Prevalence of Clinically Significant Alloantibodies among Transfusion Requiring Patients in a Tertiary Hospital in Sokoto, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IZI, EO, TCA and SAI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MI, HMA and MM managed the literature searches, analyses of the study and performed the spectroscopy analysis. Authors IZI, RTJ and FPU managed the experimental process. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: No data are available regarding the frequencies of clinically significant alloantibodies among patients requiring transfusion in Sokoto North western Nigeria. We intend to provide information on the prevalence of clinically significant alloantibodies and their specificity among patients that required blood transfusion in this region.

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Study Design: In this present cross sectional descriptive study, 229 patients consented to the study, with median ages of 26.5 years, 24.0% were males while 174 (76.0%) were females. There were 11 patients who had sickle cell disease (SCD), 10 patients had cancers (Ovarian 4, Bladder 3 and Cervical 3), while other were admitted for various diseases but pregnancy complications predominates. The study was carried out between the months of September and October 2015.

Materials and Methods: Three millilitres of whole blood was collected from each subject into an EDTA anticoagulated tubes. The plasma was collected screened for the presence of clinically significant alloantibodies by Ortho Biovue system cassettes (AHG/Coombs) technique using the Lorne Laboratories of UK antibody screen cells and panel cells.

Results: The result indicated that 14.85% of the patients had clinically significant alloantibodies in their plasma of which 64.71% were female and 35.29% were male. 100% of anti-D and anti-c and 50% of anti-E were identified in female. Anti-f, anti-Jka, anti-Leb, anti-Fyb, anti-Pi and anti-Kpa each 0.44% were identified in female plasma, anti-C^w was found in 0.44% in male while about 9.17% of the alloantibodies could not be identified. We observed that 6 out of 23 patients with chronic diseases developed alloantibodies.

Conclusion: We concluded that the prevalence of alloantibodies among the studied population was high. However, 9.17% of the formed clinically significant alloantibodies in these patients cannot be identified by the known panels of cells made from the Caucasians population. It could be that unknown antigens exist in this part of the world.

1. INTRODUCTION

Alloimmunization to red cell antigens is still a current problem in many settings in sub Saharan Africa [1]. In most hospitals, alloantibodies screening of recipient plasma are not available [1]. Red blood cell alloimmunization results from genetic disparity of red cell antigens between donor and recipients [1]. Alloimmunizations are the most important complication of transfusion and are significant especially when it involves a clinically significant alloantibody that causes haemolytic transfusion reaction (HTR) as in transfusion, haemolytic disease of the foetus and newborn (HDFN) as in pregnancy and in transplantation, red blood cell alloantibodies may raise the risk of haemolytic reactions, delayed engraftment and pure RBC aplasia [2]. It is very important that red cell antigens be correctly, and some of them routinely, typed in blood donors as well as in patients [3].

Alloantibody testing of transfusion recipients to ensure that they receive red cells negative to alloantibodies they might have developed is the best practice in managing transfusion complication and guarantee a safe transfusion therapy. This is often lacking in most settings in Africa [1], these factors all complicate transfusion practice in this region. The aim of this study therefore, was to determine the prevalence of clinically significant alloantibody among subjects indicated for red cell transfusion. The result from this study will provide an evidence based data on the prevalence of clinically significant alloantibody and autoantibodies among subjects indicated for red cell transfusion which might have developed as a result of either pregnancy related issues, transfusion events or autoantibodies. It is hoped that the information from this study will guide in policy formulation regarding blood transfusion practice in Sokoto, Nigeria.

1.1 Study Design and Study Site

The study was a descriptive cross-sectional study to determine the prevalence of clinically significant alloantibodies among subjects requiring a red cell transfusion in a specialist hospital Sokoto. Sokoto is the capital city of Sokoto state, located in the extreme Northwest of Nigeria. The median age was 26.5 years, 24.0% were males while 174 (76.0%) were females. There were 11 patients who had SCD and 10 patients who had cancers (Ovarian 4, Bladder 3 and Cervical 3). About 113 were admitted for pregnancy related anaemia and complications, 51 were admitted for medically related issues, 21 were admitted for surgical procedure and 44 were pediatrics.

