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Research Article

STUDIES ON THE DETECTION OF ANTIGEN AND PREVENTION OF RH ISO-IMMUNIZATION

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ABSTRACT
Total 68950 units of blood were collected at institute of blood transfusion medicine and immuno haematology (ibtmi) in ten months (august 2011 to May 2012) of which 68187 were voluntary at different camps and also at ibtmi and 763 were in exchange donors. In this ten month's study, rh(d)$^{-ve}$ blood was only 1997 by routine testing. D^u testing (indirect coomb test with anti - d reagent) were done on all 1997 rh (d) $^{-ve}$ bloods. 57 cases were d^u+ ve i.e. Weakexpression ofd antigen. The incidence of d^u + ve in this study is 2.85% in routine rh(d)$^{-ve}$ donors and 0.08% of total donors. By doing simple d^u testing both in donors and recipients, we can avoid delayed haemolytic disease of newborn (hdn), wastage of valuable rh^{negetive} blood, exchange transfusion in rh(d)$^{-ve}$ fetus, unnecessary anti - d ig giving in d^{u-ve} mother and also avoid rh(d) immunization from contaminated rbc of platelet concentrate, plasma, bone and renal grafts.

Key Words: *Rh (D) $^{-Ve}$, D^{u+Ve}*

INTRODUCTION
The single most important pre-requisite test for proper blood transfusion, without morbidity and mortality of the recipients is the determination of abo blood group of both donors and recipients and matching of abo blood group antigens (Mollison, 1993). Furthermore, the rh system becomes the most important blood group after abo, because exposure of rh (d)$^{-ve}$ individuals to rh (d) $^{+ve}$ red blood cells by transfusion or by pregnancy is most likely to stimulate the production of anti -d antibodies in rh (d)$^{-ve}$ individuals. The importance of the anti d antibodies is associated with the future transfusion therapy and particularly in rh(d) $^{-ve}$ pregnant women whose rh (d) $^{+ve}$ baby develop haemolytic disease of newborn (hdn). The most immunogenic of all rh – antigen is the d antigen, detection of which is done by routine testing. The d^{u+} ^{ve} bloods are actually rh (d)$^{+ve}$and it must not be given to true rh(d) $^{-ve}$ recipients and partial d variants, if it has antibodies against the epitopes missing in the d antigen (Mourant, 1993).
The objective of the study is the prevention of deleterious effects due to erroneous antigen detection and selection of appropriate donors.

MATERIALS AND METHODS
Meterial Anticoagulated (EDTA) Blood and Clotted Blood
Methods ABO blood grouping and Rh typing have been done by the standard tube method (Makroo, 1999). Forward ABO grouping has been done by Immediate Spin Technique and the reverse ABO grouping by taking serum of patients and stored cooled group A,B,O cells. Rh typing has been done by standard tube Method usuing IgM Monoclonal Anti-D (Makroo, 1999).

Tube Test Usuing Igm Monoclonal Anti-D/ Saline Aggutination Test For Rh(D) Typing
Materials
75 ✕ 10 mm tubes.
Test serum – IgM monoclonal anti-D / saline anti-D.

Test red cells
Rh positive and Rh negative control cells (for monoclonal anti D).
22% bovine albumin control (in case saline anti-D)

Method

Prepare 2-4% washed red cell suspension of test sample.
To one drop of 2-4% cell suspension, add 25 drops of anti-D serum. Mix by gently shaking the tube.
Centrifuge for 15-20 second (*I.S. Method)*or incubate at room temperature for 60 minutes. ***(Sedimentation Method)***
Look for hemolysis and agglutination against well light-back ground.
Record the results. All negative results must be checked under microscope.

D^u Testing

D^u testing has been done by following the standard method usuing Blend Monoclonal IgM and Polyclonal IgG anti-D (Makroo, 1999).
An anti-D reagent suitable for indirect antiglobulin test is used for D^U TESTING, i.e.
IgG monoclonal anti-D
Polyclonal IgG anti-D
Blend IgG monoclonal and IgM monoclonal anti-D
Blend monoclonal IgM and polyclonal IgG anti-D

Materials

75×10 mm tubes.
Suitable anti-D reagent (as above)
Test red cells
Anti-globulin (AHG) reagent
Control IgG coated red cells.

