The Professional Medical Journal
www.theprofesional.com

DOI: 10.17957/TPMJ/16.3471

ORIGINAL PROF-3471

D ANTIGEN;

CHANCES OF FINDING WEAK D ANTIGEN AND RE-EVALUATION OF ITS CLINICAL SIGNIFICANCE AS A ROUTINE BLOOD BANK PROCEDURE

nazish68@yahoo.com

Dr. Nazish Saqlain[1], Dr. Aatika Ahmed[2], Dr. Tooba Fateen[3], Dr. Nisar Ahmed[4]

1. The Children's Hospital and Institute of Child Health, Lahore.
2. The Children's Hospital and Institute of Child Health, Lahore.
3. The Children's Hospital and Institute of Child Health, Lahore.
4. The Children's Hospital and Institute of Child Health, Lahore.

Correspondence Address:
Dr. Nazish Saqlain
The Children's Hospital and Institute of Child Health, Lahore.
nazish68@yahoo.com

Article received on:
11/07/2016
Accepted for publication:
10/10/2016
Received after proof reading:
14/11/2016

ABSTRACT… Background: In 1939 Rh antigen was discovered by Levine and Stetson. Rh system antigens are very immunogenic, they can produce significant Hemolytic Disease of the fetus and Newborn as well as hemolytic transfusion reactions. There are numerous variants of D, the most common subtypes are Weak D and Partial D, now called as abnormal D antigens. The incidence of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%-1%. **Objectives:** To find out the frequency of Rh negativity and weak D antigen among the donors coming to the blood bank of The Children's Hospital & Institute of Child Health, Lahore and to review the clinical significance of weak D antigen in transfusion perspective especially its role in alloimmunization caused by Weak D antigen when transfused to Rh negative individuals. **Study Design:** Cross- sectional study. **Setting:** The Children's Hospital and Institute of Child Health, Lahore. **Period:** 1st Jan 2015 to 31st May, 2015. **Materials and Methods:** 6320 healthy donors were randomly selected. All samples were grouped for ABO and Rh-D factor by immediate spin tube technique. All samples found Rh negative, were further processed for weak D antigen with monoclonal anti D sera by using indirect Coomb's technique. The presence of macroscopic or microscopic agglutination was recorded as Rh positive. In case there was no agglutination the mixture was washed 4 times with normal saline. After the last wash, saline was decanted and 2 drops of monoclonal, polyvalent anti human globulin was added. Macroscopic and microscopic agglutination was looked for and any agglutination at this stage was recorded as weak D antigen. Positive control (check cells i.e. washed O positive cells with diluted anti D) and negative control (washed O positive cells) were always put. **Results:** Among the 6320 healthy donors, 1224(19.4%) were Rh-D negative and 5096(80.6%) were Rh-D positive. Of the 1224 Rh D negative samples, 3 (0.2%) samples found positive for weak D antigen. **Conclusion:** The frequency of Rh negative blood group was 0.2% among the healthy donors at The Children's Hospital and ICH, Lahore. Although the frequency is low but it's proven by literature that weak D antigen can produce alloimmunization if transfused to Rh-D negative subjects. At the same time the cases of hemolytic reactions reported previously with Weak D antigen have been scarce.

Key words: Anti D, immunogenic, blood donors, alloimmunization

Article Citation: Saqlain N, Ahmed A, Fateen T, Ahmed N. D antigen; chances of finding weak d antigen and re-evaluation of its clinical significance as a routine blood bank procedure. Professional Med J 2016;23(11):1395-1399.
DOI: 10.17957/TPMJ/16.3471

INTRODUCTION

In Transfusion medicine the two most important blood group systems from clinical perspective are ABO and Rh blood group systems discovered in 1939 by Levine and Stetson. As the Rh system antigens are very immunogenic, once present they can produce significant Hemolytic Disease of the fetus and Newborn as well as hemolytic reactions following transfusion.[1] Today the Rh blood group system contains over 54 different antigenic specificities but D antigen is the most commonly found. Rh- positive indicates that an individual's red cells possess the D antigen and Rh-negative indicates red cells lack D antigen.[2] In humans, Rh-system is one of the most complicated blood group system genetically, its inheritance depends on a complex interplay of two closely linked genes; RHD controls the expression of D antigen while RHCE is responsible for the expression of C, c, E and e antigens.[3]

