Original Article

Alloimmunization in multitransfused liver disease patients: Impact of underlying disease

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Abstract:

Introduction: Transfusion support is vital to the management of patients with liver diseases. Repeated transfusions are associated with many risks such as transfusion-transmitted infection, transfusion immunomodulation, and alloimmunization. **Materials and Methods:** A retrospective data analysis of antibody screening and identification was done from February 2012 to February 2014 to determine the frequency and specificity of irregular red-cell antibodies in multitransfused liver disease patients. The clinical and transfusion records were reviewed. The data was compiled, statistically analyzed, and reviewed. **Results:** A total of 842 patients were included in our study. Alloantibodies were detected in 5.22% of the patients. Higher rates of alloimmunization were seen in patients with autoimmune hepatitis, cryptogenic liver disease, liver damage due to drugs/toxins, and liver cancer patients. Patients with alcoholic liver disease had a lower rate of alloimmunization. The alloimmunization was 12.7% (23/181) in females and 3.17% (21/661) in males. Antibodies against the Rh system were the most frequent with 27 of 44 alloantibodies (61.36%). The most common alloantibody identified was anti-E (11/44 cases, 25%), followed by anti-C (6/44 cases, 13.63%). **Conclusion:** Our findings suggest that alloimmunization rate is affected by underlying disease. Provision of Rh and Kell phenotype-matched blood can significantly reduce alloimmunization.

Key words:

Alloimmunization, liver diseases, multitransfused

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Introduction

Transfusion support is vital to the management of patients with liver diseases. These categories of patients require repeated blood transfusions during the course of their illness. Patients undergoing liver transplantation also require intensive transfusion support. Repeated transfusions are associated with many risks such as transfusion-transmitted infection, transfusion related immunomodulation, and alloimmunization. Alloimmunization to red blood cell (RBC) antigens is one of the major risks of blood transfusion. It results from the antigenic disparities between the donor and the recipient. This risk depends on the recipient's exposure to the foreign antigens and their immunogenicity. Immunization may also be influenced by the number and frequency of the transfusions as well as the recipient's gender, age, and underlying disease. Recent data suggest that RBC alloantibodies may affect outcomes in orthotopic liver transplant.[1-3]

The incidence of RBC alloantibodies varies in different patient populations.[4,5] Within the general patient population, approximately, 2% of those receiving RBC transfusions develop alloantibodies.[1-3] The requirement of multiple transfusions in liver disease patients leads to a higher incidence (6–14%) of RBC alloantibody formation, adding to the difficulties in providing compatible RBCs in large quantities.[6] The impact of underlying disease on alloimmunization has not been studied.

Large retrospective studies on alloimmunization in random hospital transfusion recipients have shown that antibodies to Rh and Kell blood group antigens comprise almost 80% of clinically significant non-D alloantibodies followed by Duffy, Kidd, MNS, and other blood group systems.[7,8]

Multitransfused patients are usually at a greater risk of delayed hemolytic transfusion reactions as previously formed antibodies may become

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undetectable after some time, but show an anamnestic response on repeat exposure to the implicated antigen. Anamnestic response may occur in response to even very small quantities of the antigen and the reaction may be severe. In recent years, the Food and Drug Administration in the USA has reported irregular RBC alloantibodies as a major cause of fatal hemolytic transfusion reactions and they are considered the second major cause of transfusion-related deaths.[9]

The aim of this study was to study the impact of underlying disease on alloimmunization in liver disease patients. The study also evaluated the frequency of RBC alloimmunization and antigen specificities implicated in multitransfused liver disease patients. This will help to review current strategies of blood transfusion and inventory management in alloimmunized liver disease patients.

Materials and Methods

A retrospective data analysis of antibody screening from February 2012 to February 2014 was done at the Department of Transfusion Medicine, Institute of Liver and Biliary Sciences, New Delhi. As a part of the hospital's standard operating protocol, blood grouping and antibody screening are routinely done for all admitted patients. To determine the frequency and specificity of irregular red cell antibodies in multitransfused liver disease patients, data of 842 patients who had received two or more episodes of packed RBC transfusions were analyzed. All cases with positive antibody screen were included in the study.

