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Red cell alloimmunization in multitransfused hepatobiliary patients at hospital Selayang

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Abstract:

BACKGROUND: Transfusion support is vital for the management of patients with hepatobiliary disease. Repeated blood transfusions increase the risk of alloimmunization, i.e., the development of alloantibodies, which might lead to difficulties in blood crossmatching.

AIMS: This study aims to: (1) determine the incidence of red blood cell (RBC) alloimmunization and (2) evaluate the associations between antibody development and demographic factors among hepatobiliary patients.

METHOD: ABO blood grouping, antibody screening , antibody identification and crossmatch were done on all patients samples included in the study.

SETTINGS AND DESIGN: A cross-sectional study was conducted from February 2021 to September 2021, with a total of 132 samples from hepatobiliary patients. The relationships between RBC alloimmunization in transfused hepatobiliary patients and demographic factors (gender, age, and history of transfusion) were assessed by binary logistic regression.

RESULTS: Overall, 67.4% of the patients developed alloimmunization. The majority had a single alloantibody (75.2%) and the most frequently identified antibody specificities were anti-E (37.6%), anti-c (12.8%), anti-Mia (14.4%), and anti-Kidd (11.2%). The predominant antibodies were those against the Rh system (58.4%). Female patients recorded the highest incidence of alloimmunization (69.8%). Female patients also demonstrated a higher tendency to produce both anti-E + anti-c than male patients.

CONCLUSION: The prevalence of RBC alloimmunization is high among hepatobiliary patients and it may cause complications requiring multiple transfusions. The number of transfused packed cells has been clearly shown to be proportionally significant with the risk for alloimmunization in hepatobiliary patients. Hence, this study highlights the importance of immunohematology tests before blood transfusion.

Keywords:

Alloantibodies, alloimmunization, clinically significant, hepatobiliary, transfusion

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Introduction

Alloimmunization is a condition, in which alloantibodies are produced in response to a foreign antigens.[1] These antibodies are able to initiate transfusion reactions, such as hemolysis and even death.[2] Approximately 60% of chronically transfused patients develop alloimmunization,[3] and the alloantibodies produced might increase the difficulty of crossmatching blood.[4] Hepatobiliary disease is one of the most common conditions requiring frequent transfusions,[5] thereby patients suffering from it are at potentially higher risk of alloimmunization. Hence, this study aims to: (1) analyze the incidence of red blood cell (RBC) alloimmunization and (2) assess the relationship between

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antibody development and demographic factors among hepatobiliary patients.

Subjects and Methods

Sample collection

This cross-sectional study was conducted between February 2021 and September 2021 at the Selayang Hospital in Selangor, Malaysia. This study involved a total of 132 hepatobiliary patients who have received at least a pint of compatible ABO and Rh (D) packed cell transfusion. Data were obtained from the patient's clinical records and blood bank information system. Clinical transfusion records of the patients who fulfilled the inclusion and exclusion criteria were analyzed for demographic data.

Ethical approval

The following ethical approvals were granted from Ministry of Health, Malaysia and Universiti Kebangsaan Malaysia: NMRR-20-2211-56091 and JEP-2021-625. The procedures were performed in accordance with the Declaration of Helsinki.

Transfusion workup

The ABO blood grouping test was performed using the tube method. A gel card panel method (Diamed ID-3 panels) was used to perform the antibody screening test to detect the presence of alloantibodies in the patients' serum. Subsequently, antibody screening was conducted for samples with alloantibody-positive results using an antibody identification test (Diamed ID-11 panel), including the enzyme-treated cell (papain) at 37°C and an Antihuman globulin (AHG) phase. The criteria for antibody specificity were established according to the recommendation of the American Association of Blood Banks.

Data analysis

Data were analysed using SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 26.0 Armonk, NY: IBM Corp.). Descriptive statistics were performed using frequency distribution, whereas a Chi-squared test was applied to determine the relationship between the number of packed cell transfusions and alloimmunization. Binary logistic regression was used to determine the antibody development factors that best predict RBC alloimmunization at a significant $P \leq 0.05$.

Results

Out of a total of 132 patients who have received a repeated transfusion, 67.4% developed alloantibodies [Figure 1].

From the results presented in Figure 2, most of the alloimmunized multitransfused patients (74%) produced clinically significant alloantibodies, whereas 23 (26%) produced not clinically significant antibodies.

