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Original Article

Phenotypic expression of weak/variant D - Experience from a tertiary care center of Raipur (Chhattisgarh)

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Abstract
Introduction: *Testing for Rh system is performed as a routine test in blood banks. Rh system is one of the most polymorphic blood group systems. Rh positivity and negativity refers to presence or absence of D antigen on the red cell surface because it is most immunogenic and clinically important. Sometimes categorization of a person into definite D positive or D negative becomes difficult because variable expression of D antigen can occur. This is known as weak D/ partial D. Clinically weak D or partial D are of concern because serologically, it presents as Rh negative but for recipients they behave as Rh positive with possible risk of alloimmunization. Therefore, present study was undertaken with an aim to know the frequency of weak D antigen among donors and recipients presenting to our blood bank.*
Material and Method: *This was a one year observational hospital based study including recipients and donors. All patients were tested for Rh-D factor by commercially available monoclonal anti-D sera. The individuals who were found negative with anti-D were further investigated for weak D antigen by using indirect antiglobulin test using AHG sera by tube technique.*
Results: *During study period a total of 37,943 blood samples, including donors and recipients were tested for Rh antigen. Out of total samples tested 1,094 (2.88 %) were Rh negative and remaining (97.12%) were Rh positive. Antiglobulin test yielded positive results for Du in 6 persons (0.54% of Rh negative and 0.015% of all subjects).*
Conclusion: *Present study concluded that D^U prevalence among individuals presenting at our blood bank is 0.54%. we recommend that serologic testing of all D negative patients and donors should proceed to antiglobulin phase for identification of weak D positive donors.*
Keywords: *D^u, Weak Rh, Rh Negative.*

Introduction

Among various known blood group systems ABO and Rh blood group systems are clinically most significant.[1]ABO and Rh blood grouping is the most important test performed in blood banks to avoid mortality and morbidity.[2] Rh blood group system is most immunogenic and polymorphic with at least 45 independent antigens. Rh antigen was discovered by Weiner in 1939.[3] A person is categorized as Rh positive or Rh negative

respectively based on the presence or absence of Rh antigen on surface of RBC. Five clinically significant Rh antigen are C, c, D, E and e, but Rh positivity and negativity refers to presence or absence of D antigen on the red cell surface because it is most immunogenic and clinically important.[4]Immune reactions lead to formation of anti D in Rh negative individuals with resultant haemolytic transfusion reactions and haemolytic disease of the fetus and newborn.

Rh negative population shows 3-25%distribution worldwide.[5] Sometimes categorization of a person into definite D positive or D negative becomes difficult because variable expression of D antigen can occur due to lesser number of or altered expression of antigens on red cell surface. This is known as weak D/ partial D. Clinically weak D or partial D are of concern because serologically, it presents as Rh negative but for recipients they behave as Rh positive with possible risk of alloimmunization.[3] Therefore, present study was undertaken with an aim to know the frequency of weak D antigen among donors and recipients presenting to our blood bank. Literature was reviewed to formulate recommendations for our set up and to know the clinical relevance.

Material and Methods

Study Period: May 2017 to April 2018.

Type of study: Cross Sectional, Observational

Study Subjects: All the samples tested for Rh typing during study period

Inclusion and Exclusion criteria: Nil

Data Collection: From records of blood group typing.

Ethical considerations- Approval from institutional ethical committee.

Rh blood group typing on subjects blood samples was performed by immediate spin tube method using commercially available monoclonal Anti D (IgM + IgG) antisera from tulip Diagnostics (P) ltd. All standard operating procedures were followed. Instructions of the manufacturer were also adhered to. Negative results for agglutination were confirmed by microscopy. Samples that were negative for agglutination by spin tube method were further tested by indirect antiglobulin test for the presence of weak D antigen. For antiglobulin test cells were thoroughly washed by normal saline and antihuman globulin reagent was added. Thorough mixing was done and cells were centrifuged at 1000 rpm for 1 minute. Cells were suspended by gentle mixing and results were viewed under microscope. Samples showing agglutination were labeled as D^u positive

