Determination of Red Cell Antigen Alloimmunization and Specific Type of Antibody in Multi-Transfused Liver Cirrhosis Patient

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ABSTRACT

Background: In liver cirrhosis anemia occurs in up to 75 % of the patients and blood transfusion is the mainstay of treatment of anemia. Red cell alloimmunization is a common problem encountered in multi-transfused patient. Alloimmunization can make further blood transfusion troublesome as extensive matching of donor is required to provide antigen free blood to the recipient for which alloantibodies are formed. Antibodies to foreign red cell antigens in patient's blood results in delay of transfusion because of complex pre-transfusion tests and difficulty in finding compatible red blood cell unit. Moreover, they can also cause delayed hemolytic blood transfusion reaction. **Objectives:** 1) To determine frequency of red blood cell alloantibodies in multi-transfused liver cirrhosis patient. 2) To determine the specific type of most common alloantibodies in multi-transfused liver cirrhosis patient. **Study design:** Cross-sectional study. **Place of study:** Pathology department of King Edward Medical University with sampling from four hospitals located in Lahore, Pakistan: Mayo Hospital, Jinnah Hospital, Services Hospital and Sir Ganga Ram Hospital. **Period of study:** January 2016 to March 2016. **Methodology:** To establish alloimmunization rate in multi-transfused liver cirrhosis patient, cross-sectional study was designed. Sample size of 90 liver cirrhosis patients of all age groups and both genders who had been transfused at least 5 times were taken with the exclusion criteria of known alloantibodies or autoimmune diseases. Patients were screened for alloantibodies using tube IAT and if found positive specific type of antibody was determined using extended red cell panel. **Results:** 90 patients were screened for alloantibodies of which 3 patients were positive for alloantibodies giving alloimmunization rate of 3.3% in multi-transfused liver cirrhosis patient. All three patients had antibodies of different specificities. First patient had anti-D, second patient had anti- Le(b) belonging to Lewis blood group system and third patient had Anti-Jk(a) belonging to Kidd blood group system. **Conclusions:** This study was conducted to explore the frequency of alloimmunization in multi-transfused liver cirrhosis patients. Due to cross-sectional study design, incidence of formation of new antibodies as well as loss of antibodies over time could not be determined. Therefore, to establish red blood cell alloimmunization rate in cirrhotic patient, a large-scale prospective study should be done in which LISS IAT or enzyme treated cell should be used and antibody detection tests should be done at defined time intervals after blood transfusion. It is suggested that antibody screening test should be done twice. Once shortly after blood transfusion (may be after one month) to detect fast appearing new antibodies or anamnestic response of undetectable antibodies. Secondly after longer interval to detect slow evolving antibodies.

Keywords: Alloimmunization, red cell antibodies, liver cirrhosis

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INTRODUCTION

Liver cirrhosis is end result of hepatocellular injury which causes both fibrosis and regenerative nodule throughout liver. It is caused by variety of diseases which includes alcohol, viral hepatitis, drug-induced liver disease, metabolic liver disease, autoimmune hepatitis and cryptogenic chronic hepatitis.[1] Many other co-morbidities can occur with liver cirrhosis.[2] In chronic liver disease anemia occurs in up to 75 % of the patients. Various factors causing anemia in chronic liver disease include reduced red blood cell life span even in uncomplicated chronic liver disease due to increased lipid deposition in membrane leading to macrocytosis and hemolytic anemia. Splenomegaly occur due to portal hypertension leading to hemodilution and pooling of red blood cells in spleen and hypersplenism. Moreover, viral hepatitis is also associated with aplastic anemia.[3] Anemia is further worsened by bleeding varices due to acquired coagulation abnormalities which include decreased production of coagulation factors by liver, dysfibrinogenemia,

thrombocytopenia from hypersplenism or immune platelet destruction. Consumptive coagulopathy may be superimposed. With the improvement in medical treatment of chronic liver disease survival of individuals has greatly increased but the mainstay of treatment of anemia is blood transfusion.[4]

One of the common problem encountered in multi-transfused patients is alloantibody formation against foreign red blood cell antigens.[5] Alloimmunization can make further blood transfusion troublesome as extensive matching of donor is required to provide antigen free blood to the recipient for which alloantibodies are formed.[6] Antibodies to foreign red cell antigens in patients' blood results in transfusion delays because of complex pre-transfusion testing and difficulty in finding compatible red blood cell unit. Moreover, they can also cause delayed hemolytic blood transfusion reaction.[7]

