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Research Article

Red Blood Cell Alloimmunization among Multiple Blood Transfusions Sudanese Patients

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ABSTRACT

Objectives: This study aims to detect the alloantibody among patients treated by repeated blood transfusion for at least three or more times.

Materials and methods: Across-sectional descriptive study design applied at Eldweem and Kosti teaching hospitals, Sudan. One hundred have blood transfusion three times or more and their age between 1-70 years old recruited in this study. Three ml of venous blood samples were collected in Ethylene diamine tetra acetic acid (EDTA) anticoagulant containers from each participant. For all participants, slide method for ABO blood group and Rh factor were used. Indirect Coombs test apply to detect alloantibodies by Polly Specific antihuman globulin reagents using tube method techniques.

Results: Results indicated that out of 142 multiple transfused patients; there were 82 (57.7%) males and 60 (42.3%) females, and a total of 31 samples showing detection of RBC alloantibodies, out of 82 males, 22 (26.83%) showed presence of alloantibodies and out of 60 females, 9 (15%) showed presence of alloantibodies. The participant's mean age is 38.58 ± 20.85 years old. Patients with sickle cell anaemia represented the highest rate of detected alloantibodies (80.6%) followed by patients with renal failure and other anaemia, both with a detection rate of 9.7%. The highest occurrence of alloantibodies (5/13 (38.5%)) saw among patients who had blood transfusions more than 8 times followed by 6-8 times (11/47 (23%)), and by those having blood transfusion 3-5 times (15/82 (18%)).

Conclusion: This study has shown that frequently transfused patients are at risk of alloimmunisation, which is highly recommended to be considered when reviewing repeatedly transfused patients.

Keywords: Alloimmunisation; Multiple blood transfusion; Sudan

INTRODUCTION

The blood transfusion is a vital procedure ensuring sufficient safe and quality blood supply, especially in malignancies, chronic diseases, and hematological disorders patients who require regular blood transfusion [1]. The blood transfusion safety measurement is a core objective of transfusion medicine [2]. Alloimmunization, developed against transfused foreign RBC antigens, is a serious unfortunate and occasionally life-threatening complication among repeated blood transfusion patients, which may occur in immunocompetent blood transfusion-dependent recipients causing blood delayed transfusion reactions [3-5]. The etiology of alloimmunization might be due to multiple origins, primarily with many contributing factors including antigenic variations of RBC immune status, the diversity genetic of antigens between recipients and blood donors 'which considered as a challenging occurrence that, may increase risk of delayed hemolytic blood transfusion reactions, and complicates of the cross-matching [6-8]. The risk of alloantibodies development depends on frequency and number of blood transfusions, the ethnicity of patient, antigen immunogenicity, difference in the recipient and donor antigenic pattern, and recipient's immune response [4,9-11]. Repeated blood transfusions can cause adverse complications including, transfusion reactions and development alloantibodies red cell antigens, which caused an important

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immune-mediated hemolytic transfusion reaction. Moreover, the other factors influence alloantibody formation includes, the recipient's immune status, the blood transfusion dose, multiple or single transfusions, route of administration, age, and the immunogenicity of the antigen [12]. Multi-blood transfusions may appear with some complications such as platelet, iron overload, and RBC alloimmunization [13]. Clinically significant of RBC alloantibodies develop in 6%–36% of repeated blood transfusion patients and can cause major problems in their long-term transfusion therapy [9]. Some of the clinical antigens induce the production of the IgG alloantibodies to include the Rhesus (Rh), Duffy (Fy), Kell (K), and Kidd (JK) systems [14]. These antibodies cause hemolysis in multi-transfused patients and react at 37□C resulting in significant mortality and morbidity [15]. The Alloimmunization rates vary between 0.009% -0.6% in healthy donors, 4.24% and 1.4% in a previously transfused individual [16]. Previous studies have demonstrated alloantibodies in multi transfused patients, including different diseases in different population groups such as sickle cell disease, thalassemia, chronic renal disease and cancer patients [17,4]. Therefore, our study aimed to detect the alloantibody among patients attending Kosti and Eldweem teaching hospitals at White Nile State, Sudan and treated by repeated blood transfusion for at least three or more times.