2. MATERIALS AND METHODS

Three milliliters of whole blood was collected and the plasma was screened for the presence of

clinically significant alloantibodies by Ortho Biovue system cassettes (AHG/Coombs) technique as follows:

i. Each 50 ul of suspension of ALBAcyte CAT reagent red cells (1, 2 and 3) for antibody screening and 40 ul of the patient's serum was dispensed into the appropriate Ortho Biovue system cassettes (AHG/Coombs) reaction cambers.
ii. The cassette was incubated for 15 minutes at 37°c.
iii. The cassette was centrifuged for 5 minutes in an Ortho Biovue system centrifuge.
iv. The result was read macroscopically for red cells trapped at the top or middle of the micro tube which indicated agglutination, while when all the red cells settles at the bottom of the micro tube indicated Negative result.

Patients whose antibody screen is positive was tested against antibody identification panel cells (AHG and Enzyme panels) for effective identification of the specificity of the alloantibody.

2.1 Ortho Biovue Technique for Antibodies Identification in the Serum

i. Each 50 ul of suspension of ALBAcyte CAT papainized and nonpapainized red cells (1, 2 …11) for antibody identification and 40 ul of the patients' serum was dispensed into the appropriate Ortho Biovue system cassettes (AHG/Coombs) 11 reaction cambers.
ii. The cassette was incubated for 15 minutes at 37°c.
iii. The cassette was centrifuged for 5 minutes in an Ortho Biovue system centrifuge.
iv. The result was read macroscopically for agglutination.

2.2 Interpretation of Results

Generally, the presence of agglutination indicates a positive result while non-agglutination indicates a negative result. However the following characteristics and rules were followed in the identification of the alloantibodies or autoantibodies present in a serum. An antigram and an antibody panel which included at least 11 panel papainized and nonpapainized red cells obtained from group O red blood cells (since O cells lack ABO blood group antigens to prevent interference by group specific antibodies) were used. The bases in antibody identification are

that an antibody will only react with cells that *have* the corresponding antigen (antibodies will not react with cells that *do not have* the corresponding group specific antigen).

There were a few basic steps which were followed in interpreting panels for alloantibody identification. These include:

• Ruling out or crossing out antigens that did not react (Cross out antigens that show no reaction in any phase making sure that not crossing out heterozygous antigens that show dosage).
• Circling the antigens that are not crossed out.
• Looking for a matching pattern that enabled me conclusively identify the antibody.
• The rule of three was also met to *confirm* the presence of the antibody. That is Patient serum must; show a positive reaction with at least 3 cells with the antigens on the panel cell and show a negative reaction with 3 cells without the antigen on the panel cell.

2.3 Ethical Approval and Informed Consent

Ethical clearance was obtained from the ethical committee of the Specialist hospitals, Sokoto. While written informed consent was sought from all adult participants and from parents or guardians of underage patients in this study.

2.4 Data Analysis

The data obtained were presented in tabular forms and in proportions as the case may be, and Hypothesis was tested with statistical software (SPSS version 20) at 0.05 significant levels and 95% confidence using the 'Pearson's Chi-square test'.

3. RESULTS

Table 1 shows the prevalence of alloantibodies and their specificities among patients. It indicates that 5.24% male had alloantibodies in their plasma while 9.61% female had formed alloantibodies in their plasma, summing up to 14.85% prevalence of alloantibodies among the patients. We observed that 0.87% female had anti-D, 0.44% male and 0.44% female had anti-E, 0.87% female had anti-c, anti-f, anti-Jka, anti-

Leb, anti-Fyb, anti-Pi and anti-Kpa each 0.44% was found in female plasma, anti-C^w was found in 0.44% in male, about 9.17% of the alloantibodies could not be identified. Table 2 shows 'Pearson's Chi-square test' for age and alloantibody formation. The X^2 value was found to be 10.774 with 5 degree of freedom and the *p* value of 0.056 obtained was not statistically significant. All the variances shows no significant differences as shown in Table 3. In this study, 11 patients had SCD of which 2 developed alloantibodies and 10 had cancers (Ovarian 4, Bladder 3 and Cervical 3) of which 4 developed alloantibodies. These were the ones we classified as chronic diseases and most of them had more than two units of red cell transfusion. We observed that 6 out of 23 of the patients with chronic diseases developed alloantibodies with immunization rate of 26.09 while 28 out 206 of patients with other diseases had alloantibodies. However, the development of alloantibodies and chronic diseases showed no statistical significant difference, P = 0.110 (see Table 3). Alloantibodies prevalence among various departments was found to be statistically significant, 'Pearson's Chi-square test' value was 11.126 and p-value of 0.011.

