

## RESEARCH ARTICLE

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# Red blood cell alloimmunization in multitransfused hemodialysis renal patients in central Sudan

Mohamed Eltaib Elmobark, Asaad Adam Abbass, Eman Anwer, Usama Abdalla Elsharief, Bakri Yousif Mohamed Nour

## ABSTRACT

**Aims:** Blood transfusion is an integral part in the management of chronic renal failure (CRF). Alloimmunization is a recognized complication of red blood cell (RBC) transfusion with consequences including delayed hemolytic transfusion reactions and difficulties in getting compatible blood for future transfusion. Therefore, the current study was designed to identify the frequency of RBC alloimmunization and the specificity of alloantibodies against red cell antigen among the hemodialysis patients. **Methods:** A total of 301 patients (239 males and 62 females) with CRF who received more than two units of blood and matched for ABO & Rh(D) antigen were enrolled in this cross-sectional study which was carried out from December 2016 to December 2017. After detailed history through questionnaire conducted to the patients, antibody screening was done by 2-cell panel with homozygous expression of the antigens (ID-Diacell I-II-DiaMed). Positive samples were

further subjected to antibody identification using commercial panels of 11 cells (ID-DiaPanel-DiaMed). **Results:** Out of 301 patients 239 (79.4%) were males and 62 (20.6%) were females. The prevalence of alloimmunization was 16.9% and the most alloantibody frequent was anti-C (21.3%), anti-E (16.4%), and anti-K (14.8%). The most common alloantibodies produced were against Rh system (64%). Dual and triple antibody was observed. All alloantibodies were immunoglobulin G (IgG) class except anti-M was IgG and immunoglobulin M (IgM). **Conclusion:** This study showed that 16.9% of the multitransfused patients with chronic kidney disease (CKD) developed alloantibodies, most of which were of the Rh phenotype. These alloantibodies may lead to allograft rejection, or even poor patient survival. Thus, we advocate that renal transplant be instituted at the earliest opportunity whenever it becomes inevitable as a modality of treatment to enhance better outcomes.

**Keywords:** Alloimmunization, Anemia, Blood transfusion, Chronic kidney failure

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## INTRODUCTION

Widespread presence of CKD has increased in recent decades globally [1] due to various reasons, such as accumulated stress due to life style modifications [2], excessive intake of animal proteins [3], and severe dehydration [4]. Anemia is one of the major complications present in patients of CRF and occurs in almost 80% of such patients [5]. Majority of chronic renal disease patients continue to remain anemic during the course of their treatment involving repeated dialysis. The etiology of the anemia is multifactorial but primarily due to the reduction in production and activity of erythropoietin [6]. Although anemia in chronic renal disease responds well to the injection of erythropoietin or its substitutes, it is still a common practice to transfuse packed RBCs in resource limited settings. Repeated blood transfusions may be associated with some complications such as iron overload, platelet, and RBC alloimmunization [7]. Blood transfusion usually practiced in severe renal failure patients in dialysis unit. The safety measure of blood transfusion is a core objective of transfusion medicine since alloimmunization is an immune response developed against foreign RBC antigens after transfusion [8]. Red blood cell alloimmunization is a common complication among CRF patients who need regular blood transfusion [9]. Chronic red cell transfusions can cause unwanted complications called transfusion reactions in a patient. Development of alloantibodies to red cell antigens is an important immune-mediated delayed hemolytic transfusion reaction [10]. The other factors that influence alloantibody formation are the recipient's immune status as well as the dose, single or multiple transfusions, age, route of administration, and the immunogenicity of the antigen. The blood transfusion services play an important role and are responsible for ensuring sufficient quality and safe blood supply. Blood transfusion support is vital to the management of patient with hematologic disorders and malignancies. Many such patients require blood transfusion during their illness or may be lifetime [11]. Clinically significant RBC alloantibodies develop in 6–36% of multitransfused patients and can pose major problems in long-term transfusion therapy [12]. Therefore, in Sudan, pretransfusion testing that applied in Wad Madani and Khartoum cities which represent central Sudan is currently only limited to ABO/D grouping and cross-match. As a result, antibody screening and identification are not applicable routinely at most hospitals due to the high cost of the reagents. The lack of applying these essential tests leads to increase the possibility of the immunization against RBC antigens. Due to the classical methods applied in blood banks for packet cell transfusions, the main objective of this study is to assess the presence of RBC alloimmunization and specify of antibody in multitransfused patients with CKD in order to come out with scientific recommendations for improvement of patient management in Sudan that

undergoes multitransfusions in order to reduce blood transfusion-related hazards in the future.

