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Prevalence of Irregular Red Cell Antibodies in Healthy Blood Donors at a Stand-Alone Blood Centre in Gujarat

Dr. Jhalak Patel[1], Dr. Vishvas Amin[2]

[1]Transfusion Medicine Specialist, Deputy Director-Medical,

[2]Pathologist; General Secretary

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Corresponding author: Dr. Jhalak Patel
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Abstract

Background: Blood transfusion therapy involves multiple steps to ensure selection of safe blood component for transfusion. This includes testing for infectious markers, full ABO compatibility, free from any irregular red cell antibodies especially which are reacting at wide thermal amplitude and acceptable donor's red cell survival rates without destruction of recipient's red cells.

Objective: This study was aimed to assess prevalence and type of irregular red cell antibodies in a large number of blood donor population at a standalone blood centre in Gujarat.

Methods: This retrospective observational study included blood donor samples for antibody screening from January 2018 to March 2022. A total of 2,16,576 healthy blood donors including 2,00,493 (92.57%) males and 16,083 (7.43%) females were screened.

Results: The prevalence of irregular red cell antibodies was 0.03 per cent in our donor population. Of the total 66 donors with red cell antibodies, 52 (78.79 %) had alloantibodies, 8(12.12%) had autoantibodies and 6 donors (9.09%) showed positive antibody screening with inconclusive results. We also found one case of cold antibody.

Conclusion: Alloimmunization to red cell antigens is a challenging task for current transfusion practices. The antibody screening in blood donors may improve the quality and safety of blood transfusion in the recipients. It also reduces the risk of complications from incompatible blood transfusions.

Keywords: antibody screening, allo-antibody identification, autoantibody, safe blood transfusion, hemolytic transfusion reaction.

Introduction

Red cell antibody anti-A and anti-B are the naturally occurring antibodies that are found in the human serum. All other antibodies are called “irregular red cell antibodies.” There are two types of irregular red cell antibodies: alloantibodies and autoantibodies. Alloantibody is produced against the antigen that is lacking, whereas autoantibody is produced to an antigen that is present. Such irregular alloantibodies/autoantibodies can be encountered in healthy blood donors who are either transfused previously or in multiparous females. [1]

In some rare cases these antibodies are also naturally occurring. Such irregular red cell allo-antibodies can result in to hemolytic transfusion reaction of acute or sometimes delayed type of low intensity and most of which are underdiagnosed.

The aim of the blood transfusion service should be to provide effective blood and blood components, which are as safe as possible and adequate to meet patient's need.

For safe blood transfusion blood donors testing for infectious markers, but also for irregular antibodies should be performed for safe and compatible blood transfusion, especially for previously alloimmunized individuals.

Aims:

The present study aims to assess the prevalence of irregular red cell antibodies in healthy blood donors at a stand-alone blood centre in Gujarat.

Materials and Methods:

This retrospective study was conducted at Indian Red Cross Society, Ahmedabad District Branch which is a standalone blood centre, NABH Accredited having AABB certificate in quality and designated as regional blood transfusion centre from Jan-2018 to March 2022. Blood samples collected in ethylenediaminetetraacetic acid (EDTA) and plain vacutainer from all donors were processed for ABO and Rh (D) blood grouping as well as antibody screening on fully automated immunohematology analyzer "Qwalys-3" (Manufacturer: Diagast, France) working on the principle on erythrocyte magnetization technology. Samples giving positive antibody screen were subjected to antibody identification by extended eleven cell panel and further selective cells for confirmation if required in case of multiple antibodies or partial matches.

Results:

A total number of 2,16,576 donors donated blood at our center during the study [Table 1].