Method

To 1 drop of 2-4% suspension of test red cells, add 2 drop of anti-D.
Mix and incubate at 37°C for 45 minutes.
Look for agglutination.
If positive test, record the sample as D positive.
If positive test, i.e. no agglutination, wash the cells 3-4 times with saline and decant the last wash completely.
Add 2 drops of AHG and mix gently.
Centrifuge at 1000 r.m.p. For 1 minute.
Re suspend the cell button gently, look for agglutination and record the results.
All negative reactions should be confirmed by adding known lgG sensitized control cells, re-centrifuge and look for agglutination. The presence of agglutination confirms the test results and no agglutination indicates invalid test.

RESULTS

In the present study, 68950 units of blood were collected in ten months (August 2011 to May 2012) of this 68950 units, 68187 were voluntary and 763 were exchange collection. Of 68950 units of blood, 66953 units were Rh (D) positive (97.10%) and 1997 units were Rh(D) negative (2.8%).

Table 1: The table showing the incidence of different blood groups in Du donors

Blood Group	No of Cases (%)
A	19 (33.33%)
B	15 (26.31%)
AB	6 (10.52%)
O	17 (29.82%)

In our study, out of 68950 units of blood, 1997 units were $Rh(D)^{-ve}$. out of 1997 $Rh(D)^{-ve}$ blood, 57 units of D^{U+ve} blood (2.896%).

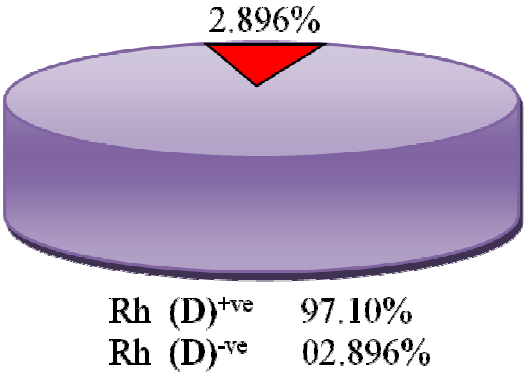

Figure 1: Pie diagram showing the Percentage of $Rh(D)^{+ve}$ and $Rh(D)^{-ve}$ blood in total 68950 donors

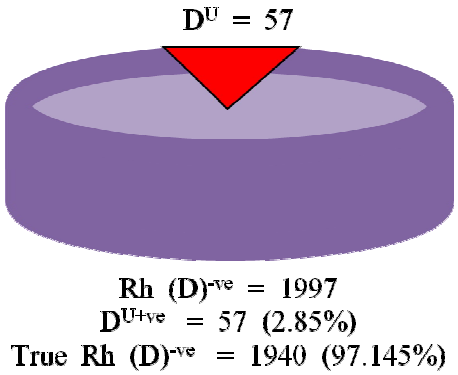

Figure 2: Pie diagram showing the Percentage of D^{u+ve} in $Rh(D)^{-ve}$ blood in total 1997

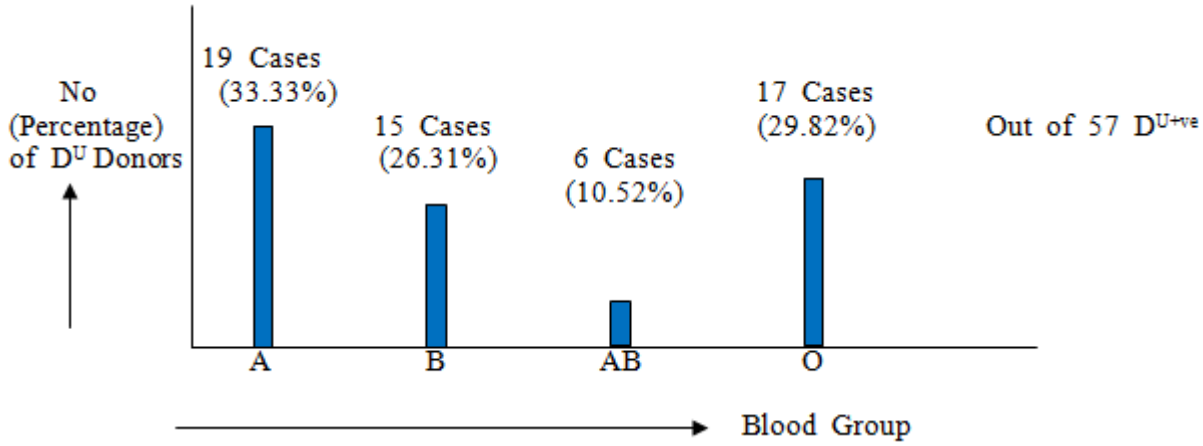

Figure 3:Bar diagram showing percentage of different ABO grouping in 57 D^{U+ve} cases

