Original Article

Frequency and Risk Factors for RBC Alloimmunization in Patients Undergoing Surgery in Tehran, Iran: the Role in Improving Type and Screening Tests

Susan Moradinasab[1], Ahmad Gharehbaghian[2*], Alireza Abdolahi[3]

1 High institute for research and education in Transfusion Medicine, Tehran, Iran.
2 Department of Hematology and Blood Bank, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3 Department of Pathology, Tehran University of medical science, Tehran, Iran

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Correspondence
Ahmad Gharehbaghian
Email:gharehbaghian@hotmail.com

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Abstract

Introduction: Alloimmunization to red blood cell antigens is one of the main complications of transfusion therapy. The appropriate pre-transfusion tests are necessary in order to minimize hemolytic reactions related to RBC alloantibodies. The aim of this study was to determine the frequency and specificity of RBC alloantibodies detected during pre-transfusion tests in addition to risk factors of alloimmunization in the hospitalized population in Tehran, Iran.

Materials and Methods: In this retrospective study, the characteristics and type of alloantibodies in 31 alloimminized patients among 6029 hospitalized patients of Imam Khomeini general hospital were examined during September 2016 to January 2017.

Results: The RBC alloantibody prevalence was 0.5% with the male: female ratio of 1:5.2. The most frequent antibodies were anti-D (30%), anti-E (24%) and anti-K (12%). Female sex, age, history of transfusion and pregnancy or abortion history were associated with alloimmunization.

Conclusion: The alloimmunization rate were relatively low in hospitalized patients in this study. Female sex, age, history of transfusion and pregnancy sound to be the risk factors of alloimmnization.

Keywords: Alloimmunization, RBC, Alloantibodies, Transfusion

1. Introduction

Nowadays, blood transfusion has become a major part of healthcare system and alloimmunization is an important adverse effect for those patients undergoing blood transfusion. The alloantibodies are the main causes of immediate and delayed hemolytic transfusion reactions (HTRs).

Frequency of blood group alloantibodies has been reported differently in various study populations including hospital-based patients, blood donors and patients with hematologic disorders. Red Blood Cell alloantibodies have been reported in up to 0.8 percent of blood donors and approximately 1-2 percent of hospital-based patients [1-4]. The higher rate of about 5 to 30 percent has been reported in transfused-dependent patients [5, 6]. The wider rates were detected in those with hemoglobinopathies (3-76%) [7-10]. It has been reported that alloimmunization rate in children with sickle cell is as high as 29% while in adults, the rate is 47% [11-13]. Red blood cell alloimmunization results from multiple risk factors, including sex,

history of blood transfusion, pregnancy, racial differences between transfusion recipients and blood donor and the number of transfusion as well as the age of onset, or antigen disparity between mother and fetus. Due to the importance of alloimmunization in patients, particularly pregnant females, and to avoid the technical and official faults of cross-match tests, antibody screening test should be carried out properly and the records must be kept and transferred with the patients.

During pre-transfusion testing, the patient's blood is screened for alloantibodies. Here, unreliable pre-transfusion tests and inappropriate methods to identify alloantibodies are the main reasons of missing immunized patients. Time courses of antibody induction (usually at least one month up to four months after first transfusion) could be considered as one of the most important factors affecting antibody screening, so testing too early or too late could lead to failure of antibody screening [14, 15]. Due to the serious consequences of undetected alloantibodies, performing appropriate pre-transfusion tests seems quite crucial. On the other hand, a truthful antibody screening test with the purpose of omitting cross match test is very important as to avoid unnecessary packed cell reservation and saving blood sources. While extended red blood cell phenotyping for all donors and patients is not affordable in developing countries like Iran, type and screen tests before transfusion must be sensitive and reliable enough to avoid alloimmunization. This retrospective study was set out to review the frequency and specificity of clinically significant RBC alloantibodies in our hospital blood bank records to evaluate how pre-transfusion tests could have impact on alloimmunization.

