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ANTI-KELL ALLO-IMMUNIZATION IN A TERTIARY CARE HOSPITAL IN NORTH CENTRAL NIGERIA

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ABSTRACT

Objective: **Although ABO and Rh are the most clinically important blood group systems, other systems are also immunogenically significant. After ABO and Rh systems, anti-K, anti-k, anti-Kpa, anti-Kpb, anti-Jsa, anti-Jsb, and anti-U1a, were the next to cause acute and delayed haemolytic transfusion reactions and Haemolytic Disease of Fetus and Newborn.**
Design: **A prospective cross-sectional study.**
Subjects: **300 adult patients who had one or more units of packed cells or whole blood for correction of anaemia.**
Interventions: **antibody screening, identification and titration was performed using commercially prepared panel of cells on the serum obtained by centrifuging 2ml of venous blood aspirated from every blood transfusion recipient 48 hours after transfusion.**
Result: **Anti-K allo-immunization in this study was 1.6%. Anaemia and bleeding associated with pregnancy and other obstetrics and gynaecology disorders, Diabetes Mellitus and cancer of the bladder were indications for transfusions in 60%, 20% and 20% respectively of the 5 anti-K allo-immunized recipients. Female sex and previous blood transfusions correlated significantly with anti-K allo-immunization while age, types of unit transfused, and types of transfusion did not. All the anti-K allo-antibodies were of the IgG while 2 were of IgG + IgM type.**
Conclusion: **Because of the high prevalence of anti-K in this study, antibodies screening and identification is recommended to improve blood transfusion safety.**

INTRODUCTION

Over 300 blood group antigens have been described on human red blood cell. Majority of these have been classified into 29 blood group systems.[1]The ABO and Rh are the most clinically important blood group systems though some other blood group systems likeKell, Duffy, Kidd and MNSsetc have also been found clinically relevant as their antibodies are able to cause haemolytic transfusion reaction to incompatible blood transfusions or giveHaemolytic Disease of Fetus and Newborn (HDFN)in incompatible pregnancies.

Kell is the third most important blood group systems in transfusion medicine. Besides, the Kell blood group antigens are not only the potent antigens but are also developed well in intra-uterine life and cause fetal anaemia that may require intra-uterine blood transfusions.[2] The Kell was taken as the index antigen 1 for immunogenicity and the other minor blood antigens were compared with it for the purpose of ranking their immunogenicity.[3] The immunogenicity of K antigen is said to be HLA-related because the frequency of H L A - DR B1*11 and H L A - DR B1*13 are higher among the anti-K alloimmunized subjects.[4]Anti-K and anti-Kp[a], have also been associated with suppression of erythropoiesis by enhancing immune destruction of erythroid progenitor cells by macrophages in the fetal liver[5]while some cases of auto immune haeamolyticanaemia have been linked with Kell antibodies[6]. The McLeod phenotype was found in Hugh McLeod, a Havard dental student, in 1961.[7]

MATERIALS, SUBJECTS AND METHODS

Study Design: A prospective cross-sectional study.

Study Area: University of Ilorin Teaching Hospital, a tertiary hospital in the North-Central region of Nigeria, serves as referral centre for the region with a total estimated population of 15.5 million.[8]ABO and Rh typing of blood donors and recipients was followed by major crossmatch. Typing for other RBC antigens were seldom performed.

Target Population: 300 adult patients (145 males and 155 females) above the age of 15 years who had requirements of one or more units of packed red cells or whole blood for correction of anaemia were included in the study. Excluded patients were those with the following clinical conditions:

1. Auto immune disorders.
2. Connective tissue disorders.
3. Lymphoproliferative disorders.
4.Therapy with immune-suppressive or immune-modulatory drugs.
4. Previous history of acute or delayed haemolytic blood transfusion reactions.
3. Unwillingness to participate in the study.

Materials: 2 ml of venous blood was collected aseptically into a plain vacutainer bottle from the patients 48 hours after transfusions. The specimen was centrifuged at 3000rpm and its contents separated.

