PREVALENCE OF ALLOIMMUNIZATION TO RED CELL ANTIGENS AMONG MULTIPLY TRANSFUSED PATIENTS WITH SICKLE CELL DISEASE IN KADUNA, NIGERIA

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ABSTRACT

Introduction: Red cell transfusion is an important modality in the treatment of complications in patients with Sickle cell disease (SCD) or Thalassaemia. However, this is associated with alloimmunization, resulting in destruction and immune-mediated clearance of the destroyed RBCs. Alloimmunization to RBC antigens complicate RBC crossmatching and delays the process of providing compatible blood for transfusion.

Aim: To determine the prevalence and pattern of alloimmunization in Multiply Transfused patients with SCD in Kaduna state.

Methods: A cross-sectional descriptive study of One hundred and thirty-one (131) individuals with SCD who had received 10 or more units of packed cells transfusions. Total Lifetime Transfusion (TLT) was defined as the total number of blood units received over a whole lifetime. Red cells were typed for ABO and Rh blood groups and Indirect antiglobulin test (IAT) for antibody screening using the saline spin tube technique and identification was done with the 3 cell Maxiscreen and 10 cell identification panel. Results were analysed using Jeffrey's Amazing Statistical Programme (JASP) version 0.14.1, Dec 2020, statistical software.

Results: The prevalence of red cell alloimmunization was 15.3% while the risk of alloimmunization per unit of blood transfused was 1.2%. Ten (10) types of alloantibodies were identified and Anti-Le[a] was the most frequently occurring antibody (3%).

Conclusion: There is a high prevalence of alloimmunization among patients with SCD. Therefore, multiply transfused individuals should undergo screening for the most commonly occurring clinically significant antibodies.

KEYWORDS: Red cell Alloimmunisation, Multiple Transfusion, Sickle Cell Disease, Total Life Time Transfusion

INTRODUCTION

Red cell transfusion is an important modality in the treatment of acute and chronic complications of Sickle Cell Anaemia (SCA).[1] It could be sessions of top-up transfusions or exchange blood transfusions (EBT). Red blood cell transfusion is indicated for the treatment of acute anaemia, and the management of heart failure, transient red cell aplasia and

acute chest syndrome (ACS).[2] Other indications for chronic blood transfusions in SCA include prevention and management of stroke, sequestration crisis and concomitant hypovolaemic shock might receive a large volume of transfusion in one episode.[2] Approximately 90% of adults with SCD would have received at least one red cell transfusion over a lifetime.[2] Some require 2-3 units per episode and may have more than one episode in a year.

Recurrent or multiple blood transfusions are not without complications or hazards. It is often said that the safest blood to transfuse is the one not given. Some non-immune related hazards of blood transfusion include the risk of infection, iron and circulatory overload.[3] Others are related to the development of allo or auto antibodies, most importantly of which is alloimmunisation to red cell (RBC) antigens. Alloimmunization to RBC antigens is a major complication of blood transfusion. These antibodies complicate RBC crossmatching and delay the process of provision of compatible blood for clinical transfusion.[4] There are several factors responsible for the rate of alloimmunization in patients with SCD. The most important is the discordance of blood group antigen expression between donor and recipient RBCs.[2] Others are the number of transfusions, degree of antigen matching, and age at first transfusion.[2,4] There is also genetic heterogeneity within the Rh blood group system, particularly among individuals of African descent.[2,4]

Alloimmunization is due to an immunologic response mounted by the recipient of the blood transfused against the donor RBC antigens.[5,6,7,8] This causes destruction and immune-mediated clearance of the destroyed RBCs. The prevalence of alloimmunization varies widely, ranging from 1% to 30% amongst different populations.[9] The most frequently seen alloantibodies in SCD are C and E (Rh system) as demonstrated in Benin, Enugu and Portharcourt, Nigeria.[5,10,11] Others are the Kell and Lewis.[2,4]

Allo and auto antibodies complicate RBC crossmatching and delay the process of providing compatible blood for transfusion. The aim of this study therefore is to determine the prevalence and pattern of alloimmunization in Multiply Transfused patients with Sickle Cell Disease (SCD) in Kaduna, Nigeria.

