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Incidence of D^U Antigen at a Blood Bank of Tertiary Care Centre: A Statistical Study.

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ABSTRACT

Background: D antigen discovered in the year 1939 was known to be the most immunogenic antigen in the complex Rh blood group system. This antigen has a lot of phenotypic variation due to gene polymorphism and genetic heterogeneity. We have assessed the incidence of Rh negative and weak D blood groups in the North Eastern Lucknow region of Uttar Pradesh, specially the population visiting the Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh. **Methods:** Rh blood group and typing of all donors and patients in blood transfusion centre under the Department of Pathology, ELMC&H, Lucknow, Uttar Pradesh, was analyzed for aperiod of 10 years from Jan 2000 to Jan 2010. There were a total of 1086 subjects enrolled in this study. **Results:** Out Of the Total 1086 All Came Out To Be Negative on Repeating the Tests for Weak D-Antigen Testing. Overall Rh negative was seen to be present in higher frequency in males than in females a negative group was more common in males while rest other groups had a slight female preponderance. **Conclusion:** Our study concluded that the incidence of Rh negative blood group was 34.5% of the population screened for presence of D^u antigen in our blood bank.

Keywords: D^U Antigen, Rh blood group, phenotypic variation, gene polymorphism, genetic heterogeneity.

INTRODUCTION

The greatest breakthrough in transfusion medicine after the discovery of ABO grouping system is the discovery of the Rh antigen in 1939[1] However after reports of conflicting results in Rh grouping, a weakly reacting D antigen was described in 1946[2].The incidence of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%- 1%[3]

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The debate over relevance of weak D antigen still goes on. The role of weak D antigens in alloimmunization and hemolytic disease of newborn cannot be undermined. Weak D represents a D phenotype in which due to decreased antigen sites, the antigen is not detected by routine grouping (using immediate spin tube methodology). Demonstration of this weakly expressed antigen requires evaluation by prolonged incubation and use of anti-humanglobulin. In pregnant women with positive weak D antigen, guidelines regarding Rh group transfusion and postpartum immuno-prophylaxis are not yet established.

Rh blood group system is a complex system comprising more than forty antigens out of which five are clinically significant. These antigens are C, c.D, E, and e. Genes for the five Rh antigens are encoded by two autosomal dominant genes RHD and RHCE on chromosome 1. The D antigen is most immunogenic and plays an important role in immunohaematology and blood banking. Consequently 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[4]. Since there is a genetic alteration associated with inheritance of the weak D antigen it was interesting for us to hypothesize a study in the Muslim predominant population visiting the hospital which has a higher chances of developing genetic abnormalities due to the culture of consangious marriages in the community. Hence in the present study we have sought the incidence of Rh negative blood group and that of weak D antigen in the North Eastern Lucknow region of Uttar Pradesh, a muslim predominant area, specially the population visiting the Era's Lucknow Medical College and Hospital (ELMCH), Lucknow.

MATERIALS AND METHODS

Rh blood group and typing of all donors and patients in blood transfusion centre under the Department of Pathology, ELMC&H, Lucknow, Uttar Pradesh, was analyzed for aperiod of 10 years from Jan 2000 to Jan 2010. There were a total of 1086 subjects enrolled in this study. Routine Rh typing was done using the immediate spin tube technique (using monoclonal anti D antisera, IgG & IgM from two different companies, Diamed and Tulip).

Samples which were negative for agglutination by both the antisera were further evaluated. Equal volume of 2-3% of washed cells and anti-D sera were mixed and incubated at 37°C for 45 minutes. The cell button was re-suspended and agglutination was looked for. In presence of macroscopic or microscopic agglutination the sample 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. The contents of the tube were mixed and centrifuged at 1000rpm for 30 seconds. 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 used. All negative results were observed for agglutination after addition of control sample.

