ORIGINAL RESEARCH PAPER

Health Science

A STUDY ON RHESUS INCOMPATIBILITY AND FREQUENCY OF WEAK D ANTIGEN AMONG BLOOD DONORS AND RECIPIENTS ATTENDING NEMBA DISTRICT HOSPITAL

KEY WORDS: Weak D, Rhesus incompatibilities, Blood donors and Recipients.

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ABSTRACT

After ABO antigens, Rhesus D antigen is the next most important in the field of transfusion medicine. There are numerous variants of D antigen; the most common subtypes are Weak D and Partial D, now called as abnormal D antigens because both terms are used interchangeably. These variants may form anti-D antibody. Weak D refers to reduced expression of D antigen on the red blood cell that requires an extended testing with indirect antiglobulin test (IAT) to get detected. The clinical significance of weak D antigen in transfusion is allo-immunization caused by Weak D antigen when transfused to Rhesus negative individuals. The objective of this study was to assess Rhesus incompatibility and frequency of Weak D among blood Donors and recipients attending Nemba District Hospital. The present study was a cross sectional study which were conducted in Nemba district Hospital Laboratory in which all blood donors and recipients samples were tested for ABO and Rhesus D by conventional tube technique by using anti-A, anti-B; anti-AB and anti- D reagent. The blood samples which were negative for agglutination by immediate spin method were further tested for weak-D using anti-human globulin reagent in the IAT (Indirect antiglobulin test). In a period of two months, a total of 129 donors and 72 recipients' blood samples were collected and analyzed for ABO and rhesus blood grouping. Among the total 201 samples; 24.3% (n=49) were of group A, 20.39 % (n=41) of group B, 47.7 % (n=96) of group O and 7.46% (n=15) were of AB group. Among all participants, 96.51% (n=194) were positive and 3.48% (n=7) were negative for D antigen. The donors and recipients blood were tested for Rhesus-D and found to be 96.15% (n=124) and97.22% (n=70) positive respectively while 3.84% (n=5) and 2.77% (n=2) were found to be negative respectively. The Weak D antigen among Rhesus-D negatives donors were 0% (n=0) that of recipients were found to be 50% (n=1). The total frequency of weak D among blood donors and recipients attending Nemba district hospital were found to be 0.49% (n=1). The inability of testing weak D antigen in the blood group may cause transfusion reaction. Some forms of weak D antigen are immunogenic and can result in production of allo-antibodies. For safe blood transfusion it should be mandatory to check the presence or absence of weak D antigen among blood donors and recipients who are rhesus D negative.

INTRODUCTION

In Transfusion medicine the two most important blood group systems from clinical perspective are ABO and Rhesus (Rh) blood group systems (Kumar et al., 2004). 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 the later produce antibodies to Rhesus D antigen due to they do not have it (Saqlain et al., 2016). The Rhesus system antigens are very immunogenic, once present they can produce significant Haemolytic Disease of the foetus and New-born for pregnant women who have Rhesus negative when the foetus is Rhesus positive; as well as haemolytic reactions following transfusion when the donor is Rhesus positive while the recipient is Rhesus negative (Tippett et al., 2010). 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 antigen 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 (Githiomi etal., 2016). The significance of weak D lies in the fact that transfusion of red cells from a weak D person to a D negative person may result in allo-immunisation and subsequent exposure to such red cell can lead to fatal haemolytic reaction or haemolytic disease of new-born in a sensitized pregnant female as this pregnant female her immune system will produce antibodies against the lacking D antigen (Denomme et al., 2005). The incidence of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%-1% (Kumar et al., 2004).

It means that these individuals who are Weak D positive could be considered as Rhesus positive but they are taken as Rhesus negative and give blood to Rhesus negative which results in major transfusion reaction which is very fatal. In Blood transfusion all the blood donors and recipients should be tested for the presence of weak D in case where the blood Donor is of Rh negative and the recipient is Rh negative (Contreras et al., 2006). The reason behind is that a Donor can be classified as Rh negative due to lack of potency of Anti-D which have failed to detect the presence of weak D in both donor and patient red blood cell. In order to find out the presence of Weak D the second step of Indirect Anti-globulin Test (IAT) could be done so that the Donors who have been named as D negative by the previous step which was unable to detect the presence of weak D be labeled as Rh positive (Flegel et al., 2002).

