

## ORIGINAL ARTICLE

## FREQUENCY OF WEAK EXPRESSION OF 'D ALLELE' AMONG HEALTHY BLOOD DONORS

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**Background:** Among different blood group systems, Rh system is second most important system. Its variants are classified in three groups. One of them is Partial D which is characterized by mutation in the extracellular domain of its gene. These variants may form anti-D antibody. Although in weak D it is not formed as it has less number of qualitatively normal antigens due to mutation. The objective of the study was to determine the frequency of Rh negatives, Rh positive and weak D individuals in the healthy blood donors. **Method:** A total of 315 blood donors were included in this cross-sectional study from Blood Bank of Jinnah Hospital Lahore, who were proven fit for blood donation. Venous blood was collected for forward ABO and Rh typing by tube method. Rh-negative blood was further tested for weak D. **Results:** The frequency of Rh-positive was 86.3% and Rh-negative were found to be 13.7% whereas, 1% individual were weak D-positive. **Conclusion:** Weak D antigen frequency is minor in comparison with Rh positive and negative. It is necessary to check for weak D status of an individual as some types of weak D can precipitate immune response in an individual who lacks Rh-D antigen.

**Keywords:** Blood groups, D antigen, Weak D, blood transfusion, blood typing, healthy blood donors

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## INTRODUCTION

Before the discovery of blood groups, all the blood groups had been thought to be the same.<sup>1</sup> ABO blood groups are first to be discovered<sup>2</sup>. Later the Rh blood group system was discovered by Landsteiner and Wiener.<sup>3,4</sup>

RHD and RHCE genes encode Rh blood group system and are present on chromosome 1.<sup>4,5</sup> There are 40 nucleotide differences in RhD and RhCE gene. There are 4 alleles of RHCE genes: RHCE, RHcE, RHcE and RHcE.<sup>6-8</sup> There are 32-35 amino acid differences in RhD and RhCE protein. The major antigen of Rh blood group system is RhD antigen.<sup>9</sup> Depending on the presence or absence of this antigen, human red cells are divided into Rh-positive and negative cells. D antigen is highly immunogenic of all Rh antigens.<sup>10</sup> Even 0.5 ml of Rh antigen exposure in Rh-negative individual can induce Rh antibody response.<sup>11</sup> After the RhD, the Rhc is most important Rh antigen. Between 14 to 21% of Rhc-positive babies whose mothers are Rhc negative require exchange transfusion.<sup>12-14</sup> Anti-C, -E, and -e can all cause HDN, but the occurrence is rare.<sup>15</sup> Rosenfield *et al* described nomenclature of Rh antigen; the numeric portion of ISBT terminology based on this.<sup>16-18</sup> According to Fisher's hypothesis there are three genes, D, C and E. At each locus there are two alternative genes present which are C and c, E and e, D and d.

Weak D antigen is present in about 0.2% to 1% of Caucasians.<sup>9</sup> Although many studies have been carried out in Pakistani population to find out the frequency of weak D, the present study will help in estimating its frequency in healthy blood donors which should be considered as RhD-positive as healthy donors

with 'weak D' can provoke synthesis of anti-D in RhD-negative recipients.

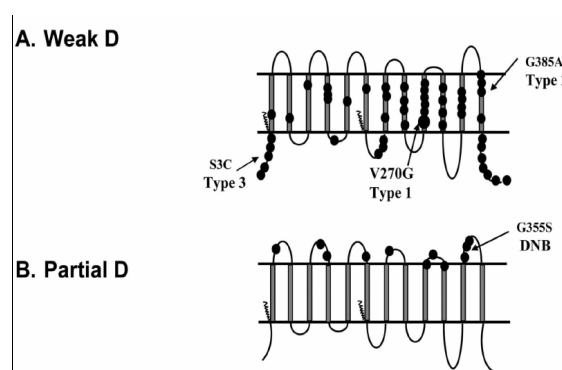


Figure-1: Structure of partial D and weak D<sup>9</sup>

## SUBJECTS AND METHODS

It was a cross-sectional study which was carried out over a period of 6 months from January to June 2014. This study was carried out at Blood Bank of Jinnah Hospital Lahore on healthy blood donors.

Sampling technique was purposive non-probability sampling. Sample size was estimated using EpiInfo to estimate a proportion with confidence level of 95% acceptable difference of 3% and assumed proportion of 0.8% to acquire a sample size is 315.

Blood sample was collected from the median cubital vein in the EDTA vial. The forward ABO and Rh typing was done by tube method as following: A 5% cell suspension of red blood cells was made by adding one drop of blood and 19 drops of normal saline. Three test tubes was taken and labelled as A, B and D. One drop of 5% cell suspension was taken in all of the three tubes labelled as A, B and D. Antisera A was

added in test tube labelled as A. Antisera B was added in test tube labelled as B. Antisera D was added in test tube labelled as D. Agglutination was observed in all tubes. When macro agglutination was not observed, thick slide of the mixture of cell suspension and anti sera and micro agglutination was checked using 40× lens of microscope. The Rh-negative blood was further tested for weak D. The negative D tube and control tube were incubated at 37 °C for 45 minutes. After incubation saline was added to fill 3/4<sup>th</sup> of the test tube and the tube was centrifuged for 1 to 2 minutes at 3,400 rpm. This process of washing was done thrice and anti-human globulin (poly specific) was added. Agglutination occurred in case of sensitization.

