Original Research Article

A retrospective study to know the prevalence of weak D(DU) among blood donors and patients at a tertiary care teaching hospital in Telangana.

Abstract

INTRODUCTION:
Rhesus (Rh) blood group system is considered the next most important after ABO system and is a clinically significant antigen in regards to transfusion and pregnancy. It is highly immunogenic and complex with numerous polymorphisms[1].

Weak D represents a D phenotype where due to decreased antigen sites the antigen cannot be detected by routine grouping (using immediate spin tube methodology).

AIMS & OBJECTIVES:
1) To determine the prevalence of weak D in this region.
2)To assess the implications and need for weak D testing.
3)To help in providing knowledge about importance of testing for weak D and thereby provide best patient care by preventing alloimmunisation in blood recipients.

MATERIALS AND METHODS:

Study design: A retrospective study for a period of one year was carried out at licensed blood bank in Malla Reddy Narayana Multispeciality Hospital under Department of Pathology , catering to patients from in and around Suraram.

Methodology: Data was collected from the bloodbank records.

Inclusion Criteria: The study group includes all donors and patients both admitted as well as attending outpatient departments who have undergone bloodgroup testing in our blood bank.

IMPLICATIONS OF THE STUDY:

The study will help in providing knowledge about weak D antigen .It helps in detecting frequency of weak D among patients and donors coming to this hospital and the need for formulating protocols for consideration of weak D serology as routine procedure to prevent transfusion related complications and misdiagnosis.

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INTRODUCTION

The major discovery in immune-hematology is the discovery of the ABO blood groups by Landsteiner in

1901 followed by discovery of Rh antigen by Levine and Stetson in 1939. The humans race was divided into, those who possessed the Rh antigen (Rh positive) and those who did not (Rh negative)[1]. Rhesus (Rh) blood group system is one of the most important as well as highly immunogenicand complex with numerous polymorphisms[2]. By the year 2015, 58 Rh antigens have been identified. The most common and immunogenic are D, C, E, c and e[3]. However, the major antigen of Rh blood group system is the Rh D antigen[4]. Subsequent to conflicting results in Rhgrouping, a weakly reacting D antigen was described by Stratton in 1946[5]. Weak D phenotypic expression arises by three mechanisms.Suppressive effect of C gene when in trans to D gene, when the part of D antigen is missing (partial D) or due to presence of aberrant form of D[6]. Weak D represents a D phenotype where due to decreased antigen sites the antigen cannot be detected by routine grouping (using immediate spin tube methodology). Demonstration of this weakly expressed antigen requires evaluation by prolonged incubation anduse of antihuman globulin[7].

MATERIALS AND METHODS

A cross-sectional study for a period of one year was carried out at licensed blood bankin Malla Reddy Narayana Multispeciality Hospital under Department of Pathology from 1st of August 2018 to 31st of July 2019 catering to patients from in and around Suraram. The study group included all donors and patients bothadmitted as well as attending outpatient departments. 2 ml blood sample was collected in EDTA vacutainers and tested for Rh typing and ABO forward and reverse grouping by conventional slide and tube agglutination methods.

The samples that turned out to be Rh negative by conventional slide and tube methods(using IgM monoclonal Anti D reagent of Tulip company) were subjected further to confirmation by

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gel card method(Matrix^{TM} Gel System).

The weakD (D^u) test was done using 1% red cell suspension in Matrix Diluent 2(LISS), prepared by dispensing 1ml of Matrix diluents2 (LISS) into clean labelled test tube to which 10µl of packed red cells is added and mixed gently. 50µl of of patients red cell suspension thus prepared was pipetted into a labelled microgel tube and 25µl of Matrix ^{TM} Anti-D IgG was added to it and incubated at 37˚C for 15min in Matrix Card Warmer.The gelcards were then centrifuged in Matrix^{TM} Card Centrifuge for 1cycle(10min). Interpretation of results were done by 2 observers independently.

RESULTS

This is a one-year cross-sectional study and a total of 4054 samples were analysed for ABO and Rh blood grouping. Out of these 3405 were Rh positive and 649 samples were Rh negative when tested by conventional slide and spin tube techniques. The blood samples which were negative on routine typing were further tested for presence of weak D and 12 of these 649 samples were found to be weak D or D^u positive.

In the present study, 84% (3405 out of 4054 samples) were Rh positive,15.71% (637 out of 4054) were Rh negative and 0.29% were found to show weak D positivity. We found that the incidence of weak D was more in blood group B (5 in 12) followed by blood group A(4in 12)and O group(3 in 12).There was no weak D expression noted in AB blood group in our study as represented in the table 1.

Table 1: Distribution of weak D(DU) among blood donors and patients

Blood Group	Rh D positive	Rh D negative	Weak D
O	1285	215	03

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A	860	136	04
B	991	232	05
AB	269	54	00
Total	3405	637	12
Percentage	84 %	15.71%	0.29%

DISCUSSION

The RHD gene polymorphism leads to phenotypic polymorphism of D variants including weak D, Deland partial D.[8] Weak D red cells have fewer D antigens per cell than normal Rh positive cells. (110 to 9000 per red blood cell).In Weak D one or more amino acid substitutes are found in the region that are presumed to be in or below the membrane and may interfere with the assembly of Rh complexes.[9] The importance of detecting weak D lies in the fact that transfusion of red cells from a person with 'weak D phenotype to a 'D Negative' person may result in alloimmunization and subsequent exposure to such 'D Positive' red cell can lead to fatal hemolytic reaction or hemolytic disease of newborn in a sensitized pregnant female. Even 0.5 ml of Rh D antigen exposure in Rh negative individual can induce antibody response[10].Considering the risk of immunogenicity, the persons with weak D phenotype are typed based on whether the person is donor or the recipient. The recipients with weak D should be considered D negative and must be transfused with D negative blood and as donors they are considered as D positive.

The incidence of Rh negativity worldwide varies between 3%-25% and that of weak D antigen ranges from 0.2%-1% [11]. The variation may be because of lack of set standards for perfoming the tests, type of reagents used (monoclonal,polyclonal,blended), objective and subjective variation in interpretation of test results. Further, it has been adequately documented that D

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epitopes distribution differs with different geographic locales and ethnicities of the populations[12]. In our study weak D constituted 0.29% of the whole study sample and 1.85% of all Rh negative samples screened. It shows the prevalence of weak D among the population in and around Suraram village, Telangana India. When compared with other studies conducted by H Kumar etal, Aslam A etal., Kabiri etal., Anshu gupta etal.,so far the prevalence of weak D% was found to be high in our study as represented in the table 2

Table 2: Distribution of Weak D percentage in other studies

S.No	AUTHOR	REGION	Weak D%	Rh positive	Rh negative
1.	H Kumar, 2005	INDIA	0.189%	93.7%	6.3%
2.	Aslam A et al, 2015	LAHORE	0.9%	86.3%	12.6%
3.	Kabiri Z, 2014	MOROCCO	0.05%	88.9%	11%
4.	Anshu Gupta et al, 2015	EAST DELHI	0.25%	96.7%	2.98%
5.	Our study		0.29%	84%	15.71%

CONCLUSION

Rh antigen being highly immunogenic and considering the significant risk of alloimmunisation even withweak D phenotype it is essential to screen all patients and donors for its identification prior to transfusionof blood and its components to ensure safe transfusion. Detection of weak D has special implication among pregnant women in whom the presence of an undetected weak D may lead to transfusion reactionsand alloimmunisation.

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