MATERIALS AND METHODS

Patients and clinical specimens

Across-sectional descriptive study applied at Eldweem and Kosti teaching Hospitals, in Sudan, from February 2019 to August 2019. One hundred and forty-two patients (Age 1-70 years old, Mean Age 38.58±20.85 years old) were recruited in this study. Patients who received multiple blood units three times or more were enrolled without any restriction on gender, age and clinical conditions, whereas patients who received two times or less multiple blood transfusion or who refused to participate were exclude from study population.

ABO blood and Rh factor test

3 ml venous blood sample were collected in Ethylenediaminetetraacetic acid (EDTA) anticoagulant universal tube. ABO blood group and Rh factor tests were applied for all participants. Two clean slides were prepared, and three drops of patients' blood samples were drop on each slide. Three drops of anti-A, anti –B, and anti –D antibodies were then put and mixed with blood drops, respectively. The agglutination reactions were observed and analysed on 40x magnitude objective lens by ordinary light microscope. Indirect Coombs test apply to detect alloantibodies by Polly Specific antihuman globulin reagents using tube method techniques.

Data collection

Data including demographic information, medical history, symptoms, laboratory findings, numbers of blood transfusion were retrieved from the patients' medical records or obtained based on data collection sheet.

Ethical approval

This study had IRB approval from the Research Ethics Committee, Faculty of Medical Laboratory, El Imam El Mahdi University, Sudan. Written informed consent was obtained from all participants according to the Helsinki Declaration principles.

Statistical analysis

The laboratory and demographic data revised, coded, and proceed to SPSS Software (Statistical Package for the Social Sciences) version-25 (IBM Corp., Armonk, NY, USA). Descriptive statistics including distribution of the frequencies, percentage, mean and standard deviation were calculated. The alloantibodies according to the diseases treated by multiple blood transfusions, number of repeated blood transfusion, and according to gender were compared between the groups using One-way ANOVA tests

RESULTS

Patients and clinical information

One hundred and forty-two patients were enrolled in this study. A total of 82 males and 60 females were participated in this study. As expected, the male patients were more than the female patients, however, the Sudanese male individuals are keen to participate in medical studies than female. Most the patients were younger than 50 years with a mean age of 38.58 ± 20.85 years old. Among these, the highest number of patients (n=24, 16%) were aged between 11-20 years old. However, 22.5% of patients (n=32) were aged between 51-60 years old and only 21 patients (14.8%) were over 61 years old. The demographic and clinical characteristics are shown in Table 1. Without any categorization of our patients in this across-sectional descriptive study, renal failure (n=70) and sickle cell anaemia (n=42) were the most common complications among the multi-transfused patients where these patients are frequently on demand of blood transfusion procedures. Blood clinical samples were collected with consent from all participants for profiling the ABO blood group, Rh factor and the presence of RBC alloantibodies. O+ blood group (n=81) were the most common type of blood group in multi-transfused patients. However, A+, B+ and AB blood groups with percentages of 21.9% (n=31), 15.5% (n=22) and 5.6% (n=8), respectively, were less common in our patients (Table 1).

Detection of RBC alloantibodies in blood samples

To assess the presence of alloantibodies against RBC in multi transfused patients, the direct Coomb test were performed for all studied patients. Patients who received two times or less of blood transfusion in their entire life were excluded from our study as mentioned before. Only patients with three-time multiple transfusion or more were being counted. As shown in Table 2, our current results indicated that, out of 142 multiple transfused patients, 31 patients (21.8%) showed detection of RBC alloantibodies, whereas 111 patients (78.2%) were negative for RBC alloantibodies.

Table 1: Demographic and clinical outcomes of multiple blood transfusions patients in study population: Age, gender, blood group distributions, diseases treated by multiple blood transfusions, and frequency of blood transfusion.

