

Prevalence of Red Blood Cell Alloimmunization among Transfused Chronic Kidney Disease Patients in Hospital Universiti Sains Malaysia

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ARTICLE INFO

Article history:

Received: 13 March 2020

Accepted: 1 June 2020

Online:

DOI 10.5001/omj.2020.95

Keywords:

Isoantibodies; Cross-Sectional Studies; Malaysia; Blood Group Antigens; Blood Transfusion; Erythrocytes; Renal Insufficiency; Chronic; Renal Dialysis.

ABSTRACT

Objectives: Red blood cell (RBC) immunization is a common complication in blood transfusion recipients. Patients with chronic kidney disease (CKD) eventually develop anemia, which is multifactorial, and requires regular blood transfusions, which exposes patients to the development of RBC antibodies. We sought to determine the prevalence and specificity patterns of RBC immunization and its risk factors among transfused CKD patients. **Methods:** We conducted a cross-sectional study over one year from January to December 2018 in the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia. A total of 249 samples were recruited from CKD patients who received a blood transfusion (at least one-pint), which only match for ABO and Rh(D) antigen. The serum was screened for the presence of the RBC antibody using the gel agglutination technique (Diamed gel cards). Samples with positive antibody screening were subjected to antibody identification. **Results:** Of the 249 transfused CKD patients, 31 (12.4%) developed RBC immunization. Thirty (12%) were alloimmunized, and one (0.4%) was autoimmunized. Anti-Mia was the most common antibody (n = 14, 46.7%) among alloantibodies, followed by anti-E (n = 7, 23.3%). There was a significant association between pregnancy history with the development of antibodies whereas, no significant association was found between sociodemographic background, stage of CKD, hemodialysis status, underlying medical illness, and number of packed cell transfusions with the development of RBC antibodies. **Conclusions:** One-eighth of our patient cohort had RBC alloimmunization, and the risk was increased in patients with a history of pregnancy. We propose Rhesus RBC phenotyping and to supply blood match Rhesus antigen in CKD patients, especially patients of reproductive age.

One of the major medical issues faced by the chronic kidney disease (CKD) population is anemia. The causes of anemia in patients with CKD are multifactorial. The most well-known cause is inadequate erythropoietin (EPO) production, which is often compounded by other factors including iron and folate deficiency, decreased red blood cell (RBC) survival, and the accumulation of toxic inhibitors of erythropoiesis.¹ Even though the use of erythropoiesis-stimulating agents has been a treatment of choice for anemia in CKD since 1990, as it is costly, many countries can not afford it. In resource-constrained settings, blood transfusion remains an important alternative for treating anemia of CKD, particularly for severely anemic

patients. Low hemoglobin levels (around 5–7 g/dL) are common, especially in hemodialysis patients. Thus, they require frequent blood transfusions to improve the symptoms of anemia.² Exposure to multiple blood transfusions inevitably lead to many undesirable outcomes, and one of the important and common outcomes is the development of RBC alloimmunization.³

Alloimmunization is defined as the development of antibodies in response to alloantigen after exposure to genetically different cells or tissue. Many factors can influence the development of alloantibodies in the blood. Studies have shown that alloimmunization is influenced by the recipient's immune status, the dose of blood transfused, and the immunogenicity of the antigen.⁴ Therefore,

ensuring that the antigens of transfused blood cells are matched with recipient blood cells is essential for safe blood transfusion. A previous study showed that the rate of RBC alloimmunizations among patients with chronic blood transfusion, such as hemoglobinopathy, aplastic anemia, myelogenous leukemia, upper gastrointestinal bleeding, and CKD was 29%, 11%, 16%, 11%, and 14%, respectively.⁵ These kinds of population are at greater risk for developing red cell alloantibodies.