Table 1 demonstrates the variety of unexpected alloantibodies developed. The most frequent alloantibodies found were anti-E (37.6%) and anti-Mia (14.4%), followed by anti-c (12.8%) and anti-Kidd (11.2%). The Rh system (anti-D, C, c, E, and e) was the most developed (58.4%) in this study. For patients that developed multiple antibodies, at least one of the antibodies was from the Rh blood group system.

As for Table 2, out of 89 patients, 30 (34.8%) developed multiple antibodies and 59 (65.2%) demonstrated single antibodies.

As shown in Table 3, male and female patients made up 65.8% and 69.8% of the participants, respectively. More alloimmunized cases (71%) were developed by those older than 60 years of age. The majority of alloimmunized patients (74.6%) were of Indian ethnicity, whereas the remaining races included Chinese, Malay, and other ethnicities. Female patients (69.8%) and those over 60 years old (71.0%) had a higher rate of alloimmunization but the difference was not statistically significant. All patients who received more than 10-pint packed cells have developed alloantibodies (100%). However, none of the affected patients developed autoantibodies. Demographic factors such as gender, age, and ethnicity were not statistically significant in terms of how alloantibodies were formed ($P > 0.05$).

Hepatobiliary patients who received over 10 pints of packed cells were more likely to develop alloimmunization [Figure 3]. The 132 patients received a total of 197 packed cell units, with the number of transfused units ranging from 1 to 38 pints of packed cells. Most hepatobiliary patients who were transfused with packed cells received fewer than 6 units of

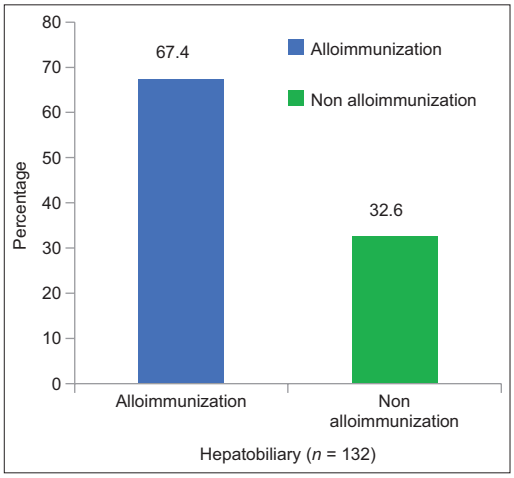

Figure 1: Alloimmunized and nonalloimmunized (n = 132)

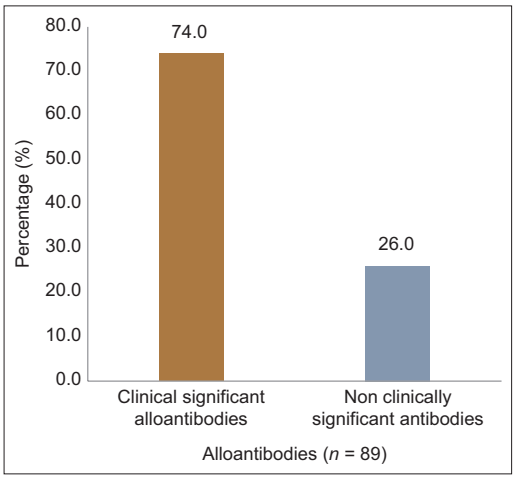

Figure 2: Types of antibodies

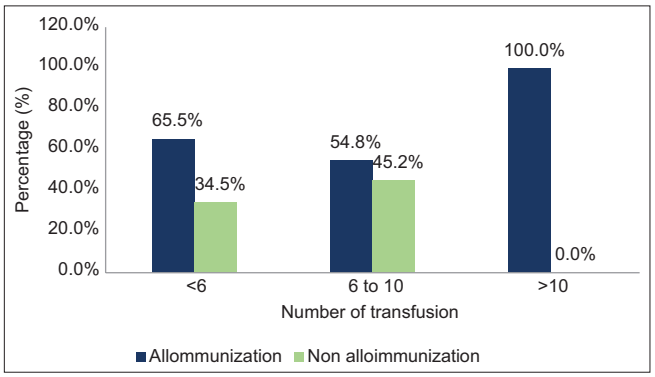

Figure 3: Alloimmunization and nonalloimmunization according to history of transfusion

