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Frequency and Distribution of RBC Alloantibodies among Transfused Patients at Ndola Central Hospital, Zambia

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Abstract: ***Background And Objectives:*** *Alloantibody formation against red blood cell (RBC) antigens is a common complication of transfusion therapy. The RBC antigens and their alloantibodies vary among different human populations and ethnic groups, and they do have a clinical significance for their adverse immunological reactions. The prevalence of RBC alloimmunization is hardly known in Zambia and hence the need to conduct this study whose main aim was to determine the frequency and distribution of RBC alloantibodies at the Blood Transfusion Unit of Ndola Central Hospital.* ***Materials and Methods****: Using a cross-sectional design, transfused patients at Ndola Central Hospital in Zambia were investigated. Demographic characteristics and transfusion histories were recorded. EDTA blood samples were obtained from the hospital blood bank and RBC alloimmunization was demonstrated using immunohaematological tests.* ***Results:*** *The overall prevalence of alloimmunization was 70 (7.0%). The majority of these had a single alloantibody 55(78.6%), whereas the remaining 15(21.4%) had multiple antibodies. The anti-E antibody comprised the most common alloantibody 19(27.0%) followed by anti K antibodies. Gender, number of blood units and ABO group A were found to be risk factors of alloimmunization in transfused patients.* ***Conclusions:*** *The prevalence of RBC alloimmunization in transfused patients at NCH is high and is mainly associated with the number of donor exposures. Women with multiple pregnancies are especially at risk of alloimmunization. We recommend the introduction of pretransfusion antibody tests in donor and patient's blood.*

Keywords: Blood transfusion, Blood group antigens, alloantibodies, antibody screening, alloimmunization

1. Introduction

Red blood cell (RBC) alloimmunization is an immune response against foreign RBC antigens; this generally occurs after sensitization due to blood transfusions and pregnancies [1] . Regular blood transfusion remains the main treatment for thalassemia, sickle cell anaemia, haematological malignancies & other haematological disorders. The multiple blood transfusions can lead to red cell alloimmunization. Red blood cell alloimmunization results from genetic disparity of RBC antigens between donor and recipients. The development of alloantibodies can significantly complicate transfusion therapy and result in difficulties in cross-matching of blood. Some alloantibodies are haemolytic and may cause, though not invariably, haemolytic transfusion reactions and limit the availability of further safe transfusion. The exact kinetics of alloimmunization is not clear [2,3]. Additionally, RBC alloimmunization investigations are often performed only after transfusion events and many alloantibodies may not be detected as no further transfusions are required or because the titer of antibodies decreases over time and reaches a non-detectable level prior to testing [4]. In cases of patients who require further transfusions and receive an antigen that had already caused sensitization, a much faster secondary immune response may occur with the possibility of a severe haemolytic reaction [5,6,7]. Over the last few years in the United States, irregular RBC alloantibodies have been linked to the majority of fatal haemolytic transfusion reactions reported to the Food and Drug Administration (FDA) and are considered the second main cause of transfusion-related deaths [8].

In Zambia antibody screening and identification tests are not done on donated blood and as a matter of fact typing of blood is only done for blood group ABO and Rhesus D antigens without determining other immunogenic antigens such as E and K antigens. Therefore it's possible that donated blood from alloimmunized blood donors may be given to patients with corresponding antigens and may result in transfusion reactions especially if the antibody titer during the compatibility tests is low to be detected. These facts motivated the present study, whose main objectives were to determine the frequency and distribution of RBC alloantibodies at the Blood Transfusion Unit of Ndola Central Hospital and to determine the risk factors associated with the development of alloantibodies in transfused patients. The knowledge of such alloantibodies that will be detected will be essential for selecting appropriate RBC products for transfusion.