Red cell alloimmunization may lead to difficulty in finding compatible blood for transfusion and might cause a hemolytic transfusion reaction if the antibodies are not detected during pretransfusion testing. The presence of alloantibodies will create significant problems in transfusion therapy and can contribute to morbidity and mortality. This will result in difficulty for cross-matching of the blood, especially during an emergency. Clinically, significant alloantibodies can cause mild to severe RBC hemolysis.⁶ Furthermore, autoantibodies that present can mask the existence of alloantibody, and this will complicate the situation.

In view of the great concern regarding alloimmunization among chronically transfused patients, many studies were conducted involving patients with thalassemia and sickle cell disease, but only a few studies assessed the risk of RBC immunization among CKD patients. Therefore, the main objective of this study was to determine the frequency of RBC immunization in transfused CKD patients. Thus, the information will provide a better approach for transfusion policy in the CKD population.

METHODS

This cross-sectional study was conducted over one year (January to December 2018) in the Transfusion Medicine Unit of Hospital Universiti Sains Malaysia. This study was approved by the Human Research Ethics Committee USM (HREC).

A total of 249 samples were collected from CKD patients who received at least one-pint blood transfusion, which only match for blood groups ABO and Rh(D) antigen. ABO blood grouping was done by using a gel card "Diaclon ABO/D+ Reverse grouping". The blood samples (serum) were screened for the presence of antibodies using the antibody screening test. Three-cells screening

panel (Diamed ID- Dia cell) was used. Samples with positive antibody screening underwent antibody identification using an eleven cell panel (Diamed ID-Dia panel, including the enzyme treated cells (papain) at 37 °C and AHG phase). RBC phenotyping of suspected antibody was done for those with positive for antibody identification. Demographic and clinical data were taken from the patient record.

Data were analyzed using SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive frequency method was used for descriptive data, and logistic regression was applied to determine the factors that best predict the development of RBC immunization with a *p*-value < 0.050 considered as significant.

RESULTS

The majority of the study subjects were elderly and aged over 60 years old (55.8%) [Table 1]. The mean age was 59.5 years old. The cohort was male dominant (55.8%) and the majority Malay (96.4%). The predominant blood group was O (37.3%) followed by A, B, and AB. All patients were Rhesus-D positive. Over half (55.8%) had hemoglobin levels between 7–9.9 g/dL, and 43.8% of samples had severe anemia with hemoglobin levels < 7 g/dL. Patients received a median of 10 pints of total transfusion, including packed cells and other blood products.

Out of 249 patients, 31 (12.4%) developed RBC immunization. The majority (96.8%) were alloantibody, and one case (3.2%) developed autoantibodies. The autoantibody was towards anti-D. Most (90.0%) had single alloantibodies, two patients developed double alloantibodies while one patient developed triple alloantibodies. Among those that developed antibodies, 19 were female and 12 were male.

The most common alloantibody was anti-Mia (46.7%), followed by anti-E (23.3%), anti-e (6.7%), anti-Le^a, -Le^b (6.7%), anti-C (3.3%), anti-K (3.3%), anti-Le^a (3.3%), anti-M (3.3%) and anti-E, -K, -Kp^a (3.3%) [Table 2]. Observation among the Rhesus group showed anti-E was the most common antibody followed by anti-e.

To determine factors associated with RBC alloimmunization in transfused CKD

Table 1: Descriptive data of transfused chronic kidney disease (CKD) patients (N = 249).

