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The Weak D Antigen: A Strong Challenge in Immunohematology

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ABSTRACT

Introduction

Rh antigen is the most clinically important blood group antigen in the field of transfusion medicine. Its phenotypic expression is complex leading to transfusion related misdiagnosis and immunological reactions. Weak D is one among its variable expressions which requires additional testing using IAT method for detection so that correct grouping of blood is ensured.

Objective

To determine the prevalence of Weak D antigen among blood donors.

Methods

This was a retrospective study conducted at a licensed blood centre in tertiary care hospital. All blood donors details over a period of 5 years was obtained from the registry. The prevalence of Rh negative blood donors tested by tube technique and Weak D antigen tested by additional tests using Antihuman globulin were tabulated and analysed.

Results

A total of 18,139 donors were included in the study among which 911 were Rh negative and Weak D antigen was found in 16 donors(0.08%).

Conclusion

The study shows the prevalence of Weak D antigen among blood donor population. This data based information concludes that it is worth detecting weak D antigen routinely to prevent alloimmunisation and Hemolytic disease of newborn.

Keywords

Immunohematology, Rhesus antigen, Transfusion, Weak D Phenotype.

INTRODUCTION

In immunohematology, Rhesus(Rh) blood group system is the most important and comparatively more complex after ABO blood group system.[1]Levine and Stenson defined Rh antigen for the first time in 1939 when they detected an irregular antibody in the serum of a mother whose fetus had Haemolytic Disease of Fetus and Newborn(HDFN).[1,2]

The presence or absence of Rh antigen determines the Rh blood grouping.[2]Molecular genetics shows that two genes RHD and RHCE on p34-p36 of chromosome one encodes 416 amino acids. One gene encodes the D antigen and the other gene codes for CE antigens in four different combinations.[1,3].

Rh antigen is a transmembrane protein in the band 4.2 having molecular weight of 15-17 kD. Unlike ABO antigens, Rh D antigens are present only on red cells. Recent studies have concluded that RH core complex is linked to the membrane skeleton through its interactions with CD47, protein 4.2 and novel Rh/RhAG ankyrin cytoskeleton connection.[1]

Stratton in 1946 described a weakly reacting D antigen as Du variant which was later renamed as weak D antigen.[3,4]Weak D is defined as the weakened expression of the normal D antigen which is an inherited characteristic.[1]Possible mechanisms responsible for weak D expression include:

1. Weak D due to transmissible gene i.e a person inherits the RHD gene which codes for weak D expression.[5]
2. Weak D due to positron effect-weakening of the D antigen by a C gene in transposition of D gene(Ceppelini effect).
3. Del- a very weak form of D expression.[1,3]

In our study we aim to determine the prevalence of weak D antigen among blood donors in a tertiary care hospital with the objective to reduce misdiagnosis and transfusion complications related to Weak D antigen.

METHODOLOGY

This is a retrospective observational study conducted at the blood centre in a tertiary care hospital in Bengaluru, Karnataka. Blood donors during a period of 5 years from January 2018 to December 2022 were included in the study. Blood grouping details regarding donors were obtained from the donor registry. The number of positive weak D antigen testing among all Rh negative donors were evaluated. A total of 18,139 donors were analysed.For all donors Rh grouping was done along with ABO grouping by tube technique.5% red cell suspension was prepared in isotonic saline. Equal volumes each of anti-D(IgM+IgG) and 5% red cell suspension was taken in the glass tube, mixed and incubated at 37^0 C for 45 minutes,then centrifuged at 1000 rotations per minute(rpm) for 1 minute. The tube was resuspended gently and observed for agglutination grossly and confirmed by microscopic examination.When no agglutination was present donor was considered RhD negative. Each Rh negative blood was further tested for weak D phenotype. Red cells were again washed twice with normal saline and 2 drops of antihuman globulin serum(Tulip Diagnostics Private Limited,India) was added and the tube was centrifuged at 1000 rpm for 1 minute.This Anti IgG reacts with any anti D IgG that became bound to the red blood cells during the initial typing test. Re-suspension of cell button was done and examined macroscopically and microscopically for agglutination. Any agglutination at this stage was recorded as weak Rh D positive.When no agglutination was noted they were typed as Rh negative.

RESULTS

Among the 18,139 donors 17,228 were RhD positive and 911 donors were Rh D negative(Chart 1), among whom 16 donors had weak D antigen. The frequency of Rh D negative and weak D antigen are tabulated in table 1.The frequency of weak D antigen was predominant in B blood group as tabulated in table 2.Most of the donors were in the age group of 30 to 45 years and predominantly males.(Chart 2)

Table 1 The frequency of Rh D negative and weak D antigen

Rh D typing	Number of donors	Frequency
Rh negative	911/18139	5.02%
Weak D positive	16/18139	0.08%

Chart 1: Rh D distribution among blood donors

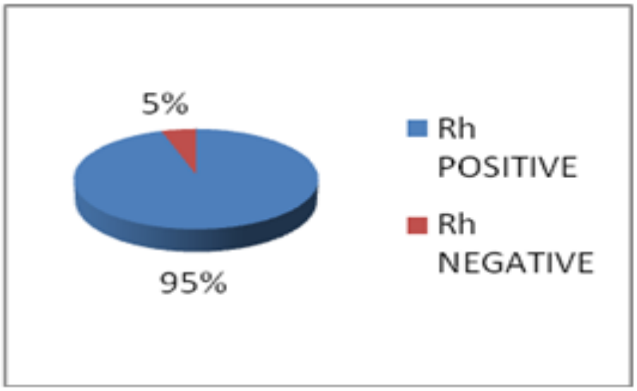
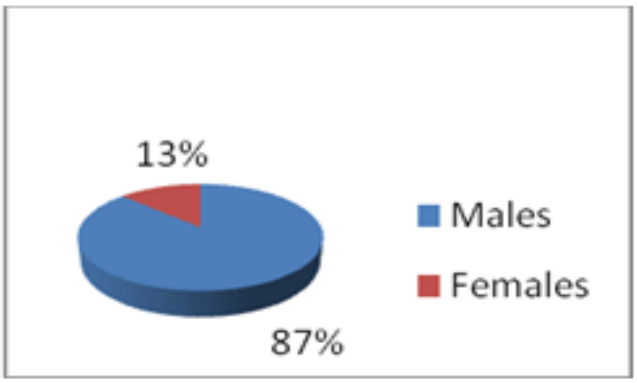


