Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Original Research Article

Prevalance of serologic weak D in Rh D negative blood donors in India: Immunohematological problems & recommendations for donors

Ashutosh Singh[1], Archana Solanki[1], Devisha Agarwal[2], Tulika Chandra[1,*]

[1]*Dept. of Transfusion Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India*
[2]*Dept. of Oto-Rhino-Laryngology, King George's Medical University, Lucknow, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 25-10-2021
Accepted 17-11-2021
Available online 28-11-2022

Keywords:
Rhesus system
weak D
Erythrocytes Magnetized Technology
Molecular testing

ABSTRACT

Background: The Rhesus (Rh) system is one of the most complex blood group systems in humans. In some individuals Rh D antigen show weaker expression on red cells. The aim of the study was to find out the prevalence of serologic weak D in north India and associated immunohematological problems.
Material and Methods: Cell and serum grouping were performed on all sample with the help of Qwalys 2 & Qwalys 3 (Diagast, France). All Rh D negatives samples in routine blood grouping were subjected to serologic weak D testing with the help of Erythrocytes Magnetized (EM) Technology.
Results: Total 65,407 whole blood donors were tested for blood grouping in the study period. Prevalence of serologic weak D phenotype in this study was 1.11% of Rh negative donors. The maximum number of serologic weak D phenotype were from B blood group, i.e. 13 (37.14%).
Conclusion: The prevalence of serologic weak D varies in different part of India as well as in the world. This study reported 1.11% prevalence of serologic weak D among Rh D negative blood donors. All serologic weak D positive individuals should give a blood group card showing their Rh D status as donor and recipient. Some European centers started routine RHD gene screening of first-time donors to eliminate the risk of Rh D sensitization. Molecular testing is very costly.For developing countries like India we required an affordable molecular testing technique to improve patient care or alternatively establish reference molecular laboratory for cost effectiveness.

1. Introduction

ABO blood group system is the most important for the blood transfusion. The Rhesus (Rh) system is second most important and one of the most complex blood group systems in humans. There are 54 antigens present in Rh system, of which Rh D is the most potent immunogenic and clinically important antigen. In some individuals Rh D antigen show weaker expression on red cells. Stratton first described these as weak D or Du in 1946.[1]

Conventational tube technique (CTT) is relatively insensitive method for Du testing. Now more sensitive methods are available for Rh D typing. Sometimes individuals who labeled as Du by CTT may found RhD positive by new sensitive methods.[1] To avoid this confusion there was a recommendation to eliminate term Du.[2]

Therefore in 2015 the work group of American Association of Blood Banks (AABB) and College of American Pathologists (CAP) give its recommendations to use the term "serologic weak D phenotype" to differentiate the result of serological test from the molecular methods.[1] The aim of the study was to find out the prevalence of serologic weak D in north India and associated immunohematological problems.

* *Corresponding author*.
E-mail address: drtulikachandra@gmail.com (T. Chandra).

https://doi.org/10.18231/j.pjms.2022.127

2. Materials and Methods

The study was conducted in Department of Transfusion Medicine, of a tertiary care center of Lucknow, India. All the donors were informed about serologic weak D testing and written consent was taken. Ethylenediaminetetraacetic (EDTA) whole blood samples routinely collected for blood grouping from all blood donors. Both cell and serum grouping were performed on all samples with the help of Qwalys 2 & Qwalys 3 (Diagast, France). All Rh D negatives samples in routine blood grouping were subjected to serologic weak D testing.

The serologic weak D testing principle is based on magnetization of donor red blood cells (RBC), which is also known as Erythrocytes Magnetized (EM) Technology. This principle uses the indirect antiglobulin test (IAT) in solid phase combined to magnetic field. First donors' RBCs are magnetized with a solution containing magnetic beads, then these magnetic RBC are mix with weak D antisera (IgG monoclonal, Clones: P3X35, ESD1, Diagast, France). When magnetic field is applied, the magnetized RBCs move to bottom of the well. The monoclonal antiglobulins present in the wells show the presence of antibodies fixed on donor RBCs. Results are interpreted as positive when RBCs form a carpet layer at the bottom of the well and negative when RBCs form a compact pellet at the bottom of the well. Direct antiglobulin test (DAT) was performed in all cases to rule out false positive cases.

