# ORIGNAL ARTICLE

# "FREQUENCY OF RH-D NEGATIVE & WEAK D IN PAKISTANI POPULATION"

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## ABSTRACT

***Introduction:***The Rh blood group system is one of the most polymorphic and immunogenic blood group systems in humans. The expression of its antigens is complex, among that Rh-D antigen is the most important antigen because of its high immunogenicity. Molecular genetic of RHD gene revealed that weak D antigen is a Rh-D phenotype that possesses less numbers of D antigen epitopes on surface of red cells. These individuals usually labeled RhD -ve by conventional testing but when transfused to RhD –ve person, it can elicit antibody production. Variableincidence of weak D worldwide,lack of awareness, proper data & multi-ethnic population of our country propelled to analyze it.

***Material and Methods:***A cross-sectional study conducted from August 2012 to August 2014. Around 48,228 healthy blood donors were tested for RhD factor. Commercially available monoclonal anti-D sera were used to detect Rh-D factor status. Individuals found negative with saline anti-D, were further investigated for weak D antigen by using indirect Coomb's technique (IAT).

***Results:***Among 48,228 healthy blood donors, 44853 (93%) were Rh-D factor positive while 3375 (7%) were Rh-D factor negative. Among these, 3375 Rh-D factor negative individuals 27 (0.8%) were found to be weak D positive.

***Conclusion***: Although frequency of weak D does not came high among our donors but is still significant enough to advocate testing of weak D in routine for all Rh –ve donors & pregnant women in order to avoid consequences of anti-D allo-immunization which can lead to serious hemato-pathological problem.

**Key words**: Weak D antigen, Rh-D phenotype, Allo-immunization.

## INTRODUCTION:

There are now formerly 38 registered blood group systems having single or very closely located more than one gene on particular locus on different chromosomes[1]. These genes can be allelic or homologous (closely-linked) controlling the specificity of these systems by coding different blood group antigens[2]. ABO & Rh system enjoy highest importance among all blood group systems because of their clinical significance in terms of transfusion & transplantation[3]. Rh blood group highlights more in relation to Hemolytic disease of fetus & newborn[4] (HDFN).

Rh blood group system comprise of over 50 Antigens. Among these antigens 5 (i.e. C, c, D, E, e) are common while D antigen being most immunogenic gains the scientific priority among them. Genes who control the Rh system antigens i.e. RHD & RHCE are located on the chromosome 1p36.13-p34.3[5, 6]. Variable prevalence of Rh D antigen is reported from different countries; it is being 93.6% in India, 99% China[7], 85% Caucasians, 92% Blacks[8]while in our country it is reported to be 92%[9]. The variation in prevalence of RhD –ve can be assessed from above figures which range from 1% to 15% but highest reported is from Saudi Arabia & Morocco

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i.e. 29%[10].

After the discovery of Rh-system antigens, variants of D antigens; mainly weak D & partial D were detected in 1946 by Stratton[11]. The weak D phenotype (formerly known as Du) is represented by a group of RHD genotypes that codes in their vast majority for altered RhD proteins associated with a reduced RhD expression on the red blood cells surface[12]. Approximately 9 D epitopes have been reported in the mosaic of RhD antigen[5].Weak D antigen is the one with all the epitopes but expressed weakly[10.] A molecularly defined weak D type is a variant of the RhD protein with an amino acid substitution in the trans-membranous or intracellular segment and expresses a decreased quantity of D antigen. Another variant "Partial D", on other hand, has decreased number of epitopes and has an amino acid substitution in at least one of the extracellular or RBC membrane surface loops[13]. Approximately 5 – 10% of weak D phenotypes in the United States are estimated to be partial D phenotypes[14].

With advances in medical therapeutic sciences & awareness; blood transfusion has become most common procedure during hospitalization. In USA, over 11 million/year RBC transfusions are given[15]. Adding to this fact are the transfusions given to chronic transfusion-dependent patients. According to a report by Lal et al 2018 published in Transfusion journal; Thalassemics constitute 34.7% of all transfusions[16.] A study by Romphruk et al 2018; which studied alloantibodies in Thalassemics, concluded that they were more prone to develop Rh antibodies as compared to Kell blood group system[17]. Although RhD testing is routine since long but some recent studies have suggested high rates of Rh antibodies[18]. This situation aggravates when we consider lack of technical facilities in majority blood banks of our country. Understanding of weak D phenotype is still not widespread in transfusion-community of our country[19]. Even a survey conducted by college of American pathologists (CAP) in 2014 gave finding of lack of standard practice for interpreting RhD type in cases of weak D phenotype in USA[20].

There is one misconception that individuals with weak D phenotypes can't make anti-D in contrast to partial D because they have low levels of complete D antigens but many detailed studies revealed that testing of weak D is significant[10] Specifically the weak D type 2 contains lowest density of epitopes. Recommendations are formulated since work of Flegel et al 2002 that weak D should be tested as part of routine immune-hematological work up[21]

The multi-ethnic population of our country, lack of awareness & lack of technical facilities deserves more work on this subject from different parts of country. The current study was designed to determine the frequency of weak D antigen in Pakistani population so that recommendations can be formulated at the district level for considering weak D serology as a routine blood bank procedure.

