Original Article

Frequency of Alloantibody with their Specification among Multitransfused Patients

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Abstract

Background and Objectives: Alloimmunization is a very common clinical problem in transfusion-dependent patients but rarely detected in Bangladesh. The risk of alloimmunization is especially high in patients who receive repeated transfusion, i.e., thalassemia patients. The objective of this study was to investigate the seroprevalence and specificity of red blood cell antibodies in multitransfused thalassemic patients. **Methods:** This cross-sectional study was conducted at a tertiary care referral center from January 1, 2013, to March 31, 2016. Blood specimens were sent from different facilities and also from daycare transfusion center of the same when cross-match difficulties occur. When the Coomb's test showed positive result, then Rhesus, Kell, Kid, Duffy, and Lewis blood group typing were done according to the patients' need. Serum was used to identify the presence of alloantibody by the indirect Coomb's test with known cell panels. An autocontrol, positive and negative control for each specimen was incorporated to detect autoantibody and correctness of the procedure done. We performed a simple percentage to evaluate the analysis. **Results:** Of 1286 cases, alloantibody was detected in 334 (25.97%) cases. Among the 334 cases, 80 (23.95%) cases were followed up by antibody identification. The remaining 254 cases were referred cases with insufficient history and were dropped from the study. The male-to-female ratio was 1:2.2. The age group of 21–30 years showed the maximum antibody specificity (7.48%). Among the 80 samples with alloantibodies, Rh antibodies were noted in 80% (64) samples. Other antibodies detected belong to Kell, Kid, Duffy, and Lewis systems. **Conclusion:** Multitransfused (thalassemic) patients are more vulnerable to develop alloantibody. Therefore, routine antibody screening of donors and patients and the provision of antigen negative blood is recommended.

Keywords: *Alloantibody, antibody specificity, multitransfused, thalassemia*

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Introduction

Blood transfusion, despite being life-saving process, is associated with inherent risks of alloimmunization against red-cells antigens. Red blood cell (RBC) alloimmunization occurs due to genetic disparity between donor and recipient red-cells antigens.[1] The development of alloantibodies and autoantibodies against RBC antigens causes laboratory difficulties during RBC cross-matching, shortens *in vivo* survival of transfused red cells, delays provision of safe transfusions, and may accelerate iron overloading.[2,3] The term "clinically significant" in relation to alloantibodies may refer to an antibody that causes an obvious, clinical hemolytic transfusion reaction (fever, chills, hemoglobinuria, etc.) or an antibody that does not cause any overt clinical symptoms but is associated with laboratory signs of hemolysis (increased bilirubin, decreased haptoglobin, etc.) or an antibody that is not associated with any clinical or laboratory signs of hemolysis, but RBCs incompatible with it survive less than normal lifespan.[4]

In addition, the factors for alloimmunization are complex and involve at least three main contributing elements: the RBC

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antigenic difference between the donor and the recipient, the recipient's immune status, and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system.[5] As blood is routinely matched with respect to major blood group antigens-ABO and Rh D, there is a high probability that the donor will have minor blood group antigens not present in the recipients, which will result in alloimmunization.

Clinically, significant blood group antibodies are those directed against Rhesus, Kell, Duffy, and Kidd system and may cause hemolytic transfusion reactions.[6]

Blood transfusion support is vital to the management of patients with hematologic disorders and malignancies. Many such patients require blood transfusion during their illness or may be lifetime.[7] Blood group antigens and alloimmunization occur when an incompatible antigen introduced in an immunocompetent host evokes an immune response. Clinically, significant RBC alloantibodies develop in 6%–36% of multitransfused patients and can pose major problems in long-term transfusion therapy.[8]

The most important RBC alloantibodies in daily transfusion practice, in terms of frequency of occurrence, are directed toward Rhesus (anti-D,-C,-E,-c, and-e), Kell (anti-K), Duffy (anti-Fy^a and-Fy^b), Kidd (anti-Jk^a and-Jk^b), and MNSs (anti-M,-N,-S and-s) blood group systems. Of these, the D-antigen is the most immunogenic, resulting in more than 80% of immunocompetent D-negative persons becoming alloimmunized after a transfusion of D-positive erythrocytes. There are no published studies on RBC alloimmunization from Bangladesh.[9]

The risk of developing RBC alloantibodies depends on the age, sex, and genetic makeup of the patient, as well as the number and frequency of transfusion.[9] The development of RBC alloantibodies complicates the long-term transfusion therapy.[10] If antibody screening is positive, additional tests are carried out to specify the identity of antibody using the antibody identification panel and RBC antigen typing.

Materials and Methods

The present study was conducted in the transfusion medicine department of a tertiary care referral center equipped with a day care blood transfusion center. Blood specimens from thalassemic children who had difficulties in cross-matching were sent from different medical college hospitals and other medical service delivery centers. The present study was conducted from January 1, 2013, to March 31, 2016. As this was a tertiary care referral center, all samples were coming from other institutions and had invariably received medication or transfusion before their referral. Records of all known multitransfused patients were maintained at our institution. Multitransfused patients were tested for alloimmunization, and antigen-negative blood issued where necessary.

