Wiley and Sons, Ltd. 2016: Pp. 252-55.
[12] Flagel WA. How I manage donors and patients with weak D phenotypes. In: Victor Hoffbrand, Daniel Catovsky, Edward GD, Tuddenham, editors. Post Graduates Haematology, 5th ed. Oxford: Blackwell Publishing. 2008;70.
[13] Kumar H, Mishra DK, Sarkar RS, Jaiprakash M. Difficulties in Immunohematology: The weak D antigen. Med J Armed Forces India. 2005;61(4):348-50.
[14] Gupta A, Mirza S, Khurana S, Singh R, Chaturvedi S, Singh B. Enigmatic weak D antigen: An experience in a tertiary care hospital of East Delhi. J Clin Diagn Res. 2016;10(6):12-15.
[15] Srivastava AR, Dhote WD, Singh I. A retrospective study on the prevalence of weak D antigen (Du) in a blood bank in a tertiary care hospital in Maharashtra India. MGM Journal of Medical Sciences. 2021;8(4):410-14.
[16] Gundrajukuppam DK, Vijaya SBK, Rajendran A, Sarella JD. Prevalence of principal Rh blood group antigens in blood donors at the blood bank of a tertiary care hospital in Southern India. J Clin Diagn Res. 2016;10(5):07-10.
[17] Deepthi Krishna G, SreedharBabu KV, Arun R, Jothibai DS. A study on Rh incompatibility and frequency of weak D among blood donors and patients at a tertiary care referral teaching hospital in Tirupati, Andhra Pradesh. J Clin Sci Res.2015;4:281-84.
[18] Kanwar R, Awasthi S, Dutta S. Detection of weak Rh D (DU) phenotype among blood donors. Ann Int Med Den Res. 2018;4(2):31-33.
[19] Singh A, Solanki A, Agarwal D, Chandra T. Prevalance of serologic weak D in Rh D negative blood donors in India: Immunohematological problems & recommendations for donors. Panacea J Med Sci. 2022;12(3):686-89.
[20] Kotwal U, Raina TR, Sidhu M, Dogra M. Distribution of ABO & Rh (D) blood groups among blood donors of Jammu region with respect to various ethnic groups. J Med Thesis. 2014;2(1):03-06.

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