DISCUSSIONS

The Rh blood group system is very important clinically because the antigen D of the system is highly immu nogenic being at least 20 times more immunogenic than " C, " the second most potent Rh antigen (Haque, 1999). If a unit of D^{+ve} blood is transferred to a $(D)^{-ve}$ recipient, the recipient forms Anti D in 80-90% cases and thereafter cannot safely be transfused with D^{+ve} red blood cells.

A weakly reacting form of D is described as D^{u} or weak D. D^{U} red blood cells carry relatively small number of D antigen sites and are evidently D^{+ve}. The weak expression of D antigen can be explained by their different mechanisms. :

1. Genetic weak D – The inherited D genes that code for a weak expressions of D antigen , appear to be complete but few in numbers , Genetic D^{U} is most frequently seen in blacks, rarely in white, inheritance is vertical.
2. C Trans - (position effect or gene interaction effect)

In individuals showing the gene interaction D^U , the allele carrying D is trans (or in the opposite haplotype) to the allele carrying C antigen (eg Dce/dCe) Through the Rh antigen on the RBC is normal, but steric arrangement of the C antigen is relationship to the D antigen appears to interfere with the expressions of D antigen. This interference with D expressions does not occur when the C gene is inherited in the cis position to D (e.g. Dce/dce). It is not possible to distinguish the genetic weak D from the position effect weak D serologically (Denis, 3rd edition).

3. D mosaic (Now considered as variant of D, but not included in D^U)

In this mechanism, one or more of the eight epitopes present on D antigen are missing. If an individual lacks any epitope of the total D antigen, allo antibody can be made to the missing fraction(s), if the individual is exposed to red blood cells that possess the complete D antigen (Denis, 3rd edition) .

In D^U testing, this D mosaic will be represented as DU^{+ve}. Except this D mosaic/variants, other D^{U+ve} recipients can be given Rh^{+ve} blood without hazards. For this reason, DU testing in recipient's blood should be done. It is important to know that there is no such thing as a D^u test that is one that will distinguish between D and D^u . D^u test here is done on Rh negative blood which is negative by routine testing (Ray M and others, 2006).

$D^U$is much less antigenic in comparison to D. D^U red cells may be destroyed if transfused to a person, already having anti D. So D^U donor unitsare labeled as Rh D^{+ve}.

If D^U testing of the Donor Blood is not done, true Rh D^{-ve} mother can be sensitized by giving D^{U+ve} blood thinking it as $Rh(D)^{-ve}$. This sensitization may cause HDN of the fetus of that mother in future pregnancies.

If D^U test of the newborn of an $Rh(D)^{-ve}$ sensitized mother is not done, HDN can be missed and the exchange transfusion of the jaundiced newborn is neglected or delayed.

The importance of D^U testing also lies with the determination of recipients of anti D immunoprophylaxis with Rh immunoglobulin (Rhlg). Rhlg is recommended for the $Rh(D)^{-ve}$ mothers, of D positive or D^u positive fetus in order to prevent potential immunization (DGHS, 1991).

If D^U test of the mother is not done, Unnecessary immunoprophylaxis of the D^{U+ve} mothers is done.

D^U testing of different blood components also help in avoiding Rh(D) immunization by red cell present as contaminants in platelet concentrate, plasma, renal transplantation , bone graft. Approximately 0.37ml RBC is present in each platelet concentrate unit (Tippet Petrica, 1992).

The random use of platelet concentrate from $Rh(D)^{+ve}$ or DU^{+ve} donors to $Rh(D)^{-ve}$ women, who have not yet reached menopause, needs injection of anti D immunoglobulin to suppress primary Rh(D) immunization. The survival of platelets from D^{+ve} or D^{U+ve} donors is not impaired, as platelets do not carry Rh (D) antigen.

Transfusion of liquid stored plasma which may contain small number of red cell may cause both primary and secondary responses. It is possible that traces of stroma in frozen plasma from D^{+ve} or D^{U+ve} donors can stimulate a secondary response to D antigen.

This is a pilot study which warrants long term prospective study to strengthen this view.

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