Table 1: Profile of The Donors Tested for Antibody Screening

Group	Male	Female	Total
Total donors	2,00,493	16,083	2,16,576
Antibody positive	**47**	**19**	**66**

Of these, 66 (0.03%) were positive for antibody screening on fully automated immunohematology analyzer "Qwalys-3" (Manufacturer: Diagast, France) working on the principle on erythrocyte magnetization technology. Samples giving positive antibody screen were subjected to antibody identification by extended eleven cell panel. Among them, 69.86% were males and only 30.14 % were females. Donors within the age group of 18-25 years showed maximum number (n = 19; 28.79%) of antibody screen positivity. [Tables-2 and 3]

Table 2: Age-Wise Distribution of Donors with Antibody Positive Screen Results

Age-group (Years)	Number of positive donors
18-25	19
26-30	10
31-35	10
36-40	9
>40	18
Total	**66**

Table-3 The Characteristic of Donors with Positive Antibody Screen

Group	*n* (%)
Total	66 (0.03%)
Gender	
Male	47 (71.21%)
Female	19 (28.79%)
ABO blood Group	
A	14 (21.21%)
B	27 (40.91%)
O	10 (15.15%)
AB	15 (22.73%)
Rh Blood Group	
Positive	49 (74.24%)
Negative	17 (25.76%)

On identification, 8(12.12%) donors were screen positive with autoantibody. 52 (78.79%) had allo-antibodies (80.33%) alone in their plasma. 6 (9.09%) samples were having positive antibody screen with in-conclusive results. Anti-D (*n* = 15; 22.73%) was the most common antibody identified, followed by Anti-M and Anti-E (*n* = 6; 9.09%) and Anti-C and Anti-K (n=5, 7.58%) [Table 4].

Table-4: Frequency of Alloantibody among Antibody Screen Donors

Alloantibody	Frequency (%)
Anti-D	15 (22.73%)
Anti-M	06 (9.09%)
Anti-E	06 (9.09%)
Anti-C	05 (7.58%)
Anti-K	05 (7.58%)
Anti-c	03 (4.55%)
Anti-S	02 (3.03%)
Anti-s	02 (3.03%)
Anti-e	02 (3.03%)
Anti-Kp^{a}	01 (1.52%)
Anti-Fy^{a}	01 (1.52%)
Anti-Jk^{b}	01 (1.52%)
Anti-Lu^{a}	01 (1.52%)
Anti-N	01 (1.52%)
Cold antibody	01 (1.52%)
Positive antibody screen with in-conclusive results	06 (9.09%)
Auto-antibody:	08 (12.12%)

Discussion:

Various worldwide literature calls out the importance of donor irregular antibody screening. Since in our study, most of antibodies detected had potential to cause hemolytic transfusion reaction, irregular red cell antibody screening should be an imperative element in assuring transfusion safety. Several studies have reported that the rate of allo-immunization in blood donors varies from 0.32% to 2.4%. [2,3] This large variation may be due to the different screening method used, and characteristics of the population studied.

The prevalence noted in the present study is 0.03%, which is comparable with the study done by Pahuja *et al.* [3] The highest frequency of alloantibodies was identified in blood donors aged between 18-25 years in our study, which is comparable to the study done by Gayatri *et al.* [4] On the contrary study by Macroo *et al* showed highest frequency in age group of 26-30 years. In the present study, we found a predominance of male donors compared to female donors, which is comparable with a study done by Garg *et al*. [5]

This can be due to a greater number of male donors compared to female donors.

A study on the frequency of allo-immunization in the Kuwaiti population describes that five most frequently identified alloantibodies were Anti-D, Anti-E, Anti-K, Anti-Le^a and Anti-Le^b in the descending order.[6] Malaysian study on prevalence and specificities of red cell alloantibodies among blood recipients shows that Anti-E antibody comprised the most common alloantibody followed by the Anti-Le^a antibodies and the Anti-M antibody.[7] A study done in the Southern Thai population showed the following antibodies frequency wise as most common – Anti-Mia, Anti-E, Anti-Le^a, Anti-c and Anti-Le^b. [8]

Several Indian studies mostly in the Northern population are available in the literature. 0.09% prevalence of unexpected red cell antibodies was noted among healthy donors in the Delhi population.[5] The most common alloantibodies were of the MNS system (39.13%) followed by Anti-D (13%) and Anti-Lewis (10.8%). Another similar study involving blood donors in northern India showed the prevalence of 0.05% alloantibodies was observed.[9] A tertiary care center in northern India reported positive screening cells and pooled O cells in 0.27% of donors, 66.08% of these donors had autoantibodies, 1 had autoantibodies with underlying alloantibody Anti-Jk^a (0.001%), and 0.09% had alloantibodies alone in their plasma. Anti-M was the most common antibody (18.94%) identified, followed by Anti-D (9.25%).[2] In the Indian scenario, female blood donations are fewer when compared to male blood donations and hence this may be a reason that the female population with positive red cell alloimmunization maybe the tip of the iceberg phenomenon.