## MATERIALS AND METHODS

### Ethical consideration

This study was conducted in accordance with the ethical standards within the political borders of the Sudan. All the participants involved in this study have read, understood, and signed a written consent form. This study was approved by ethical committee of University of Gezira. During the distribution of the questionnaire, participants were informed that the information collected would be kept anonymous and participation was totally voluntary. Across sectional study design was performed at renal dialysis center, Khartoum and Wad Madani, Sudan from December 2016 to December 2017. A total of 301 patients among them 239 males and 62 females of various age groups were diagnosed as chronic renal failure who had received more than two units of RBC from the donors. Clinical and transfusion records of all the patients were written and maintained throughout study.

### Inclusion and exclusion criteria

Diagnosed chronic renal failure patients received more than two units of blood were included, in this study patients with previous transfusion due to disease other than chronic renal disease, autoimmune disease, and chronic renal failure associated with other disease were excluded.

### Sample collection

Five milliliters of venous blood was collected from each patient using plain containers and stored at  $-20^{\circ}\text{C}$  for further experimental work.

### Laboratory investigation

After detailed clinical history, antibody screening was done using 2-cell panel with homozygous expression of the antigens (ID-Diacell I-II-DiaMed, Switzerland) as follows (Saline R.T tube/saline  $37^{\circ}\text{C}$  tube/AHG tube/enzyme tube). The positive sample was subjected for antibody identification using commercial panels of 11 cells (ID-DiaPanel-DiaMed, Switzerland). Results were interpreted as per manufacturer's instructions.

### Statistical analysis

The statistical analyses were performed by Microsoft Excel 2007, MS Office, USA and Statistical Package SPSS (Statistical Package for Social Sciences) software version 20 was used for data entry and analysis. Data was summarized by appropriate statistical tools such as mean,

median, standard deviation, frequencies, and proportion. The statistical significance was tested using the chi square and Fisher's test. P values less than or equal to 0.05 were considered as statistically significant.

**RESULTS**

A total of 301 patients were included in the study and screened for the presence of any antibodies. This included 239 males and 62 female patients. The patients were between 12 and 79 years of age with a mean age of 43.6 years. Number of blood units transfused per patient ranged from 3 to 16 with a total of 1636 transfusions and an average of 5.5 units per patient. Alloantibodies were detected in 51 out of 301 patients, that is 16.9% of total patients (Figure 1). The mean age of patients with alloantibodies was 41.8 years. There were 44 males and 7 female patients with alloantibodies. Of the total of these patients, 43 patients (84.3%) developed single antibody while 6 (11.8%) patients developed dual alloantibody and 2 (3.9%) triple in their sera. On alloantibody type identification, the most common type found was anti-C (21.3%), anti-E (16.4%), anti-K (14.8%), anti-c (9.9%), anti-e (8.2%), anti-D (8.2%), anti-M (4.9%), anti-Le<sup>b</sup> (3.3%), anti-Jk<sup>a</sup> (3.3%), anti-S (3.3%), followed by anti-FY<sup>a</sup> (1.6%), anti-FY<sup>b</sup> (1.6%), anti-Le<sup>a</sup> (1.6%), and anti-N (1.6%) in one patients each as shown in Figure 2. It also showed that no correlation between age, sex, and alloimmunization the p value (p = 0.334\*), (p = 0.254\*), respectively, meaning that age and sex are not risk factors influence the occurrence of alloimmunization.

On the other hand, there was significant correlation between number of blood transfusion and alloimmunization (p < 0.05) as shown in Table 1. The most common blood group among our patients was

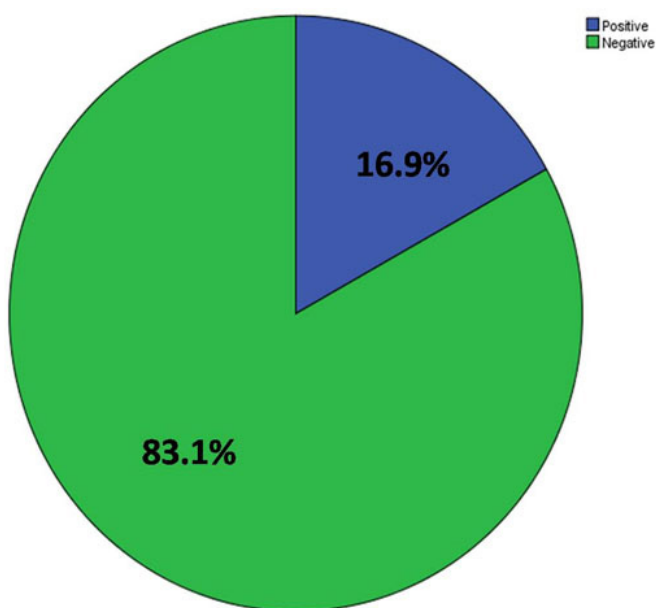


Figure 1: Frequency of red blood cell alloantibodies.