Results

During study period a total of 37,943 blood samples, including donors and recipients were tested for Rh antigen (Table -1). Out of total samples tested 1,094 (2.88 %) were Rh negative and remaining (97.12%) were Rh positive. Antiglobulin test yielded positive results for Du in 6 persons (0.54% of rh negative and 0.015% of all subjects). (table-2)

Table 1 Total collection during study period

	Male	Female	Total
Donor	17,292	927	18219
Recipient	8599	11125	19724
Total	25891	12052	37943

Table 2 Frequency of weak Rh positive among Rh negative individuals

	Rh Negative		Weak Rh Positive	
	Number	%	Number	%
Male	1016	92.87	6	-
Female	78	7.12	-	-
Total	1094	100	6	0.54

Table 3- Prevalence of weak D among Rh negative and overall study population

S. No	Year	Author	Region	Weak D %		Rh –ve (%)	Rh+ve (%)
				In D -ve	In all subjects		
1.	2018	Present	Chattisgarh	0.54	.015	2.88	97.12
2.	2015	Gupta A.[14]	East Delhi	7.6	0.25	2.98	96.7
3.	2014	Kotwal U.[15]	Jammu	0.14	.0075	5.48	94.5
4.	2014	Pahuja S.[7]	Delhi	0.2	.009	5.4	94.6
5.	2015	Ryhan R.[16]	Kashmir	0.2	.01	5.4	94.6
6.	2013	Agarwal N.[17]	Uttarakhand	.09	.005	5.2	94.8
7.	2017	Lamba H.S[18]	Punjab	-	.06	6.49	95.51
8.	2018	Gujar R.[19]	Madhya Pradesh	-	.04	4.11	95.89

Discussion

In present study we found Du prevalence of 0.54% among Rh negative persons and Rh negative prevalence of 2.88%. Prevalence of Rh negative is in slight variance with a study conducted at the same center previously showing Rh negative prevalence of 3.15%.[2] This difference may be due to smaller sample size in present study or due to study population in both studies being of recipients and donors. For definite prevalence of various blood groups population based studies with larger sample sizes are required.

In 1939 the first Rhesus antigen (D antigen) was described. D antigen positive patients were termed Rhesus-positive. Du or weak D is a quantitative variant of D antigen with weak expression of D antigen on Red blood cell surface. Detection of weak D requires testing through antiglobulin phase. It was described in 1946 by Stratton and was labeled D^U. [5] The term D^U was later replaced by a more appropriate term Weak D. Qualitative variants of the D antigen also known as partial D variant are positive for the D antigen, but they can form anti-D also.[6] These are also known as variant D.[7]

Rh protein on red cell surface are coded by two genes (RHD, RHCE) located in close proximity on chromosome 1. These genes respectively carries the the D antigen and CE antigens in various combinations (ce, Ce, cE, or CE)[8-10]

Genetic studies mechanisms are suggested for acquisition of Weak D. one postulate suggests inheritance of Rh gene coding for weak expression of D antigen. Another mechanism suggested is weak expression of D antigen due to presence of C antigen in the trans position on the opposite chromosomes such as Dce/dCe genotype. When one or more epitopes of the D antigen are missing from surface part of Rh antigen, partial D phenotype results and these individuals may be alloimmunized if transfused with D positive blood possessing the missing epitope.[5,11,12]

Molecular basis of Rh phenotype has been elaborately discussed by Flegel W.A.[3]In Rh D negative phenotype there is complete absence of Rh D protein in the erythrocyte membrane. Complete absence of RhD protein in a Rh negative person accounts for strong antigenicity of Rh D protein. Apart from lack of Rh D protein the phenotypic changes in D antigen can also occur from a series of molecular changes in Rh D protein. Depending on these molecular changes phenotypically partial D, weak D or DEL can result.[3]

Rh D protein is a trans membrane protein with a membranous part and an extra membranous portion exposed on cell surface. Substitution of an aminoacid on surface part of Rh protein can result in loss of single or multiple epitopes of the D antigen or some new antigen can be formed. This leads to weak D phenotype.[3]

