

# Red blood cell alloimmunization in multi-transfused patients with chronic kidney disease in Port Harcourt, South-South Nigeria

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## Abstract

**Background:** Serological safety is an integral part of overall safety for blood banks.

**Objectives:** The aim of the study was to determine the prevalence and specificities of red blood cell alloimmunization in multi-transfused patients with chronic kidney disease (CKD).

**Methods:** A cross-sectional case-control study carried out at the University of Port Harcourt Teaching Hospital in which 186 patients with CKD were enrolled consecutively, 124 had received multiple transfusions (more than one unit of blood in one month, or at least 10 units within 3 months), while 62 had never been transfused. Antibody screen test was performed by the gel agglutination technique. RBC antibody identification was performed on the sera of those that tested positive to antibody screening test.

**Results:** Out of the 124 multi-transfused patients (total of 789 transfusions), 4 (3.2%) were alloimmunised. The alloimmunised patients received a higher mean number of  $17.5 \pm 12$  blood units, compared to  $6 \pm 6$  units by the non-alloimmunised multi-transfused patient ( $p = <0.001$ ). Six clinically significant alloantibodies were identified with all of the alloimmunised patients forming more than one antibody. Anti-E was detected in all alloimmunised patients.

**Conclusion:** The prevalence of RBC alloimmunisation in multi-transfused CKD patients was 3.2% with anti-E being the most frequently identified antibody.

**Keywords:** Red blood cell alloimmunization, chronic kidney disease, Port Harcourt, South-south Nigeria.

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## Introduction

Blood transfusion is an integral part of the management of patients with chronic kidney disease (CKD) as severe anaemia is a feature of the late stages<sup>1</sup>. Chronic Kidney disease (CKD) is defined either as kidney damage/injury for  $\geq 3$  months and/or glomerular filtration rate (GFR)

$< 60\text{ml/min per } 1.73\text{m}^2$  for  $\geq 3$  months with or without kidney damage. It is usually accompanied by features of uraemia, and a need for renal replacement therapy (which includes haemodialysis and/or kidney transplant) in its later stages.<sup>2,3,4</sup> Management of renal anaemia include the use of erythropoiesis-stimulating agents (ESAs), blood transfusion as well as replacement of iron and other nutritional supplements. The decision to treat a patient with either an ESAs or blood transfusion is made on the threshold for transfusion. A high threshold includes a low haemoglobin level, usually less than  $10\text{g/dl}$  or the presence of cardiac decompensation.<sup>3,4</sup> In severe anaemia, immediate management may be blood transfusion to correct the anaemia in the short term. However, blood

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transfusion especially when given repeatedly may increase the risk of red cell alloimmunization.<sup>5</sup>

Red cell alloimmunization, is a humoral immune response which occurs when antibodies bind to foreign red cell antigens due to antigenic disparity between donor and recipient. Red cell alloimmunization may be induced by blood transfusion, pregnancy or organ transplantation. The consequences include delayed haemolytic transfusion reaction, difficulties in getting compatible blood for future transfusions, and a delay or failure in getting a kidney transplant.<sup>6,7</sup> The incidence of red cell alloimmunization ranges between 1-6% in single-transfused and up to 30% in multi-transfused patients (e.g, sickle cell disease, thalassaemia, haematological malignancies and ESRD).<sup>8,9,10,11,12</sup> In Nigeria, where multiple ethnicities contribute to genetic heterogeneity among the population, a wide variety of antibodies are encountered. A study in Northern Nigeria reported a red cell alloimmunization prevalence of 8.8% in multi-transfused patients with sickle cell anaemia.<sup>13</sup>

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<sup>14</sup>. Further studies have shown that prospective organ transplant recipients who received blood transfusions have an increased chance of having a high panel-reactive antibodies (PRA) score of greater than 80% compared to those who have never received blood transfusion. PRA is defined as the percentage of human leukocyte antigen (HLA) antigens singly or in combination out of a panel reacting with the patient's serum and may reflect the percentage of donors expected to react with the patient's serum. This finding has been associated with a longer wait to find a compatible donor and have completely precluded transplantation in some patients.<sup>3,15</sup>

Pre-transfusion compatibility testing (PCT) in this environment does not include antibody screening and titration which determines the patient's antibody status as well as the severity of alloimmunization when present. Identification of the offending antibody can prevent further development of red blood cell alloantibodies as the patient is subsequently transfused with blood units free of the offending antigen.