Frequency (%)	Category	Parameter
Gender	Male	82 (57.7)*
	Female	60 (42.3)
	Total	142 (100)
Age distributions	1-10 years old	18(12.7)
	11-20 years old	24 (16.9)
	21-30 years old	7 (4.9)
	31-40 years old	13 (9.2)
	41-50 years old	27 (19)
	51-60 years old	32 (22.5)*
	61-70 years old	21 (14.8)
	Total	142 (100)
Blood group distributions	A +	31 (21.9)
	B+	22 (15.5)
	O+	81 (57)*
	AB+	8 (5.6)
	Total	142 (100)
Diseases caused multiple blood transfusions	Renal failure	70 (49.3)*
	Sickle cell Anemia	42 (29.6)
	Other Anemia	20 (14.1)
	Cancer	4 (2.8)
	Diabetes mellitus	3 (2.1)
	Hepatitis	3 (2.1)
	Total	142 (100)

*:Denotes highest frequency

Table 2: Detection of alloantibodies in patients' samples.

Frequency (%)	**Category**	**Parameter**
Number of detected Alloantibodies	31 (21.8)	+ve alloantibodies
	111 (78.2)*	-ve alloantibodies
	142 (100)	Total

*: Denotes highest frequency

Increase frequency of RBC alloantibodies in multiple blood transfusion patients

To assess the relationship between the frequency of the presence of RBC alloantibodies and number of blood transfusion process, we categorize our patients to three groups: patients who had 3-5 times blood transfusion, patients who had 6-8 times blood transfusion and patients who had more than 8 times blood transfusion. In our study population, most patients (n=82) had 3-5-times blood transfusion whereas only 9.2% of patients (n=13) had more than 8 times multiple blood transfusion and the rest of patients (n=47) had 6-8 times blood transfusion. As shown in Table 3, the high occurrence of alloantibodies (5/13(38.5%)) saw among patients who had blood transfusions more than 8 times followed by 6-8 times (11/47 (23%)), and by those having blood transfusion 3-5 times (15/82 (18%)). It is clearly that the frequency of the presence of RBC alloantibodies in patients proportionally increases with the number of blood transfusion procedures.

Increase of alloantibodies according to sex and some medical conditions

To assess the presence of alloantibodies in patients with different clinical outcomes. The demographic data were analyzed in context of frequency of alloantibodies detection. As shown in Table 3, patients with sickle cell anemia represented the highest percentage of frequency of detected alloantibodies (59%), followed by patients with other anemia category who present 12%. On other hand, less than 5% were detected in other clinical conditions (Renal failure, Cancer, Diabetes-mellitus and Hepatitis) patients. Our perspective study also showed the number of males developed alloantibodies were more than females. 22 patients (26.83%) out of the total of 82 participant males showed presence of alloantibodies whereas only 9 female patients (15%) had alloantibodies against red blood cells.

Table 3: Distribution the number of detected Alloantibodies according to the diseases treated by multiple blood transfusions, number of repeated blood transfusion, and according to gender.

Parameter	Category	Number of detected Alloantibodies Frequency (%)	p- value
Diseases treated by multiple blood transfusions	Renal failure	3 (9.7)	0.000*
	Sickle cell Anemia	25 (80.1)*	
	Other Anemia	3(9.7)	
	Diabetes mellitus	0 (0)	
	Cancer	0(0)	
	Hepatitis	0(0)	
	Total	31(100)	
Patients developed alloantibodies regarding number of repeated blood transfusion	3-5 times	15/82 (18)	0.044*
	5-8 times	11/47 (23)	
	More than 8 times	5/13(38.5)*	
	Total	31/142(21.8)	
Patients developed alloantibodies according to gender	Male	22 (71)*	0.000*
	Female	9 (29)	
	Total	31 (100)	