Sample size in prevalence study is calculated by[3]

$$n = \frac{z^2 P(1-P)}{d^2}$$

Where Z = level of confidence

P = 0.189%, prevalence of Weak D in blood donors.[4]

d = Precision ($1/5^{th}$ of prevalence)

Then, minimum sample size required to be n = 52,277 blood donors.

This study was approved by Institutional Ethics committee.

3. Results

Total 65,407 whole blood donors were tested for blood grouping in the study period. All samples which were negative for Rh antigen in routine blood group testing were subjected to serologic weak D testing. On further testing 35 whole blood donors were found to be serologic weak D antigen positive.

Prevalence of serologic weak D phenotype in this study was 0.054 % of total whole blood donors and 1.11% of Rh negative (Rh D-) donors. (Table 1)

Table 2 shows the distribution of serologicweak D phenotype in various ABO blood groups. The maximum number of serologic weak D phenotype were from B blood group, i.e. 13(37.14%) followed by A, O and AB blood groups, i.e. 12(34.29%), 9(25.71%) and 1(2.86%) respectively.

Table 1: Serologic weak D prevalence in blood donors

	Number	Percentage
Total whole blood donors	65,407	
Rh positive (Rh D+) donors	62,254	95.18
Rh negative (Rh D-) donors	3,153	4.82
Serologic weak D (Rh Du) donors	35	0.054 (of Total whole blood donors) 1.11 (of Rh negative (Rh D-) donors)

Table 2: Distribution of serological weak D phenotype in various ABO blood groups

Blood group	Number	Percentage
A	12	34.29
B	13	37.14
AB	1	2.86
O	9	25.71
Total	35	100

Out of 35 serologic weak D phenotype 26 (74.29%) were Hindu and 9 (25.71%) were Muslims. (Table 3)

Table 3: Ethnic distribution of serologic weak D.

Ethnic group	Number	Percentage
Hindu	26	74.29
Muslims	9	25.71
Total	35	100

4. Discussion

In 1946 Stratton found that red blood cells (RBCs) of a blood donor not agglutinate with 20 anti D sera, but react with variable intensity with 12 other anti D sera. He describe this D variant as weak D or 'Du'.[1]

Various genetic studies classify D antigen into Weak D, Partial D, Weak Partial D and Del.[16]

Weak D: This is due to substitution of amino acid in transmembrane or intracellular segment of Rh D protein. This substitution leads to decrease expression of D antigen i.e. quantitative reduction. There are 147 types of weak D have been discovered, of which types 1,2,3 are common. These persons do not make anti D.[17]

Partial D: This is a qualitative defect. Some epitopes are missing in D antigen. This is due to substitution of amino acid in extracellular segment of Rh D protein. These persons are prone to form anti D when exposed to Rh D positive RBCs.[18] There are 105 types of partial D have been discovered, of which DVI is the most common.[1]

Weak Partial D: This is variant of weak D along with qualitative changes in epitopes. So, this variant has both quantitative as well as qualitative changes. The common

