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# Prevalence of irregular red cell antibody in transfusion recipients vis-a-vis healthy blood donors attending a tertiary care hospital in North India

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## Abstract:

**CONTEXT:** Alloimmunization by foreign red cell antigens is a matter of concern as it may lead to hemolysis in transfused patients as well as fetus of pregnant females.

**AIMS:** This study aimed to perform a comparative analysis of prevalence and type of irregular antibodies in healthy donors, vis-a-vis blood transfusion recipients.

**SETTINGS AND DESIGN:** Blood samples of 4000 individuals comprising healthy donors, exposed patients, and nonexposed patients were collected and were analyzed for irregular antibodies.

**MATERIALS AND METHODS:** Commercially available three-cell antigen panel was used for the antibody screening. The samples positive in antibody screen were further subjected to an extended 11-cell panel for antibody identification in low-ionic strength saline with and without enzyme.

**STATISTICAL ANALYSIS:** Statistical analysis was done using SPSS for Windows 15.0 program. Chi-square test was used for detecting statistical significance of exposure to red blood cell antigens in the formation of alloantibodies.

**RESULTS:** Of the 4000 samples, antibodies were identified in 105 (2.6%) samples. Overall, nonexposed group showed a seropositivity of 0.36%, while the exposed group showed a seropositivity of 9.4%. Anti-D was the most common antibody found in 38 patients (33.3%). Anti-E was the most common antibody in males, while anti-D was the most common antibody in females.

**CONCLUSIONS:** Since the risk of alloimmunization is more common in multitransfused patients, it is advisable to screen at least those cases for irregular antibodies.

## Keywords:

Alloimmunization, antibody identification, antibody screening, indirect Coombs test, seropositivity

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## Introduction

Blood transfusion though is essential in many situations to prevent loss of life or functional capacity of an individual, it carries certain inherent risks associated with it.<sup>[1]</sup> Red blood cell (RBC) alloimmunization is a common complication among the

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packed RBC and whole blood transfusion recipient and is induced due to genetic differences between the blood donor and the recipient.<sup>[2]</sup> This red cell alloimmunization may lead to difficulty in finding compatible blood for transfusion or even can cause severe hemolytic transfusion reaction.<sup>[3,4]</sup>

Usually, ABO- and Rh (D) antigen-matched blood is provided by the blood banks, so the

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risk of alloimmunization to minor blood group antigens is very high. The most important irregular RBC alloantibodies are directed toward Rh (anti-D, -C, -E, -c, and -e), Kell (anti-K), Duffy (anti-Fy<sup>a</sup> and -Fy<sup>b</sup>), Kidd (anti-Jk<sup>a</sup> and -Jk<sup>b</sup>), and MNS (anti-M, -S, and -s) blood group systems.<sup>[5]</sup>

Repeated blood transfusion can result in the development of alloantibodies against one or more red cell antigens. The risk of alloimmunization is high in patients receiving multiple transfusions such as patients having thalassemia major, aplastic anemia, sickle cell disease, hematologic malignancies, chronic renal failure, and cancer patients receiving chemotherapy. One of the most important determinants for pretransfusion testing is to exclude the presence of clinically significant alloantibodies in the patient's blood before selecting RBC for transfusion.<sup>[6]</sup>

## Materials and Methods

This study was conducted in the blood bank of a tertiary care center in Dehradun over a period of 2 years from 2017 to 2019. A total of 4000 samples were analyzed during the course of the study. Of these, 2000 samples were of the control group (healthy donors) and the remaining 2000 samples were from the study group (patient samples). Of the 2000 patient samples, 1000 patients did not have any history of previous antigenic exposure, i.e., they were nontransfused male patients or primigravidae females having <24 weeks pregnancy. The remaining 1000 patients had a previous history of antigenic exposure, of which 750 patients had a history of transfusion, pregnancy, or both, but the number of such exposures was <4; the rest 250 patients had a history of 4 or more exposures.

Ethylenediaminetetraacetic acid blood samples were used for direct Coombs test, while plain samples were used for antibody screening and identification in both blood donors and admitted patients.