2. Materials and Methods

The study was conducted at Ndola Central Hospital, a third level referral hospital for Copperbelt and Northern part of Zambia. It is located in Ndola, the provincial headquarters of the Copperbelt Province. The hospital has a bed capacity of 851. The study was conducted between January 2013 and March 2014. The purpose of this study was explained to adult patients who had received a blood transfusion at Ndola Central hospital. Post-transfusion blood samples were drawn in EDTA containers from those who consented. A total of 1000 patients were enrolled in the study and ABO and rhesus forward and reverse grouping was done in all participants so as to confirm the blood group. Antibody screening was done using DiaCell I and II reagents

(Diamed-Biorad) [5, 6, 9]. For the samples with positive serological results, the alloantibodies were identified, utilizing a commercial panel of 11 papainized and 11 non-papainized RBCs (Diamed-Biorad) [5, 6, 9].Information on gender, age, type of acute disease or medical emergency, history of transfusion and pregnancies and the number of transfusions were obtained from each participant using a structured questionnaire.

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) software Version 16.0. The study was approved by the University of Zambia research ethics committee.

3. Results

The initial investigations showed that the majority of the study participants were female patients. The mean age of the participants was 38.5 ± 15.5 years old. Of all the records investigated, only 70 patients (7.0%) were found to be positive for alloantibodies to RBC antigens (95% CI: 5.8-9.5). The medical histories were widely distributed, though dominated by pregnancies 25(35.7%) and haemoglobinopathies 16 (22.8%) [Figure1]. Blood grouping results among those who had alloantibodies showed that 25 patients had blood group A, Rhesus positive (35.7 %), 23 patients had blood group B, Rhesus positive (32.8%), 18 patients with blood Group O, Rhesus positive (25.7 %), and 3 patients with blood group AB, Rhesus positive (4.3 %). In addition, there was one patient with blood group O, Rhesus negative 1 (1.4 %) [Table 1] of the 70 patients with alloantibodies, 53 patients (75.7%) had a single alloantibody, whereas 17 patients (24.3%) had multiple alloantibodies [Table 2]. Investigating the alloantibody specificities revealed mostly Anti-E and Anti-K were most prevalent [Table 3].

Figure 1: A bar chart showing sample distribution in participants with alloantibodies according to medical histories

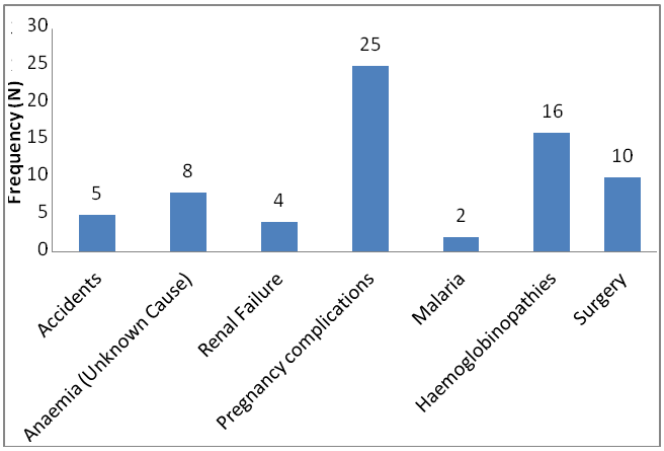

Table 1: Distribution of blood groups among alloimmunized study participants (N=70)

Blood group	Frequency N (%)
O,Rh positive	18 (25.7)
A, Rh positive	25(35.7)
B,Rh positive	23(32.8)
AB,Rh positive	3(4.3)
O,Rh negative	1(1.4)

Table 2: Distribution of RBC alloantibodies based on number of alloantibodies in each study subject

Variable	*N (%)*
Single	55 (78.6)
Multiple	15 (21.4)

Table 3: The distribution of RBC alloantibody types detected

Variable	*Frequency (n)*	*Percentage*
Anti-c	2	2.9
Anti-D	1	1.4
Anti-E	19	27.0
Anti-E +Anti-Jka	1	1.4
Anti-E +Anti-K	7	10.0
Anti-Jkb	2	2.9
Anti-K	18	25.8
Anti-Lewis (a)	7	10.0
Anti-Lewis (a+b)	4	5.8
Anti-Lewis (b)	4	5.8
Anti-M	7	10.0
Total	70	100.0