The sera were used for antibody screening by tube method using commercial 3 cells panel 'Maxiscreen 3'and if positive, the antibody identification was performed using 10 cells panel (Identicell 10) of the Lorne Laboratories Limited, Berkshire, UK. The reagents were supplied as 2.8±0.2% suspension in Alsevers solution.

Age and gender of the recipients, ABO and Rh blood groups of the transfused blood products and that of the recipients, history of previous transfusion and indications for transfusions were extracted from the records.

Laboratory tests:

Allo-antibody Screening and identification: tests were performed by tube method in low ionic

strength solution, albumin, and AHG phases according to the manufacturer's instructions.
*Allo-antibody Titration:*was also performed.
Ethical approval: Ethical approval was obtained from University of Ilorin Teaching Hospital Ethical Research Committee, with assigned number NHREC/02/05/2010. The issues of voluntariness, consent, and confidentiality were extensively discussed with every patient.
Data analysis/calculations: Data was analyzed using Statistical Package for Social Sciences (SPSS) program, version 20.
The data was summarized as frequencies and percentages. Comparison of multiple subgroups was done using one-way Analysis of Variance (ANOVA). Significant result was interpreted as $p<.05$.

RESULTS

A total of 300 blood transfusion recipients were recruited into this study, including 155 (52%) females and 145 (48%) males. Age wise break up included 78 (26%) patients between 15-30 years, 140 (47%) between 31-50 years and remaining 82 (27%) were above 50 years.One hundred and fifty (50%) each had stored whole blood and packed cells transfusions, Table 1.

Table 1
Clinical and laboratory features of all 300 blood transfusion recipients

Clinical and Laboratory features	**N(%)=300(100)**
AGE (YEARS):	
15-30	78(26)
31-50	140(47)
>50	82(27)
SEX:	
Male	145(48)
Female	155(52)
TYPES OF UNIT/S TRANSFUSED:	
Stored Whole Blood	150(50)
Packed Red Cells	150(50)
PREVIOUS TRANSFUSIONS:	
Yes	184(61.3)
No	116(38.7)
TYPES OF TRANSFUSION:	
Group identical	102(34)
Group compatible	198(66)

Indications for transfusions included anaemia and bleeding episodes associated with pregnancy and other obstetrics/gynaecology disorders 88 (29.3%), infections 68 (22.7%), haematology disorders 47 (15.7%), oncologies 44 (14.7%), endocrine disorders 12 (4%) and others 41 (13.7%), Figure 1.