A commercially available three-cell antigen panel (ID-Diacell I-II-III; Biorad) was used for the antibody screening procedure. The patient's serum was incubated at 37°C for 15 min with reagent red cells using low-ionic strength saline (LISS) Coombs gel card. The cards were and then centrifuged for 10 min. If the antibody screen with the three-cell antigen panel was positive, an extended 11-cell panel was used for antibody identification in LISS with and without enzyme (ID-Diapanel Biorad).

## Statistical analysis

IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp was used to perform Chi-square test to calculate the association of number of exposures (4 or more) to the risk of alloimmunization.

## Results

Of 4000 samples studied, the total male subjects in our study were 2390 comprising 59.8% of the total population. The male: female ratio was highest in healthy donors and lowest in the study group having <4 antigenic exposures. Irregular antibodies were detected in 105 samples comprising 2.6% of the total samples. However, the prevalence of antibodies in different groups was highly variable. Healthy blood donors showed the lowest seropositivity of 0.3%, followed by 0.5% in nonexposed patients and primigravidae females. The study group having <4 exposures showed a positivity of 6.1%, while the highest seropositivity (19.2%) was found among the group having 4 or more exposures [Table 1]. Overall, nonexposed group showed a seropositivity of 0.36%, while the exposed group showed a seropositivity of 9.4% [Table 2]. Chi-square test showed that the association of number of exposures to the risk of alloimmunization was highly significant ( $P = 6.843e-9$ ).

Overall, anti-D was the most common antibody found in 38 patients (33.3%). Anti-D was also the most common antibody detected in females (34/71, 47.9%); however, in males, anti-E was the predominant antibody (8/34, 23.5%). In the study group having 4 or more exposures, anti-D and anti-E were the most common antibodies. In other patient groups (patients having <4 exposures or no exposure), anti-D was the most common alloantibody. In healthy donors, anti-D, anti-M, and autoantibodies were detected in equal proportions [Table 3].

Overall, females showed a higher seropositivity of 4.5% than males who exhibited a seropositivity of 1.4%. The group showing the highest % seropositivity was males having 4 or more exposures (20.2%), followed closely by females having 4 or more exposures (18.4%). Seropositivity rates in all other female groups were higher than their male counterparts [Table 4].

## Discussion

Irregular antibodies are antibodies present in an individual's serum apart from anti-A and anti-B and are usually acquired due to previous antigenic exposure via transfusion or pregnancy.<sup>[7]</sup> Their prevalence in donors and multitransfused patients has been studied by many Indian and International scholars; however, studies comparing the prevalence of irregular antibodies in healthy donors with nonexposed as well as multitransfused patients are limited. Thus, this study was undertaken with an aim of detecting the prevalence and type of irregular antibodies among donors as well as transfusion recipients.

The overall male-to-female ratio in our study was 1.5:1. The ratio rose to 4.6:1 in case of blood donors. Thus,

**Table 1: Distribution of negative and positive results for irregular antibodies in different study groups**

| Group            | Subgroup            | Number of subjects | Antibodies detected | Percentage |
|------------------|---------------------|--------------------|---------------------|------------|
| Nonexposed group | Healthy donors      | 2000               | 6                   | 0.3        |
|                  | Nonexposed patients | 1000               | 5                   | 0.5        |
|                  | Total               | 3000               | 11                  | 0.36       |
| Exposed patients | <4 exposures        | 750                | 46                  | 6.1        |
|                  | 4 or more exposures | 250                | 48                  | 19.2       |
|                  | Total               | 1000               | 94                  | 9.4        |
| Grand total      |                     | 4000               | 105                 | 2.6        |