The frequency of anti-D in our study was found to be 22.73%. Of the donors with anti-D (n = 15), 12 were females and 3 were males. 10 of the 12 female donors had a history of previous blood transfusion during antenatal period, the remainder had unknown transfusion history. 2 of the 3 males gave a history of previous blood transfusion, whereas one did not remember about any previous transfusions. The frequency of other Rh antigens anti-E and anti-C was found to be 9.09% and 7.58% respectively and anti-c and anti-e was 4.55% and 3.03% respectively.

We found combination of allo-antibodies which had 2 cases of anti-D and anti-C and one case of anti-Jk^b and anti-M. We also found 6(9.09%) cases of positive antibody screen with in-conclusive results.

Other alloantibodies found in our study are anti-M (9.09%), anti-K (7.58 %), and anti-S (3.03%), anti-s (3.03%), anti Kp^a, anti-Fy^a, anti-Lu^a, anti-Jk^b, anti-N is 1.52%.

Apart from this we also found one case of cold antibody (1.52%). Cold autoantibodies are often detected in healthy individuals, with the majority being benign which react more strongly at 0°C–4°C than at warmer temperatures. Pathological cold autoantibodies are characterized by wide thermal amplitude and/or high antibody titers.[10] Joshi et al. state that cold autoantibodies observed in their study were found among blood donors with no apparent health problems and may be due to exposure to infectious agents as antibody stimulants in past was open as the population in the city of Surat was exposed to vector-borne infections such as malaria, gastroenteritis, and infective hepatitis during the contemporary period of this study.[11] The cold antibody detected in present study was reactive at room temperature and negative at 37^0C.

In the present study, there were 8 cases of auto-immunization or DAT positivity in a total healthy blood donors studied. Issitt and Anstee[12] reported that of blood donors with a positive DAT and IgG coating the RBCs, 5%–10% will develop AIHA, 20%–25% will become DAT negative over time, and 60%–70% will remain DAT positive, but hematologically normal. The incidence of a positive DAT among the healthy blood donors was 0.04% in an Indian study done in the northern Indian population by Tiwari et al. [13] Makroo et al. [2] state that in their study, the prevalence of DAT positivity among healthy donors was 0.18% and in Kaur et al. study, it was 0.05%.[14] We had a DAT positive rate of 0.003% which was lowest in comparable to other studies.

In current study it was also noted that the majority of blood donors belonged to blood group B followed by AB, A and O of ABO blood group system. The reason may be because of high frequency of B blood group in population but the reason of AB being the next highest cannot be explained due to lack of comparative studies. Also, it was noticed that the allo-antibodies were more seen in Rh positive individuals compared to Rh negative. This can be attributed to the high frequency of Rh Positive in population compared to Rh negative.

Conclusion:

Red cell antibody screening of the donors is a simple test, adds a layer of safety in transfusion and reduces the need for minor cross matching. In addition, we recommend that in cases where the antibody is found in blood donor, they should be informed, so that in future if they require any transfusion, they can inform the blood centre prior. It is also seen here that MNS blood group is system is also second most common blood group system after Rh blood group system to have high number of irregular antibodies significantly acting at 37^0C temperature and this cannot be neglected. Although the prevalence of unexpected antibodies is not that high in our study but to offer safe blood to patients; extended phenotyping is the approach that should be given a thought for issuing blood in cases of positive antibody screen of patients as well as reducing the rate of antibody positivity by giving phenotypically matched blood units.

We found that the overall prevalence of unexpected RBC alloantibodies was 0.03% with anti-D, anti-E and anti-M being the most frequently identified alloantibodies in blood donors at our center followed by antibodies to other Rh antigens. Positive antibody screen was more seen in Male blood donations and in age group of 18-25 years. B blood group donors were identified to have high number of positive antibody screen followed by AB group. We also found 3 cases positive of multiple unexpected antibodies along with 08 cases of DAT positivity and one case of cold antibody.

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