Therefore, the main objective of this study was to assess the prevalence of red blood cell alloimmunization in multi-transfused patients with CKD in Port Harcourt, Nigeria.

## Methodology

### Study population

The study population consisted of 186 adult patients with chronic kidney disease defined by patients who have GFR  $<60\text{ml}/\text{min}/1.73\text{m}^2$  for  $\geq 3$  months<sup>1</sup>, calculated using the modified diet in renal disease (MDRD) formula who had received multiple blood transfusions (more than one of blood within one month or at least 10 units of blood within 3 months), and adult patients with chronic kidney disease who had no history of blood transfusion. The study population were recruited consecutively from the nephrology out-patient clinic, medical outpatient clinics, as well as medical inpatients in UPTH.

Ethical approval was obtained from the hospitals' Ethics Committee and written consent was obtained from every patient before recruitment into the study. All the patients were routinely screened for HIV.

**Inclusion criteria for the cases:** Patients with CKD who were eighteen years and above; who had received multiple transfusions (more than one unit of whole blood within one month or at least 10 units of whole blood within 3 months).

**Exclusion criteria for the cases:** Patients with chronic kidney disease who were HIV positive and those with a history of kidney transplant.

**Inclusion criteria for the controls:** Patients with chronic kidney disease who were eighteen years and above; who have never been transfused.

**Exclusion criteria for the control population:** Patients with chronic kidney disease who had had a kidney transplant; and those who were HIV positive.

**Sample analysis:** Two milliliters of venous blood was collected into plain Vacutainer® bottles for Antibody screening, Antibody identification and titer determination. The serum was separated and stored in the freezer at  $-20^{\circ}\text{C}$  and analyzed weekly. The stored sera were thawed

and used for antibody screening by gel technology using commercially made panel of cells – “ID-Diacell I-II-III®” lot number 45184.91.x for antibody screening and set of 11 “ID-DiaPanel®” Lot number 45161.40.x for antibody identification manufactured by Diamed GmbH, Pra Rond 23, 1785 Cressier FR, Switzerland.

**Antibody screening:** Antibody screening test was performed on all study participants. Antibody screening test is not routinely done at UPTH as part of pre-transfusion testing. The recipient’s serum was combined with group O reagent red cells to allow for antigen/antibody interaction in the upper chamber of the microtube containing antiglobulin and low ionic strength saline (LISS) reagent. They were incubated at 37°C. The gel card method is based on the principle of differential passage of agglutinated and free red cells through a dextran-acrylamide gel microtube column during controlled centrifugation. Agglutinated red cells become trapped in or above the gel. Unagglutinated red cells travel through the gel particles and form a pellet at the bottom of the microtube as shown in figure 1<sup>17</sup>.

Three panel of reagent red cells were used for antibody screening, and the antigenic specificity of the reagent red cells used included Rh (D,C,E,c,e,Cw); Kell (K,k,Kpa,kp-b,Js,a,Js,b); Duffy (Fya, Fyb); Kidd (Jka,Jkb); Lewis (Lea,-Leb); P (P1); MNS (M,N,S,s); Lutheran (Lua,Lub); and Xg (Xga).