Variables	n (%)	Mean (SD)	Median (IQR)
Age, years		59.5 (14.5)	62.0 (17.0)
< 18	2 (0.8)		
18–40	22 (8.8)		
> 40–60	86 (34.5)		
> 60	139 (55.8)		
Gender			
Male	139 (55.8)		
Female	110 (44.2)		
Race			
Malay	240 (96.4)		
Chinese	8 (3.2)		
Indian	1 (0.4)		
Blood group			
O	93 (37.3)		
A	77 (30.9)		
B	66 (26.5)		
AB	13 (5.2)		
Hemoglobin level, g/dL			
< 7	109 (43.8)		
7–10	139 (55.8)		
> 10	1 (0.4)		
Blood component transfusions, units		15.2 (15.6)	10.0 (15.7)
≥ 10	125 (50.2)		
< 10	124 (49.2)		
Number of packed cell transfusions, pints		9.3 (8.3)	7.0 (10.0)
≥ 7	125 (50.2)		
< 7	124 (49.8)		
Stage of CKD			
1	0 (0.0)		
2	3 (1.2)		
3	21 (8.4)		
4	75 (30.1)		
5	150 (60.2)		
Hemodialysis			
No	23 (9.2)		
Not regularly	41 (16.5)		
Regularly	185 (74.3)		
Primary causes of CKD			
Diabetes mellitus	178 (71.5)		
Hypertension	225 (90.4)		
Gouty arthritis	21 (8.4)		
Polycystic kidney disease	3 (1.2)		

IQR: interquartile range; SD: standard deviation.

Table 2: Spectrum of unexpected RBC alloantibody in CKD patients (n = 30).

RBC Alloantibody	Frequency (%)
Number of antibodies identified	
Single	27 (90.0)
Multiple	3 (10.0)
Total	30 (100)
Antibody specificity	
Single	27 (90.0)
Anti-C	1 (3.3)
Anti-E	7 (23.3)
Anti-e	2 (6.7)
Anti-K	1 (3.3)
Anti-Le ^a	1 (3.3)
Anti-M	1 (3.3)
Anti-Mia	14 (46.7)
Multiple	3.0 (10.0)
Anti-Le ^a , -Le ^b	2 (6.7)
Anti-E, -K, -Kp ^a	1 (3.3)

RBC: red blood cell; CKD: chronic kidney disease.

patients, seven variables were selected in preliminary main effect models and backward likelihood ratio (LR) variables was done. In the final model, history of pregnancy had significant association with RBC immunization. This signifies that patients with a history of pregnancy had 2.65-times greater odds of RBC immunization (95% confidence interval: 1.20–5.91, $p = 0.017$) compared to those with no pregnancy history, when adjusted to diabetes status and dialysis status as shown in Table 3.

The backward LR multiple logistic regression model was applied. Multicollinearity and interaction term were not found. The Hosmer-Lemeshow test ($p = 0.725$), classification table (overall correctly classified percentage = 87.6%), and area under the ROC curve (71.0%) were applied to check the model fitness.

DISCUSSION

Blood transfusions are a lifesaving measure in CKD due to the risk of anemia facing these patients. Alloimmunization and transfusion-transmitted infections are potential complications, especially in multi-transfused patients. The presence of RBC alloantibodies may make finding compatible antigen-negative RBC units difficult, especially if multiple alloantibodies present and increase the risk of

Table 3: Associated factors for RBC immunization by multiple logistic regression model (n = 249).

Variable	Crude odds ratio (95% CI)	p-value	Adjusted odds ratio (95% CI)	p-value
Gender				
Male	1	0.044	-	-
Female	2.21 (1.02–4.78)			
History of pregnancy				
No	1	0.029	1	0.017
Yes	2.34 (1.09–5.03)		2.65 (1.20–5.91)	
Stage of CKD				
Late	1			
Intermediate	1.92 (0.60–6.19)	0.273		
Early	3.85 (0.34–43.91)	0.278	-	-
Dialysis				
No	1	0.074	1	0.133
Regular	0.36 (0.12–1.11)	0.984	0.42 (0.14–1.30)	0.133
Not regular	1.01 (0.29–3.49)		1.36 (0.38–4.91)	0.636
Diabetes				
No	1	0.183	1	0.089
Yes	0.59 (0.27–1.28)		0.49 (0.21–1.12)	
Blood group				
O	1	0.248	-	-
A	1.69 (0.70–4.09)	0.976		
B	0.99 (0.35–2.74)	0.736		
AB	0.69 (0.08–5.90)			
Number of packed cells transfusions, pints				
> 7	1	0.074	-	-
≤ 7	2.11 (0.93–4.79)			