Table 1 shows the prevalence of alloantibodies and their specificities among patients. 4.37% and 4.80% of unknown alloantibodies was found in male and female respectively,

4. DISCUSSION

At the end of this present study, we observed an overall prevalence of alloantibodies of 14.85% (5.24% males and 9.61% females). This finding is consistent with that found among sickle cell disease patients in Saudi Arabia as reported by Bashawari [4]. In his study, 48 out of 350 SCA patients (13.7%) formed clinically significant alloantibodies. Our obtained value of 14,85% is also within the range of the alloimmunization rate of sickle cell patients reported in literature [5]. But the prevalence of alloimmunization in our study is much higher than the prevalence of allantibodies reported in other studies done in Nigeria: 8.8% in Kano [6] and 3.2% in Lagos [6], Sourthern part of Nigeria; [7] in Uganda [8] and in Zimbabwe [9]. The lower values reported in the studies were studies mostly carried out among antenatal women and may have resulted from pregnancy related issues, while our study was carried out on heterogeneous population composing of children, adult, pregnant women and transfusion dependent patients. It has been reported that recipient sex and age, history of pregnancy, number and timing of blood transfusions, recipient clinical diagnosis and treatment, genetic factors related to the antigenic response, and racial differences between donors and recipients [10]. Transfusion dependent (chronic diseases) diseases and pregnancy are usually characterized by high alloimmunization frequencies.

Table 1. Prevalence of alloantibody and their specificity among the patients

	Gender		Total
	Male	Female	
Anti-D	0	2	2
Anti-E	1	1	2
Anti-c	0	2	2
Anti-f	0	1	1
Anti-Jka	0	1	1
Anti-Leb	0	1	1
Anti-Fyb	0	1	1
Anti-Pi	0	1	1
Anti-Kpa	0	1	1
Anti-C^w	1	0	1
Unknown antibodies	10	11	21
Total	12(5.24%)	22(9.61%)	34(14.85%)

Table 2. 'Pearson's chi-square test' for age and alloantibody development

Age group	Alloantibody		Total	X^2	df	*P*
	Pos.	Neg.				
< 10	9	26	35	10.774	5	0.056
11 – 20	6	34	40			
21 – 30	15	64	79			
31 – 40	4	42	46			
41 – 50	0	11	11			
≥51	0	18	18			
Total	34	195	229			

'Pearson's Chi-square test' for age and alloantibody formation. The X^2 value was found to be 10.774 with 5 degree of freedom and the p value of 0.056 obtained was not statistically significant

Sensitivity (semi-automated gel technology) of the method used in this study as opposed to manual method used in other studies may be another reason. The use of Coombs reagents with enzymes treated cells which were used in this study enhances reactivity of sensitive antibodies such as Rh, P, I, Kidd and Lewis, explaining the higher detection rate of the antibodies as compared with other studies, given a true prevalence of alloantibodies compared to manual tube method. The results obtained using these reagents were; 12.5%, using Coombs reagent with enzymes, compared to Coombs

reagents only 0.8% and enzymes only 9.2%, which implies a variation in reactivity.

Our finding is however, lower than other findings among SCD patients around the world such as; 29% in US, 21% in UK, 30% in Oakland US, 12.0 and 41% respectively in two separate reports from Brazil and 18.7% in Enugu, Nigeria [11,12,13]. We attributed the reason for the higher alloantibodies prevalence in the above studies as being carried out on SCD patients, a chronic disease that is transfusion dependent and is usually characterized with higher risk of alloimmunization frequency. In this study, 11 patients had SCD (six of them below the age of seven) of which 2 developed alloantibodies and 10 had cancers (Ovarian 4, Bladder 3 and Cervical 3) of which 4 developed alloantibodies. It thus indicated that 6 out of 21 of the patients with chronic diseases developed alloantibodies giving an alloimmunization rate of 0.29 while 28 out 208 of patients with other diseases had alloantibodies giving an alloimmunization rate of 0.14. Therefore, the immunization rate of chronic disease in our study is two times more than in

other diseases. This is because these patients often receive more than one unit of red cell transfusion. However, the development of alloantibodies and chronic diseases showed no statistical significant difference, P = 0.110 (see Table 3), and this was attributed to the small number of patients with chronic diseases. 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 [14]. A Study of Cozac [15] 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. We also observed that Obstetrics and Gynaecology (O&G) had the highest alloantibodies prevalence. Surgical and paediatric departments had 6 patients each with alloantibodies in their plasma out of 21 and 44 patients respectively. The alloantibodies prevalence among various departments was found to be statistically significant, 'Pearson's Chi-square test' value was 11.126 and p-value of 0.011 (see Table 4).