Serological work up

Blood grouping was done using the standard test tube technique. Antibody screening was done by column agglutination test using a commercial three cell panel (ID-Diacell, Bio-Rad, Switzerland). The screening cell panel covered most antigens against which clinically significant antibodies are formed. An autocontrol was put up to rule out autoantibodies. Direct antiglobulin test was done to detect sensitization of red cells. In cases with a positive antibody screen, further testing for antibody identification was done using a commercial 11 cell panel (ID-Diacell, Bio-Rad, Switzerland). Advanced work up included assessment of thermal amplitude of antibodies, serial alloadsorptions, autoadsorption, and adsorption elution techniques as per case requirement. Extended phenotyping was also done to confirm the specificity of the alloantibody, using the commercially available rare antisera.

Transfusion policy

In patients with alloimmunization to RBC antigens, antigen-negative and cross-match compatible units for transfusion were given whenever possible. Where the alloantibody could not be identified, cross-match compatible units were given and close clinical monitoring was advised. Results of antibody screening and identification were documented and notified to the clinician.

Statistical analysis

The Chi-square test and Fisher's exact test were used for determining categorical variables and Student's – *t*-test was used for determining continuous variables with normal curve. All analyses were performed using IBM SPSS Statistics version 21.0 (license authorization wizard) software. All tests were two-tailed and $P < 0.05$ was considered to indicate statistical significance.

Results

A total of 842 patients were included in our study. Patients' blood samples were screened for the presence of unexpected antibodies as a part of routine serological work up. The study included 661 (78.5%) males and 181 (21.49%) females with age ranging from 1 to 80 years. Antibody screening was positive in 44 patients. This yielded an alloimmunization rate of 5.22%. Our patient population included patients with liver diseases. Over half the patient population had either alcoholic liver disease (32.5%) or viral hepatitis (24.10%). Other liver diseases prevalent in our study population were nonalcoholic steatohepatitis, liver cancer, cryptogenic cirrhosis, cholestatic liver disease, genetic liver disorders, liver damage due to drugs/

Table 1: Underlying liver disease in patient population and alloimmunization

Liver disease	Patients' *n* (%)	Nonalloimmunized (%)	Alloimmunized patients' *n* (%)	OR (95% CI)	*P*	Mean RBC transfusions in nonalloimmunized (range)	Mean RBC transfusions in alloimmunized (range)	*P*
Alcoholic liver disease	274 (32.54)	265 (96.7)	09 (3.28)	0.517 (0.25-1.09)	0.097	3.48 (2-16)	5.44 (2-12)	0.110
Viral hepatitis	203 (24.10)	193 (95.08)	10 (4.92)	0.998 (0.41-2.41)	0.097	2.90 (2-7)	4.70 (2-10)	0.102
Nonalcoholic steatohepatitis	115 (13.65)	109 (94.79)	06 (5.21)	0.99 (0.41-2.41)	1.00	3.21 (2-10)	7.67 (3-19)	0.120
Liver cancer*	90 (10.68)	84 (93.34)	06 (6.66)	1.34 (0.55-3.26)	0.61	3.18 (2-8)	4.50 (2-8)	0.019
Cryptogenic cirrhosis	65 (7.71)	57 (87.70)	08 (12.30)	2.88 (1.28-6.5)	0.01	3.18 (2-11)	5.88 (2-11)	0.032
Cholestatic liver disease	48 (5.70)	48	-	-	0.10	2.81 (2-5)	-	
Genetic liver disorders	22 (2.61)	22	-	-	0.40	2.77 (2-5)	-	
Liver damage due to drugs and toxins	19 (2.25)	17 (89.48)	02 (10.52)	2.18 (0.78-9.78)	0.26	2.65 (2-5)	2.5 (2-3)	0.821
Autoimmune hepatitis	06 (00.71)	3 (50)	03 (50)	19.39 (3.79-99.0)**	0.002	2.67 (2-3)	2.33 (2-3)	0.519

*Also includes liver metastasis;**CI is very high and unstable due to very small sample. CI: Confidence interval, RBC: Red blood cell, OR: Odds ratio

toxins, and autoimmune hepatitis [Table 1]. The underlying liver disease had an overall statistically significant impact on the rate of alloimmunization ($P < 0.001$). Higher rates of alloimmunization were seen in patients with autoimmune hepatitis, cryptogenic liver disease, liver damage due to drugs/toxins, and liver cancer patients. Patients with alcoholic liver disease who comprise a major part of our patient population had a low rate of alloimmunization. The mean number of transfusions received was higher in alloimmunized patients as compared to nonalloimmunized patients in most categories although the difference was not significant [Table 1].