D antigen has many variants. Broadly two categories are described, Weak D (previously Du term devised by Stratton in 1946) and Partial D, these terms are however used interchangeably and clinically are of little significance. Weak D cells express all epitopes of D antigen but at a low level and are not able to stimulate anti-D production, whereas on partial D red cells some epitopes of D are missing. An individual with Partial D red cells when immunized by a complete D antigen, can make antibodies to the D epitopes they lack. However, things are not as simple as theoretically proposed, some individuals with Weak D found to produce anti-D and some with Partial D antigens phenotypes led to no anti-D production as the epitope expression was very weak. According to the recent recommendations the term Abnormal D is introduced, taking Weak D and Partial D types under its umbrella.[4]

The incidence of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%-1%.[1] When red cells positive for weak D are transfused to a Rh negative recipient it may lead to alloimmunization to the Rh D antigen. Such blood transfusion to an already immunized individual may result in speedy RBCs destruction or Hemolytic Transfusion Reactions (HTR).[5]

The current opinion is that the weak D individuals particularly the women in the child bearing age group should be considered as RhD negative if they require blood transfusion for any medical or surgical reason. There is a potential risk of antibody formation against the missing epitopes when transfused with Rh positive red cells. Alloimmunization of females with weak D while in the reproductive years of life can be life threatening and can lead to the hemolytic disease of the fetus and newborn. The most important individuals to be typed for weak D antigen are donors because if they found to have Weak D they should be labelled as Rh D positive. Studies with molecular analysis should be conducted to formulate a cost-effective national policy as the knowledge of blood group phenotype distribution in that particular population is very important for safe transfusion services.[5]

The current study was conducted with the objective to determine the frequency of weak D antigen in donors coming to our blood bank so that the suggestions can be drawn. There is a strong need to formulate a national policy about performing weak D serology as a routine blood bank procedure or not and to review the literature to study the possibility of alloimmunization caused by Weak D antigen when transfused to Rh negative individuals.

MATERIALS AND METHODS

It was a cross- sectional study conducted at The Children's Hospital and Institute of Child Health, Lahore, from 1st Jan 2015 to 30th June, 2015.The objective of the study was to find out the frequency of Weak D antigen in Rh negative donors coming to the blood bank of The Children's hospital, Lahore and to review the importance of Weak D in terms of alloimmunization.6320 healthy donors were randomly selected. ABO and Rh-D typing was done on all the samples by commercially available anti-sera using immediate spin tube technique. All samples found Rh negative, were further processed for weak D antigen with monoclonal anti D sera by using indirect Coomb's technique. Equal volumes of 3% of washed cells and anti D sera were mixed and incubated for 45min at 37°C.The cell button was resuspended and looked for agglutination or hemolysis. The presence of macroscopic or microscopic agglutination was recorded as Rh positive. In case there was no agglutination the mixture was washed 4 times with normal saline. After the last wash, saline was discarded and 2 drops of monoclonal, polyvalent anti human globulin was added. Macroscopic and microscopic agglutination was looked for and any agglutination at this stage was recorded as weak D antigen. Positive control (check cells i.e. washed O positive cells with diluted antiD) and negative control (washed O positive cells) were always tested.

DISCUSSION

Rh blood group system comprises of more than 54 antigens out of which five are clinically significant.

RESULTS

Gender	Number (n)	Frequency (%)
Male	6315	99.9
Female	05	0.08
Socioeconomic status		
High	05	0.08
Middle	2025	32.04
Low	4290	67.9

Table-I. Demographic representation of data

Age Range	16-26yr	27-36yr	37-46yr	47-56yr	57-60yr
Number (n)	338	4297	1685	0	0
Frequency (%)	5.3	68.01	26.7	0	0

Table-II. Age distribution among donors Mean Age± SD: 30.50±14.25 years

RH Status	Number(n)	Frequency (%)
Rh positive	5096	80.6
Rh negative	1224	19.4
Total Donors	6320	100

Table-III. Rh Status Among Donors of the 1224 Rh D negative samples, 3 (0.2%) samples found positive for weak D antigen.

