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Prevalence of Weak D (Du) among blood donors in South-West region of Maharashtra- An Observational study

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Abstract
Background: ABO was the first blood group system to be discovered by Landsteiner in 1901 and then along with Weiner he discovered Rh antigen in 1939 which farther led to description of haemolytic disease of new born by Levine and Stetson. The term Rh positive and Rh negative refers to the presence or absence of D antigen. The incidence of Rh negativity varies between 3%-25% and that of the weak D antigen range from 0.2-1%. This study aimed to find the prevalence of Weak D among blood donors.
Materials and methods: ABO and Rh Blood Group of donors was analysed in our blood bank from 2018 to 2020 in South West region of Maharashtra, India. Rh negative individuals were further processed for detection of weak "D" antigen by gel card method using LISS/Coombs reagent.
Results: A total of 21823 blood samples were analysed during the period 2018 to 2020. Out of these 20746 (95.06%) were Rh D positive and 1077 (4.93%) were Rh D negative. Out of 1077 Rh negative blood samples 45 (4.1%) were found positive for weak D antigen.
Conclusion: The prevalence of Rh-negative blood group is 4.93% and weak D positive is 4.1% among the blood donors of south west region of Maharashtra.

Keywords: Rh antigen, weak D (Du) antigen, Indirect antiglobulin test, gel card method

Introduction

ABO was the first blood group system to be discovered by Landsteiner In 1901 and then along with Weiner he discovered Rh antigen in 1939 which was further followed by the description of haemolytic disease of new born by Levine and Stetson [1]. However after reports of conflicting results of Rh grouping a weakly reacting antigen was described in 1946 [2]. The incidence of Rh negativity varies between 3%-25% and that of the weak D antigen range from 0.2-1% [3]. The term Rh positive and Rh negative refers to the presence or absence of D antigen. Upwards of 50 Rh antigens have been characterized albeit the five principal antigens – D, C, c, E & e are responsible for most of the of clinically significant antibodies [4]. When D antigen is weakly expressed on RBC's, it cannot be detected by routine monoclonal anti-D sera. It requires testing by Indirect Antiglobulin Test (IAT). The RBCs that were found positive after IAT are referred to as Weak D. Though the number of weak D is less but its detection helps in safe blood transfusion [5]. It has been also noted that prevalence of ABO and Rh blood grouping varies from region to region. Therefore, we intended to find the prevalence of ABO and Rh-negative individuals as well as weak D positive individuals among the blood donors in south region of Maharashtra.

Materials and Methods: ABO and Rh Blood Group of donors was analysed in our blood bank from 2018 to 2020 in South West region of Maharashtra, India. Determination of ABO typing was done by forward and reverse grouping by tube method and examined for agglutination macroscopically as well as microscopically. For determinations of Rh typing immediate spin tube method was used. Result was examined for agglutination macroscopically as well as microscopically. Rh negative individuals were further processed for detection of weak "D" antigen by gel card method using LISS/Coombs reagent. 50ul of IgG & IgM blend monoclonal reagent is added to 50ul of 1% suspension of donor/ recipient

red cells. Then the gel card is incubated at 37^0 C for 15 minutes and centrifuged for 10 minutes. If there is red cell button at the bottom of microtube then it is negative for Weak D antigen and if the red cell agglutinates are trapped in get matrix it is interpreted as weak D (Du) positive. Data was extrapolated in MS Excel 2007 version and was analysed for percentage prevalence.

Results: A total of 21823 blood samples were analysed during the period 2018 to 2020. Out of these 20746 (95.06%) were Rh D positive and 1077 (4.93%) were Rh D negative as shown in table1. These negative samples were further tested for weak D antigen. Out of 1077 Rh negative blood samples 45 (4.1%) were found positive for weak D antigen as showed in table 2.

Table 1: Frequency of Rh antigen status (N=21823).

Blood Group	Rh Positive	Rh Negative	Total
A	6327	351	6678
B	6220	295	6515
AB	2122	109	2241
O	6077	322	6399
Total	20746	1077	21823
Percentage	(95.06%)	(4.93%)	(100%)

Table 2: Frequency of Weak D positivity in Rh Negative samples (n=1077).

Blood Group	Du Positive	Du Negative	Total
A	14	337	351
B	12	283	295
AB	11	98	109
O	8	314	322
Total	45	1032	1077
Percentage	4.1%	95.9%	100%

In our study the frequency of ABO blood group by gender was calculated. In both genders, Blood group A was most common, followed by group B and group O. In male participants frequency of ABO blood group with respect to Rh factor positive was calculated, which showed a slightly higher frequency of "A" positive (30.54%) followed buy 'B' positive 29.98%, "O" positive 29.20% and "AB" positive 10.27%. In the case of Rh factor negative male individuals, "A" negative showed slightly higher prevalence (32.19%), followed by "O" negative 29.81%, "B" negative 27.73% and "AB" negative 10.25%.

In female participants, frequency of ABO blood group with respect to Rh factor showed a slightly higher frequency of 'O" positive 33.64%, followed by "B" positive 29.98%, "A" positive 28.30%, and "AB" positive 8.12%. In the case of Rh-negative females, "A" Negative was 50% as compared to other blood group type "O" negative 33.33%, "B" negative 12.5% and "AB" negative 4.1 % (Table 3).