The recipient could also be tested for the presence of weak D

for those recipients who have been labeled as Rh negative and the second step of IAT reveal that they have weak D and later on be labeled as Rh positive. In hospital many lab technicians do not take into consideration the second step of doing IAT in order to find out if there is the presence of Weak D antigens in blood Donors and recipients they only perform immediate spin cross match and do not proceed to the second step of IAT (Makubi et al., 2012).

The reason behind this is that to perform IAT is time consuming as it require long period of time for incubation, and also apart from time consuming the second step of IAT it can lead to cost expenses as it needs some reagents and different materials. The failure of not doing IAT on all blood donors who are Rh negative can lead to allo-immunization if their blood is transfused to Rh negative recipients which could results in Hemolytic transfusion reaction, it can also cause Hemolytic disease of new born (HDN) for women of child bearing age if these women are Rh negative and get transfused with weak D positive blood suspected of being Rh negative due to failure of doing IAT (Brar et al., 2019). The recipients also suspected of having Rh negative buttruly are Rhesus positive due to the presence of weak D, performing of IAT can remove unnecessary stress of looking Rh negative blood to be transfused as the recipients with weak D can be considered as Rh positive. This research is coming to aware the laboratory technicians about weak D in order to avoid transfusion reactions caused by it.

MATERIALS AND METHODS

Study Area

This study was conducted at Nemba Hospital located in Northern Province, Gakenke District, Nemba sector, Gacaca cell. It is located on the main road Musanze-Kigali at one Kilometer from Gakenke center. This hospital in the laboratory service is equipped with all materials necessary for performing the similar study.

Study Design

This was a cross sectional study in which blood samples from donors and recipients were analyzed in Immunohematology for blood grouping and Rhesus typing. The samples which were Rhesus negative were further analyzed for the presence of Weak D. The study was carried out in a period of two months, from 10th September to 10th November, 2020.

Study Population

The targeted populations were blood donors and recipients who needed blood transfusion in the period of this study.

Sample Size

This study included 201 participants; where Donors and recipients were 129 and 72 respectively. The recipients who need blood transfusion and their donors were taken into consideration. The recipients with others cases other than blood transfusion were not taken into account in this study.

Sample Collection

Gloves were worn, donor's samples were obtained from Immunohematology laboratory fridges; donors blood usually came from Transfusion Center and the recipients' sample were collected bynurses in EDTA tubes in different Hospitals' wards and then were transferred in laboratory department.

Sample Analysis

Six test tubes were brought to the bench and divided into two series each containing three test tubes. Then by using a permanent markers each of the three tubes in each series was labeled as anti-A, anti-B and the last anti-D. In tube labeled anti-A one drop of an anti-A was added and in tube labeled as anti-B one drop of anti-B was added and also in tube labeled as anti-D, one drop of anti-D was added.

In the three donors' tubes one drop of donor blood was added in each by using pastor pipette and also in the three recipient tubes one drop of whole blood was added in each tube by using another pastor pipette. All six tubes were well mixed one by one and then observe for the presence or absence of agglutination. In tube labeled anti-A if the agglutination occurs in it but not in anti-B tube the person was blood group A. In tube labeled anti-B if agglutinations occurs but not in Anti- A the person was blood group B, if agglutinations occur in all anti-A and anti-B the person was AB blood group and also if there is no agglutination in both anti-A and anti-B the person was blood group O. In tube labeled anti-D if agglutination occurs the person was Rhesus positive if not the person was Rhesus negative.

The samples (Both Donors and Patients) that were found to be Rhesus negative, were further processed for weak D antigen with monoclonal anti D sera by using indirect Coomb's technique i.e. IAT in the following steps:

Preparation of five percentile (5%) of red cell suspension

One to 2 ml of anticoagulated blood was placed in a test tube. The tube was filled with saline and gets centrifuged. The supernatant saline was decanted. The latter two steps were repeated until the supernatant saline was clear. In another clear test tube 10 ml of saline was added. In that tube 0.5 ml of the packed cell button prepared was added. The tube was covered until time of use. Immediately before use, the cell suspension was mixed by inverting the tube several times until the cells were in suspension.

Perform the first step of indirect Anti-globulin test

Equal volumes of 5% of washed red cells suspension and anti D sera were mixed and incubate for 45min at 37°C. The cell button was suspended and looks for agglutination or haemolysis. The presence of macroscopic or microscopic agglutination was recorded as Rh positive.