Red cell antibody identification was done using an anti-gram provided by the manufacturer. Eleven panel of reagent red cells were used for antibody screening with the above antigenic specificity.<sup>18</sup>

**Antibody titration:** Eleven tests tubes were labelled according to the serum dilutions: 1, 2, 4, 16, 32, 64, 128, 256, 512, 1024 and 2048. After preparing the master dilutions, titration was done using the gel cards following the same procedure above.<sup>19</sup> To each of the gel card column, 50µL of well mixed reagent cells (for which the patients’ serum tested positive during the antibody screening) was added. This was followed by 25µL of diluted serum sample. Incubation was done at 37°C and the gel cards were thereafter read using the Banjo® reader

**Statistical analysis:** The 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 186 patients with chronic kidney disease participated in the study. They comprised of 124 patients with history of multiple blood transfusions and sixty-two (62) patients without a history of blood transfusion. The cases and controls were well-matched for sex and age as shown in table 1.

**Table 1: AGE AND SEX DISTRIBUTION OF PATIENTS WITH CHRONIC KIDNEY DISEASE**

AGE RANGE (YEARS)	MULTI-TRANSFUSED CKD PATIENTS (n=124)		NON-TRANSFUSED CKD PATIENTS (n=62)	
	MALE (n/%)	FEMALE (n/%)	MALE (n/%)	FEMALE (n/%)
<20	5(7.4)	0	0	0
20-29	7(10.3)	9(16.1)	2(5.3)	3(12.5)
30-39	6(8.8)	15(26.8)	7(18.4)	5(20.8)
40-49	20(29.4)	7(12.5)	14(36.8)	9(37.5)
50-59	14(20.5)	13(23.2)	2(5.3)	3(12.5)
>60	16(23.5)	12(21.4)	13(34.3)	4(16.6)
<b>Total</b>	<b>68(100)</b>	<b>56(100)</b>	<b>38(100.0)</b>	<b>24(100.0)</b>

Among the multi-transfused patients, the mean serum creatinine and urea were  $906.2 \pm 631.2$  mmol/l and  $28 \pm 9.8$  mmol/l respectively compared to  $608.9 \pm 392.9$  mmol/l and  $20.4 \pm 12.4$  mmol/l respectively for the controls. The differences in creatinine and urea in the subjects and controls were not statistically significant as shown in table 2. The mean estimated GFR for the subjects was 24 mls/min/1.73 m<sup>2</sup> compared to 62 mls/minute/1.73 m<sup>2</sup> for the controls. This value was statistically significant (p-value <0.005).

Seven hundred and eight-nine [789] units of blood were received by the multi-transfused patients. The number of units transfused per patient ranged from 2 to 34 with a mean and standard deviation of  $6 \pm 6$  units.

**Prevalence of red cell alloimmunization:** Antibody screening was positive in five of the multi-transfused CKD patients. None of the controls had a positive antibody screen. Alloantibodies were identified in four of the multi-transfused patients and one of them had an autoantibody.

TABLE 2: Baseline characteristics of patients with chronic kidney disease.

VARIABLE	MULTIPLY TRANSFUSED CKD PATIENTS	NON-TRANSFUSED CKD PATIENTS	P-VALUE
WEIGHT (KG)	68.7 ± 14.5	77.8 ± 12.5	0.112
HEIGHT (CM)	170.8 ± 12	171.2 ± 13	0.904
Creatinine (µmol/L)	906.2 ± 631.2	608.9 ± 392.9	<0.0001*
Urea (mmol/L)	28 ± 9.8	20.4 ± 12.4	<0.0001*
eGFR (mls/min/1.73m <sup>2</sup> )	24	26	<0.0001*

eGFR: estimated glomerular filtration rate.

Values are expressed as mean ± SD

\*Indicates difference is statistically significant (p<0.05)

The alloimmunized multi-transfused patients had a higher mean number of units transfused ( $17.5 \pm 12$  units) compared to the non-alloimmunized multi-transfused patients which had a mean of  $6 \pm 6$  units, and this was statistically significant (p= 0.001).

Among the subjects, females had a higher alloimmunization rate of 2.4% (n = 3) compared to the males 0.8% (n=1). Females were 2.2 times more likely to develop alloantibodies than the males, however this association was not significant (odds ratio = 2.2, 95% Confidence Interval = 0.193-26.154. Of the 3 females who were alloim-

munised, one was nulliparous while the other two were multiparous.

