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ORIGINAL ARTICLE

Occurrence of anti D alloantibodies among weak D and D negative patients.

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ABSTRACT… Objective: To estimate the frequency of anti-D alloantibodies in patients with weak or negative D. **Study Design:** Cross-sectional study. **Setting:** Under CIP/IRB/1111 in the Blood Bank of CHUGHTAI Institute of Pathology. **Period:** May 2022 to October 2022. **Material & Methods:** Patients who booked their blood group testing at this laboratory were tested for their ABO RH blood type. The EDTA and yellow top serum samples were taken from patients. Blood group was identified using tube method. Patients with Rh-D negative blood type and weak D were included in this study. Those patients were screened for alloantibodies by screening cell (Bio-Rad), and those patients whose screening came out positive were tested for antibody identification using an extended 11cell panel (Bio-Rad). The Statistics calculator was used to determine the sample size. Software SPSS version 23 was used to conduct all statistical analyses. **Results:** 14103 blood groups were tested. The age of patients on average was 51±22 years. The males and females were 44% and 56%, respectively. Out of n=14103, 9.5% came out Rh-negative blood type. The blood group distribution was as follows, B negative 46% O negative 23% A negative 20% AB negative 11%, and weak D 0.02% only. Total 7.1% participants with negative RH-D blood type had Rh alloantibodies detected. Patients with weak D had no Rh alloantibody. **Conclusion:** Blood group O RH-D negative was the most prevalent with positive anti D-alloantibodies. Prevalence of positive antibody was 7.1%.

Key words: Anti-D Alloantibodies, Alloimmunization, Bio-Rad, Rh-D, Weak–D.

INTRODUCTION

With at least 45 distinct antigens, the Rh blood group system is one of the most immunogenic and polymorphic systems in humans and is the most clinically significant system in transfusion medicine.[1] There are two extremely homologous genes in the Rh blood group system. The five primary Rh antigens in the Rh system are D, C, c, E, and e.[2] A individual is either Rh positive or Rh negative depending on whether the Rh antigen, commonly referred to as the Rh factor, is present or absent on the cell membranes of red blood cells (RBCs).[3] The D antigen can appear in more than 200 forms, which are currently referred to as D variants, in addition to regular D positive.[4] The term "weak" refers to decreased D antigen expression," whereas a "partial D" type may be indicated if typing to the D antigen elicits weaker than usual reactions.[5]

The population's genetic variety affects the frequency and specificity of red blood cell (RBC) alloantibodies.[6] Red cell alloimmunization leads to future hemolytic transfusion reaction or hemolytic illness of the unborn and newborn later in pregnancy. Red blood cell alloimmunization happens when a pregnant woman is exposed to foetal blood cells that are Rh positive.[7] Based on the patient's immune response, the smallest quantity of foetal blood required to induce alloimmunization ranges from 0.1 ml to 1 ml.[8] Pregnancy is especially vulnerable during parturition (birth), when anti-D alloantibodies can develop.[8]

Rh-Ig, a substance made from human plasma that contains IgG antibodies to the D antigen, is typically used to protect D negative people from getting immunized with the D antigen. Rh-Ig is most frequently used to stop the production

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of anti-D in D-negative people who receive D-positive blood products as well as in pregnant and nursing D-negative mothers. Each Rh-Ig dose lessens the immune response to a specific number of D-positive red blood cells.[3,9,10] Rh-Ig administration during pregnancy has been shown to considerably reduce the chance of HDFN and lower the risk of anti-D production in D-negative women carrying D-positive foetuses. Because of its scarcity, high price, or inadequate public health organization or resources, anti-D prophylaxis is sporadic or nonexistent in many countries.[11,12]

Many people around the world routinely use D-typing and D-negative matched transfusions. Caucasians are more likely to have the D-negative phenotype than Africans.[13] D-negative blood is generally in limited supply in Asia because of the shortage of donors. The demand for Rh-D negative and blood type O units is higher than the supply offered by blood facilities in many tertiary care institutions.[14] Red cell alloimmunization also occurs when a Rh-negative person receives a transfusion of Rh-positive blood products, which is most common in massive blood transfusions after trauma, accidents, and major bleeding.[15] The mainstay of this resuscitation is massive transfusion (MT), which entails the speedy administration of huge volumes of blood products, including red blood cells (RBC), plasma, platelets (PLT), cryoprecipitate (CRYO), and whole blood (WB)[15] When platelets from Rh-D positive donors are transfused into Rh-D negative patients, the Rh-D antigen may be alloimmunized to. The development of anti-D alloantibodies can result from platelet transfusions from Rh-D positive donors to recipients who have anti-D antibodies, despite the fact that these transfusions do not result in hemolysis since the platelets contain few red blood cells.[4,16,17] The risk of Rh-D alloimmunization only applies to people who receive platelets from donors who have Rh-D positive blood.[18]