Perform the second step of indirect Anti-globulin test

In case there was no agglutination the mixture were 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 anti-D) and negative control (washed O positive cells) were always tested in order to make sure that the procedure was done well.

The sample which was positive after performing IAT (weak D) was taken as Rhesus positive for blood donors and Rhesus negative for recipients. Those samples which were Rhesus negative after IAT were taken as Rhesus negative irrespective of being either donor or recipient.

Statistical Analysis

The descriptive statistics was used for describing data obtained in immunohematology laboratory. The Categorical values were expressed as frequency and percentage. The presentation of results was done using tables.

Ethical Consideration

Prior to this study, official approval to conduct this study was obtained from Nemba District Hospital by the Hospital Research and Ethic Committee. The principle of confidentiality and patient's privacy were respected by using code for donors and recipients the results will only be used for academic purpose.

RESULTS

The distribution of Blood group and Rhesus D antigens among Blood donors and Recipients

The obtained results include a total of 129 donors and 72 recipients' blood samples were analyzed for ABO and Rhesus blood grouping. Among the total 201 samples, 24.3 %

[(49×100)÷201] (n=49) were of group A, 20.39 % [(41×100)÷201] (n=41) of group B, [(96×100)÷201] 47.7 % (n=96) of group O and 7.46% [(15×100)÷201] (n=15) were of AB group. For Rhesus D antigen 96.51 % [(194×100)÷201] (n =194) were positive and 3.48% [(7×100)÷201] (n = 7) were negative.

Table 1 : Blood Group Distribution among Blood Donors and Recipients

Blood Group	Donors		Recipients		TOTAL
	Rh Positive	Rh Negative	Rh Positive	Rh Negative	
Group A	30	2	16	1	49 (24.3 %)
Group B	24	3	13	1	41 (20.39 %)
Group O	60	0	36	0	96 (47.7 %)
Group AB	10	0	5	0	15 (7.46%)
TOTAL	124	5	70	2	201 (100%)

Table 1 is showing the distribution of blood grouping and rhesus typing among the donors and recipients attended Nemba district hospital in the period of this study. The outcome is that, there is a higher number of Rhesus positive and lower number of rhesus negative. The blood group O individuals were counted in high number compared to others blood groups.

Weak D frequency among rhesus negative donors and recipients

The total population of the rhesus negative individual in the study were found to be 7 in blood both donors and recipients. In these individuals one recipient was found to be weak D positive which contain the 14.28% of the total population of rhesus negative blood donors and recipients.

Table 2 : Frequency of weak D among rhesus negative blood Donors and recipients

No of Recipients and Donors with rhesus negative	N0Weak D among Donors and Recipients	Total % of weak D
7	1	14.28%

The total frequency of Weak D among blood donors and recipients

During the period of study, 201 blood samples were analyzed. A total of 129 blood donors samples were analyzed and found to be positive for Rh-D in 96.12% (n = 124); negative for Rh-D in 3.87% (n= 5). Out of Rh-D negatives among donors 0% (n =0) were found to be weak D i.e no Weak D were found among blood donors. Among the 72 recipients screened; 70 (97.22%) were Rh-D positive and 2 (2.77%) were Rh-D negative. Of the Rh-D negative persons among recipients 1 (1.38%) turned out to be weak D positive. The total weak D antigen among blood donors and recipients were found to be 1 (0.49%).

Table 3 : Total frequency of Weak D Positivity among Blood Donors and recipients

Variable	Donors	Recipients	Total
Rh Positive (N0)	124	70	194
Rh Negative [N0 (%)]	5 (3.84)	2 (2.77)	7 (3.48)
Weak D Positive [N0 (%)]	0 (0%)	1 (1.38%)	1 (0.49%)
Total	129	72	201

Table 3 indicate the frequency of weak D among blood donors and recipient attending Nemba district hospital were found to be 0.49% this frequency is low as the weak D is subjected to be tested in the rhesus negative individuals which is low.

DISCUSSION

During blood transfusion, the determination of weak D (and other D variants) is important to ensure blood safety. The term Du was coined by Stratton (Garratty, G., 2005). The other research were done on this antigen and showed that it was an inherited characteristic. The currently preferred term for Du is weak D. The incidence of weak D and other D variants varies worldwide. More than 100 variant of D types have been reported in literature (Wafi et al., 2016).

Although various authors have given the prevalence of weak D and other D variants in their populations, the comparative analysis becomes difficult due to the lack of set standards and the type of reagents used (monoclonal / polyclonal, single / blended).