Alloimmunization targets red cell antigens but most have low antigenicity. Several Blood group antigens have been identified up till now, some of them are capable of alloimmunization

resulting in hemolytic transfusion reaction.[8] It has been observed that alloantibodies do not develop in all the individuals exposed to foreign red cell antigen in the form of blood transfusion. Exact underlying mechanism which determines whether alloimmunization will occur or not in an individual after exposure to antigen is still not known.[9] However certain factors are associated with increased chances of alloimmunization of which frequency of blood transfusion[10] and inflammatory state[11] of recipient are most important. Female sex and history of pregnancy is also a risk factor. Racial difference between donor and recipient is also important factor causing alloimmunization.[12]

Transfusion reactions due to alloimmunization are quite rare in most patient. However, frequently transfused patients due to chronic diseases or hematological disorders, these events can be a major concern. Currently there is no therapeutic intervention available to prevent the alloimmunization. Multi-transfused individuals continue to generate alloantibodies against multiple red cell antigens, making it increasingly more difficult even impossible in some cases to find a compatible red cell unit. Phenotypic matching between donor and recipient red cells is performed in some cases, which may help to avoid further alloimmunization.[13, 14]

The effect of alloimmunization can be significant resulting in increased difficulty in finding compatible blood for patients; added cost to pre-transfusion testing and inconvenience to the patient; and causes hemolytic transfusion reactions which might be life threatening at times. Alloimmunization can cause acute or delayed hemolytic reaction. Acute hemolytic reaction is usually caused by blood group incompatibility between recipient and donor. In some patients these reactions are not associated with signs or symptoms; whereas, in others symptoms range from mild to severe even resulting in death.[13, 15]

METHODOLOGY

Study Design: Cross-sectional study.
Place of Study: This study was performed in pathology department of King Edward Medical University with sampling from four hospitals located in Lahore, Pakistan: Mayo Hospital, Jinnah Hospital, Services Hospital and Sir Ganga Ram Hospital.
Duration of Study: January 2016 to March 2016.
Consent was obtained from each participant after the purpose of the study was explained to him/ her. Those who agreed were enrolled in study. Study was conducted on 90 diagnosed liver cirrhosis patients who had been transfused more than 5 times and were admitted.
Methods:
For each enrolled patient, personal data and obstetric history, i.e. information about her previous pregnancies and babies, as well as information about any previous blood transfusion was collected using a questionnaire. Then blood sample was collected under aseptic conditions. Collected blood samples were tested within six hours of collection to minimize variations due to sample aging.
Tests Performed:

All the samples collected were screened for irregular antibodies, and, if positive, the antibody specificity was determined.
Antibody screening:
The commercially available screening red cell panel was employed to detect irregular antibodies in tested sera.
Method:
3 tubes were taken and labeled with patient's ID and label I, II & III for antibody screening. The screening cell were brought to room temperature (20 -24°C) for 15 – 20 minutes. After mixing the vials of screening cell, 50 micro liters of screening cell were taken and add to correspondingly labeled glass tube I, II & III. 2 drops of patient's serum were added in all the labeled tubes. Tubes were mixed and centrifuge at 3400 RPM for 15 seconds and observed for any agglutination/ hemolysis and result were recorded. Tubes were incubated at 37°C for 45 minutes. After incubation they were mixed and centrifuged at 3400 RPM for 15 seconds and observed for any agglutination/ hemolysis and result were recorded. If no agglutination occurred and free cells were present, the tubes were gently mixed and washed three times with 0.9% NaCl (normal saline). 2 drops of Coomb's reagent was added and mixed gently. The tube was centrifuged at 3400 RPM for 15 seconds and observed for any agglutination/ hemolysis. Test results were interpreted, graded and recorded in an antigram. Cross out the negative results and rule in the antibodies.