ABO Blood Group	Number(n)	Frequency (%)	Rh negative		Weak D positive	
			n	%	N	%
A	1663	26.3	399	6.3	1	0.08
B	2182	34.5	216	3.4	1	0.08
O	1480	23.4	386	6.1	1	0.08
AB	995	15.7	223	3.5	0	
TOTAL	6320	100	1224	19.4	3	0.2

Table-IV. Distribution of ABO/Rh negative blood group & weak d among donors

These antigens are D, C, c, E and e. The two autosomal dominant genes RHD and RHCE on chromosome 1 encodes these five antigens.[6] The D antigen is most immunogenic making it an important part of immunohematology and transfusion practices. Rh positivity and negativity imply presence or absence of the D antigen on the surface of red blood cell. There is a lot of polymorphism in the D antigen phenotype because of variations due to deletions and missense mutations. There is an evidence to indicate that the D antigen is a mosaic of many epitopes.[5]

Majority of the world population is D- positive with the approximate figures of 85% of Caucasians, 95% among Africans and over 99% of eastern Asians.[4] Depending on ethnic group about 3-25% lack Rh D antigen worldwide. The incidence of Weak D antigen is different in different geographical areas; however, in Caucasians it is found to be 0.2%-1%.[10] A study done by Urbaniak and his colleague found that frequency of Weak D antigen in Caucasians is 0.2%-1%.[11] 0.3%-0.5% Indian population found to have Weak D, while incidence in Europe is 0.23%-0.5% and 3% in USA.[10]

A study done by Krishna et al reported prevalence of Weak D antigen to be 0.06% in Indian population.[5] In Uttarkand northern hilly areas of India, Agarwal et al in 2013 found it to be 0.005%.[1] In Pakistani Population a study done in Lahore, Punjab found the prevalence of Rh negative to be

13.7% and among them 1% are Weak D positive and a local study done in Swat found 9.87% to be Rh negative.[10] A study done in Baqai Medical University, Karachi among 48,228 healthy donors 3375(7%) found to be Rh negative and among them, 27 donors (0.8%) were Weak D antigen positive.[3] Our study revealed that out of 6320 cases, 1224(19.4%) subjects were Rh negative and 3(0.2%) of negative subjects expressed the weak D antigen, which is consistent with the study done at The Baqai Medical University, Karachi.

Wagner et al in 1999 indicated that an amino acid change in the transmembrane and intracellular regions of the D antigen affecting its insertion and hence density on the surface of RBC is due to point mutations in the RHD gene.[7] Using flow cytometry it was established that the weak D subjects had at least ten times lower expression of the antigen as compared to D positive individuals. In contrast to the normal D+red cells which bear about 10,000 (R1r) to 30,000 (R2R2) D antigen sites per cell, Du red cells have only 300 to 9000 per cell.[8]

There are three genetic mechanisms postulated for the acquisition of weak expression of the D antigen. These are:

1. Individuals inherit the RHD gene which codes for a weakly expressed D antigen.
2. D antigen may be weakly expressed due to presence of C antigen in the trans position on the opposite chromosomes such as Dce/dCe genotype. This is seen fairly commonly in blacks.
3. When one or more epitopes of the D antigen are absent a weak D phenotype may be seen. This is termed as partial D antigen and these individuals may be alloimmunized if transfused with D positive blood bearing the missing epitope.

At times partial D antigens may present as normal D types and may remain undetected unless they form anti D.[8]

As D antigen is highly immunogenic, a significant antibody response is seen when a D negative patient receives weak D positive blood. So, individuals with weak D phenotype are typed depending on whether the person is donor or the recipient; so recipients with weak D are considered D negative and must be given D negative blood and donors are considered as D positive. If the mother is Rh negative and fetus has weak D phenotype, then the mother must receive Anti-D prophylaxis as passage of weak D red cells from fetus to mother may result in sensitization and causes Hemolytic Disease of Fetus and Newborn.[9] Theoretically, when weak D positive blood is transfused to a D negative person it may lead to alloimmunization. But this is debated because there are not enough cases to support this theory.[12]