MATERIALS AND METHODS

Study Population

This is a cross-sectional study involving patients with SCD attending the haematology clinics of Ahmadu Bello University Teaching Hospital (ABUTH) and Kaduna State University (KASU) who were recruited from November 2020 and March 2021. Ethics approval was obtained from the hospital's Health Research Ethics Committee. One hundred and thirty one (131) SCD participants who had been multiply transfused were consecutively enrolled after an informed written consent. Multiple transfusion (MT) was defined as the transfusion of 10 or more units of ABO and Rh compatible blood and /or blood products in previous transfusions.[11] A questionnaire was used to collect information on socio-demographics and blood transfusion history.

Sample Analysis

Five (5) ml of venous blood was drawn from a suitable antecubital vein into an EDTA anticoagulated sample bottle from each participant according to standard methods adopted from Dacie and lewis.[13] This was centrifuged and serum stored at -20^0C until analysis. Red cells were typed for ABO and Rh blood groups and indirect antibody testing (IAT) was carried out using 3 cell Maxiscreen and saline spin technique. Antibody identification was done using commercially produced 10 cell identification panel and manufacturer specific Identigram (antigram) all by Lorne Laboratories Limited United Kingdom.

Statistical Analysis

Data generated were analysed with Jeffrey's Amazing Statistics Programme (JASP) (2020), version 0.14.1. Statistically significant levels were set at $P \leq 0.05$.

RESULTS

A total of 131 participants (who had received ≥ 10 units of red cells) with SCD were studied out of which 93 (71.0%) were females but there was no statistically significant relationship between gender and alloimmunisation. The median (IQR) age of the participants was 24 (30-21) years.

The Median (IQR) number of total lifetime transfusion (TLT) for all participants was 10 (10,10). One participant received 130 unit of red cell transfusions.

The mean age of participants with alloimmunisation was higher than those without alloimmunisation (31.5 ± 10.0 years versus 25.4 ± 7.4 years, t-statistic = -3.189, P = 0.002). Figure 1.

Only 20 (15.3%) of participants were alloimmunised and had positive antibody screening results. Participants who were alloimmunised had received a higher number of previous blood transfusions compared to those who were not alloimmunised, Mann Whitney U test of association. [16.5 (25.0,108.0) units vs 10.0 (10.0,10.0) units, MWU 359.500, P = < 0.001].

Ten (10) different alloantibodies were identified in the 20 participants with positive antibody screen and the severity represented by titre is shown in Table 2.

The prevalence of alloimmunisation was 15.3% (20/131 x 100%) and the risk of alloimmunisation per unit of blood transfused was 1.2% (The risk of alloimmunisation per unit of blood transfused is the total number of alloantibodies detected (20) divided by the TLT (1721) multiplied by 100).[11]

Table 1 shows the antibodies identified were to the Lewis, Kidd, MNS, Kell, and Duffy antigen groups. Anti-Le^a was the most frequently occurring antibody at 3%. One participant had double alloimmunization with S and K antibodies. Table 2 shows the various titres of the identified antibodies also with S and K having the highest.

Table 1: Antibodies Identified and Frequencies

Type of Antibody/ies	Frequency n=20	Percentage
C	2.290	15%
E	0.763	5%
Fy^a	0.763	5%
JK^a	2.290	15%
K	0.763	5%
Le^a	3.053	20%
N	1.527	10%
S	2.290	15%
S, K	0.763	5%
Total	20	100%

Table 2: Antibodies Detected and Titres

Serial	Antibody	Highest Tire	Lowest Titre
1.	S	128	32
2.	Le^a	32	8
3.	C	32	4
4.	JK^a	16	4
5.	K	128	64
6.	N	32	16
7.	s	16	16
8.	Fy^a	8	8

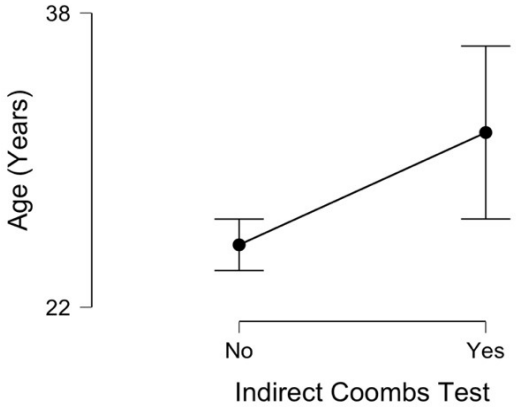

Figure 1: Descriptive Plot for Chronological Age and Alloimmunization

DISCUSSION

Alloimmunisation to red cell antigens despite being a common phenomenon, studies on it were found to be few in Nigeria. Even though the prevalence ranges widely as reported in literature, the specificities were mostly similar. This study also showed a statistically significant relationship between chronological age and alloimmunisation. This is because older participants were observed to have had more alloimmunizations. This is also in keeping with findings by Adewoyin (2016)[5] in Benin, Kangiwa et al (2015)[10] in Enugu and Olujohungbe et al (2001)[14] in the UK and Jamaica.