RBC: red blood cell; CKD: chronic kidney disease; CI: confidence interval.

developing a delayed hemolytic transfusion reaction. This restricts clinicians' ability to transfuse RBCs rapidly and safely. The majority of our patients were elderly and had late-stage CKD similar to the findings of a previous study.⁷ The frequency of alloantibodies among transfused CKD patients in our population was 12.4%. This incidence was higher than those reported by Shukla et al,⁸ (9.8%) and Obi et al,⁹ (3.2%). The lowest reported incidence among multi-transfused hemodialysis patients was 1.72%.¹⁰ A study from Sudan reported the incidence as 13.1%.¹¹ The incidence of RBC alloantibody among CKD patients varied between the studied population. Factors that contributed to the alloimmunization rate were the heterogeneity of the donors and blood recipients of studied population, antigenicity of the antigen, practice in pretransfusion testing, sample size of each study population, and sensitivity of the test method.

Studies among patients with sickle cell disease showed a higher prevalence range from 19% to 43% followed by myelodysplastic syndrome (15–59%),¹² thalassemia (5–45%),¹³ and autoimmune conditions (16%).^{4,14} The incidence rate was dependent on the patient's age, RBC exposure, and extent of antigen matching for blood groups other than ABO and Rhesus.¹⁵ A lower rate of RBC immunization was reported in areas with homogeneity of the donor and the recipient.³ Patient populations with lower RBC alloimmunization rates than predicted based on the transfusion burden included those with leukemia undergoing chemotherapy.¹⁶ Patients treated with steroids or other immunosuppressive agents are also less likely to become alloimmunized.¹⁷

This study showed majority of patients had single rather than multiple alloantibodies. These findings were agreeable with results obtained by Shukla in India and Al-Joudi et al.^{8,18} Of the single antibody,

anti-Mia was most commonly observed (14/30, 46.7%). Similar results were also noted in Kuala Lumpur, Malaysia, among the transfusion recipients, in which of 24 263 transfusion recipients, 30.4% had anti-Mia specificity.¹⁹ Another study also reported anti-Mia as the commonest antibody detected among regular blood donors.²⁰ Similar findings were found in studies from Taiwan, Thailand, and Hong Kong.²¹⁻²³ In contrast, the prevalence of anti-Mia among Caucasians is rare with an incidence of < 0.01%.²⁴ These findings support that anti-Mia have much higher incidence among east-Asian populations.

The second most common alloantibody detected in this study was Rhesus group antibodies: 23.3% developed anti-E. This finding was comparable with study among thalassemia patients and among a transfused Kelantan population.^{18,25} Two other studies done in Eastern Taiwan and in the Arabian gulf region also showed similar findings.^{21,26} These findings remark that anti-E was common among various populations, and is expressed differentially among individuals. Furthermore, it implies that E antigen is highly immunogenic and patients with negative E antigen are prone to sensitization if they receive blood transfusion from E antigen positive donors.²⁷

Other Rhesus antibodies found in our study were anti-e (2/30) and anti-C (1/30). A similar study also reported the commonest alloantibody detected in CKD patients among the Rhesus group were anti-c (27.3%) and anti-C (18.2%).¹¹ Rhesus group antibodies is the second most important blood system after the ABO group. The immunogenicity of Rhesus antigens listed in order of decreasing immunogenicity are D > c > E > C > e. All Rhesus group antibodies are clinically significant with varied presentation ranging from mild to severe hemolysis.

Anti-K was implicated in one patient in our study. K antigen stimulates the formation of anti-K in about 10% of K-negative patients when they receive blood that is positive for antigen K. It also has been reported to cause fatality due to severe hemolysis.²⁸ We found one patient developed anti-M and no features of hemolysis were observed in this patient nor problems in blood cross-matching. Anti-M is commonly a naturally occurring antibody but can also be immune related.