*:Denotes highest frequency

DISCUSSION

Alloantibodies may develop among a various number of patients with multiple blood transfusions. This causes complications in subsequent RBC transfusion therapy such as difficulties in obtaining compatible blood and immune-mediated hemolytic transfusion reactions. When planning the support of transfusion-dependent patients, these immunological complications need to be considered. This study aimed to characterize alloantibody formation within the patient population of White Nile state, Sudan, on those that have had three or more blood transfusions. This was carried out among patients attending Kosti and Eldweem teaching hospitals. Donor-recipient racial discrepancy can be ameliorated since this study was carried out in a racially identical donor-recipient population. 142 patients were enrolled in the study, with a total of 31 samples showing detection of RBC alloantibodies (Table 2). The data showed an overall alloantibody frequency of 21.83% which was consistent with reported rates of 22.7% [18] and in Saudi 22.6% [19]. However, compared with previous international studies, this study displayed a high rate of alloimmunisation in multiple transfused patients, where studies showed rates ranging between 5.5%-7.98% [20-23,10]. Lower rates of alloimmunisation may reflect high phenotypic compatibility between patient and donor. The relatively high rate of alloantibodies in this study could partially be due to the diverse ethnicity in the study patient population. Out of 142 multiple transfused patients, there were 82 males and 60 females. Out of 82 males, 22 (26.83%) showed presence of alloantibodies, out of 60 females, 9 (15%) showed presence of alloantibodies in Tables 1 and 2. This study showed that the rate of alloimmunization was higher in males (26.83%) compared with females (15%). Contrary to this, previous international studies have shown that the rate of RBC alloimmunization was higher in females comparable with males [24,25,10]. This may be biologically plausible because females are exposed to alloantibodies during pregnancy. Other studies have shown no statistical significance between gender and alloimmunization. In this study, the age of multiple transfused patients ranged from 1 year to 70 years with a mean age and SD of 38.58 ± 20.85 years old. Most patients were aged between 51-60 years old (22.5%) followed by those aged between 11-20 years old (16.9%). The rate of alloimmunization may also vary with diseases status. Patients with sickle cell anemia require repeated blood transfusions and this group saw the highest rate of detected alloantibodies (80.6%). Patients with renal failure and other anemia category, both with a detection rate of 9.7% (Tables 1 and 3). Although previous studies have shown that Sickle Cells Dieses (SCD) patients are susceptible to alloantibody formation, lower alloimmunization rates have been reported but this has varied from 8-76%. Expectedly, there was a high occurrence of alloantibodies in this patient population. Those having blood transfusions more than 8 times saw the highest rate of detected alloantibodies (5/13(38.5%)) followed by 6-8 times (11/47 (23%)), and by those having blood transfusion 3-5 times (15/82 (18%)). The findings from Table 2 are consistent with the previous study results shown that the higher number of blood transfusions, the higher the risk of alloantibodies [23].

CONCLUSION

Various factors such as gender, disease status, and the number of blood transfusions contribute to the production of alloantibodies in multiple transfused patients. In future, looking at antibody

specificity would have also been useful to identify the most common alloantibodies in multiple transfused patients. This study has shown that frequently transfused patients in the White Nile state, Sudan are at risk of alloimmunization. Alloimmunization needs to be considered when reviewing repeatedly transfused patients. Identifying alloantibodies and typing beforehand will allow for better patient management. It is therefore essential to introduce a policy for antigen-matched blood in order to reduce the rate of alloimmunization and hemolytic transfusion reactions. However, this presents expenses and high feasibility costs in some medical centers.

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None

CONFLICT OF INTEREST

The authors declared no conflict of interests.

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AUTHORS' CONTRIBUTIONS

Ahmed M Elkhalifa, Dania Z Ahmed and Abozer Elderdery: write the final manuscript, and Supervision; Hadia AM. Ahmed, Mohammed Abd Allah, Shaima E Meirghani and Abdulaziz H. Alhamidi samples collection and analysis; Nada Yassin, Anass M. Abbas and Manar G. Shalabi Supervision and Editing

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