The relationship between chronological age and frequency of transfusion may be explained by the fact that patients with SCA are likely to be exposed to increasing episodes of blood transfusion due to complications such as chronic anaemia, nephropathy and sickle cell cerebral syndrome as they grow older. This is expected as blood transfusion is the mainstay of management for SCA and many of its complications.

Although majority of participants in this study were female there was no statistically significant relationship between gender and alloimmunisation. This finding was found to be in line with that by Rashmi et al (2013)[7] India, Kangiwa et al (2015)[10] Enugu and Obi et al (2018)[11] Portharcourt. This is despite the fact that females are more exposed to some risks of alloimmunisation like pregnancy and adverse obstetric experiences.

The positive trend of alloimmunisation with dose of transfusion is in line with the observation by Olujohungbe et al (2001).[14] Therefore, our findings of higher TLT with higher risk of alloimmunisation than those with a lower TLT is not unexpected. Several studies support this observation.[5,11, 12, 15]

The prevalence of alloimmunization (15.3%) observed in this study is higher compared to the reports 4.3% by Adewoyin (2016)[5] in Benin City and 3.2 % by Obi et al (2018)[11] in Portharcourt . It is however lower than 18.7% by Kangiwa et al (2015)[10] in Enugu. Studies show that alloimmunization rates differ widely depending on the population,[15] and certain factors are responsible for this. The RBC antigenic variability within a population may be a contributing factor.[15] Also, the absence or lack of adherence to blood transfusion policies and practices that are aimed at preventing alloimmunization may be considered as additional contributory factors, as well as the differences in samples sizes; Adewoyin and Obi et al had sample sizes of 55 and 124 respectively. Another factor may be that our study was on patients with SCA which is a chronic inflammatory condition and a risk factor of alloimmunisation. Thus, a higher alloimmunisation rate is not unexpected as compared to the study of patients with CKD due to other causes by Obi et al.[11] It is also instructive to note that the rate of 15.3% in this study is similar to those documented by Patel et al (2009)[12] in India with 19.6% and 15.5% for multi transfused and multiparous patients respectively.

The frequency distribution of the clinically significant antibodies identified in this study is identical to those previously described by other authors.[5,10,11] The clinical significance of an alloantibody is a function of its ability to cause in vivo destruction of RBCs.[16]

Contrary to the findings from other studies, Anti Le^a was the most frequently occurring followed by Anti C, JK^a and S. Other studies in Nigeria identified similar antibodies; Rh, Kell and Lewis by Adewoyin (2016) in Benin, Rh, Kell, Duffy by Kangiwa et al (2015) in Enugu, and Lu while Obi et al (2018) Portharcourt identified Rh, Kidd Duffy and MNS among multiply transfused patients. The most frequently occurring antibodies in these studies were in the Rh blood group system with anti- D, -C, -E, and -e specificity. One participant had double alloimmunization with anti-S and -K. This suggests a wide variation in exposure to non-self-antigens among patients with SCD in this study. This wide variation in alloimmunisation could be attributed to both genetic and epigenetic factors. Genetic factors may be differences in HLA and variants of RBC antigens exposure while acquired epigenetic factors include inflammatory conditions, splenectomy, multiparity and adverse obstetric events. In addition, pre-transfusion practices which incorporate phenotype red cell matching and RBC filtration in order to achieve leucoreduction are known to impact alloimmunisation. Routine red cell phenotyping and leukoreduction are not widely practised in Nigeria.

SUMMARY

There is a high prevalence of alloimmunisation among multiply transfused patients with SCD with a wide variation in the exposure to non self-antigens. The chronological age and dose of transfusion though independent, have a significant relationship with the rate of alloimmunisation. Therefore, multiply transfused individuals should undergo screening for alloimmunisation of the most commonly occurring clinically significant antibodies such anti- Rh (D, C, E) Le^a, MNS (M, N, S), -Kidd (JK^a), -Kell (K) and -Duffy (Fy^a).

Competing Interests

The authors declare no competing interests.

Authors' contributions

AA, UA, BA and AS designed the study. MS and NJ collated data, AA and AS analysed the data. All authors took part in writing, reading and approving the final manuscript.

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