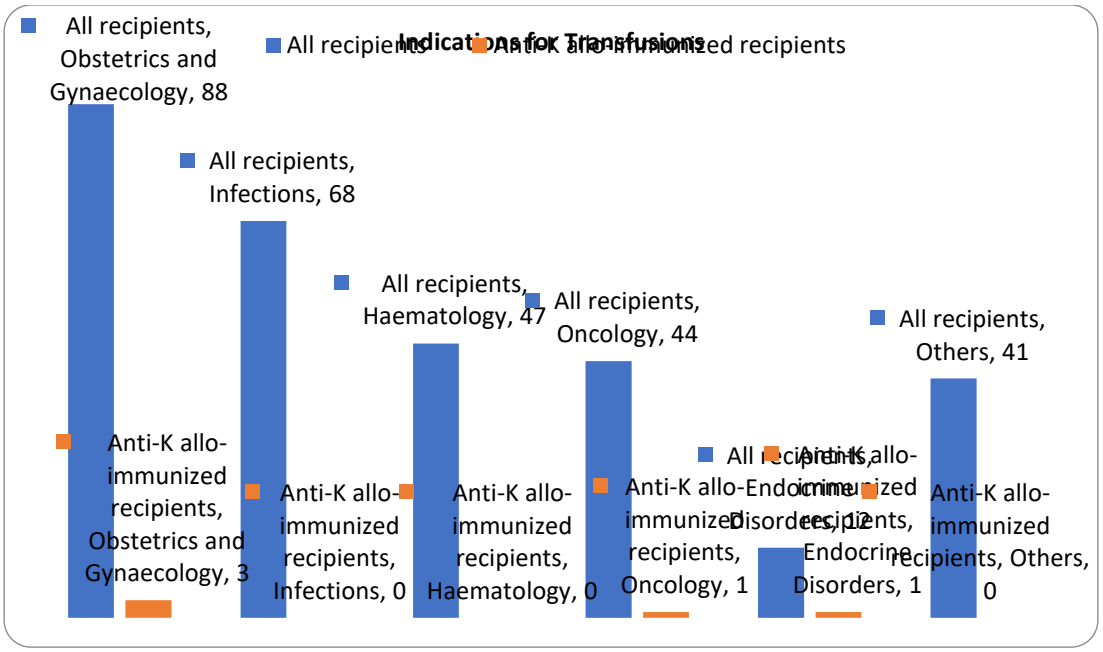

Figure 1: Indications for transfusions in all 300 recipients and anti-K allo-immunized recipients

Fourteen (4.6%) of all the 300 blood transfusion recipients were allo-immunized of which 5 (1.6%) were due to anti-K allo-antibodies. Three (60%), 1 (20%) and 1 (20) respectively of these five patients had blood transfusions due to anaemia and bleeding associated with obstetrics and gynaecology disorder, diabetes mellitus and cancer of the bladder. Two (40%) each were of the age range 15-30 and 31-50 years while one (20%) was above 50 years (X^2=2.34. p=.723). Four (80%) were females while 1 (20%) was a male recipient (X^2=0.334, p=.0023). Two (40%) and 3 (60%) received stored whole blood and packed cells respectively (X^2=3.112, p=.094). Four (80%) and 1 (20%) had and had no previous blood transfusions (X^2=0.921, p=.003) while 2 (40%) and 3 (60%) had group compatible and group identical transfusions (X^2=0.764, p=.063), Table 2.

Table 2

'Pearson's Chi Square Tests' for Age, Sex, Types of blood unit transfused, Previous transfusions and Types of transfusions

Clinical and Laboratory Features	Anti-K (n=300)		Total	X^2	p-value
	Pos	Neg			

AGE:				2.344	.723
15-30	2	76	78		
31-50	2	138	140		
>50	1	81	82		
Total	5	295	300		
Sex:				0.334	.0023
Male	1	144	145		
Female	4	151	155		
Total	5	295	300		
Units Transfused:				3.112	.094
Stored whole blood	2	148	150		
Packed red cells	3	147	150		
Total	5	295	300		
Previous Transfusion:	4	98	102	0.921	.003
Yes	1	197	198		
No	5	295	300		
Total					
Types of Transfusion:	2	185	187	0.764	.063
Group Compatible	3	110	113		
Group Identical	5	295	300		
Total					

Considering the serological properties of the ant-K allo-antibodies, all the samples showed marked agglutination at 37°C, albumin and anti human globulin phases. Two samples (40%) also showed moderate agglutination at immediate spin phase. When the sera were diluted, all of them showed marked agglutination up to dilution ratio 1 in 64, while 3 (60%) and 2 (40%) samples showed moderate and mild agglutinations at dilution ratio 1 in 256 and 1 in 1024 respectively, Table 3 and Figure 2.

Table 3

Serological Properties of anti-K developed

Properties	**Degree of Reaction**	**N (%)=5(100)**	**Antibody characteristics**
Thermal Range:			
Room Temperature	++	2(40)	Anti-K (IgM)
37°C	+++	5(100)	Anti-K (IgG)
Albumin:	+++	5(100)	Anti-K (IgG)
AHG:	+++	5(100)	Anti-K (IgG)