Alloimmunization was higher in females, i.e., 12.7% (23/181) as compared to males, i.e., 3.17% (21/661) in males (odds ratio, 0.204; 95% confidence interval, 0.11–0.38; $P < 0.001$). Fifteen of the 21 females with alloantibodies had a positive obstetric history. Out of total 44 cases, eight cases (19.51%) revealed autoantibodies with one or more underlying alloantibody/ies. Two patients among these eight cases showed cold antibodies with a broad thermal amplitude (0–30°C) reacting optimally at 4°C in addition to alloantibodies. In four samples, the alloantibodies could not be accurately identified. Of the 32 cases in which definitive alloantibody identification was possible, 22 (68.75%) cases had a single antibody and ten (22.72%) cases had multiple antibodies. A total of 44 antibodies were identified.

Antibodies against the Rh system were the most frequent with 27 of 44 alloantibodies (61.36%) belonging to this blood group system. The most common alloantibody identified was anti-E (11/44 cases, 25%), followed by anti-C (6/44 cases, 13.63%). The frequency and specificities of the various alloantibodies identified are provided in Table 2. Antibodies against the Rh system were over-represented in females whereas four of the five antibodies against Kell antigen were found in males. Among the alloimmunized patients, there was one case of a 45-year-old female with chronic viral hepatitis typing as O Rh D negative, which on initial antibody identification mimicked anti-D + C, but on further evaluation with serial double adsorption elution revealed Anti-C + G antibodies.

Discussion

The liver is the site for synthesis of most coagulant and anti-coagulant factors and therefore plays a prime role in hemostasis. Liver disease leads to deregulation of this fragile balance resulting in hypocoagulable or hypercoagulable states. Routine tests for coagulation such as the platelet count, prothrombin time, and activated partial thromboplastin time are often abnormal in these patients. Transfusion of blood components to correct these abnormal parameters is often a standard practice although there are controversies regarding the benefits of this practice.

Table 2: Irregular alloantibodies detected in liver disease patients

Blood group system	Antibody	Number/ frequency (%)	Antibodies in Males	Antibodies in Females
Rh (RH)	Anti-D (RH1)	4 (9.09)	-	4
	Anti-C (RH2)	6 (13.63)	1	5
	Anti-E (RH3)	11 (25)	3	8
	Anti-C (RH4)	3 (6.81)	1	2
	Anti-E (RH5)	2 (4.54)	1	1
	Anti-G (RH12)	1 (2.27)	-	1
Kell (KEL)	Anti-K (KEL1)	5 (11.36)	4	1
Duffy (FY)	Anti FY^a (FY1)	1 (2.27)	-	1
	Anti FY^b (FY2)	2 (4.54)	-	2
Kidd (JK)	Anti JK^a (JK1)	2 (4.54)	-	2
	Anti JK^b (JK2)	2 (4.54)	1	1
Lewis (LE)	Anti-LE^a (LE1)	1 (2.27)	1	-
MNSs (MNS)	Anti-M (MNS1)	2 (4.54)	2	-
	Anti-S (MNS3)	1 (2.27)	-	1
	Anti-S (MNS4)	1 (2.27)	1	-

In the present study of 842 patients, the alloimmunization rate was 5.22% in multitransfused patients with liver diseases. This is in keeping with studies on other multitransfused patients in India which have repeatedly shown a lower rate of alloimmunization as compared to studies from other regions of the world which have reported alloimmunization rates ranging from 9.4% to 23%.[4,6,10,11] Rate of alloimmunization in multitransfused ranges from 3% to 10% in most reports from India.[12-14] Lower rates of alloimmunization may be explained by supposedly high prevalence of phenotypic similarity between blood donors and transfusion recipients in the Indian population.[15] Studies are required to see if human leukocyte antigen (HLA) alleles which confer protection against alloimmunization are over-represented in our population.