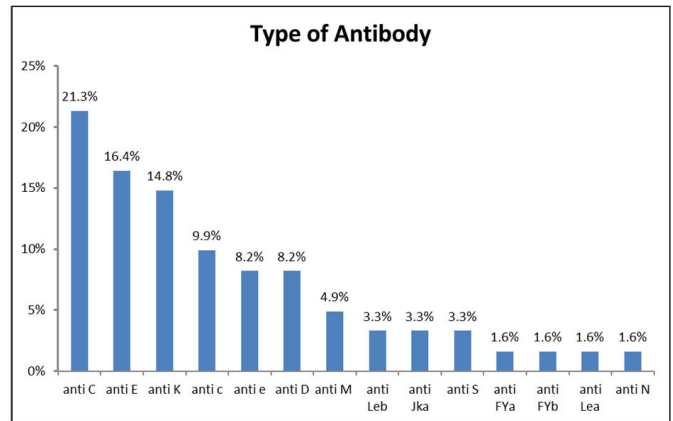


Figure 2: Prevalence of alloantibodies among multitransfused chronic kidney disease patients.

A positive which is detected in 21 patients (41.2%). Distribution of specificity of unexpected alloantibodies depends on blood group system. The most common alloantibodies produced were against Rh system (64%) followed by Kell system, MNs system, Lewes system, Duffy system, Ked system illustrated in Table 2. The risk of alloimmunization per unit of blood, defined [2, 13] as the total number of alloantibodies detected (51), divided by the total number of transfused units (1936)

Table 1: Relationship of alloimmunization with number of units transfused

Antibody screening	No. transfusion unit			Total	Chi square p value
	(3-6)	(7-10)	(Above 10)		
Positive	26	19	6	51	0.000*
Negative	203	47	0	250	
Total	229	65	6	301	

Significant correlation p value < 0.001 (statistically significant).

Table 2: Rh and other blood groups distributions

Blood Group System	Alloantibodies	Percent
Rh system	Anti-C: 21.3%, anti-E: 16.4%, anti-c: 9.9%, anti-D: 8.2%, anti-e: 8.2%	64
Kell system	Anti-K: 14.8%	14.8
MNs system	Anti-M: 4.9%, anti-N: 1.6%, anti-S: 3.3%	9.8
Lewes system	Anti-Le <sup>a</sup> : 1.6%, anti-Le <sup>b</sup> : 3.3%	4.9
Duffy system	Anti-Fy <sup>a</sup> : 1.6%, anti-Fy <sup>b</sup> : 1.6%	3.2
Ked system	Anti-Jk <sup>a</sup> : 3.3%	3.8
Total		100



multiplied by 100, was 3.2% for the multitransfused CKD patients.

## DISCUSSION

Blood transfusion may be lifesaving in chronic kidney disease (CKD) as it promptly improves the oxygen-carrying capacity in patients with symptomatic anemia. However, transfusion-transmissible infections (TTI) and alloimmunisation are potential complications especially in multitransfused patients. In our study, there were more males with CKD compared to females similar to previous findings [14]. Therefore, the reported frequency of alloantibody formation is highly variable in different parts of the world ranging from 1.13% to 40.4% [15]. The risk of alloimmunization in this study was 3.1% and this is within the reported range of 0.5–5.9% seen in the literature [16–18]. The rate of alloimmunization observed in the present study was (16.9%) which is similar to a study conducted by [3, 19]. They reported that the overall rate of alloimmunization to erythrocyte antigens was (18.6%). Antibodies to the C and E antigens of the Rh group, the Kell antigen and the Lewis antigens were most common and also similar to the result (19.5%) obtained by [4, 15] which is done in Egypt for multiple transfusions thalassemia patient. As a comparison, the result of our current study was found to be higher in its value than other studies achieved in different times [5, 20] which came out with (6.1%), and [6, 18] was done in port Sudan was resulted in 13.1%. This difference could be due to the variation in the study populations, and the frequency of RBC alloantibodies varies considerably depending upon numerous factors, such as demographics, number of transfusions, pregnancy, genetic constitution, immune competence, disease factors, time and frequency of screening, and sensitivity of the methodology [7, 21].