2. Materials and Methods

2.1 Subjects Studied

In this study, records on Red blood cell clinically significant alloantibodies in blood bank database of Imam Khomeini hospital, the referral center and the biggest multi-disciplinary general teaching hospital in Iran with 1300 active bed were examined retrospectively. The total of 6029 hospitalized patients (3984 female and 2045 male) in Imam Khomeini hospital of Tehran were retrieved for analysis from September 2016 to January 2017. An extended red cell antigen panel was performed to detect alloantibodies. For alloimmunized patient, demographic characteristics including sex, age, medical history, transfusion history, pregnancy history and abortion history were collected from hospital medical records. Records with missing data were excluded. In order to ensure that the same patients were not included in the study repeatedly, in case a patient had multiple screening results during the hospitalized period, the record would be entered into database only once. Clinical characteristics which were studied were the underlying disease, previous transfusion and the reason for transfusion, pregnancy and abortion history. Transfusion-dependent patients were excluded from this study.

2.2 Serological testing

Pre-transfusion tests were carried out for all the patients who applied for blood transfusion in the hospital. Pre-transfusion testing included ABO group and Rh-D type, antibody screening and compatibility testing. Antibody screening was performed by testing a patient's plasma against a reagent of O red blood cell. Albumin was used to enhance the antigen antibody reactions. When the alloantibody screening was positive, patient's plasma was examined over again for alloantibody with commercial 3-cell panel of reagent O RBCs, including the RBC's frequent antigens such as Rh, KELL, Duffy, Kidd, Lewis, MNS, P and Lutheran blood group system (Immunohematology reference laboratory of the Iranian Blood Transfusion Organization in Tehran, Iran, Lot N: 16IP3C105). In those samples with positive

antibody screening test, antibody identifications were done with 11-cell panel, covering the US Food and Administration-recommended RBC antigens in LISS (DiaLISS, Bio-Rad) with and without papain. Antibody screenings were also done with the commercial anti-globulin gel card (Invitrogel AHG Coombs). The criteria for antibody specificity were based on American Association of Blood Banks.

2.3 Statistical analysis

Analyses and data management were performed using statistical software SPSS version 23.0 and Excel 2016, Microsoft Corporation. Continuous variables with were compared with a t-test. Comparison of categorical variables was carried out, using chi-square test or Fisher's exact test. P values less than 0.05 were considered significant.

3. Results

After excluding multi-transfused patients, such as hemophilia, thalassemia, and leukemia from the present study, the total data from 6029 hospitalized patients (2047 males and 3982 females) with the age of 1 year to 90 years were analyzed. Totally, 50 clinically significant alloantibodies were found in 31 patients, demonstrating the alloantibody prevalence of 0.5 percent in this study. Out of the 31 patients with alloantibodies, 20 (64.5%) had single antibody, whereas the remaining 11 patients (35.4%) had multiple alloantibodies, 5 patients had two antibodies, 4 patients had three antibodies and 2 patients had more than three antibodies). Among patients with single antibody, those with anti-D were the most (50%) and among the patients with multiple antibodies, the combination of anti-D and anti-C had the highest ratio. Overall, the most coexisting alloantibodies were seen in Rh system antibodies. The 8 frequently identified alloantibodies were the anti-D 15 of 50 (30%), anti-E 12 of 50 (24%), anti-K 6 of 50 (12%), anti-C 5 of 50 (10%), anti-c 4 of 50 (8%), anti-e 4 of 50 (8%), anti-jk ^{b} 2 of 50 (4%) and anti-s 2 of 50 (4%). Antibody specificity and frequency in 31 alloimmunized patients were shown in table1.