A comprehensive study on alloimmunization especially in Pakistan to determine the exact frequency of anti-D alloantibodies has not been done before, and available data is limited to select patient populations like multi transfused, or pregnant women. It's critical for effective blood bank management and secure blood transfusion services to understand the distribution of ABO and Rh blood groups at the local and regional levels. This study's objective is to assess the prevalence of anti-D alloantibodies in individuals with a weak D blood type or ABO Rh-D negative blood in the general population.

OBJECTIVE

To estimate the frequency of anti-D alloantibodies in patients with weak or negative D.

MATERIAL & METHODS

This six-months cross-sectional study was carried out at the Blood Bank of CHUGHTAI Institute of Pathology Lahore Pakistan from May 2022 to October 2022. The Statistics calculator was used to determine the sample size. A sample size of 14,103 was included by using a 1% margin of error and a percentage of negative D that is 12.1%.[19]

All samples were taken by using simple random sampling. EDTA and yellow top serum samples were taken. The blood group was identified using the tube method, Patients were screened for alloantibodies by using 3 cell screening panel (Bio-Rad), and those patients whose screening came out positive were tested for antibody identification using an extended 11cell panel (Bio-Rad) (IAT). Software SPSS version 23 was used to conduct all statistical analyses. The frequency of Anti-D alloantibodies was expressed in percentage.

Inclusion Criteria

All patients with Rh-D negative and Weak D blood type.

Exclusion Criteria

Those patients who were transfusion dependent and Rh-D Positive blood type.

RESULTS

A total of 14,103 blood groups were tested. The average age of the patients was 51.28 ± 22.96 years (19-75 years). There were (n=6205) 44% men and (n=7898) 56% women. Of the n=14103 patients, RH was positive in the highest

percentage (n=12766) 90%, (n=1334) 9.45% were Rh negative, and (n=03) 0.02% were weak-D. (Table-I) (Table-II). Rh alloantibodies were detected in 7.1%, including 5.4% of participants who were positive for anti-D alloantibodies, and 1.49% were positive for other Rh alloantibodies such as (C, c, E). Of the (n=1337) patients who were Rh-negative or weak-D, (n=584) 43.7% were male and (n=750) 56.2% were female. Of the 95 patients who were positive on IAT (three-cell panel antibody screening), 18 (18.36%) were male and 77 (81.0%) were female. (Table-III) Anti-D alloantibodies were detected in 65 (68.4%) females who were pregnant, in 4 (4.21%) females who had received transfusions, and in 6 (6.31%) males who had received transfusions. Other (Rh) alloantibodies were detected in 21.0% (10 men+ 10 women). No significant alloantibodies were detected in Rh D variant. Table-IV and V:

	Number	Percentage
Total sample tested	14103	100%
Rh Positive	12766	90.5%
Rh Negative	1334	9.45%
Weak D	03	0.02%

Table-I. Distribution of Rh blood group

ABO RH Group	Number	Percentage
B Rh Negative	614	46%
O Rh Negative	306	23%
A Rh Negative	266	20%
AB Rh Negative	145	11%

Table-II. Frequency of ABO group type among RH Negative blood groups

Antibody Screening IAT	Number		Percentage
Positive IAT	95	Male – (n=18)	7.1%
		Female (n=77)	
Negative IAT	1239		92.8%

Table-III. Results of antibody screening

Anti D alloantibodies	75	5.62%
Anti c alloantibodies	7	0.52%
Anti E alloantibodies	8	0.59%
Multiple Rh alloantibodies	05	0.37%

Table-IV. Results of alloantibody identification.

	Number	Percentage
Total cases with Rh alloantibodies	95 (male=18) (female=77)	7.1%
Due to pregnancy	65	4.87%
Due to transfusion	28 (male=18) (female=12)	2.09%

Table-V. Causes of anti D alloimmunization.