We collected 5 ml of whole blood specimens in the sterile plain test tube. Serum and RBC were separated within 30 min of specimen collection. We performed routine ABO and Rh D blood grouping. Serum specimens were processed for antibody screening and identification. We performed initial testing using indirect anti-human globulin (IAT/ICT) tests using three cell panels (R_1R_1, R_2R_2, and rr). All tests were performed according to the manufacturer's instructions. (Lorne Laboratories Ltd., Great Britain) by tube test. All specimens that tested positive were followed up for antibody identification by an 11-cell panel.(Lorne Laboratories Ltd., Great Britain). For positive and negative control R_1r (CDe/cde) cells were used with the incorporation of anti-D and normal saline, respectively. For auto control, recipients own cell and serum were used.

Statistics: we performed simple percentage analysis.

Results

Out of 1286 cases, alloantibody was detected in 334 (25.97%) cases. Among the 334 cases, 80 (23.95%) cases were followed for antibody identification. Remaining 254 cases were dropped as these were referred from different centers with incomplete data, and no patient consent could be taken. Female cases showed more antibody specificity than male cases [55 (16.47%) vs 25 (7.48%)] [Table 1]. In age group-wise specificity cases, the age group of 20–31 years (7.48%) showed maximum antibody specificity followed by 11–20 years (6.29%) [Table 2]. We found alloantibody anti-c in 31 (38.75%) cases followed by anti-E 20 (25.00%), anti-C (5.00%), and anti-e (1.25%) cases. Other combinations of Rhesus alloantibodies were anti-C and anti-e (3.75%), anti-C and anti-E (3.75%), and anti-c and anti-E (2.50%) cases. Among the non-Rhesus cases, anti-Jk^a (2.50%), anti-Le^a (2.50%), anti-Fy^a (2.50%), and anti-K (1.25%) were observed. Out of the eighty cases of alloimmunization, antibody specificity could not be detected in 9 (11.25%) cases, and in more than 80% of cases, the alloantibodies belonged were Rhesus specific [Table 3].

Discussion

Alloantibodies produced by an individual against a foreign antigen present in another individual (i.e., donors) usually by multiple transfusions or by pregnancy. Alloimmunization to RBC antigens is usually stimulated by the transfusion of blood products and is one of the complications of RBC transfusion. Other than RBC alloimmunization, immunological complications of repeated RBC transfusions include difficulties obtaining compatible blood, development of antibodies, acute or delayed hemolytic transfusion reactions, and hemolytic disease of the newborn.[11]

Of 1286 cases, alloantibody was detected in 334 cases giving an overall prevalence of 25.97%, which is similar to the other reports 22.8% by Hussain *et al.*,[12] 22.7% by Hassan *et al.*,[13] and 22.6% by Spanos.[14] In another study, the overall frequency of alloantibody formation was found to be 22.06% in 68 multitransfused patients.[15]

Alloimmunization of 22% in patients with thalassemia was observed in the patients of Asian descent living in the United States by Singer *et al.*[16]

Much lower rates of alloimmunization frequencies observed in studies 5.5% by Philip *et al.*[17] and 5.3% by Karimi *et al.*[18]

Lower rates of alloimmunization frequencies also observed in studies by Pahuja *et al.*[19] (3.79%) and Bhatia *et al.*[20] (4.97%).

The high incidence of anti-c (38.75%) and anti-E (25.00%) in our study is comparable to other studies done in ethnic Asian peoples.[6,18,21] Thakral *et al.*[22] on 531 multitransfused patients found that anti-c was the most common specificity (38.8%), followed by anti-E (22.2%), anti-M (11.1%), anti-Le^a (11.1%), anti-Jk^a (5.6%), and anti-Le^b (5.6%).

Azarkeivan *et al.*[23] in a multicentric study conducted on 835 patients also observed double antibody. Anti-E and anti-c were the most common multiple alloantibodies in combination showed in Thakral *et al.*[22] and Yousuf *et al.*[24] studies.

Conclusion

Alloimmunization poses a higher risk to multitransfused patients and difficulties in pretransfusion testing. We recommend the inclusion of antibody screening test in the routine pretransfusion testing protocol for those who are at higher risks for antibody formation and long-term transfusion-dependent patients like those with thalassemia. We further recommend the use of antigen-negative cross-matched blood products to multitransfused patients.

Table 1: Distribution of sex of the antibody-specificity cases (*n*=80)

Gender	Frequency (%)
Male	25 (7.48)
Female	55 (16.47)
Total	80 (23.95)

Table 2: Age group-wise distribution of antibody-specific cases (*n*=80)

Age category (years)	Frequency (%)
0-10	15 (4.49)
11-20	21 (6.29)
21-30	25 (7.48)
31-40	16 (4.79)
41-50	3 (0.90)
Total	80 (23.95)

Table 3: Distribution of cases according to alloantibody specificity

Name of antibody	Frequency (%)
Anti-c	31 (38.75)
Anti-E	20 (25.00)
Anti-cE	2 (2.50)
Anti-C	4 (5.00)
Anti-e	1 (1.25)
Anti-Ce	3 (3.75)
Anti-CE	3 (3.75)
Anti-Jk^a	2 (2.50)
Anti-K	1 (1.25)
Anti-Le^a	2 (2.50)
Anti-Le^b	2 (2.50)
Specificity not detected	9 (11.25)
Total	80 (100.00)

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Conflicts of interest

There are no conflicts of interest.

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