Original Research Article

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

A comparison study to determine the prevalence of weak D in donor and patients at a tertiary care teaching hospital in Telangana

Abstract

INTRODUCTION:

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

The weak D phenotype (D^u) is a weakened form of the D antigen ~~Weak D represents a D phenotype where due to decreased antigen sites the antigen~~ that cannot be detected by routine grouping (using immediate spin tube methodology).

AIMS & OBJECTIVES:

1) To determine the prevalence of weak D in this region. ~~Is it region or a specific Hospital?.~~

2)To assess the implications in terms of alloimmunisation ~~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.~~ To provide knowledge on the weak D status and enhance the importance of weak D determination in the donor and patient population

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 Multi specialty 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 included ~~s~~both ~~all~~ donors and patients who were ~~both~~ admmitted and those ~~as well as~~ attending outpatient departments who were identified for a blood grouping test ~~have undergone blood~~ group ~~testing in~~ at the ~~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

The study will provide information on the prevalence of weak D among the donor and patients population in the tertiary care teaching hospital in Telangana

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Comment [Ma2]: Add DU definition

Comment [Ma3]: IIs it region or a specific Hospital?.
add the words terms of alloimmunisation

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Comment [Ma11]: A retrospective study

Comment [Ma12]: Are these hospital blood banks

Comment [Ma13]: Methodology indicates blood banks and inclusion criterial indicates outpatient also- Kindly clarity is required

Comment [Ma14]: The study will provide information on the prevalence of weak D among the donor and patients population in the tertiary care teaching hospital in Telangana
It will also support in the development of testing protocols as well as guidelines for weak D determination both in donors and patients

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protocols for consideration of weak D serology as routine procedure to prevent transfusion related complications and misdiagnosis.

INTRODUCTION

Themajordiscoveryinblood group systems was associated with immune-hematologyisthediscoveryoftheABOin 1900 by Karl Landsteiner. This was followed by the description of Rh blood system ~~bloodgroupsbyLandsteinerin 1901 followed by discovery of Rh antigen~~ by Levine and Stetson in 1939. The Rhesus system discovery divide the humans race was dividedinto two~~,~~those who were ~~thosewhopossessed the~~RhD antigen(D ~~Rh~~ positive)~~andthosewhodid not~~(RhD negative)[1].Rhesus~~(Rh)~~bloodgroupsystemis among the clinical significant only second to ABO. ~~is~~It isalso ~~oneofthemostimportantaswellas~~highlyimmunogenic~~and complex with~~ numerous polymorphisms[2]. By the year 2015, 58 Rh antigens have beenidentified and documented. Rh system has RhD antigens and RHCE types. RhD has the D antigen while RHCE has ~~The most common and immunogenic are D,~~ C, E, c and e[3]. However, the most immunogenic is the RhD antigen in regard to transfusion and pregnancy.~~majorantigen of Rh blood group system is the Rh D antigen~~[4].In 1946, Stratton described ~~Subsequent to conflicting results in Rhgrouping,~~aweaklyreacting D antigen. The weak D phenotype is a weakened form of D antigen that in routine D antigen testing will react with some anti-D but not with others (when 37 C incubation or an immediate spin is given). Weak D RBC has D antigen but fewer in number as compared to normal Rh D-positive red cells.~~wasdescribedbyStrattonin1946~~[5].WeakDphenotypicexpressionarisesbythreemechanisms.Suppressive effect of C gene when in trans to D gene, when the part ofD antigen ismissing(partialD) ordueto presenceof aberrantformof D[6] (The citation is very old 1988).~~Weak D represents a D phenotype where due to decreased antigen sites the antigencannotbedetected byroutinegrouping (usingimmediatespintubemethodology).~~Demonstration of this weakly expressed antigen requires evaluation by prolonged incubation anduseof antihumanglobulin, enzymes, extended phenotyping and

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genotyping[7].

MATERIALS ANDMETHODS

Across-sectionalstudyforaperiodofoneyear wascarriedoutatlicensedbloodbankin Malla Reddy Narayana Multi~~speciality~~specialty Hospital under Department of Pathology from 1st ofAugust2018 to31st of July2019 cateringtopatientsfromin and aroundSuraram.Thestudygroupincludedalldonors andpatientsbothadmittedaswellasattending outpatientdepartments. 2 ml blood samplewas collected in EDTA vacutainers and tested for Rh typingand ABO forward and reverse grouping by conventional slide andtube agglutinationmethods.

The samples that turned out to be Rh negative by conventional slide and tube methods(usingIgM monoclonalAntiDreagentofTulipcompany)weresubjectedfurtherto confirmationbygelcardmethod(Matrix™GelSystem).

The weakD (D^u) test was performed ~~done~~ using 1% red cell suspension in MatrixDiluent 2(LISS), that was prepared by dispensing 1ml of Matrix diluents2(LISS) into clean labelled testtube and adding ~~to~~ which 10µl of packed red cells ~~is added~~ and mixed gently. 50µl of ~~of~~ patients red cellsuspension ~~thus prepared~~ was pipetted into a labelled microgel tube and 25µl of Matrix ™ Anti-D IgG was added ~~to it~~ and incubated at 37˚C for 15min in Matrix Card Warmer.Thegelcardswere then centrifuged in Matrix™ Card Centrifuge for 1cycle(10min), and results interpreted.~~Interpretation of resultsweredoneby~~ by 2 observersindependently.