**Frequency, specificity and titre of red cell alloantibodies:** A total of 6 alloantibodies were detected in the alloimmunised, three patients developed double alloantibodies while one developed triple alloantibodies [table 3]. Anti E was detected in all four multi-transfused alloimmunised patients while the other antibodies detected were anti Jka, anti C, anti D, anti Fya and anti M respectively [table 3].

Table 3: Characteristics of alloimmunized multi-transfused chronic kidney disease patients.

S/NO	AGE	SEX	TRANSFUSIONS RECEIVED (N)	ALLOANTIBODIES IDENTIFIED (N)	ANTIBODY SPECIFICITY
1	40	Male	9	2	Anti-Jka, Anti-E
2	38	Female	10	2	Anti E Anti- Fya
3	32	Female	34	2	Anti-E Anti-M
4	22	Female	17	3	Anti-C Anti-E Anti-D

Figure 1: Antibody screening using the gel card

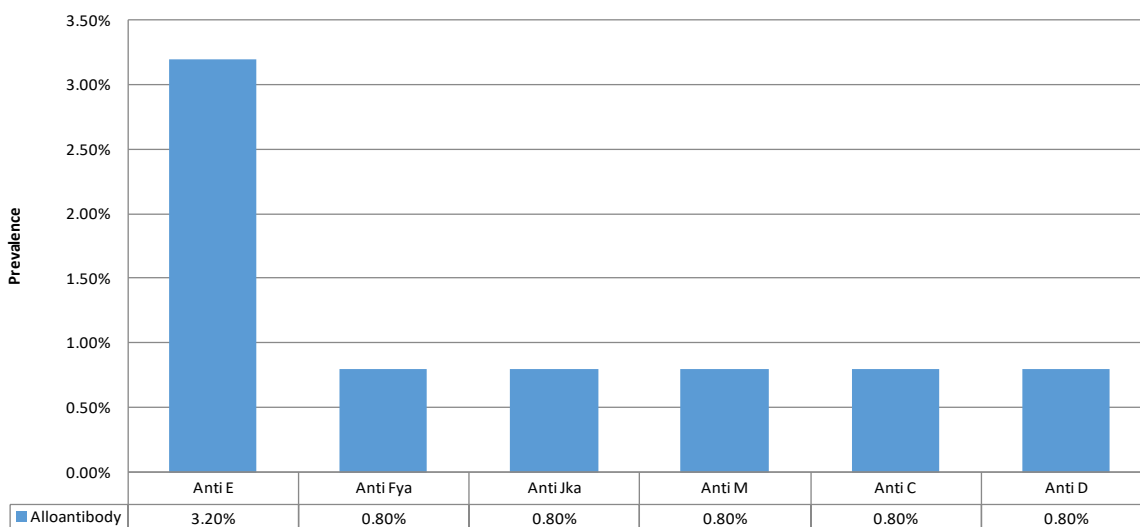


The frequency of anti E among the multi-transfused ESRD patients was 3.2% while the other antibodies each showed frequency of 0.8% each as shown in figure 2. Two of them had a titre of 1024 while the others had titres of 512 and 32 respectively. The multi-transfused pa-

tients with the highest titre developed antibodies to anti E, anti- M and anti Fy.a

The risk of alloimmunization per unit of blood, defined<sup>19</sup> as the total number of alloantibodies detected (6), divided by the total number of transfused units (789) multiplied by 100, was 0.76% for the multi-transfused CKD patients.

**Figure 2: Prevalence of alloantibodies among multi-transfused chronic kidney disease patients**



### Discussion

Blood transfusion may be life-saving in chronic kidney disease (CKD) as it promptly improves the oxygen-carrying capacity in patients with symptomatic anaemia. However, transfusion-transmissible infections (TTI) and alloimmunisation are potential complications especially in multi-transfused patients. In our study, there were more males with CKD compared to females similar to the findings of Iseki et al.<sup>20</sup>