Chi-square analysis showed that there was an association between gender and the prevalence of alloantibodies with 45(4.5%) of female patients having alloantibodies than the male patients 25(2.5%) respectively (Table 4). Infact the odd of female patients developing alloantibodies was 2.31 times that of male patients with 95% CI (1.31-4.80) (Table 6). History of blood transfusion was also associated with the prevalence of alloantibodies with patients who had received 2 or more units of blood having a higher prevalence of alloantibodies than those who had received less than one unit of blood 60(6.0%) and 10(1.0%) respectively (Table 5). In addition, patients with histories of previous blood transfusions of more than 2 units were 10.51 times more liable to developing alloantibodies compared to those who have had no transfusion or less than one unit of blood transfused. A higher proportion of blood group A-positive patients 25(35.7%) had more alloantibodies than any other ABO blood group. The odds of developing alloantibodies among blood group A patients was twice as compared to patients with other ABO blood groups [Table 6].

Table 4: Association between gender and alloantibody

Gender	*% alloantibody*		*P-value*
	Yes	**No**	
Male	25(2.5%)	516(51.6%)	
Female	45(4.5%)	414(41.4%)	

Table 5: Association between history of blood transfusion and alloantibody

History of transfusion	*% alloantibody*		*P-value*
	Yes	No	
0-1 Units	10 (1.0%)	690 (69.0%)	
≥ 2 units	60 (6.0%)	240 (24.0%)	

Table 6: Factors associated with the development of RBC alloantibodies

Factor	OR	95% C.I
Gender		
Female®	Ref	
Male	2.31	1.31-4.80
History of transfusion		
0-1 Unit®	Ref	
≥2 Units	10.51	6.20-
Blood Groups		
Blood Group (Other than	Ref	
Blood group A	1.99	1.08-.2.36

4. Discussion

In this study the frequency of alloimmunization was higher than that found by other authors [5,10].The prevalence of alloantibodies could have been higher if individuals were not only studied during hospitalization but also followed up to allow sufficient time for the development of alloantibodies[4].This study shows that majority of the study subjects had single rather than multiple alloantibodies of which anti-E was the most common alloantibody found followed by anti-Anti K. The higher occurrence of alloantibodies against antigens of Rh and Kell systems accords most studies. Both systems have highly immunogenic antigens [5,9,10,11]. In a previous study, evaluating patients with chronic and acute diseases, 53.76% and 13.87% of the alloimmunized patients produced antibodies against Rh and Kell antigens, respectively [11]. A Study of Cozac [12] carried out in 722 patients, most of whom had oncohematological diseases and haemoglobinopathies, reported 59.42% and 21.01% of alloimmunization against Rh and Kell antigens, respectively. In other words, the absence of antigen E may render a recipient prone to sensitization by the E antigen that comes from an E-positive donor [13]. This explanation entails the necessity for RBC phenotyping to stop unnecessary sensitization to RBC antigens, and to aid in avoiding unwanted clinical consequences. In this study, as in most other studies, the incidence of alloimmunization among females is more predominant than in male patients, possibly because most of the blood recipients are females, especially those with histories of eventful pregnancies. Hence, immunization through pregnancy could be one main reason for the high incidence of RBC alloimmunization among female patients. [5,6,10]. Santos et al [5] reported a significantly higher rate in women and suggested that the risk of alloimmunization might be influenced by the gender of the recipient, in particular due to gestations. Furthermore, the proportion of individuals who were alloimunized was higher among patients who had haemoglobinopathies probably because most individuals who have these conditions normally are on life-long blood transfusions support and therefore liable to alloimmunization due to exposure to foreign antigens. [2, 14-16].

As expected, there was a higher occurrence of alloimmunization in individuals with history of transfusion. In this study patients who had experienced blood transfusions were found to be more liable to developing alloantibodies than those who never experienced a blood transfusion. Similar findings have been indicated in other works [15, 17].

In our study individuals whose blood group was A were more at risk of developing alloantibodies than other ABO blood groups. The reason could be that among the ABO blood group system, group A has more sub groups than other blood groups within the ABO system. However, the results obtained in this study are in conflict with other studies in which no significance difference was found among various blood groups within the ABO system [5].

5. Conclusions

The prevalence of RBC alloimmunization in transfused patients at NCH was high and mainly associated with the number of donor exposures and pregnancy. The study also emphasizes the need to carry out antibody screening and identification among all the blood donors.

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