Table 4: Comparison table from various study

S.No.	Authors	City	Total blood donors	Rh D negative donors	P in Rh D negative donors (%)	Routine blood group Method	Du confirmation Method
1	Dhot PS et al[5] (1998)	Pune	NA	5042	0.43	IST	NA
2	Makroo RN et al[6] (2010)	New Delhi	184072	13253	0.12	IST	Tube (AHG phase)
3	Agrawal N et al[7] (2013)	Dehradun	58,614	3048	0.09	Microplate	CAT
4	Ryhan R et al[8] (2015)	Srinagar	15680	847	0.2	IST	CAT
5	Pratima K et al[9] (2015)	Imphal, Manipur	17544	346	0.578	IST	Tube (AHG phase)
6	Sadaria T et al[10] (2015)	Ahmedabad	38962	3360	0.65	Microplate (Diagast)	CAT
7	Krishna GD et al[11] (2015)	Tirupati	46654 (Donor+patient)	2883	1.04	IST	CAT
8	Lamba HS et al[12] (2017)	Jalandhar	13043	847	0.95	IST	CAT
9	Sehgal S et al[13] (2018)	New Delhi	NA	1149 (Donor+patient)	0.96	NA	CAT
10	Srivastava RK et[14] al (2018)	Ranchi	1,66,338	2013	0.35	IST	Tube (AHG phase)
11	Brar RK et al[15] (2020)	Port Blair	6415	330	1.51	IST	Tube (AHG phase)
12	Present Study	Lucknow	65407	3153	1.11	Microplate (Diagast)	Microplate (Diagast)

P=prevalence, NA= not available, IST= immediate spin tube technique, AHG= anti human globulin, CAT= column agglutination technique

weak partial D types are 4.2, 11, 15, and 21. These variants are prone to develop anti D.[19]

Del: D antigen expression is too weak on RBCs surface. This variant is Rh D negative in routine anti D and weak D testing. For detection adsorption elution tests or molecular tests are required.[1]

There are two genes, RHD and RHCE for Rh blood group system. The possible mechanisms which give rise to serologic weak D phenotypes are:[5]

1. RHD gene present in, an individual has a weak expression of D antigen.
2. Two genes interact and modify each other, leads to decrease expression of D antigen.
3. RHD gene may not encode all the epitopes of D antigen.

Prevalence of serologic weak D in India is approximately from 0.0075 to 0.189% of total blood donation. In present study it was 0.054%. The Rh D typing discrepancies may be due to various reasons:[20]

1. Testing methods (tube, microplate, column agglutination technique).
2. Saline or Coombs' phase of testing.
3. Specificities and avidity of anti D sera.

The conventional tube technique is although considered as a gold standard but is relatively insensitive.

Therefore, observed prevalence of serologic weak D is increased when a routine blood group typing is done with manual tube technique.[1] In the present study microplate technique is used for routine blood grouping and confirmation of serologic weak D. This study reported 1.11% prevalence of serologic weak D among Rh D negative blood donors. Prevalence of serologic weak D in Rh D negative blood donors is ranges from 0.09 to 1.51% (Table 4).

In ABO blood groupwise distribution, we found the maximum number of serologic weak D in B group (37.14%) followed by A group (34.29%) while in a previous study it was maximum in O group (68.3%) followed by A group (22%).[5]

The prevalence of serologic weak D varies in different part of India as well as in the world. All serologic weak D positive individuals should give a blood group card showing their Rh D status as donor and recipient, i.e. Rh D positive as a donor and Rh D negative as a recipient.

5. Conclusion

Rh D typing should be done with two monoclonal anti D sera, one for DVI and clinically significant partial D, and second for normal Rh D. In case of discrepancy,

use molecular methods for confirmation.[1] Some European centers started routine RHD gene screening of first-time donors to eliminate the risk of Rh D sensitization. Molecular testing is very costly.[21] For developing countries like India we required an affordable molecular testing technique to improve patient care or alternatively establish reference molecular laboratory for cost-effectiveness.[1]

6. Source of Funding

None.

7. Conflicts of Interest

There is no conflict of interest.

