

Evaluation of Alloimmunization Rate and Necessity of Blood Type and Screening Test among Patients Candidate for Elective Surgery

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ABSTRACT

Introduction: Alloimmunization is a reaction of the immune system to foreign antigens. For prevention of alloantibody formation, performing of type and screen test is necessary on a patient's blood specimen as part of pre-transfusion testing.

Materials and method: In this cross-sectional study, type and screen test done for 1420 patients with elective surgery for detection of alloantibody in Imam Khomeini hospital in Ardabil.

Results: Prevalence of alloantibody in this population was 0.92% (13 patients) and 99.2% (1407 patients) showed no alloantibody in their serum. The most prevalent alloantibody was anti-K, anti-E and anti-c. No significant relationship observed between sex and alloimmunization rate.

Conclusion: performance of type and screen test play an important role in reducing the rate of alloimmunization, and also, could reduce the demands for blood reservation in hospital blood banks.

KEY WORDS: Alloimmunization, Type and screen test, Elective surgery

INTRODUCTION

Type and Screen (T&S), done pre-operatively to prevent complications from blood transfusion incompatibility between donor and recipient for existence of alloantibody. Alloantibody screening is a laboratory method for detection of broad range unexpected (usually clinically significant, IgG) an antibody(s) against an antigen(s) (usually the minor blood group) in sample sera that blood recipient lacks. Indeed if the blood recipient recognizes the

donor RBC surface antigens as foreign, the host will mount an immune response to the donor RBC's.¹⁻²

Those unexpected antibodies are usually results of RBCs stimulation including history of blood transfusion, pregnancy or history of miscarriage. Although, naturally occurring alloantibody(s) may be present in some patient sample.¹⁻² Clinically significant alloantibody(s) affected by IgG immunoglobulin could be associated with hemolytic disease of new born (HDN), hemolytic transfusion

reaction (HTR) and notable decreases in the survival of transfused RBCs.^{2,3} According to similar studies, the prevalence of clinically significant alloantibodies has been reported from less than 0.5% to up to 60% of samples depending on the population study and the test method sensitivity, while in those patients receiving blood products regularly consisting of patients suffering from different type of malignancy, hemoglobinopathies or hematological disorders the rate of unexpected antibodies is significantly higher compared to those patients underwent elective surgery or those women had a history of pregnancy or miscarriage.^{2,3} This procedure (T&S) is much reliable and even less expensive than full cross matching and gives the same immunohematological safety.¹ In the T&S test, each recipient's blood sample is typed for its ABO and Rh D blood groups and screened for unexpected but clinically significant antibody(s) that could lead to Red Blood Cell (RBCs) alloimmunization. The recipient's serum is incubated with two or three different group-O screening red cells (not pooled) in 2-5% RBCs suspension, which carry important and representative blood group antigens as homozygote including D, C, E, c, e, M, N, S, s, P1, Lea, Leb, K, k, Fya, Fyb, Jka, and Jkb antigens.^{2,4} Alloimmunization is a reaction of the immune system to foreign antigens. It is one of the most important complications of blood transfusions. In the presence of alloantibody, the life span of red blood cells is shortened and the patient's need for blood increases. Identification of the types of antigens present and transfusion of fully compatible blood may prevent alloimmunization.⁴

So, in this study, we evaluated relative frequencies of alloantibody against RBC in a general population of Ardabil, (Ardabil province in North West of Iran with Azeri race) whose referred to Imam Khomeini hospital for elective surgery and may be candidate to receive blood transfusion.

MATERIALS AND METHODS

In this cross-sectional study, type and screen test done as a pre-transfusion test for 1420 patients of Imam Khomeini hospital in Ardabil that underwent elective surgery between March 2012 and February 2013. Blood group typing done by Anti-A, Anti-B

and anti-D reagent (Iranian Blood Research and Fractionation Holding Company, Tehran-Iran) in Tube test procedure for each patient. Screening test, Also done by Antibody screening kit contains three vials of RBC with known surface antigen (Iran blood transfusion organization, Iran, Lot No: 11Ip3C40-13Ip3C65) according to manufactory method in room temperature and 37°C. Briefly, Add 2 drops of patient plasma/serum to be tested to each labeled tube. Then, add one drop of thoroughly mixed reagent red cells to the appropriate labeled tube. Afterwards, 2 drops of Albumin 22% added to each tube. After mixing the contents of each tube, incubate all tubes at room temperature and 37°C ($\pm 1^{\circ}\text{C}$) for 10-30 minutes. Then proceed directly with the antiglobulin test phase following incubation. Wash tubes a minimum of 3 times with isotonic saline. Completely decant saline after final wash to obtain a "dry" red cell button. Add two drops Anti-IgG to each tube. Centrifuge tubes in 3000 RPM for one minute. Immediately re-suspend the cells by gentle agitation; examine the tubes macroscopically for agglutination. Grade and then record the results. Patients with positive results (presence of antibody in their serum) continued by Antibody identification test (Iran blood organization, Iran, Lot No: 11Ip11C40-13Ip11C65). This kit contains 11 cell panels and had a similar method to antibody screening test (described above). DAT (direct anti-globulin test) done for patients with Positive auto-control.