## MATERIAL & METHODS

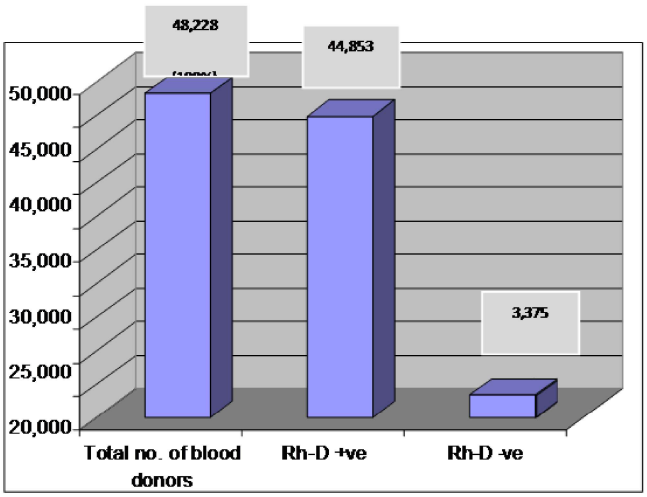
This multi-centercross-sectional study was performed at the Baqai Institute of Hematology, Fatima Hospital, Baqai Hospital Nazimabad, Husaini Institute of Hematology and Oncology Trust and Muhammadi Blood Bank, Karachi from August 2012- August 2014. Test population was healthy blood donors who were registered after informed written consent. All samples were grouped for ABO and Rh-D factor using commercially available anti-sera. All samples found negative with saline anti D, were further tesed for weak D antigen using indirect Coomb's technique. The results were analyzed using SPSS statistical software version 21.

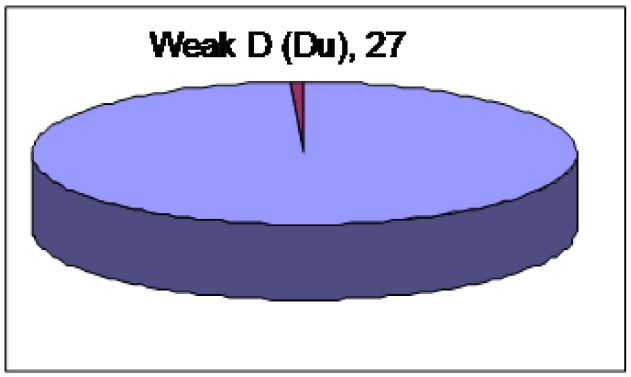
## RESULTS:

During this study, 48,228 healthy blood donors were tested for Rh-D factor status. The results are depicted graphically in figure 1 and 2. Among these, 44,853 (93%) were Rh-D factor positive while 3,375 (7%) were Rh-D factor negative. Out of these 3,375 Rh-D factor negative individuals, 27 (0.8%) were determined as weak D positive.

## DISCUSSION:

Weak D is a phenotype with either a qualitative or quantitative difference in the RhD moiety resulting in a weakened expressed of D antigen. Depending




upon the ethnic group about 3-25% of human population lacks RhD antigen[11]. Importance of Weak D antigen surface when person having Rh D –ve phenotype requiring blood transfusion receives blood from donor having Weak D phenotype & typed as RhD –ve this sparkle more when occurs in pregnant women[13].

Although recommendations for testing Du (currently "Weak D") was found even in first edition of AABB standards published in 1958[22], it declared donors having Weak D as "RhD positive" while recipients having Weak D as "RhD negative" with recommendation of IAT for donors & DAT for recipients, that policy prevailed for around 50 years. The 30$^{th}$edition of standard of AABB (published in 2016) renders Weak D testing optional for recipients and advocate molecular testing[23]

Frequency of weak D antigen is observed **0.8%** in our study. The finding which is quite comparable with studies from different countries. A study by Dehapriya et al from India reported 0.215% frequency among donors (n = 1,528) same study compared their results with German population whose frequency was 0.44%[24]. A multicenter study from Kenya reported 2.1% frequency among blood donors with sample size of just 384[25]. Even back in 2005; study from Toronto, Canada reported findings of 0.96%[26] while similar findings (i.e. 0.96%) in a study conducted in Dutch donors[27].

Another study from India (Uttarkhand) having large sample size (n = 58,614) concluded frequency of 0.09%[28]. Frequency of 0.03% being reported from China; a study by Xu Zhang with sample size of 132,479[29] another report of China few years back concluded 0.015 & 0.012% in Han population from Shanghai. Talking of Europe, studies from Poland & Denmark concluded 0.2% & 0.3% respectively[30.] The China having lowest because they have lowest RhD negative percentage.

Till 2017 around 147 weak D types were listed on Rhesus database[31] which makes it worthy to be tackled at all levels of healthcare. Although molecular tests are the ultimate answer to resolve discrepancy of weak D and D variants but in under developed countries at their rural district level, anti-human gamma globulin test to detect "weak D" has still got its worth especially for donors and women of child bearing age and efficacy of anti-human gamma globulin in detecting weak D antigen is well accepted[32]. Although the use of different commercial anti D sera are debatable but laboratories should follow guidelines of the particular country for patient and donor typing and select reagents accordingly.

## CONCLUSION:

Frequency of Weak D although low but is comparable with worldwide data makes it significant enough to be recommended as routine test in all RhD negative donors & women of child bearing age.

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