Besides ABO grouping, if agglutination was observed in D tube the blood group was typed as Rh-positive. If agglutination was not observed in D tube initially, and after adding anti-human globulin there was still no agglutination, it was typed as Rh-negative. If after addition of the anti-human globulin the agglutination was observed then it was typed as weak D. Statistical analysis was done using SPSS-20. Categorical values were expressed as frequency and percentage.

## RESULTS

Out of 315 blood donors, there were 162 females (51.43%) and 153 males (48.57%) with mean age 35±10 years. The distribution of ABO and Rh blood groups among males and females is shown in Table-1 and 2, while distribution of weak D among male and female donors is shown in Table-3 and 4 respectively.

**Table-1: ABO and Rh/D distribution in male donors [n (%)]**

ABO Group	Rh Antigen		Total
	Positive	Negative	
A	22 (16.2)	3 (17.6)	25 (16.3)
B	57 (41.9)	7 (41.2)	64 (41.8)
O	41 (30.1)	6 (35.3)	47 (30.7)
AB	16 (11.8)	1 (5.9)	17 (11.1)
<b>Total</b>	136 (100)	17 (100)	153 (100)

**Table-2: Distribution of ABO and Rh/D in female donors [n (%)]**

ABO Group	Rh Antigen		Total
	Positive	Negative	
A	21 (15.4)	6 (23.1)	27 (16.7)
B	55 (40.4)	5 (19.2)	60 (37.0)
O	42 (30.9)	11 (42.3)	53 (32.7)
AB	18 (13.2)	4 (15.4)	22 (13.6)
<b>Total</b>	136 (100)	26 (100)	162 (100)

**Table-3: 'Weak D' distribution in male donors**

ABO Group	Weak D positive	%	D Negative
A	0	0	3
B	0	0	7
O	1	16	6
AB	0	0	1

**Table-4: 'Weak D' distribution in female donors**

ABO Group	Weak D positive	%	D Negative
A	1	16	6
B	0	0	5
O	1	9	11
AB	0	0	4

## DISCUSSION

This study gives an outline of importance and frequency of weak D in Pakistan. In another Pakistani study conducted in Swat<sup>19</sup>, the total Rh-negative individuals were 9.87%, which was slightly lower than the current study (13.7%), however, in their study amongst Rh-positive males blood group B was found to be the most common group followed by group O which is similar to the sequence observed in the current study. In Rh-positive females, blood group B was again the commonest and group O was second commonest. In Rh negative males blood group O was the commonest blood group while group B followed it (2.88%). Frequency of group A was 2.25%. Least common was group AB (0.88%). This sequence showed deviation from our study as group B was the commonest group among Rh-negative males in our study, whereas in Rh-negative females in that study the sequence was in consistence with our study.

A study conducted in Northern India, reported 4.29% subjects to be Rh-negative out of a total of 23,320 male and female individuals.<sup>20</sup> Makroo *et al*<sup>11</sup> conducted a study in India. They study a total of 184,072 donors, among these 13,253 (7.2%) were Rh-negative. Out of these 16 (0.12%) were weak D positive.

In another study from India<sup>21</sup>, in Rh-positive male subjects blood group B was found to be the commonest group followed by group O. Next most frequent blood group was group A and then group AB like in our study. Amongst Rh-positive female subjects, the sequence is different from our study. This deviation may be due to diverse ethnicity.

Another study at Uttarakhand India<sup>22</sup>, tested 58,614 donors, out of which 55,566 (94.8%) were found to be Rh-positive and 3048 (5.2%) were Rh-negative. The frequency of weak D was 0.09%; even less than that found in our study (1%).

A Pakistani study<sup>23</sup> reported 7% of Rh-negative individuals, out of which 0.8% were weak D positive. Depending upon the ethnic group about 3–25% of human population lacks Rh/D antigen. The incidence of weak D is different in different ethnic groups. It is from 0.2 to 1% in Caucasians.<sup>1</sup> In India, frequency of weak D antigens turned out to be 0.3 to 0.5%.<sup>1,15</sup> The occurrence of weak D antigen is about 0.23 to 0.5% in Europe and 3% in USA.<sup>12,13</sup>

## CONCLUSION & RECOMMENDATION

Out of 13.7% 'Rh-negative donors', 1% were found to be 'Weak D positive' which is substantial. Not testing for the Weak D antigen in the blood group may cause transfusion reaction. Some forms of Weak D antigen are immunogenic and can result in production of allo antibodies. For safe blood transfusion it should be mandatory to check the Weak D antigen.

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