Table 1. Frequency and specificity of alloantibodies in the alloimmunized patients*

Type of alloantibody	n=50 (%)
Anti-D	**15** (30)
Anti-E	**12** (24)
Anti-K	**6** (12)
Anti-C	**5** (10)
Anti-c	**4** (8)
Anti-e	**4** (8)
Anti-jk^{b}	**2** (4)
Anti-s	**2** (4)

Of all alloimmunized patients, 28 patients (90.3%) had the history of transfusion and 3 patients had no transfusion history. In 26 alloimmunized females, 23 patients (88.4%) had history of pregnancy or abortions. The alloimmunized patients were categorized according to age, sex, transfusion history and history of pregnancy or abortions (Table 2).

Table2. Demographic characteristics of immunized and non-immunized patients

Characteristic	Alloimmunized (n=47)	Non-immunized (n=25)	*P*-Value
Mean age in years (median; range)	38.77 (34; 6-78)	40.0 (37; 1-90)	<0.05
Sex			<0.05
Female	26	3958	
Male	5	2040	
Pregnancy/abortion	23	199	<0.001
Transfusion History	28	429	<0.001

These data were collected in order to find out if there would be any relations between these factors and alloimmunization. Among 31 alloimmunized patients, 26 were females. Females registered the higher rates of alloantibody formation (83.8%), in comparison to males (16.1%) (Particularly anti-D). This is possibly because of more exposure to immunizing events through pregnancy. Overall, the male: female ratio of alloimmunization was 1:5.2 (5 males, 26 females). The most clinically significant alloantibodies in females with positive screening test were anti-D and anti-E, respectively. In male, anti-K was the most prevalent antibody. Alloimmunized patients were between 6-78 years of age. Interestingly, the prevalence of alloantibody detection was significantly different between various age groups. The highest frequency was identified in patients aged 21-30 years and among the other patients, the higher rate of alloimmunization was seen in patients between 31-40 years old. Patients more than 70 years old were the least alloimmunized (Chart1).

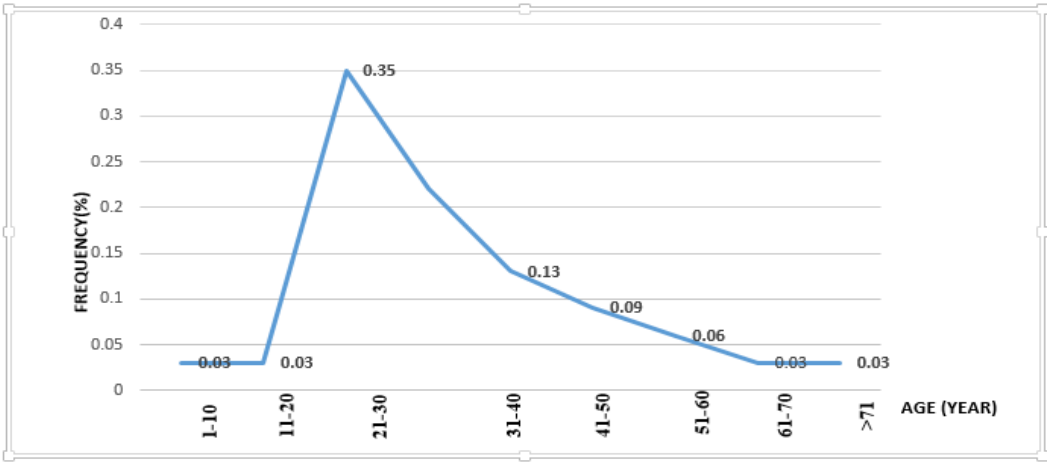

Figure 1. Alloimmunization between different age groups

4. Discussion

In this multi-variant retrospective study, the frequency of clinically significant alloantibodies in hospitalized patientsm and the factors influencing incidence of alloimmunization were investigated. Age, sex, transfusion history and pregnancy proved to be as the most important factors,

and were the indicators of alloimmunization against red blood cell antigens.