In the present study the high prevalence of alloantibodies observed was actually because of many reasons. First of all, most blood banks in the area of our study depend on cross-match technique only for blood transfusion which alone is not enough to be used as a mean for safe blood transfusion according to the WHO and the other international standards. Second, most blood banks did not do antibody screening and identification because no facilities in blood banks and/or high cost of these techniques. Third, antigen phenotype assay is not applicable in most of the blood banks in the area of the study excluding even Rh and Kell antigen. Fourth, more over the issue is so complex in terms that even if these techniques are available they are not always be achieved unless the doctors request the application of these techniques after suffering from incompatible of blood in their hospitals. In concordance with other studies, the most common alloantibodies produced were against Rh system (64%) flowed by Kell system (14.8%), MNs system (9.8%), Lewes system (4.9%), Ked system (3.3%), Duffy system (3.2%) illustrated in Table 2. Similar to that

observed in other study [8, 14] had shown that rhesus system antigens were among the most immunogenic ones (Rh antibodies frequency was 55.5%). In our study, 43 patients (84.3%) developed single antibody while 6 (11.8%) patients developed dual alloantibody and 2 (3.9%) triple in their sera. On alloantibody type identification, the most common type found was anti-C (21.3%), anti-E (16.4%), anti-K (14.8%), anti-c (9.9%), anti-e (8.2%), anti-D (8.2%), anti-M (4.9%), anti-Le<sup>b</sup> (3.3%), anti-Jk<sup>a</sup> (3.3%), anti-S (3.3%), followed by anti-FY<sup>a</sup> (1.6%), anti-FY<sup>b</sup> (1.6%), anti-Le<sup>a</sup> (1.6%), and anti-N (1.6%) in patients each as shown in Figure 2, similar to the study reported by [9, 22]. The authors explained that antibodies to the C and E antigens of the Rh group, the Kell antigen, and the Lewis antigens were found to be the most common. The most frequent antibodies anti-C and anti-E of rhesus blood group system, as shown in Figure 2, may be subjected to the following justification. The frequency of C and E antigens generally found to be the lowest in black abut (21%), as such multitransfusion patients who were hither to lacking the C and E antigens were likely to develop anti-C and anti-E [10, 22]. There were alloimmunized patients formed more than one alloantibody agreed with the study of [11] and [8, 14, 23], as each of them has found more than one alloantibodies.

The study also showed that there is no significant relationship between gender and the incidence of alloimmunization ( $p = 0.254^*$ ), and this result was in this agreement with the findings of [11, 23],  $p$  value (0.864\*). The authors justified that gender was not a risk factor for alloimmunization.

Findings of our study showed that there is no significant relationship between the age and the incidence of alloimmunization ( $p = 0.334^*$ ) and this also agreed with results obtained by [12, 24] in which they proved that age was not a risk factor for alloimmunization.

On the other hand, our study found that there is significant relationship between the incidence of alloimmunization and the number of transfused units ( $p = 0.000^*$ ) which was in agreement with the finding of [5, 20]. They found that there is a significant relationship between number of units transfused and incidence of alloimmunization ( $p = 0.01^*$ ).

## CONCLUSION

Chronic renal failure needs special precautions to minimize any possible alloimmunization. Many factors may have contributed to the high immunized ratio observed in this study include heterogeneity of the population living in Sudan, lack of better-matched donors for those patients, misuse of leukodepleted blood and antibody screening, and identification which are not applied in most of the hospitals. Based on the results of the study, we recommend that the application of antibody screening and identification is crucial in Sudanese hospitals to minimize the risk of alloimmunization. There

are many recent developments that can be used in the genetic profiling of the blood cell that can be exploited to facilitate the identification of RBCs matched with the antigen to improve blood transfusion for chronic renal patient. The study also showed that there was no significant relationship between age, gender, but significant relationship between number of units of blood transfused and the development of alloantibody.

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## Author Contributions

Mohamed Eltaib Elmobark – Conception of the work, Design of the work, Acquisition of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Asaad Adam Abbass – Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Eman Anwer – Conception of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Usama Abdalla Elsharief – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Bakri Yousif Mohamed Nour – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that

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### **Guarantor of Submission**

The corresponding author is the guarantor of submission.

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### **Consent Statement**

Written informed consent was obtained from the patient for publication of this article.

### **Conflict of Interest**

Authors declare no conflict of interest.

### **Data Availability**

All relevant data are within the paper and its Supporting Information files.

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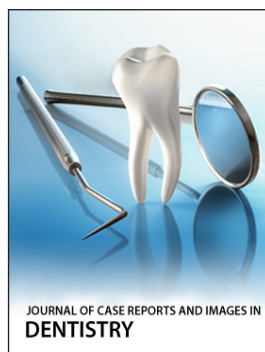
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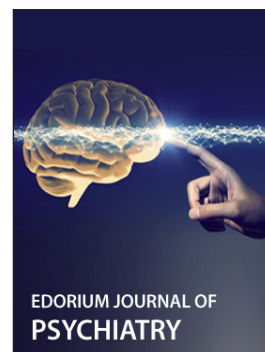
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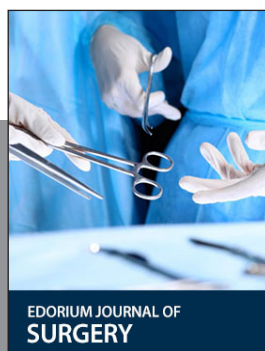
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