RESULTS

In this study, of 1420 patients, 842 (59.3%) were male and 578 (40.7%) were female. Patient's age ranged between 5 and 82 year and had mean age of 43.2 ± 24.7 year. Prevalence of alloantibody in this population was 0.92% (13 patients) and 99.2% (1407 patients) showed no alloantibody in their serum. Of 13 alloimmunized patients, 7 patients (8% of male population) were male and 6 patients (1% of female population) were female. There was not any significant relationship between sex and prevalence of alloantibody ($p > 0.05$). The identified antibodies contains: K, E, c, Lea, C, Fya and D (Table 1). Anti-K and anti-Lea, was the most prevalent clinical significant, and non-significant antibodies, respectively. The mean age of alloimmunized group

was 45.2 year and in non-alloimmunized group had mean age of 43.8 year. No significant correlation found between age and prevalence of alloantibody among patients ($p>0.05$).

Table-1: Prevalence of Alloantibody among Patients Ready to Elective Surgery

Alloantibody	Male	Female	Total
K	2	2	4 (30%)
E	1	1	2 (15%)
C	0	2	2 (15%)
C	1	0	1 (8%)
Le ^a	2	0	2 (15%)
D	0	1	1 (8%)
Fy ^a	1	0	1 (8%)
Total	7	6	13 (100)

Of total 1420 under study patients, 1318 patients (93%) had no history of blood transfusion and 7% of patients had blood transfusion in past. Among patients with positive antibody screening test, 10 patients had history of blood transfusion and a significant relationship found between history of transfusion and alloimmunization ($p<0.05$). During the study, observed that demands for reservation of blood reduces significantly than before establishment of TS policy ($p<0.05$).

DISCUSSION

Blood banks will provide enough compatible blood for surgical procedures, particularly elective ones. A reason for this assurance is the successful use of maximum surgical blood order schedules, which guide clinicians and blood banks alike in predicting the number of units that will need to be prepared for commonly performed surgical procedures.⁵⁻⁶ After the introduction of the indirect anti-globulin test by Coombs in 1945, which added a new dimension to the safety of blood transfusion, there was a rapid increase in the identification of alloantibody that caused transfusion reactions or hemolytic disease of the newborn.⁷ Factors for immunization are complex and involve at least three main contributing elements. This includes RBC antigenic difference between the blood donor and the recipient, the recipient's immune status and immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system.⁸ In our study, prevalence of alloantibody was 0.9%. In a

study conducted in Minnesota region on normal population between 1975 and 1995, the rate of prevalence of alloantibody against Red cell antigens was less than one percent. The most prevalent antibody was against E, Le and K antigens, respectively.⁹ In another study in Tehran (central part of Iran) between 1997 and 1998, prevalence of alloantibody reported less than one percent (0.97%).² These efforts beside our study data and more similar reports in worldwide indicated that in normal population, incidence of occurrence of alloimmunization is less than one percent. History of blood transfusion and pregnancy can lead to formation of alloantibody. Beside alloantibody, also autoantibody can be a source of potential risk in blood transfusion and must be regarded in antibody screening tests. In our study, we did not find any correlation between formation of alloantibody and gender. Like our study in similar study in Tehran, also no relationship found between gender and prevalence of alloantibody.² While, in a study in Kuwait occurrence of alloantibody was higher in females.¹⁰ Like Fluit et al., in our study, antibody against K, E and c antigens with 30%, 15% and 15% prevalence, respectively, had higher rate among the other RBC antigens.¹¹ In Western Europe and the United States, the most frequently reported alloantibody in alloimmunized patients were alloantibody against Rh and Kell antigens. In a study in Minnesota, also anti-E and anti-K had higher rate than the other. Prevalence of a specific antigen in region under study and also application of different methods and reagents can be considered as an explanation for this difference.² Alloantibody against these antigens has ability to mediate an intermediate to strong hemolytic reaction in recipients. Thus, identification of these antigens in donors associated with precise performance of antibody screening and antibody identification tests have a significant role in reducing the risk of a hemolytic reaction. In our study, as expected, history of blood transfusion has a direct correlation with production of alloantibody especially in males, because women due to pregnancy have higher ability to produce alloantibody than men.

CONCLUSION

The T&S test provides many benefits to patients and the main one is that, blood issued is safe and compatible. In cross-match test, blood units are randomly tested without information about the patient's antibody status. For cross-match test, blood units must reserve for a designated patient and this led to overestimate the number of units that would be required. In addition, as a repeat cross-match required at least another 1 to 2 hours and increase the workload of the blood bank staff. By T&S test, blood units are no longer reserved for a patient if the results from the antibody screen are negative and instead, a validity period is given to an individual for their negative antibody screen status. Therefore, alloantibody screening must to preformed for patients needing blood transfusion, pregnant women, cases of blood cells transfusion reactions by blood bank laboratory in hospitals and finally for blood and plasma donors by blood transfusion establishments.

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