**Table 2: Profile of antibodies in males versus females**

| Antibody            | Males                         | Females                       | Total                           |
|---------------------|-------------------------------|-------------------------------|---------------------------------|
| Anti D              | 4                             | 34                            | 38                              |
| Anti E              | 8                             | 13                            | 21                              |
| Anti Kell           | 5                             | 7                             | 12                              |
| Anti C              | 5                             | 9                             | 14                              |
| Anti M              | 3                             | 3                             | 6                               |
| Anti N              | 2                             | 2                             | 4                               |
| Anti c              | 5                             | 3                             | 8                               |
| Anti lutheran       | 2                             | 0                             | 2                               |
| Autoantibodies      | 4                             | 5                             | 9                               |
| Multiple antibodies | 4                             | 5                             | 9                               |
| Total               | 38 occurrences in 34 patients | 76 occurrences in 71 patients | 114 occurrences in 105 patients |

**Table 3: Profile of antibodies in different study groups**

| Antibody            | Healthy donors | Nonexposed patients | <4 exposures                 | 4 or more exposures          | Total                           |
|---------------------|----------------|---------------------|------------------------------|------------------------------|---------------------------------|
| Anti-D              | 2              | 3                   | 20                           | 13                           | 38                              |
| Anti-E              | 0              | 0                   | 8                            | 13                           | 21                              |
| Anti-Kell           | 0              | 0                   | 4                            | 8                            | 12                              |
| Anti-C              | 0              | 1                   | 8                            | 5                            | 14                              |
| Anti-M              | 2              | 0                   | 2                            | 2                            | 6                               |
| Anti-N              | 0              | 1                   | 1                            | 2                            | 4                               |
| Anti-c              | 0              | 0                   | 4                            | 4                            | 8                               |
| Anti-lutheran       | 0              | 0                   | 1                            | 1                            | 2                               |
| Autoantibodies      | 2              | 0                   | 2                            | 5                            | 9                               |
| Multiple antibodies | 0              | 0                   | 3                            | 6                            | 9                               |
| Total               | 6              | 5                   | 50 antibodies in 47 patients | 53 antibodies in 47 patients | 114 occurrences in 105 patients |

**Table 4: Seropositivity in different study groups**

| Group               | Seronegative males | Seropositive Males | Percentage positivity | Seronegative females | Seropositive females | Percentage positivity |
|---------------------|--------------------|--------------------|-----------------------|----------------------|----------------------|-----------------------|
| Healthy donors      | 1639               | 3                  | 0.2                   | 355                  | 3                    | 0.8                   |
| Nonexposed patients | 387                | 1                  | 0.3                   | 608                  | 4                    | 0.7                   |
| <4 exposures        | 240                | 6                  | 2.4                   | 464                  | 40                   | 7.9                   |
| 4 or more exposures | 91                 | 23                 | 20.2                  | 111                  | 25                   | 18.4                  |
| Total               | 2357               | 33                 | 1.4                   | 1538                 | 72                   | 4.5                   |

in our study, females constituted 17.9% of total blood donors. This result is in consonance with the findings of Halder *et al.* where females comprised 20.16% of total donations.<sup>[8]</sup> Rajendra *et al.* also found female participation in blood donation at 16.2%.<sup>[9]</sup> It is a common practice in a patriarchal society such as India that males participate more often than females in activities perceived to be associated with masculinity such as blood donation.

In our study, the seropositivity rate among healthy blood donors was 0.3%. Pahuja *et al.* showed the prevalence of 0.05% among 7756 whole blood donors.<sup>[10]</sup> Garg *et al.* reported a prevalence of 0.09% among 47,450 whole blood donors.<sup>[11]</sup> On the contrary, Giblett had reported a 0.32% incidence of irregular RBC antibodies in blood donors.<sup>[5]</sup> In our study, the incidence of irregular antibodies in healthy donors is comparable to the study

by Gilbertt but higher than the prevalence recorded by Pahuja *et al.* and Garg *et al.* This can be due to higher percentage of female donors in our study group as female donors have more chances of alloimmunization due to pregnancy. In our study, the seropositivity rate among females (0.5%) was higher than men (0.2%) in the healthy donor group. Similar results were reported by Sachan *et al.*<sup>[12]</sup>

In our study, the incidence of irregular antibodies in the exposed group was 9.4% which can be compared to the following studies. A similar study by Patel *et al.* done on multitransfused patients also reported a prevalence of 7.0% (14/200) which is in concordance with the present study.<sup>[13]</sup> In a similar study by Agarwal *et al.* on multitransfused patients, they documented a prevalence of 2.71% (7/258).<sup>[14]</sup> A slightly higher prevalence of alloimmunization in this study can be due to the fact that 25% of our study population comprises patients having history of multiple transfusions, namely thalassemic, oncology, and chronic kidney disease patients.