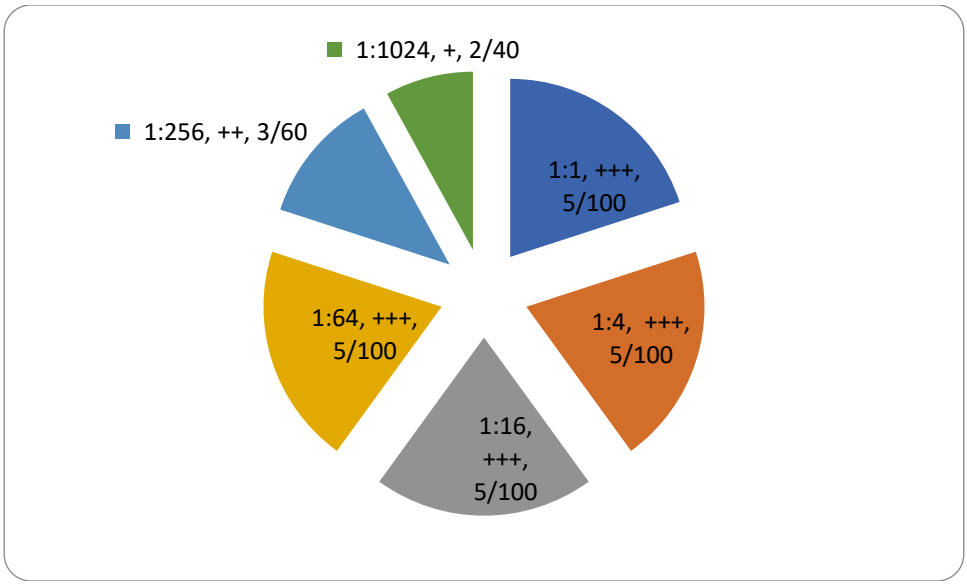

Figure 2: Showing the titre levels, degree of agglutinations and the number/percentage of the 5 samples with anti-K allo-antibodies.

LEGENDS:
+ Mild agglutination.
++ Moderate agglutination.
+++ Marked agglutination.

DISCUSSION

Kell system is characterized by a high degree of genetic polymorphism. There are about 25 Kell antigens, all encoded by the *KEL* genes on chromosome 7, at 7q33. Kell system consists of one triplets and four pairs of allelic antigens; Kp^a, Kp^b and Kp^c; K and k; Js^a and Js^b; K11 and K17; K14 and K2. K/k (KEL1/KEL2) is the most important, followed by Kp^a/Kp^b (KEL3/KEL4) and Js^a/Js^b (KEL6/KEL7). K and k are the two primary, co-dominant alleles of the *KEL* gene, which encode for K (Kell 1) and k (Kell 2 or Cellano) antigens, respectively.

K antigens are rare, with an estimated prevalence in the general population ranging from 0%[9]-2%[10,11] in Nigeria and up to 8.8%[12] among the Whites (K+k- in 0% blacks and 0.2% Caucasian, K+k+ in 2% blacks and 8.8% caucasian). On the other hand, k antigen (K-k+) is common, occurring in up to 98% of blacks and 91% of whites[12] while Js^b antigen is found in almost 100 percent of whites and 80 percent of blacks. Hence anti K is the commonly developed antibody to the Kell glycoproteins. The K and k antigens differ by a single amino acid shift from methionine 193 (in the K antigen) to threonine 193 (in the k antigen) in the Kell glycoprotein of about 732 amino acids

Antibodies to antigens in the Kell blood group system are usually IgG, IgM being far less. Anti K has been reported in few studies where incidences of alloimmunization has been estimated to range from 1 in 1000 pregnancies[13,14], 0.5 in 1000 antenatal women (0.5%)[15] and 10 out of 75 alloimmunized women[13]. Being IgG, anti K is expected to react maximally at 37^0C and it does not bind complement, therefore hemolysis is predominantly extravascular.

In the blood banks in most developing nations, as in our case, typing for other RBC antigens

after ABO and Rh and antibodies screening are not routinely performed before blood transfusion. Also, in Nigeria, where multiple ethnicities contribute to genetic heterogeneity among the population, in this case among blood donors and blood transfusion recipients, a wide variety of allo-antibodies are expected. Because of these, we decided to assess the degree of red cell allo-immunization from K antigen of Kell system which is the third clinically important blood group system in blood transfusion medicine.