If an aminoacid is substituted from trans membrane or cytoplasmic part of the Rh D protein this results in Weak D phenotype. Resultant phenotype shows quantitative weakening of the D antigen. Qualitatively there is no change.[3]

DEL phenotype of the Rh D protein shows particular weak expression of the D antigen. It is detected by demonstration of antibodies in the elute after separating them from erythrocytes. Molecular changes show incomplete integration of Rh D protein into the cell membrane.[3]

Prevalence of Rh negativity worldwide is 2.5-8.5% in india and neighbouring countries.[2]Some

communities in India like Parsis, Chitrapur Saraswats and Goans showed Rh negative prevalence of 15-17%. [5,12,13]. Worldwide it shows prevalence of 3- 17%.[4]

Various studies from different parts of India in different ethnic groups have shown weak D prevalence in range of .0075-0.25% among all persons and .09-7.6% among D negative persons (table-2).[14-19] Present study showed 0.54% weak D prevalence among Rh negative persons and .015% among all persons. Present study showed slightly higher weak D prevalence among Rh negative individuals. This may be due to larger sample size of present study.

Correct identification of Rh status of a blood donor is important because transfusion of D variant blood to a Rh negative recipient can initiate immune response. It is essential to ascertain Rh D antigen status of an individual to prevent Rh alloimmunization of Rh D negative pregnant women and for safe transfusion of blood to Rh D recipients. However in individuals with variant D definite categorization of Rh antigen status is very often difficult as partial or weak D variants give inconsistent results with commercially available anti D reagents, especially monoclonal ones. Kulkarni et al has highlighted the fact that reagent selected for anti d testing should identify the majority of D variants prevalent in our populationby simple serological techniques.[20]

For recipients Rh variant can be safely labeled as Rh negative. World health organization (WHO) recommendations state that when testing patients, it is not necessary to perform a test specifically to detect weak D if the routine anti-D reagent(s) give a negative result. But for the testing of donors test for Du should be performed when samples give a negative result with the test anti-D. Any donor found to be Du has a weakly expressed D antigen and is therefore regarded as RhD positive. There is no harm if a Du patient is typed as D negative, they will receive D negative blood without adverse effects. Our blood bank is following the WHO recommendations.

Proper identification of weak D gives following advantages clinically- Firstly Rh negative blood products are saved, secondly D negative women are saved from alloimmunization, thirdly administration of anti D to weak D positive pregnant women is saved thus saving costs and possible complications.[3] In serologically discrepant results molecular analysis may be more accurate. Recommendations state that issues related to weak D phenotype should be undertaken in conjunction with molecular studies to formulate beneficial, cost effective standardized guidelines

Now a day's genotype testing is strongly recommended for definitive typing of weak/partial Rh.[6] Genetic testing is commercially available for blood group typing since 2000. It involves extra costs initially but can help to avoid potential side effects and reduces cost in long run for individual patient.[3]

With evolution of methods terminology has also evolved from DU to weak D now with molecular and genetic testing being commercially available American association of Blood Banks (AABB) and College of American Pathologists recommended use of term "Serologic weak D phenotype" based on serologic weak D testing using antiglobulin sera in clinical laboratories versus the Genotyping of RhD for weak D based on molecular methods.[21]

Conclusion

Present study concluded that D^A prevalence among individuals presenting at our blood bank is 0.54%. we recommend that serologic testing of all D negative patients and donors should proceed to antiglobulin phase for identification of weak D positive donors. This will save potential complications in Rh negative pregnant women and transfusion recipients. In low resource health services genetic and molecular testing is not available everywhere hence every transfusion service should develop standardized guidelines for serologic testing weak D detection. It will prove beneficial and cost effective.

Limitations

The main limitation of present study is that it is a blood donor and recipient based and observational study. Elaborate population based studies with larger sample size are recommended to know the real prevalence of weak D.

Other limitation is that ours being a low resource facility we were using tube method for Rh D typing. Better methods like gel card or spectrophotometric methods can be used for Rh testing.

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Conflict of interest- None

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