A recent study shows that the most important D variant, from a transfusion point of view, is DVI ('D six'). Although most common, but as it lacks most of the D epitopes therefore most commonly associated with the production of anti-D. According to the British Society of Haematology protocols the anti-D reagents used for typing patients (recipients) should not detect DVI. The situation will be reversed in the case of donor typing. So the anti-D reagents used for typing donor red cells should detect DVI and such donors will be considered as D-positive. Hence DVI persons should be typed D-negative patients, but D-positive donors. Weak D types 1,2,3 are three most common D variants and almost never associated with anti-D production.[4]

In 1962, Schmidt et al (cited by Jones et al) did an experiment with 45 Rh negative recipients who were transfused with small amounts of Du blood, none-produced anti-D, although anti-E and anti-K were found in two individuals. Critically evaluating the particular work of Schmidt et al, Contreras suggested that if such weak D antigens do not lead to antibody stimulation, there is weak evidence to carry out such time consuming and cost bearing test.[8] In some countries the indirect antiglobulin test is still carried out for Weak D typing but a molecular test is mandatory in Switzerland for confirming D-negative status of donors.[7]

CONCLUSION

Although the Weak D antigen can produce alloimunization but in our Blood Bank as well

as in other studies the frequency of Weak D is too low and the procedure to carry out with antiglobulin test is too lengthy and time taken, so it's questionable how significant it is to make Weak D testing as a part of Routine blood banking. There is a high need to review the local guidelines for blood bank procedures keeping in view of the local population characteristics and financial restraints.

REFERENCES

1. Agarwal N, Chandola I, Agarwal I. **Prevalence of weak D in northern hilly areas of Uttarakhand, India.** Asian Journal of Transfusion Science 2013; 7(1): 90-91.
2. Harmening DM. **Modern Blood Banking & Transfusion Practices, 6th ed.** Philadelphia: F. A. Davis Company; 2012.
3. Usman M, Rizwan M, Waheed U, Moinuddin, Saboor M, Anjum S. **Prevalence of Weak 'D' Antigen in Pakistani Population.** Journal of Public Health and Biological Sciences 2013; 2(1): 169-172.
4. Hoffbrand AV, Higgs DR, Keeling DM, Mehta AB. **Postgraduate Haematology, 7th ed.** Hoboken, New Jersey, United States: John Wiley & Sons, Ltd; 2016.
5. Krishna G D, Babu KVS, 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.** Journal of Clinical and Scientific Research 2015; 4(4): 281-284.
6. Pratima K, Barilin P, Avila S, Memtombi DK, Rachandra SK, Nando SK et al. **Study of Rhesus Status among Blood Donors in RIMS Hospital, Imphal.** International Journal of Health Sciences and Research 2015; 5(7): 111-114.
7. Wagner FF, Gassner C, Muller TH, Schonitzer D, Schunter F, Flegel WA. **Molecular Basis of Weak D Phenotypes.** Blood 1999; 93(1): 385-393.
8. Tayyab M, Malik AR, Khan AS. Du **Phenotype - A Review.** Journal of Ayub Medical College, Abbotabad 2000; 12(3): 41-44.
9. Kumar H, Mishra DK, Sarkar RS, Jaiprakash **M. Difficulties in Immunohaematology: The Weak D Antigen.** Medical Journal of Armed Forces of India 2005; 61(4): 348-350.
10. Aslam A, Azmi R, Sheikh MZ, Javaid I. **Frequency of weak expression of 'D ALLELE' among healthy blood donors.** Pakistan Journal of Physiology 2015; 11(3): 22-24.
11. Urbaniak SJ, Robertson AE. **A successful program of immunizing Rh-negative male volunteers for anti-D production using frozen/thawed blood.** Transfusion 1981; 21(1): 64-69.
12. Tippet P. **Subdivisions of the Rh Antigen D, review.** Medical Laboratory Scientist Journal 1988; 45(1): 88-91.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Nazish Saqlain	Conception of ideas, Data analysis, drafting author and editing	
2	Dr. Aatika Ahmed	Perfamance of tests data analysis, drafting of author	
3	Dr. Tooba Fateen	Drafting of author and editing of author	
4	Dr. Nisar Ahmed	Drafting of author and final approval All author are overtable for every aspect of this patwlar author	