Table 1: Development of alloantibodies

	n (%)
Anti-E	47 (37.6)
Anti-C	5 (4)
Anti-c	16 (12.8)
Anti-e	3 (2.4)
Anti-D	2 (1.6)
Rh system	73 (58.4)
Anti-Kidd	14 (11.2)
Anti-Duffy	2 (1.6)
Anti-Lewis	11 (8.8)
Anti-Mia	18 (14.4)
Anti-MNS	5 (4.0)
Anti-P	2 (1.6)
Total	125 (100)

Table 2: Percentage of single and multiple antibodies

	n(%)
Single Antibody	59 (65.2)
Multiple Antibodies	30 (34.8)

blood [Table 3]. There was a statistically significant association between alloimmunization and the number of pint transfusions ($\chi^2 = 0.005$, $P \leq 0.05$).

Predictors of alloimmunization were determined using maximum likelihood estimation and by selecting three variables, namely, gender, age, and history of transfusion. Table 4 depicts that gender is an important predictor of alloimmunization compared to age and history of transfusion. Our results also revealed that female patients had 18.72 (95% confidence interval 1.6–223. 0) higher odds of alloimmunization compared to male patients. Female patients also have a higher tendency to produce anti-E and anti-c antibodies compared to male patients. Therefore, hepatobiliary patients with a history of more than 6 pints of transfusion are more likely to produce multiple alloantibodies.

Discussion

Multiple blood transfusions are associated with numerous adverse effects, especially the development of alloantibodies.[1] Alloimmunization rates of approximately 5%–30% have been previously reported among multiple transfusion patients in Malaysia.[6] In addition, multiple transfused patients are typically more susceptible to hemolytic transfusion reactions.[7] The high rate of alloimmunization among hepatobiliary patients at Selayang Hospital was associated with the patient's diagnosis requiring multiple transfusions. This finding is consistent with a previous study[8] that reported a high prevalence of alloimmunization among hospitalized patients. Hepatobiliary patients are among the hospitalized patients who must undergo surgery that necessitates extensive blood transfusions to reduce the risk of excessive bleeding, thereby resulting in oxygen depletion in the systemic circulation and body's systems.[9] The number of transfusions increased the risk of developing alloimmunization.[1,10] Previous research has also demonstrated that repeated blood transfusions are the primary cause of the formation of alloantibodies in hepatobiliary patients.[5,9,11]

The percentage of patients with multiple antibodies were found to be as high as 45% in liver transplantation studies.[12] In another study,[13] more than 60% of alloimmunization patients were found to have received repeated transfusions for the treatment of hemoglobinopathy, hematological diseases such as sickle cell anemia, cancer, organ transplants, and renal failure. Meanwhile, a study conducted in West India revealed that women have a 72.7% higher risk of developing alloimmunization compared to men.[14] Another report suggested that the high risk of alloimmunization among women may also be contributed by those with a history of pregnancy complications that required blood transfusions.[4] The present study's finding is also consistent with a previous research that indicated female patients produce

Table 3: Analysis of the relationship of alloimmunization with gender, age, ethnicity, and history of red blood cell transfusion

Hepatobiliary	*n*	Alloimmunization, *n* (%)	Nonalloimmmunization, *n* (%)	*P*
Gender				
Male	79	52 (65.8)	27 (34.2)	0.632
Female	53	37 (69.8)	16 (30.2)	
Age (years)				
≤59	70	45 (64.3)	25 (35.7)	0.414
≥60	62	44 (71.0)	18 (29.0)	
Ethnic				
Malay and others	65	39 (60.0)	26 (40.0)	0.073
Chinese and Indian	67	50 (74.6)	17 (25.4)	
Pack cell transfusion				
<6	84	55 (65.48)	29 (34.5)	
6-10	31	17 (54.84)	14 (45.16)	
>10	17	17 (100)	0	

*The significance value tested based on chi square, $P \leq 0.05$

Table 4: Analysis of factors involved with red blood cell alloimmunization

	B	Wald	Exp (B)	95% CI		Significance
				Lower	Upper	
Age	0.02	0.346	1.01	0.96	1.08	0.556
Gender	2.84	5.372	18.72	1.55	223.03	0.02*
History of transfusion	0.72	2.33	2.29	0.71	6.65	0.127