DISCUSSION

This study's primary goal was to find out whether the study population had Rh- D alloantibodies. Incidence rates of weak-D antigen and Rh negativity ranges from 0.2% to 1% and 3 to 25%, respectively, globally. 90.6% of the participants in this cross-sectional study over a period of six months were Rh-positive, 9.45% are Rh-negative, and 0.02% are weak-D. In total, women made up 57.7% of the Rh-negative study group. Rh D negativity has a very unequal distribution around the world; among Caucasians, it is more than 14%, although it is more common among various sub-Saharan African ethnic groups, ranging from 2.4 to 4.5%.[20] The prevalence of Rh-negative blood group was 4.1% in Bangladesh[20], 6.49% in Indians[21], 7.75% in Africans[22], 10.86% in Albanian population[23] and 11.1% in Palestinian population.[24] The prevalence of weak-D antigen is 0.01% in India, 0.19% in Bangladesh, 0.14% in Albania, 0.5% in Europe, 3% in USA, and 0.8% in Brazil.[20,21,22,23,24]

The most prevalent blood group in our study was type B Rh-negative and the least common was AB Rh-negative. This is consistent with studies in India, which also showed the predominant group to be B Rh-negative and the least common to be AB Rh negative.[25] In present study blood group O Rh-D negative was the most common blood group with positive anti-D alloantibody. In our study, 7.1% of patients had Rh alloantibodies, including 5.4% with positive anti D alloantibodies. These findings are in accordance with a number of previous studies among Africans and Asians, which found an Rh-D prevalence of <7.2%. A study conducted in Tehran, 7.4% of patients had red cell alloimmunization due to Anti D alloantibodies.[22] Another study found that 2.87% of Iranian patients with thalassemia had alloimmunization.

In nine individuals, they found 12 alloantibodies, all directed against the antigens of the Rh blood group system (D, C and E). Anti D alloantibodies were the most common (88.88%).[26]

Alloimmunization occurred more frequently in females, Rh negative blood type and splenectomized patients.[27] In the current study, we discovered a noticeably greater percentage of women who had positive anti-D alloantibodies, and the majority of those women were of childbearing age, out of 19% (254/1334) pregnant Rh-D negative women, 25.5% (65/254) developed anti-D alloantibodies. Alloimmunization rate among Rh-D negative pregnancies was 6.9%.[28] According to GedikÖzköse et al 0.40% of pregnancies were affected by Rh alloimmunization, while 0.34% of those were affected by anti-D alloimmunization.[8] Another study in pregnant women showed that the prevalence of a positive antibody screen was 0.36%.[9] A study conducted in Karachi showed frequency of anti-D alloantibodies among pregnant ladies were 0.9% posing a risk for HDFN.[29] According to Mbalibulha, Y,et al a total of 12.12% of pregnant patients tested positive on antibody screening, demonstrating the presence of anti-D alloantibodies.[24] Comparing these findings to those of a comparable study conducted in Kampala, where a frequency of 5.5% was discovered, shows a higher prevalence of these antibodies.[30] This variation in the prevalence of Rh-D alloantibodies between the study population and the general population is primarily due to the fact that the study groups consisted primarily of pregnant women while our study covered the general community with the Rh negative blood group. The variation may be explained by geographic variations in the populations studied Rh antigen frequencies, transfusion protocols, and the frequency of antibody screening in different countries.

Reportedly, up to 19% of D-negative recipients experienced D-alloimmunization as a result of these D positive erythrocytes. However, careful reanalysis of data from these studies indicates that this number may be as low as 7%.[14,15,16] In our study 28/1334(2.09%) had developed anti D alloantibodies due to transfusion. Out of which 17/1334(1.27%) were due to platelet transfusion. 12.3% of people who received red blood cells that were Rh-D positive but not Rh-D negative experienced alloimmunization as a result of the transfusion.[31]

CONCLUSION

To reduce the risk of recipients developing alloimmunization, it is recommended that the detection of weak D in individuals who are Rh-negative should be taken into consideration as an important component of the workup before blood transfusions, antenatal testing, and to guide clinicians regarding Immuno prophylaxis of Rh D-negative women.

RECOMMENDATIONS

The pregnant woman's screening and laboratory testing should include paternity genotyping. Large-scale studies shall be conducted to evaluate D antigen-causing alloimmunization to prevent the risk of complications due to alloimmunization.

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5	Tayyab Noor	Literature search.	
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