Table 3. 'Pearson's chi-square test' for transfusion event, pregnancy, miscarriage and stillbirth statuses on the development of alloantibody

	Alloantibody		Total	Pearson's Chi square test value	Degree of freedom	p-value
	Positive	Negative				
Transfusion event						
Yes	24	143	176			
No	10	52	62	0.110	1	0.740
Nature of disease						
Chronic disease	6	17	23			
Non chronic disease	28	178	206	2.555	1	0.110
Pregnancy status						
Yes	25	127	152			
No	9	68	77	0.916	1	0.339
Miscarriage status						
Yes	16	49	65			
No	18	146	164	6.850	1	0.009*
Stillbirth status						
Yes	11	31	42			
No	23	164	187	5.235	1	0.022*

'Pearson's Chi-square test' for transfusion event status, nature of disease pregnancy status, miscarriage status and stillbirth status and alloantibodies formation. The p-values obtained were 0.740, 0.110, 0.339, 0.009 and 0.022 respectively.
* = statistical significant differences

Table 4. Alloantibodies prevalence among various departments

Departments	Alloantibody		Total
	Positive	Negative	
O & G	21	92	113
Medical	1	50	51
Surgical	6	15	21
Paediatrics	6	38	44
Total	34	195	229

Obstetrics and Gynaecology (O&G) had the highest alloantibodies prevalence and the least was Medicine

We also observed that out of the 34 that developed alloantibody, 64.71% were female. In this study, we also shoed like most other studies, has shown that the incidence of alloimmunization among females is more predominant than in male patients, possibly because most of the patients were females, Women also are believed to receive more blood recipients than the males as a result of pregnancy related complications, pregnancy itself is an alloimmunaztion event and can also be attributed to the high prevalence among females, especially those with histories of eventful pregnancies [16,17,18,19]. Santos et al. [17] 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 alloimmunized 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 [20,21,22].

Alloimmunization in pregnant women had been found to range from 0.4% to 2.7% worldwide [23,24,25]. It has been reported that 1.5%– 2% of pregnant women shows atypical blood group sensitization. Verduin et al. [26] review showed that women with sickle cell disease were at a higher relative risk (27.0%) of developing RBC alloimmunisation compared to men with the same disease. Other groups of patients, also showed that the risk of RBC alloimmunisation appeared to be similar among men and women. However, they observed that RBC antibodies were slightly more present in women [26]. Saverimuttu et al. [27] evaluated 27,968 RBC antibodies screens from 15,966 patients and found 457 patients with RBC alloimmunisation (2.9%) and among them 304 (1.9%) had clinically significant RBC antibodies. According to their observations the prevalence of RBC antibodies increased with age and was higher in females.

We therefore recommend that antibody screening for these alloantibodies in antenatal women and the routine administration of pregnant women with anti-D immunoglobulin as done in the developed world be implemented to prevent HDFN.

The specificity of the alloantibodies found in our study as shown in Table 1, indicated that 0.87% female had anti-D, 0.44% male and 0.44% female had anti-E, 0.87% female had anti-c, anti-f, anti-Jka, anti-Leb, anti-Fyb, anti-Pi and anti-Kpa each 0.44% was found in female plasma and anti-C^w was found in 0.44% in male. Our findings also indicated that all the anti-D and anti-c and 50% of anti-E all of which are alloantibodies against the Rh red cell antigens were found in female. Koelewijn JM et al. [28]. In the Netherlands, observed a prevalence of 1.232% of all, 0.328% of non RHD patients; Al-Ibrahim et al. [29] in Saudi Arabia, observed the prevalence of 1.92% with 52.38% of the Rh group, 2.38% Kell, 2.38% Kidd, 2.38% Lewis, 2.38% Duffy, 4.76% non-specific, 33.33% autoantibodies. Gottvall et al. [30] in Sweden observed the prevalence of 0.4% with Anti D-60%, Fya-10%,c-7%, K-4%, Lurie et al. [31] in Tel Aviv, Israel observed the prevalence of 0.2%; RHD negative women 0.9%, and reported that routine antibody screen in Rh positive women is not warranted, so was the report of Lee et al. [32] in China, who observed the prevalence of 0.79% Clinically significant 0.27%, Anti Mi -57.6% Anti E -19.7% and conclude that routine antenatal antibody screening for Chinese women may not be worthwhile. Sangeeta et al. [33] in Delhi, India observed the prevalence of 1.25% RhD contributed to 78.4% of all the antibodies formed.