The alloimmunization rate in hospitalized patients of the present study was 0.5%. In two other previous studies in Iran on transfusion-independent patients undergoing elective surgery, the prevalence of alloantibodies were 1% and 0.92% [1, 3]. This study's results also revealed the lower alloantibodies in comparison to the studies in Malaysia (0.76% and 1.4%)[16]. However, the rate of alloimmunization in Chinese hospitalized population were reported differently from 0.21% to 0.5%, according to heterogeneity of the studied population [17, 18]. These different findings might be due to heterogeneity of the population and different screening and detecting methods. The relatively lower rate of alloantibodies in this study might be related to insensitive pre-transfusion tests or the lack of post transfusion screening tests, so the antibodies with short lifespan and low titer would disappear during the period of time after transfusion or due to their lower titer, they would merely be detected through tests with high sensitivity. It is known that LISS can detect the low titer antibodies better than Albumin; consequently, using Albumin as an enhancer in blood bank might lead to missing the low titer antibodies. The rate of alloantibodies might be underestimated as the result of the lag before antibody induction [4, 14] or due to a short life span of some antibodies, they could become undetectable within months after the formation [15]. The other factor that might impress the results of antibody detection was sensitivity of the testing methods. The lower rate of alloimmunization has shed light on the necessity of improvement in antibody screening tests in order to avoid hemolytic reactions.

The higher prevalence of alloimmunization among female was seen in our study with male: female ratio of 1:5.2. The predomination of female in alloimmunization was seen in many other studies [19, 20] but not all studies confirm this fact [21]. The logic behind this result is that females are more exposed to alloantigens during the pregnancy. It should be considered that the most of the population of the present study were females and many of them were pregnant due to the active section of Gynecology/Obstetrics in the hospital. In earlier studies done in Iran, there was not any correlation between alloantibody formation and sex. The most frequent antibodies in this study's population were anti-D, anti-E and anti-K respectively. It is noteworthy that although anti-D prophylaxis in obstetric women were performed in Iran, the most frequent antibody in females were anti-D and due to the predominant population of females than males in our study, anti-D is the most frequent antibody in this study similar to the other study done in Egypt [20] and different from some other studies with the predominance of anti-E [7, 18, 22] and predominancy of anti-K [1, 4]. It can be postulated that this difference might be contributed to the study population or identification methods and enhancement methods. It is important to note that the high titer anti-D in the pregnant women in the present study would have been screened easier by the insensitive methods. The other reason of anti-D predominance in this study could be related to the transfusion of Rh-D positive platelet products for D-negative patients due to the lack of blood products in emergency cases. Anti-D formation might be stimulated due to residual RBCs or RBC-drives micro particles in platelet blood products [23].

The other finding of this study was the association of age with alloimmunization. Results are comparable with the study by Zhuaning and colleagues [24] and Abdelrazik and colleagues [20] demonstrating the association of age with alloantibody detection. The highest frequency of alloimmunization were seen in patients aged 21-40, relatively, which is

similar to the study on hospitalized Chinese population [18]. However, in transfusion-dependent patients such as major thalassemia, due to regular transfusions, the highest frequency of alloantibodies were seen in patients aged 1 to 17 [24]. In this study, females constitute the higher portion of patients and many of them who required transfusion had the history of pregnancy or abortion before.

5. CONCLUSION

Alloantibody formation as an adverse effect of blood transfusion is still a concern of most clinicians. In this study on non-transfusion dependent hospitalized patients in Iran, multi-variant factors affecting alloimmunization like age and sex and underlying disease were considered. However, due to retrospective design of the study, the other factors affecting alloimmunization, like the number of receiving units, leuko-reduced units and kinetics of antibody induction were not feasible for the purpose of the study. Further studies are required to assess the other predicators of alloimmunization.

Compliance with Ethical Standards

This article does not contain any studies with animals performed by any of the authors.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and national research committee of Iranian Blood transfusion organization.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Conflict of interest

The authors declare no conflict of interest.

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