The prevalence of red cell alloimmunization among multi-transfused patients with CKD in this study was 3.2%. None of the controls had alloantibodies. This prevalence is lower than that reported by Shukla et al.<sup>21</sup> (9.8%) and Babiker et al.<sup>22</sup> (13.1%). The difference could be due to varied geographical locations of the studies as prevalence of antigens varies with geographical locations.<sup>23</sup> A higher prevalence may also have been seen in this study if serial antibody screening tests were done on these study participants. Red blood cell non-ABO blood group alloantibodies has been shown to evanesce over time confounding compatibility testing and predisposing patients to delayed haemolytic transfusion reactions.<sup>23</sup> The mean number of blood units transfused in this study was significantly higher in individuals that developed RBC alloantibodies. This might be because the multi-transfused patients were exposed to more donors with different genetic makeup. Natukunda et al.<sup>12</sup> and San-

tos et al.<sup>24</sup> also reported higher prevalence of alloantibody formation in recipients who received the more transfusions. The mean number of blood units received by the cases was  $17.5 \pm 12$ , which is in concordance with reports that multi-transfused patients who developed received 16 to 25 units of blood.<sup>24,25</sup> The risk of alloimmunization in this study was 0.76%, and this is within the reported range of 0.5 to 5.9% seen in the literature.<sup>24,25,26</sup>

In our study, the rate of alloimmunization was not significantly influenced by sex which is contrary to what was reported by Babiker et al.,<sup>27</sup> Silvia et al.<sup>28</sup> and Zaman et al.<sup>29</sup> who found a higher prevalence in women, but similar to the findings of Shukla et al.<sup>30</sup> We also observed that all the alloimmunised patients developed multiple antibodies contrary to the findings of Shukla.<sup>30</sup> This may be attributed to genetic differences, and lack of routine pretransfusion antibody screening test in this environment which increases the tendency to develop an additional alloantibody after subsequent transfusions.

In concordance with other studies, the most frequent red cell antibodies in our study were against the E antigen (figure 2) of the Rh blood group system.<sup>31,32,33</sup> The frequency of the E antigen is generally low in blacks (about 21%), as such multi-transfused patients who were hitherto lacking the E antigen are likely to develop anti-E from transfusions.<sup>34</sup> In addition, anti-E has been found

to be the commonest alloantibody during routine screening and this is probably because half the anti-E are weak, naturally occurring Ig M antibodies<sup>35</sup>. A study in Kano, Nigeria showed a low distribution of Rh E (34%), which further suggests a higher likelihood of recipients developing alloantibodies to it.<sup>36</sup>

The other identified antibodies which showed the least combination were anti-M, anti-Jka and anti-Fya and this is similar to other reports.<sup>37</sup> Anti M is a naturally occurring cold antibody and is often not reactive at 37°C and with anti-human globulin [AHG] reagent, except when present in very high titre.<sup>38</sup> However, a small percentage of anti-M is of the immune type and can elicit haemolytic transfusion reactions.<sup>39</sup> Studies show that two-thirds or more of blacks are negative for Duffy antigen Fy (a-b-), and in Nigeria, the prevalence of anti-Fya is about 4.3%.<sup>39</sup> Similarly, the prevalence of anti JKa was reported as 4.9% in Nigeria.<sup>40</sup>

The highest antibody titre observed in this study was 1024 while the least was 4. One of the multi-transfused patients developed an autoantibody and this can lead to shortened red cell life span, predisposing the patient to further transfusions<sup>28</sup>. One of the multi-transfused patients that was Rh D positive, developed antibodies to the Rh D antigen. This suggests that this patient might have had a partial D. Persons of African origin have been reported to show the presence of numerous Rh variants.<sup>41,42</sup> In addition, patients in this environment are not usually phenotyped for the partial-D variant; hence there may be a propensity to develop antibodies to the Rh D antigen. Studies have shown that HLA alloimmunization is associated with RBC antibodies in multiply transfused sickle cell patients. This may result in graft rejection during stem cell or organ transplantation.<sup>43</sup>

### Conclusion

This study showed that 3.2% of the multi-transfused patients with 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.

### Conflict of interest

Authors have declared there are no conflicting interests.

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