In recent times, there has been a better understanding of the factors influencing RBC alloimmunization. These include recipient factors such as genetic disparity between donor and recipient, ability of recipient's HLA to present foreign antigens, genetic predisposition to respond, health status at the time of antigen exposure, prior exposures, and impact of the underlying disease.[16] Evidence suggests that recipient's inflammatory status affects alloimmunization rates in transfusion recipients.[17] One study suggested that viral-type inflammation leads to enhanced alloantigen specific immunity.[18] Another study found a high rate of alloimmunization to low incidence and low immunogenicity antigens in individuals with autoimmune disorders.[16] Our study has also shown significant differences in the rates of alloimmunization in various liver diseases with highest rates in autoimmune hepatitis and cryptogenic cirrhosis [Table 2]. Patients with alcoholic liver disease showed a much lower rate of alloimmunization, this may be due to depressed immune response (decreased T cells, B cells, natural killer cells, and monocytes) in patients with chronic alcoholism.[19] All patients with alcoholic liver disease were male, due to cultural inhibitions regarding alcohol intake by females in India; this too may have contributed to the lower rates of alloimmunization in these patients. Patients on immunosuppressive therapy also tend to have lower rates of alloimmunization.[20] In our study, alloimmunization was not seen in patients with genetic disorders and patients with cholestatic liver disease. In both categories, the number of RBC units transfused was low as compared to other patient groups. The antibody response to RBC antigen may be affected by the HLA II genotype of the patient. There is an increased risk of RBC alloimmunization in persons with HLA-DRB1*1503 allele, whereas HLA-DRB1*0901 may be protective against alloimmunization.[21] HLA type of a person affects alloimmune response irrespective of the antigen specificity. The majority of studies show higher rates of alloimmunization among females. A recent review has shown an equal risk of alloimmunization in both genders and has attributed the higher rates of alloimmunization in female to greater exposure to immunizing events such as pregnancy and transfusions.[22] The numbers of RBC units transfused was overall significantly higher in alloimmunized patients; this has been corroborated in many studies.[13] Donor factors include genetic factors, length of RBC storage, presence of white cells and platelets, and antigenic dose.[16]

Most common alloantibodies were against the Rh and Kell blood group systems, 61.36% and 11.36%, respectively. The most common antibody encountered was anti-E in 25% of the patients, followed by anti-C in 13.63% and anti-K in 11.36%. This is similar to the previous reports of alloimmunization.[6,23,24] As is evident from these findings, antibodies against the Rh and Kell system are the most commonly implicated alloantibodies. This re-iterates the need for extended Rh and Kell phenotype-matched RBCs in patients requiring repeated transfusions to prevent RBC alloimmunization. At our institute, we match Rh and Kell phenotype selectively for multitransfused patients, not for all cases.

Alloimmunization may lead to clinically significant transfusion reactions and decreased survival of RBCs. This results in increased transfusion requirements. Pretransfusion testing is often a cumbersome and time-consuming process in alloimmunized patients. It is, therefore, important to prevent the formation of these alloantibodies by providing extended phenotype-matched RBCs. Leucodepletion of blood components can also significantly reduce alloimmunization. Regular antibody screening should be done in all cases where RBC transfusions are anticipated, this will facilitate availability of antigen-negative, compatible RBC units for patient when needed.

Conclusion

This study highlights the fact that the underlying disease may also be an important factor in determining the susceptibility of a patient to alloimmunization. This should also be taken into consideration while planning provision for transfusion support. Patients at higher risk of alloimmunization should be given priority for phenotype-matched RBC units and the same should be done for patients expected to require a higher number of transfusions. Phenotype matching of Rh and Kell blood groups would prevent alloimmunization in most patients.

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Conflicts of interest

There are no conflicts of interest.

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