Table 3: ABO and RH status by gender

ABO	Gender	Rh antigen		Total
		Positive	Negative	
A	Male	6205 (30.54%)	339 (32.19%)	6544 (30.62%)
	Female	122 (28.30%)	12 (50%)	134 (29.45%)
B	Male	6091 (29.98%)	292 (27.73%)	6383 (29.87%)
	Female	129 (29.93%)	3 (12.5%)	132 (29.01%)
AB	Male	2087 (10.27%)	108 (10.25%)	2195 (10.27%)
	Female	35 (8.12%)	1 (4.1%)	36 (7.91%)
O	Male	5932 (29.20%)	314 (29.81%)	6246 (29.23%)
	Female	145 (33.64%)	8 (33.33%)	153 (33.62%)
Total	Male	20315 (95.07%)	1053 (4.92%)	21368 (100%)
	Female	431 (94.72%)	24 (5.27%)	455 (100%)

Discussion

The rhesus (Rh) system is one of the most complex blood group system in humans. It has more than forty antigens of which five are clinically significant. These antigens are C, c, D, E and e. Genes for the five Rh antigens are encoded by two autosomal dominant genes RHD and RHE on chromosome 1. The Rhesus D (Rh D) antigen is the most important antigen because of its immunogenicity. Thus, Rh positivity and Rh negativity indicate presence or absence of the weak D antigen on the surface of the red cells respectively [6]. There are many variants of Rh D antigen and the most important variants of D antigen are weak D, Partial D, Rh null. Weak D red cells have fewer D antigen per cell than normal Rh-positive cells. In weak D one or more amino acid substitutes are found in the region that are supposed to be within or below the membrane and may interfere with assembly of Rh complex [7]. They react with anti D only after extended testing with the indirect anti-globulin test. It is conventional to consider weak D subjects as Rh-negative when they are recipients of transfusion and Rh positive when they are blood donors [8].

The clinical importance of detection of weak D is consequence of the fact that Rh negative individuals are easily stimulated to form Rh antibodies if transfused with weak D Rh positive blood or in pregnant woman if exposed to foetal Rh-positive cells, leaked through placenta into maternal circulation. So, identification of Du positive donors and recipients can prevent transfusion reaction and reduce the incidence of unnecessary Rh negative blood transfusion. Identification of weak D Positive individuals is also beneficial for patients with frequent transfusion requirements such as patients with HIV/AIDS, chronic renal failure, advanced malignancies, aplastic anaemia, bone marrow failure, sickle cell anaemia and other causes of chronic anaemia [9].

The use of modern sensitive gel card system for ABO and Rh typing has given concordant result when compared with the conventional blood grouping system [10]. The enhanced

sensitivity of anti-D antisera is also accountable for the lowered frequency of the weak D phenotypes. In our study, weak D antigen comprised 0.2% of all study sample and 4.1% of all Rh D antigen negative samples. The serological method has not distinguished between weak D & other D variants in our study. This study shows prevalence of weak D antigen in our blood donor population who are representatives of South West region of Maharashtra State.

Table 4: The prevalence of Rh D negative and weak D antigen is variably reported in different studies.

Sr. No.	Year	Studies	Weak D (%) in		Rh- negative (%)	Rh- positive (%)
			Rh D Negative	Total donors		
1.	2020	Present study	4.1	0.2	4.93	95.06
2.	2018	Sindhu R [11]	0.2	-	6.64	93.36
3.	2018	Shivali Sehagal [12]	0.96			
4.	2017	Lamba H S [13]	0.9	0.07	6.3	93.50
5.	2016	Rahmani MT Hamid [14]	0.98	-	8.0	92.0
6.	2015	Anshu Gupta [15]	7.6	0.25	2.98	96.7
7.	2015	Pratima K [16]	0.578	0.0114	1.97	98.03
8.	2015	Deepthi Krishna G [17]	1.04	0.6	-	-
9.	2014	Kabiri Z [18]	5	0.05	11	88.9

Results of our study were comparable with results obtained by Kabiri Z [18] and Anshu G [15]. Therefore, the data of Rh-D negative and weak D positive patients in this study would be useful in several following ways: Primarily, the individuals who have weak expression of D antigen with decreased number of D antigens on red cells surface, can be transfused with Rh-D positive donor blood without prior sensitization. Furthermore, Rh-D negative blood would be preserved for genuinely Rh-D negative patients preventing misutilization of scarce Rh-D negative blood. Finally, a weak D positive female does not require administration of anti-D immunoglobulin's in case of Rh D positive off springs. So, this would aid in the organization and establishment of more efficient blood transfusion service that would meet the ever-increasing demand for safe blood and blood products.

Conclusion

Prevalence of Rh-negative blood group was 4.93% and weak D positive was 4.1% among the blood donors of south west region of Maharashtra. In our study we also observed that the commonest ABO blood group was group "A" followed by "B" and "O". D antigen is most immunogenic and plays an important role in immunohematology and blood banking. Detection of weak D positive blood donors and recipients would remarkably reduce the incidence of transfusion reactions and save on the scare O negative blood for emergencies. Not testing for the weak D antigen in the blood group may cause transfusion reactions and allo-immunisation. It also stresses the need to identify individuals with variants of D rather than weak or partial D and to inform them about their status as donors and recipients of blood or organs.

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