Three patients were identified as having multiple alloantibodies in our study. One patient had three

types of alloantibodies which were anti-E, anti-K, and anti-Kp^a. Two patients had two concomitant alloantibodies: anti-Le^a and anti-Le^b. It was reported that E, K, Le^a, and Le^b were among the 10 most potent antigens to stimulate RBC alloimmunization and these can stimulate multiple antibodies in antigen-negative patients.²⁹ A combination of Rhesus system (anti-E, -D, -C) with anti-K were the most common specificities found in one study.³⁰ Because of this significant finding, the authors suggested that patients who have potential for multiple transfusion should be phenotyped for at least E and K antigens in addition to the ABO and RhD groups. The blood to be supplied also needed to match for these antigens to reduce the incidence of RBC alloimmunization.³⁰

We found that history of pregnancy had significant association with development of antibodies towards RBCs in transfused CKD patients. Other factors such as gender, underlying clinical conditions (e.g., diabetes mellitus), frequency of dialysis, history of blood component transfusions, blood group, and the number of packed red cell transfusions were not associated with development of RBC antibodies. Each pregnancy has the risk for RBC alloimmunization, therefore the greater the number of pregnancies the higher risk for immunization. This occurs because of exposure of the paternally derived foreign RBC antigen during pregnancy triggers the immune system to produce antibodies towards the respective antigen.

There is still conflicting results for sex differences and RBC immunization difference as some studies have reported a high risk for women and other showed no risk difference between the sexes.³¹⁻³³ One study reported development of RBC alloimmunization is independent of disease states such as diabetes, atherosclerosis, and orthopedic fracture.³⁴ However, diabetes mellitus seems to be a risk factor for development of RBC alloimmunization.³⁵ In our study, the majority of patients with CKD were elderly whereby in other studies their population was younger.^{36,37} The uremic status of the patients also will alter the humoral and cell mediated immune response of individual.

The number of packed cell transfusions has been shown to pose a significant risk for alloimmunization in thalassemia patients who received ≥ 10 or more units of packed cell transfusion.³⁸ However, our study did not show significant association between number of transfusion and RBC immunization. This is

probably due to smaller number of transfusions among CKD patients compared to thalassemia patients.

In many younger dialysis patients who were ultimately hoping to receive a kidney transplant, there was a concern that blood transfusions could increase sensitization to HLA antigens, which reduces the chance of a patient receiving a transplant or increase the waiting time for kidney transplantation. Overall, approximately 28% of transfused patients developed HLA antibodies.³⁹ The presence of RBC alloimmunization on top of HLA alloimmunization will make the condition more problematic. As a result, RBC alloimmunization may limit the availability of compatible blood for future transfusions, whereas the development of leukocyte alloimmunization often makes it necessary to postpone transplantation. A previous study on human leukocyte antigen (HLA) alloimmunization in pediatric sickle cell disease patients who receive primarily leukoreduced RBC transfusions found that HLA alloimmunization tendency was associated with antibodies to RBC antigens.⁴⁰ Knowing the risks and benefits of RBC transfusions will help clinicians make more informed decisions regarding the need for RBC therapy for patients with end-stage renal disease on dialysis.

CONCLUSION

RBC immunization is a common complication among CKD patients who require blood transfusion and we found a high prevalence of 12.4%. We observed that the Rhesus group antibody was the second most common alloantibody that developed among CKD patients and pregnancy was a significant risk factor. We propose at least Rhesus phenotype (C, E, c, e) testing pretransfusion and to supply matched Rhesus phenotype blood to avoid RBC immunization for better care of patients with CKD.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

Acknowledgements

We would like to thank all staffs of Transfusion Medicine Unit, Hospital Universiti Sains Malaysia for their help and support in carrying out this study.

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