Koelewijn *et al.*, in their study to assess the efficacy of a universal antibody screening program for pregnant females, found a total alloimmunization rate of 1.2%.<sup>[15]</sup> Al-Ibrahim *et al.* found a 2.0% alloimmunization rate, while Howard *et al.* detected clinically significant antibodies among 1.0% of all pregnant women.<sup>[16,17]</sup> In our study, the overall seropositivity of females is 4.5%. This increased seropositivity can be attributed to the fact that a significant proportion (575 of total 1538 females) had a history of multiple exposures due to pregnancy or transfusion.

In the present study, the most common irregular antibody was anti-D, accounting for 33.3% of total irregular antibodies. In a study conducted by Pahuja *et al.*, anti-D antibody contributed to 78.4% (40/51) of total alloimmunizations in the study which is in accordance with the present study.<sup>[10]</sup> Similarly, a study conducted by Ameen *et al.* documented that anti-D was the most common antibody accounting for 27.3% of the total antibody-positive cases.<sup>[18]</sup> A study conducted by Makroo *et al.* showed that the majority of alloantibodies were anti-M and anti-D, accounting for 56.57% (43/76) and 27.63% (21/76), respectively, of all positive antibody cases in healthy donor population.<sup>[19]</sup>

The high incidence of anti-D in our study can be due to the fact that a large number of antenatal patients admitted in our hospital come from far-flung remote areas of Garhwal where they are not educated about anti-D prophylaxis. Anti-D was also detected in two healthy donors as well as four males in the multitransfused category. Both the donors showing anti-D were females and might have acquired alloantibodies due to previous pregnancy

information regarding which was not revealed during the questionnaire. The four males in which anti-D was detected all belonged to multitransfused category and had a history of transfusions (both RBCs and platelets) from outside blood banks and might have received Rh D-positive or weak D-positive transfusion of either RBCs or platelets.

Apart from anti-D, many significant antibodies were detected; foremost among them are anti-E, anti-K, and anti-C. All these antibodies are warm reacting and can lead to hemolysis as well as hemolytic disease of fetus and newborn.

Anti-Kell was reported in 12 subjects, all belonging to the exposed category (0.4% in <4 exposures and 0.8% in more than 4 exposures). The proportion of anti-Kell in all antibodies detected was 10.5% (12 out of 114 occurrences). This high ratio of anti-Kell can be due to a higher K antigen prevalence in North India.<sup>[20]</sup>

Anti-M and anti-N were detected in a total of 10 cases (in exposed as well as nonexposed group). They are generally naturally occurring alloantibody which do not react at 37°C and are not clinically significant for transfusion but can cause a problem in pretransfusion testing. In cases where they are detected at 37°C, cross-match compatible antigen-negative blood should be given to prevent any hemolytic transfusion reaction.

Autoantibodies (DAT positive) were detected in 0.2% of our nonexposed subjects. Our results are in consonance with the results of Makroo *et al.* who detected a seroprevalence of 0.18% in their donor population.<sup>[19]</sup> The DAT-positive bags were discarded as per the institutional policy. No DAT-positive donor showed any evidence of hemolysis on follow-up.

## Conclusions

We recommend the inclusion of antibody screening tests in routine pretransfusion testing protocol, especially those at requiring frequent transfusions and having risk of alloimmunization. As anti-Rh and anti-K antibodies are most potent as well as frequent, antigenic typing for Rh and Kell must be carried out for multitransfused patients.

## Limitations

This test may not be cost-effective for all transfusion recipients in our country; hence, reference centers should be developed to provide antigen-negative blood to patients requiring repeated transfusions.

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## Conflicts of interest

There are no conflicts of interest.

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