Antibody identification:
All sera which gave a positive antibody screening test were tested against a panel commercially available selected group O red cells samples with known antigen composition for the major blood groups.
Method:
12 tubes were taken and labeled with patient's ID and label I to XI for antibody screening and tube XII for auto-control. After mixing the vials of identification panel, 50 micro liters of screening cell were taken and add to correspondingly labeled glass tube I to XI. 50 micro liter of patients own red cells were added in tube labeled XII for auto control. 2 drops of patient's serum were added in all the labeled tubes. Tubes were mixed and centrifuge at 3400 RPM for 15 seconds and observed for any agglutination/ hemolysis and result were recorded. Tubes were incubated at 37°C for 45 minutes. After incubation they were mixed and centrifuged at 3400 RPM for 15 seconds and observed for any agglutination/ hemolysis and result were recorded. If no agglutination occurred and free cells were present, the tubes were gently mixed and washed three times with 0.9% NaCl (normal saline). After last wash, the last drop of saline was removed by inverting the tube on a piece of tissue paper. 2 drops of Coomb's reagent was added and mixed gently. The tube were centrifuged at 3400 RPM for 15 seconds and observed for any agglutination/ hemolysis. Test results were interpreted, graded and recorded in an antigram.
Interpretation of results:
Once results were recorded on the sheet, ruling out technique is used to determine the specificity of the alloantibody. If red cell contains an antigen but test serum did not cause agglutination

means that corresponding antibody was not present; i.e. exclusion technique or rule-out or cross-out. After all antigens present on that cell were crossed-off, this was applied to all cells and additional specificities were excluded.

Next, the cells reactive with the serum were evaluated. The pattern of reactivity for each non-excluded specificity was compared to the pattern of reactivity obtained with the test serum; i.e. inclusion technique. A pattern that matches exactly was the specificity of the antibody in the serum under test.

RESULTS

In this study alloimmunization rate of 3.3% was found among multi-transfused liver cirrhosis patient. In the study 48 males and 42 females were studied having mean age of 52.6 years with average of 7.72 transfusions per person. Alloantibodies were detected in 3 patients among 90 multi-transfused liver cirrhosis patients. Anti- D was detected in 55-year-old cirrhotic female who was transfused 6 times and had history of 4 children. As it is routine to match RhD before each transfusion, this anti-D found in patient's serum might be due to exposure to D antigen during pregnancy as her husband was RhD positive and she also had history of one abortion (cause of abortion was not known to the patient or her attendants and they were not aware whether Rh immunoprophylaxis was done or not, raising the suspicion of hemolytic disease of fetus and newborn). However, the possibility of incompatible RhD transfusion due to inability to detect D antigen in donor's blood because of weak D, partial D, less potent anti-D sera used for blood grouping or by human error could not be ruled out. Anti-Le(b) of Lewis system was detected in the serum of 60-year-old cirrhotic female with 11 pints of transfused blood and had 5 children. Anti-Jk(a) belonging to Kidd system was detected in the serum of 55-year-old cirrhotic male with 15 blood transfusions in last 2 years.

Table 1: Summarizing parameters of patients having alloantibodies

Patient	1	2	3
Age (Years)	55	60	50
Gender	Female	Female	Male
No. of transfusions	06 pints	11 pints	15 pints
No. of pregnancies	4	5	N/A
Antibody specificity	Anti-D	Anti -Le (b)	Anti-Jk(a)
Antibody against blood group system	Rhesus System	Lewis System	Kidd System

DISCUSSION

Every red blood cell transfusion introduces a wide range of foreign antigens but still majority of blood transfusion recipients do not allo-immunize against foreign red blood cell antigens. Alloimmunization is a multi-factorial immune event requiring several risk factors to be present simultaneously. Fundamentally, each individual requiring blood transfusion has a unique and specific clinical profile and essentially has been exposed to certain environmental factors resulting in immune modulation and own a unique set of genes controlling the immune response. Therefore, only exposure to foreign antigen does not result in an immunologic response and other factors such as frequency and duration of exposure to foreign antigen, immune function and genetics, etc. may play role in determining antibody response.

Cirrhosis causes varying level of immune dysfunction and is referred to as cirrhosis associated immune dysfunction syndrome (CAIDS). Diverse immune dysfunction involving regulatory mechanisms, antigen recognition and effector response of both adaptive and innate immune system occur in cirrhotic patients. B cells become hypo-responsive to activation, less secretion of TNF-β along with decreased IgG production. CD4+ T lymphocyte proliferation stimulation by cirrhotic B lymphocytes is also decreased. Due to dysfunction of B cells in liver cirrhosis, antibody production against foreign red cell antigens may be impaired leading to decreased rate of alloimmunization but it needs to be proved by further research. This low alloimmunization rate of 3.3% in present study could be due to many contributing factors. One of which is fewer number of blood transfusions in this study. A mean of 7.5 transfusions was done in this study population. Higher rates of alloimmunization are usually reported in haemoglobinopathies in which transfusion therapy starts in first year of life. A higher number of transfusions will increase the possibility of encountering a foreign antigen. In a study by Hoeltge et al in general transfused population showed association between mean number of red blood cell transfusions and number of alloantibodies. Alloimmunization rate increases exponentially as numbers of transfused pints increase. In sickle cell disease alloimmunization rate of 7% to 47% has been documented[16,17] whereas, in thalassemia patient alloimmunization rate of 4% to 50% is reported in different studies in different populations.