RESULTS

Out of the total 1086 samples analysed for weak D-antigen all came out to be negative with a slight male preponderance for the male gender having a negative blood grouping [38.1%] [Table 1, 2].

Out Of the Total 1086, all came out to be Negative on repeating the test for weak D-Antigen Testing.

Table 1: Frequency of Blood Groups.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	A NEG	150	13.8	13.8	13.8
	A-B NEG	66	6.1	6.1	19.9
	B NEG	265	24.4	24.4	44.3
	O NEG	230	21.2	21.2	65.5
	RH NEG	375	34.5	34.5	100.0
	Total	1086	100.0	100.0	

Table 2: Sexwise Cross tabulation.

			Sex			
			Not mentioned	F	M	Total
Result	A NEG	Count	0	20	130	150
		% within Sex	0%	16.8%	13.5%	13.8%
	A-B NEG	Count	0	11	55	66
		% within Sex	0%	9.2%	5.7%	6.1%
	B NEG	Count	1	47	217	265
		% within Sex	20.0%	39.5%	22.6%	24.4%
	O NEG	Count	3	34	193	230
		% within Sex	60.0%	28.6%	20.1%	21.2%
	RH NEG	Count	1	7	367	375
		% within Sex	20.0%	5.9%	38.1%	34.5%
	Total	Count	5	119	962	1086
		% within Sex	100.0%	100.0%	100.0%	100.0%

Overall Rh negative was seen to be present in higher frequency in males than in females a negative group was more common in males while rest other groups had a slight female preponderance.

DISCUSSION

Detection of the D antigen is done routinely by immediate spin tube method. A weak expression of the D antigen described by Stratton in 1946 was called the D^u antigen. This term was abandoned in 1984 and replaced by the weak D antigen. This is detected by use of anti human globulin. A study indicated that point mutation in the RHD gene results in an amino acid change in the trans-membrane and intracellular regions of the D antigen affecting its insertion and hence density on the surface[5,6].Incidences of D negative and weak D antigen are variably reported around the globe. Incidence of Rh negativity is 3- 25% worldwide depending upon the ethnic group. Approximately 5% of the Indian population is negative for the D antigen, though the incidence varies from community to community[7-9].Rh blood group system

is a complex system comprising gmore than forty antigens of which five are clinically significant. These antigens are C, c.D, E, and e. Genes for the five Rh antigens are encoded by two autosomal dominant genes RHD and RHCE on chromosome 1. The D antigen is most immunogenic and plays an important role in immune hematology and blood banking. Consequently Rh positivity and negativity imply presence or absence of the D antigen on the surface of red blood cell. There is an evidence to indicate that the D antigen is a mosaic of many epitopes[4].

There are three genetic mechanisms studied for the acquisition of weak expression of the D antigen. The first being that the, Individuals inherit the RHD gene which codes for a weakly expressed D antigen. Another mechanism held responsible is that the 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. When one or more epitopes of the D antigen are missing 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[4].At times partial D antigens may present as normal D types and may remain undetected unless they form anti-D. Using molecular techniques, DNA genomic samples of D positive, D negative, weak D and partial D individuals have been extensively studied. These studies showed that some serologically Rh negative individuals had an intact RHD gene but for a point mutation which caused the negative phenotype[7]. There is heterogeneity in the inheritance of a weak D phenotype. Some studies revealed that RHD gene alteration leads to amino acid substitution in the cellular and transmembranous part of the D antigen resulting in a weak D phenotype[5]. Other studies done observed that a normal RHD gene with a severely reduced messenger RNA transcript can also cause a weak expression of the normal polypeptide[8]. Using flow cytometry it was seen that the weak D individuals had at least ten times lower expression of the antigen as compared to D positive individuals[6].