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RESULTS

A total of 4054 bloodsamples were analyzed during the study period ~~This is a one year cross-sectional study and atotal of4054 samples were analysed~~ for ABO andRhD antigens. ~~blood~~grouping. A total of ~~Outof these~~3405 (84%) were found to be RhD positiveand637 ~~49~~ (15.2%) sampleswereRhDnegativewhen tested by conventional slide and spin tube techniques.The blood samples which werenegative on routine typing were further tested for presence of weak D and 12 of these 649samples werefound to beweakDor D^u positive.

In the present study, 84% (3405 out of 4054 samples) were Rh positive~~,15.71~~, 15.71%(637 out of 4054) were Rh negative and 0.29% were found to be ~~show~~ weak D positive~~positivity~~. The study also revealed that ~~We foundthattheincidenceof~~weakD was more common inbloodgroupB(5 in 12)compared with ~~followedby~~bloodgroupA(4in 12)andO group(3 in 12), while ~~.Therewas noweakD expressionnotedin~~AB~~blood~~phenotype was not assocated with RhD phenotype ~~groupinourstudy~~ as ~~represented~~shown in the table 1.

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Table 1: Distribution of weak D(DU) among blooddonorsandpatients

Blood Group	Rh Dpositive	RhD negative	WeakD
O	1285	215	03
A	860	136	04
B	991	232	05
AB	269	54	00
Total	3405	637	12
Percentage	84 %	15.71%	0.29%

DISCUSSION

TheRHD gene polymorphismleads to phenotypic polymorphism ofD variants including weak

D, Deland partialD.[8]Weak D red cells have fewer D antigens per cell than normal Rh positive cells.(110 to 9000 per red bloodcell).In Weak D one or more amino acid substitutes are found in the region that are presumed to be in orbelowthemembraneand may interferewith theassemblyofRh complexes.[9]The importance of detecting weak D lies in the fact that transfusion of red cellsfrom a person with 'weakD phenotypeto a 'D Negative' person may result in alloimmunization andsubsequent exposure to such 'D Positive' red cell can lead to fatal hemolytic reaction or hemolytic diseaseof newborn in a sensitized pregnant female. Even 0.5 ml of Rh D antigen exposure in Rh negativeindividualcaninduce antibodyresponse[10].Consideringtheriskofimmunogenicity,thepersonswithweak D phenotype are typedbased on whether the person is donor or the recipient. The recipients withweakDshouldbeconsideredDnegativeandmustbetransfusedwithDnegativebloodandasdonor stheyareconsidered asD positive.

The incidence of Rh negativity worldwide varies between 3%-25% and that ofweak D antigen ranges from 0.2%-1% [11].The variation may be because of lack of set standards for~~perfoming~~performing the tests, type of reagents used (monoclonal,polyclonal,blended), objective and subjectivevariation in interpretation of test results. Further, it has been adequately documented that D epitopesdistributiondifferswith differentgeographiclocales andethnicities ofthepopulations[12].In our study weakD constituted 0.29%of the whole study sample and1.85% ofall Rhnegative samples screened. It shows the prevalence of weak D among the population in and aroundSuraramvillage, TelanganaIndia. When compared with other studies conducted by H Kumar etal, Aslam A etal.,Kabirietal., Anshuguptaetal.,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

Comment [Ma17]: Discuss the study outcome in relation to other similar studies – the introduction of this part should focus on the study outcome not the clinical significance of RhD antigen

S.No	Author	Region	WeakD%	Rhpositive	Rhnegative
1.	HKumar, 2005	INDIA	0.189%	93.7%	6.3%
2.	AslamAetal, 2015	LAHORE	0.9%	86.3%	12.6%
3.	Kabiri Z, 2014	MOROCCO	0.05%	88.9%	11%
4.	AnshuGuptaetal, 2015	EAST DELHI	0.25%	96.7%	2.98%
5.	Our study		0.29%	84%	15.71%

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CONCLUSION/Recommendation

RhDantigen ~~being~~ ishighly immunogenic. It is associated with immunological responses (immunization) once introduced to those who are RhD negative via pregnanacy ,transfsusion or transplantation. . ~~and considering the significant risk of alloimmunisation even~~ .Therefore, it is essential to screen all donors and reciepients for the presence of RhD antigens as well as ~~with~~ the weak 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 and prevention of haemolytic disease of the fetus and the newborns (HDFN). Detection of RhD and the weak phenotypes in all clinical settings(national blood services, hospital blood banks and laboratories) is vital toward the reduction of alloimmunisation at the time ensuring safer transfusion practices ~~Detection of weak D has special implicationamong pregnant women in whom the presence of an undetected weak D may lead to transfusion reactionsandalloimmunisation.~~

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Comment [Ma22]: Most of the references used are very old- Need reference current publications

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