There are three genetic mechanisms postulated Saqlain et al., (2016) for the acquisition of weak expression of the D antigen. These include the fact that, primary individuals inherit the RHD gene which codes for a weakly expressed D antigen; secondly 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. In addition to that another mechanism is 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 allo-immunized if transfused with D positive blood bearing the missing epitope. Further, it has been adequately documented that D epitopes distribution differs with different geographic locales &ethnicities of the population. It is being felt that the reagents produced in western countries may not be suitable for Indian population as D antigen is genetically controlled and major variations may exist in the D antigen profile of the populations (Devi et al., 2010). In our study which comprises 14.28 % of all D antigen negative samples, 0.49 % of weak D in all study samples as shown in Table 2 and 3 respectively. The frequency of Rhesus incompatibility was 0% due to the fact that weak D obtained in the study was from the recipient who could be considered as rhesus negative. The problem could arise in case where the weak D antigen was obtained in the blood donors and if the donor blood is transfused to recipient who is rhesus negative this could lead to rhesus incompatibility. This study showsthe prevalence of weak D antigen in our blood donor and their recipient's population required transfusion in the period of study at Nemba district hospital. The obtained results in this study are comparable to other studies conducted in different areas as stated below.

In the study conducted in Tertiary Care Hospital in Srinagar, Kashmir a total of 15680 donor blood samples were analyzed in a period of 18 months for ABO and Rh blood grouping. Among the total 15680 samples 94.6 % (n =14833) were Rh-D positive and 5.4 % (n = 847) were Rh-D negative. All the Rh-D negative (847) samples were subjected to weak D testing. Of the Rh-D negative samples 0.2 % (2/847) were weak D positive and of all the test samples 0.01 % (2/15680) turned out to be weak D positive (Ryhan et al., 2017).

In the study taken place in Tirupati Andhra Pradesh tertiary care referral teaching hospital in India their results are comparable to the ones obtained in Table 2 in this study. A total of 46,654 blood samples were tested (22,326 donors and 24,328 patients) during the period January 2012 to August 2014. Among these 43,771 (93.82%) were Rh D positive and remaining 2,883 (6.18%) were Rh- D negative. A total of 30 individuals (16 donors and 14 patients) were weak D positive constituting 1.04% of Rh-D negatives and 0.06% of total individuals screened (Krishna et al., 2015).

The obtained results in the following countries are also comparable to the results that were obtained by this study including the one conducted in China where 0.016% were obtained to have weak D antigen (Mak et al., 2001). Another study conducted in India has found that the prevalence of weak D antigen in the population was 0.01% (Makroo et al., 2010). There is also a study conducted in India showed that the frequency of weak D in the population of Delhi was 0.009% this results shows that the weak D antigen is low in that population (Pahuja et al., 2014). In 2016 another study was

conducted and found that the weak D in the total population was 0.014% (Devi et al., 2010).

In order to prevent allo-immunization to occur the current opinion is that weak D and other D variant subjects should be treated as rhesus D positive as donors to prevent the allo-immunization which could takes place if accidently transfused to D negative recipients. For the case of the patients with Partial D who need blood transfusion they should be considered as D negative, else they will form antibodies against the missing epitopes of the D antigen when transfused with D positive blood (Pahuja et al., 2014).

CONCLUSION & RECOMMENDATIONS

CONCLUSION

In this study out of 201 blood donors and recipients, 0.49% were found to be 'Weak D positive' which could be substantial in case where this recipient is a donor and give his or her blood to recipient who is Rhesus negative. In routine testing we must also concern on weak D antigen as it may lead to transfusion reaction in case it is not taken into account. Some forms of Weak D antigen are immunogenic and can result in production of allo-antibodies therefore for safe blood transfusion; it should be mandatory to check the Weak D antigen.

Recommendations

This study is invaluable in the domain of biomedical laboratory sciences and could serve as research tool for further studies in this domain. This study met with two major's limitation including financial means and the time limit. The recommendation can be given on this study where further research should be done to investigate the frequency of weak D and Rhesus incompatibility among blood donors and recipients with a very large sample size. Due to the fact that the Rhesus negative antigen comprise 15% of the total population in the world it could be better to make this study in a longer period in order to find out many people with rhesus negative antigen which could be tested for the presence of weak D.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no conflicts of interest.

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