A review of literature showed no study for the incidence of Rh negative blood group in the North Eastern Lucknow region of Uttar Pradesh, specially the population visiting the Era's Lucknow Medical College and Hospital. A similar study has been done in Garwal Uttarakhand region where they found just one case positive for the weak D antigen out of the 5855 cases they studied for a period of 4 and a half years. We on the other hand found none positive out of the 1086 cases we studied in our population over a period of 10 years. The incidence of weak D antigen ranges from 0.2%-1%, worldwide. Studies conducted in India showed an incidence of 0.189% and 0.15%[3,10]. In the studies which have detected weak D antigen, the use of potent monoclonal antisera with a high antibody titre may be responsible for detecting the Rh D positive cells that would be otherwise difficult to detect with less sensitive polyclonal reagents[11].The D antigen is highly immunogenic and a significant antibody response is seen when a D negative patient receives D positive blood. Hemolytic disease of the newborn is also caused by an already sensitized pregnant D negative female with a D positive fetus. Hence these females are given anti D immunophylaxis for safety. However, even after so many years of the discovery ofthe weak D antigen, its clinical significance, immunogenicity and guidelines are controversial. Therefore, the blood banks evaluate all D negative subjects for weak D antigen by AHG, though the cost effectiveness of the same has never been studied. In a study it was seen that in child bearing women who expressed weak D antigen, 10.2% institutions transfused D negative blood components while approximately 90% transfused D positive components 14. Theoretically, transfusion of weak D positive blood to a D negative patient may lead to alloimmunization. However there are not enough cases to substantiate this contention. In a follow-up of 45 Red negative cases who received weak D blood, none developed anti- D antibodies even though the weak D positive erythrocytes remained in the circulation for hundred days in 34 cases[12]. Only two case reports have reported alloimmunization of D negative patients following transfusion with weak D blood[13]

A study recommended that obstetric patients who test positive clearly for weak D by AHG (2+ macroscopic agglutination) can be safely regarded as D positive and transfused with D positive blood components. Most of the institutions recommend that a weak D status of gravidae should avoid postpartum or ante partum anti-Dimmuno prophylaxis[14]. This is contradictory to the decision of American Association of Blood Banks in 2003 that it is no longer necessary to test for weak D antigen in obstetric patients[15]. The reason behind this was that the present day blood typing reagents are more potent. They recommend that patients should be typed either as D positive or D negative by immediate spin tube method. The clinical implication of this being that a few women who actually have a weak expression of the D antigen will receive Rh immunoglobulin which has no adverse outcome. Newer techniques like UV spectrophotometric approach to blood group typing and molecular analysis may be more accurate[16]. Issues related to weak D phenotype should be undertaken in conjunction with molecular studies to formulate beneficial, cost effective standardized guidelines[17].

In our blood bank the incidence of weak D is very low, probably due to routine use of two potent monoclonal anti-D blood typing antisera. Weak D individuals are treated as D positive when they are

donors and D negative when recipients of blood transfusion. However the effort, time and money put in these testing needs to be evaluated further and should be clinically justified.

CONCLUSION

Our study concluded that the incidence of Rh negative blood group was 34.5% of the population screened for presence of D^u antigen in our blood bank. We found 0% weak D-antigen positivity. This 0 % could be due to the rarity of presence of this antigen as well as difficulty in its detection as has been seen that the incidence of occurrence of D^u is < than 0.1 %[11]. Clinical relevance of weak D is debatable however it becomes relevant in cases of pregnant females where the presence of an undetected weak D antigen may cause transfusion reactions as well as reaction in the next pregnancy.[18,19] Still the exact implication of having a weak D antigen is debatable with few studies calling its presence a significance finding while few protocols like that of the American association of blood bank not considering it significant[20,21]. Since there is a genetic alteration associated with inheritance of the weak D antigen it was interesting for us to hypothesize that in the Muslim predominant population visiting the hospital which has a higher chances of developing genetic abnormalities due to the culture of consangious marriages in the community. Though in our hospital, we evaluate all D negative patients and donors for weak D antigen, studies with molecular analysis should be conducted to formulate a cost effective policy.

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