Table 2: The distribution of weak D antigen among different blood groups

ABO blood group	Weak D positive	% (n=18139)
A	04	0.02
B	08	0.04
AB	-	0
O	04	0.02

Chart 2: Gender distribution among blood donors



DISCUSSION

The clinical significance of Rh blood group system stems from the fact that Rh antigen is the most polymorphic and immunogenic.[6]As Rh system is well developed at birth, Hemolytic disease of fetus and newborn may occur in Rh D negative mother with Rh D positive fetus and also Rh antibodies may develop in Rh negative patients if exposed to Rh positive blood.[1]

The Weak D antigen detection requires additional steps in testing to demonstrate the presence of the D antigen.[1] The most common approach in identifying weak D antigen is by extended incubation and use of Antihuman globulin(AHG). Most serological weak D phenotypes are detected when RhD typing gives a weaker reaction. Problems in transfusion medicine occurs when blood donors are wrongly typed as Rh negative inspite of having trace amounts of RhD antigen (weak D)which can lead to alloimmunisation.[3]

The global incidence of Rh negative blood group is between 3-25%,[2,7,8]while the prevalence of weak D among Indian blood donor population is 0.01%.[1,9].

The prevalence of weak D antigen among blood donors in our study is 0.08%.The frequency of Rh D negative and weak D antigen reported in different research studies across India and different territories of the world are tabulated in table 3 and 4 respectively.As observed two studies conducted in Karnataka shows similar incidence as our study. Studies conducted in Northern parts of India showed a lower incidence of weak D antigen comparatively ,while there was slight variation in the incidence of weak D in different countries of the world probably due to difference in ethnicity and methods of detection. A study done by Maryam et al using molecular genotyping of RHD gene

in Nigerian population showed a high prevalence of 10.6 %. The molecular analysis and characterisation of serologic weak D phenotypes was contributory in their study.

Clinical aspects of Weak D phenotype

Inspite of its low frequency, weak D antigen is clinically important due to its strong immunogenicity. Its detection and confirmation helps:

1. To avoid transfusion of weak D positive blood to Rh negative recipients and hence prevent alloimmunisation.
2. Weak D positive women do not require anti-D immunoglobulin in case of Rh D positive fetus.[5

3. In diseases requiring multiple blood transfusions like Sickle cell anemia, Thalassemia and HIV/AIDS, failure to detect weak D antigen results in alloimmunisation and subsequent transfusion reactions.
4. Transfusion discrepancies in women of child bearing age group have immunological and clinical consequences as alloimmunisation can lead to HDFN during pregnancy.

Hence it is recommended to consider individuals with weak D antigen as Rh positive donors and as Rh D negative as recipients.[1,12]

CONCLUSION

Though the prevalence of weak D antigen among donors is low, its highly immunogenic property validates evaluation and detection of weak D antigen among all Rh D negative donors and recipients to minimise misdiagnosis and transfusion related discrepancies. Knowledge of blood group phenotype distribution and its clinical significance among health care workers becomes important to ensure safe transfusion services.

Table 3

Sl.no	Year of study	Authors	Region	Total study poulation	Study duration (years)	No. of Rh negative blood	No. ofweak D antigen	% of weak D antigen (in total population)
1	2023	Our study	Bengaluru	18139	5	911	16	0.08
2	2021	Sreelakshmi S[10]	Manipal	82824	1 1/2	5714	75	0.09
3	2013	Das S[11]	Kolar	15666	1	2000	25	0.15
4	2014	Deepthi K[6]	Tirupathi	46654	2	2883	30	1.04
5	2021	Srivastava AR[2]	Maharashtra	17262	3	1864	52	0.02
6	2002	Kumar H[12]	Delhi	34942	5	2201	66	0.18
7	2014	Pahuja S[13]	Delhi	64234	1	3481	6	0.009
8	2017	Lamba HS[14]	Punjab	13043	3	847	8	0.06
9	2014	Kotwal U[15]	Jammu	13281	1	728	1	0.0075
10	2016	Devi G[16]	Rewa	7019	2	232	1	0.01
11	2010	Makhroo RN[9]	Delhi	184072	9	13253	16	0.0086

Table 4

Sl.no	Year of study	Authors	Region	Total study poulation	Study duration (years)	No. of Rh negative blood	No. ofweak D antigen	% of weak D antigen (in total population)
1	2023	Our study	Bengaluru	18139	5	911	16	0.08
2	2021	Afroz T[3]	Bangladesh	1,77,702	5	7359	14	0.19
3	2021	Maryan DU[17]	Nigeria	4482	1	189	20	10.6
4	2019	Hameed M[18]	Pakistan	4361	1	575	3	0.06
5	2019	Brar RK[19]	Andaman	6415	1	330	5	1.51
6	2019	El Housse[20]	Morocco	4458	NA	420	23	0.52
7	2014	Xhetani M[21]	Brazil	2007	NA	239	16	0.8
8	2016	Xu Xhang[22]	China	132471	5	495	45	0.034

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