Another important factor is the degree of genetic dissimilarity between donor and recipient. Some diseases which are restricted to certain communities e.g. sickle cell disease, exhibit higher rate of alloimmunization especially when the patients are transfused in countries where the ethnic group is underrepresented in the donor population. But this is not the case in this study population because in Pakistan usually blood donations are taken from the family members or close relative of the patient. Therefore, ethnic similarity may also be a contributing factor to low alloimmunization rate in this study.

Another factor which affect alloimmunization detection rate is different time interval taken by antibodies of different specificities to develop after transfusion. When antibody test was done within one month of transfusion, more than 20 % of antibodies were against Kidd system. Whereas when the antibody test was done after 3 months of transfusion, anti-Kidd frequency decreased to 3-6 percent. Similarly, anti-Duffy antibodies appear in 5-8% of patients when antibody test was done within 6 months of transfusion but increases up to 27 % when test is done after 6 months. This frequency difference can be explained by slow development of anti-duffy antibodies along

with time dependent undetectability of other antibodies.[17, 18] In a study performed by Schonewille et al,[14] it was found that anti Kidd antibodies were predominant when antibody test was done within 3 months, whereas anti-K and anti-Fy(a) were predominant when antibody test was done after 5 years of transfusion. Some of the red blood cell alloantibodies become undetectable on one or more occasions after their first detection. Patient's age and sex had no effect on disappearance of different antibodies. Kidd antibodies are detectable for the shortest time. Kidd antibodies are very difficult to detect but they are often involved in delayed hemolytic transfusion reactions. Among common antibodies, anti-D persisted for the longest duration. Study by Schonewille et al, showed that approximately 25% of antibodies cannot be detected with passage of time. Therefore, time duration between antibody test and transfusion is related to red cell alloantibody detection. Generally, antibody tests are not performed at fixed interval after blood transfusion and are usually done when new transfusion is required. As a result, many alloantibodies can be missed if no new transfusion is required. Moreover, if the test is performed early, there is chance that the antibody has not developed yet. On the other hand, if the test is performed at delayed interval, there is chance of antibody becoming undetectable. As this study is cross-sectional, the incidence of formation of new antibodies as well as loss of antibodies over time could not be determined.

The sensitivity of the antibody detection technique is another factor which affects the rate of alloimmunization detection. Enhanced detection of many antibodies including Rh and Lewis systems occurs when enzyme treated red cells are used. While column LISS IAT is more sensitive as compared to tube IAT and can detect low titer of antibodies because antibodies are quickly taken up at low ionic strength. In this study tube IAT is used to detect alloantibodies which might miss low titer antibodies resulting in lower alloimmunization rate.

Limitation in this study is selection bias due to patient's refusal due to multiple reasons including trauma of phlebotomy, extra loss of blood (3-5 ml) and lack of interest in research outcome. Moreover, proper transfusion records are not maintained in the hospital or by the patients. Therefore, the history of number of blood transfusion and time lapsed after transfusion are solely depends on that given by patient or attendants of patient and is subjected to recall bias.
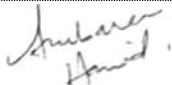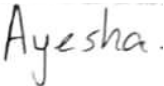
CONCLUSION

Due to aforementioned limitations of this study in establishing red blood cell alloimmunization rate in cirrhotic patient, a large-scale prospective study should be done in which LISS IAT or enzyme treated cell will be used and antibody detection tests should be done at defined time intervals after blood transfusion. It is suggested that antibody screening test should be done twice. Once shortly after blood transfusion (may be after one month) to detect fast appearing new antibodies or anamnestic response of undetectable antibodies. Secondly after longer interval to detect slow evolving antibodies.

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