The type of blood donation been practiced in this locality may also be another reason for the high prevalence among patients indicated for transfusion. A report on the pattern of blood donation in the locality where this study was carried out, indicated a predominance of family replacement where the relation insists in the use of the blood donated by their relation. This can potentially cause alloimmunization of women who receives blood from spouse or spouse's relation if she lacks an antigen present in the spouse or spouse relation's blood, this can also increase the risk of haemolytic disease of newborn (HDN) in pregnant women. Aside from all this, it is noteworthy that transfusion practiced in Sub-Saharan African is plagued with lots of problems which include but not limited to; poor infrastructure, lack of effective functional national

blood transfusion service, lack of adequately trained and qualified laboratory staff, inter-professional rivalry in the health sector, reliance on poor and insensitive tube techniques and above all absence of both pre and post transfusion testing of patient and donor blood for clinically significant antigen and alloantibodies.

In this study also, about 9.17% of the alloantibodies could not be identified in this our study. We attributed the reasons for these high unknown alloantibodies to the fact that the use of screening cells prepared from foreign donors still leaves a possibility where the antibodies, especially the ones against minor antigens and possibly a 'local' antigen may go undetected, and account for high percentage of nonspecific and inconclusive antibodies [29]. AlSaeed, reported that 4.76% of the alloantibodies found among his subject were non-specific [34].

We observed in this study that the age group 21 – 30 years shows the highest number of patients who had alloantibodies, followed by < 10 and then 31 – 40 years. However, the alloimmunization rate was highest among age group < 10 years with alloimmunuzation rate of 0.26 followed by age group 21 – 30 years with 0.19, 11 – 20 years with 0.15, 31 – 40 years with 0.09 while age groups 41 -50 and > 50 years had zero alloimmunization rate. Although alloimmunization was reported to be considerably lower in patients in whom blood transfusion is started before the age of 3 than in those in whom it is started after that age (20.9 vs. 47.5%, p less than 0.0001, probably due to immature immunity, [35] reported that age of first transfusion of < 10 years appeared to be significant determinant of alloantibody formation among SCD patients [36]. We attributed the reason why age group < 10 years in this study had the highest alloimmunization rate as due malaria infection which is common cause of anaemia and hospitalization among this age group in this area. The high alloimmunization rate among age group 21 – 30 years and age group 31 – 40 years may have resulted from high blood transfusion due to road traffic accident, terrorist attacks, HIV infection and pregnancy related complications among women to which these age groups are exposed.

We observed a significant relationship between miscarriage and stillbirth statuses and the development of alloantibodies. 'Pearson's Chi-square test' for transfusion status, pregnancy status, and miscarriage status and stillbirth status

with alloantibodies formation showed respective P-values of 0.740, 0.339, 0.009 and 0.022. Trans-placental haemorrhage is the source of alloimmunization among pregnant women during childbirth, stillbirth or miscarriage. Threatened miscarriage is the most common complication of early pregnancy. Approximately 4% of women who have a therapeutic or complete miscarriage was reported to have a trans placental haemorrhage of > 0.2 millilitres of foetal red cells and of these patients, 4% - 5% will become sensitized having introduced to foetal antigen which the mother may lack [37].

It is obvious that the additional testing of blood donors for clinically significant red cell antigens as well as alloantibody screening of all transfusion recipients as well as pregnant women in which red cell transfusion is indicated and a strict transfusion policy and guidelines especially for pregnant women is essential in order to facilitate uneventful pregnancies, should be implemented as a routine to prevent as far as possible the incidence of alloimmunization. It would also be cost-effective if such screening is carried out before transfusion, bearing in mind the fact that there are laborious and expensive laboratory testing needed to carry out in order to provide compatible blood for alloimmunized patients. Extended blood typing should be implemented for some categories of poly-transfused patients as well. This strategy is another step forward to improving the safety of blood transfusion in Africa.

5. CONCLUSION

We conclude that the prevalence of alloantibodies among the studied population was high. However, 61.8% of the formed alloantibodies in these patients cannot be identified by the known panels of cells made from the Caucasians population. It could be that unknown antigens exist in this group of patients and in this part of the world which may require further investigation.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee

has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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