CI=Confidence interval. *The significance value tested based on logistic regression, $P \leq 0.05$

more clinically significant alloantibodies.[15] Despite alloimmunization was found to be more common in female patients, the present study's finding was not statistically significant, which is similar to those reported in previous studies.[11,16,17]

Clinically significant antibodies consist of immunoglobulin G alloantibodies associated with various blood group systems, including the Rhesus (Rh), Kell (K), Duffy (Fy), and Kidd (Jk) systems. In the present study, 74.0% of the patients were more likely to develop clinically significant antibodies after receiving repeated blood transfusions. This result corroborates the outcomes from previous relevant research.[15,18,19] These antibodies can react at 37°C, causing hemolysis in transfused patients and a subsequent increase in morbidity and mortality.[8] In terms of the antibody specificities, the most prevalent were potentially hemolytic[2,7,10] comprising anti-E (37.6%), anti-c (12.8%), and anti-Kidd (11.2%). According to available data, the majority of hepatobiliary patients were over the age of 60 years.[12] Previous research has also shown that most alloimmunization cases in the kidney (55.8%) were associated with people aged 60 years and above.[1] Moreover, the risk of alloantibodies formation increases in elderly individuals with repeated transfusions in hepatobiliary patients.[20] In comparison to men, approximately 80% of pregnant women had a higher risk of developing alloantibodies if their age was over 45 years old.[14]

Several studies similarly found a correlation between the number of transfused packed cells and the formation of alloantibodies, whereas other studies documented no correlation between blood transfusions and the rate of alloimmunization. All 17 patients in this study who received over 10 transfusions had developed alloimmunization. With each blood transfusion, chronically transfused patients are more likely to develop clinically significant alloantibodies. In a study conducted by[21] the frequency of alloimmunization following blood transfusion was estimated at 2.8%. Furthermore, hepatobiliary patients who had received a blood transfusion were more likely to produce alloantibodies than those who had never received a blood transfusion.[4] According to,[15] the development of alloantibodies in hepatobiliary patients receiving RBC transfusions was estimated to increase by 0.1%–0.2%. Approximately 6%–14% of liver transplant recipients develop alloantibodies, delaying the transfusion process, which requires substantial amounts of compatible packed cells. In many previous studies, anti-Kell and anti-E were recorded as the most prevalent antibodies in hepatobiliary patients. However, in the present investigation, no Kell antibodies were detected.

According to,[22] the antigens most frequently implicated in alloimmunization include the Rh blood group, Kell, Kidd, Duffy, Lewis, and MNS. Significant amounts of these antibodies can also result in multiple organ failures, electrolyte disturbances, coagulopathy, and death in certain circumstances. Based on a study conducted by[7] the most prevalent alloantibodies target the Rh and Kell blood group systems at 61.36% and 11.36%, respectively. Meanwhile, earlier investigations[10,23] revealed that anti-Rh and -K antibodies were the most prevalent in hepatobiliary tissue. These findings differed slightly from those of other studies. Consequently, the incidence of alloantibodies may vary depending on clinical practice, immunosuppression, transfusion assistance, patient age, and ethnic diversity.

Anti-Mia is the most frequently detected antibody in the Malaysian population, followed by Rh antibodies,[24] although the rate was slightly lower than in a previous investigation, in which 18 out of 89 patients produced anti-Mia. Nevertheless, after their initial sensitization, there is a likelihood that alloantibodies will vanish rapidly.[25] One study revealed that one-fourth of induced alloantibodies were likely to be undetectable after 1 month of their initial identification, with 50% vanishing within 6 months.[21]

Conclusion

The prevalence of RBC alloimmunization is high among hepatobiliary patients and it may cause complications among patients requiring multiple transfusions. The number of transfused packed cells has been clearly shown to be proportionally significant with the risk for alloimmunization in hepatobiliary patients. Anti-E and anti-c were observed to be the most prevalent antibodies among hepatobiliary patients and are positively associated with a significantly higher risk of alloimmunization. Hence, it is recommended that pretransfusion Rhesus phenotype (C, E, c, and e) testing should be performed before the crossmatching testing and transfusion to reduce the risk of alloimmunization.

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Conflicts of interest

There are no conflicts of interest.

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