References

1. Sandler SG, Chen LN, Flegel WA. Serological weak D phenotypes: a review and guidance for interpreting the RhD blood type using the RHD genotype. *Br J Haematol*. 2017;179(1):10–9. doi:10.1111/bjh.14757.
2. 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):86–7. doi:10.1046/j.1537-2995.1992.32192116441.x.
3. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. *Gastroenterol Hepatol Bed Bench*. 2013;6(1):14–7.
4. Kumar H, Mishra DK, Sarkar RS, Jaiprakash M. Difficulties in Immunohaematology : The Weak D Antigen. *Med J Armed Forces India*. 2005;61(4):348–50.
5. Dhot PS, Machave YV. Study of weak d phenotype in heterogeneous population. *Med J Armed Forces India*. 1998;54(4):309–10.
6. Makroo RN, Raina V, Chowdhry M, Bhatia A, Gupta R, Rosamma NL, et al. Weak D prevalence among Indian blood donors. *Asian J Transfus Sci*. 2010;4(2):137–9.
7. Agarwal N, Chandola I, Agarwal A. Prevalence of weak D in northern hilly areas of Uttarakhand, India. *Asian J Transfus Sci*. 2013;7(1):90–1.
8. Ryhan R, Handoo S, Reshi R. Prevalence of Weak D among Blood Donors at a Tertiary Care Hospital in Srinagar, Kashmir. *Int J Sci Res*. 2017;6(6):1697–700.
9. Pratima K, Barilin P, Avila S, Memtombi DK, Rachandra SK, Nando SK, et al. Study of Rhesus Status among Blood Donors in RIMS Hospital. *J Family Med Prim Care*. 2015;5(7):111–4.
10. Sadaria T, Goswami HM, Patel S, Patel K, Bhatnagar N, Gajjar MD, et al. Prevalence of Weak D. *BJKines-NJBAS*. 2015;7(2):1–5.
11. Krishna GD, Babu K, Arun R, Joyhibai 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 Journal of clinical and scientific research*. 2015;4:281–84.
12. Lamba HS, Kaur K, Kaur K, Vij AS. Prevalence of Weak D (Du) in Blood Donors in a Referral Teaching Hospital. *Int J Med Dent Sci*. 2017;6(2):1509–12. doi:10.18311/ijmds/2017/18841.
13. Sehgal S, Chatterjee P, Kumar R, Pathak C. How many of the rhesus D-Negative cases are actually weak D positive? *Glob J Transfus Med*. 2018;3(1):75–81. doi:10.4103/GJTM.GJTM_59_17.
14. Srivastava RK, Prasad D, Halder D. Study of Prevalence of Weak D Antigen (Du) Amongst Supposed Rh Negative Blood Donors in a Tertiary Care Hospital of Jharkhand. *Int J Scientific Res*. 2018;7(8):69–70.
15. 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. doi:10.4103/tcmj.tcmj_222_18.
16. Subramaniyan R. Prevalence of D variants in the Indian donor population. *Hematol Transfus Cell Ther*. 2019;41(2):190–3. doi:10.1016/j.htct.2018.09.004.
17. Flegel WA. Molecular genetics and clinical applications forRH. *Transfus Apher Sci*. 2011;44(1):81–91. doi:10.1016/j.transci.2010.12.013.
18. Westhoff CM. Review: the Rh blood group D antigen. . .dominant, diverse, and difficult. *Immunohematology*. 2005;21(4):155–63.
19. Mcgowan EC, Lopez GH, Knauth CM, Liew YW, Condon JA, Ramadi L, et al. Diverse and novel RHD variants in Australian blood donors with a weak D phenotype: implication for transfusion management. *Vox Sang*. 2017;112(3):279–87. doi:10.1111/vox.12488.
20. Roback JD, Grossman BJ, Harris T, Hillyer CD. Technical manual - American Association of Blood Banks. 17th ed. Bethesda: AABB Press; 2011. p. 407.
21. Hussein E, Teruya J. Weak D Types in the Egyptian Population. *Am J Clin Pathol*. 2013;139(6):806–11.

Author biography

Ashutosh Singh, Associate Professor

Archana Solanki, Associate Professor

Devisha Agarwal, Junior Resident

Tulika Chandra, Professor & Head

Cite this article: 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):686-689.