Antibody screening and identification are fundamental to blood transfusion practice, upon which selection of suitable blood and blood products for transfusion, after ABO/Rh typing and cross-matching, is hinged. Equally important is haemovigilance, which is simply record of all activities (including adverse effects) surrounding blood donations and transfusions. Antibody screening and identifications are not routinely performed in the blood banks in most developing countries and also a recent review of blood safety in sub-Saharan African indicated that many countries in Africa do not have an effective haemovigilance system.[16] In the absence of these, blood transfusion safety in developing countries will be a mirage.

There are few studies on the prevalence and characteristics of Kell antigens and allo-antibodies in Nigeria. Kell antigen prevalence of 0-2[9-11]and anti-Kell frequency of 1.0%[17] was reported in Nigeria. Prevalence of anti-Kellallo-antibodies in this study was 1.6%, slightly higher than what was reported by previous researchers locally,[17] much higher than the ones reported outside Nigeria where Kellallo-immunization was find in 1 out of 1000 pregnancies[12,13] and 0.5 in 1000 antenatal women (0.5%)[14] but lower than that reported amongst 10 out of 75 (13.3%) alloimmunized women.[12]Prevalence of K-k+ phenotype has been reported to be as high as 98% among blacks and 91% among the caucasian populations[11] while K+k- is 0% in blacks and 0.2% in Caucasian and K+k+ is 2% in blacks and 8.8% in caucasian.

Development of anti-K in this study was seen commonly amongst recipients less than 50 years of age. This could be a co-incidence since age is not known to be a factor for development of allo-antibodies. In this study as in most other studies, the frequency of anti-Kell was higher among female blood transfusion recipients[18]. This is not unexpected as most of the female blood transfusion recipients are multiparous. Immunization through pregnancy could be one main reason for the high incidence of RBC alloimmunization among female patients. There was no significant difference in the development of anti-K with transfusions with stored whole blood or packed red cells in this study. It is a known fact that incidence of allo-antibodies is not affected by pre-storage manipulations.

Previous blood transfusion was significantly associated with the development of anti-K in this study. This is similar to what was reported by previous researchers in Nigeria where red cells allo-immunization prevalence of 8.8% was reported in multi-transfused patients with sickle cell anaemia[19] and chronic kidney disease.[20] Previous blood transfusion is a major means of been sensitized with foreign antigens. As with types of blood units, no significant association was noticed also with types of blood transfusions (group compatible or identical) and development of anti-K. This is because allo-antibody formation is directly proportional to the foreign antigen loads which are presumably same in stored whole blood or packed red cells transfused as group compatible or group identical method.

All the five anti-K in this study gave marked agglutinations at 37^0C (IgG type) while two also reacted moderately at room temperature (IgG+IgM type). All the samples also reacted with marked agglutination at albumin and Anti-Human Globulin phases. The anti-K allo-antibody could be highly lytic as up to 40% of the samples are still reactive (with mild degree of agglutination) at a dilution of 1 in 1024. These reactions are typical of anti-K being the most immunogenic after antibodies in ABO and Rh systems.

Limitations to the study

The two samples that were IgG+IgM type could not be further proven or characterized as IgM using Dithiothreitol method because of paucity of fund.

Also the five patients that developed anti-K should have been phenotyped for the absence of K antigen by performing their K/k phenotype as well as the K/k phenotype of the blood units they received but for the paucity of fund.

CONCLUSION

Knowledge of red cell antigens and their allo-antibodies frequencies in a population is fundamental in order to develop a donor data base and also to be able to provide red cells antigen negative compatible blood for blood recipients with multiple allo-antibodies. Acute and delayed haemolytic blood transfusion reactions and haemolytic disease of foetus and newborn due to anti-K can have serious consequences. Because of the high prevalence of anti-K found in this study, it is suggested that expanded blood grouping including K antigen be implemented in this environment. This would be another strategy to improve blood transfusion safety.

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