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Original Research Article

Prevalence and Specificities of Immune Red Cell Antibodies in Adult Patients with Sickle Cell Anaemia and Blood Donors in Uyo, South-South Nigeria: A Case-Control Study

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Abstract

Background: Sickle cell anaemia (SCA) is a major public health issue in Sub-Saharan Africa, including Nigeria. Transfusion of red blood cells is an essential therapeutic modality in SCA. Repeated RBC transfusions can cause alloimmunization resulting in haemolytic transfusion reactions, transfusion refractoriness among other complications. ***Aims and Objectives:*** The study aimed to determine the prevalence and specificities of immune erythrocyte alloantibodies among adult patients with SCA compared with healthy HbAA blood donors in Uyo, South-South Nigeria. ***Materials and Methods:*** All participants were interviewed using a structured questionnaire to obtain information on bio-data, blood transfusion history and other relevant SCA history. Antibody screening and identification were carried out using tube agglutination method with commercially made panel of cells. ***Results:*** A total of 160 subjects were studied. They were made up of 80 SCA patients and 80 healthy HbAA blood donors. Prevalence of red cell alloimmunization among multi-transfused patients with SCA was observed to be 11.3%. Alloantibodies identified were mainly against Rh antigens contributing 66.7% (anti-E 22.2%, anti-C 22.2%, anti-D 11.1% and anti-e 11.1%). Antibodies directed against Kell and Lutheran blood group antigens together constituted 33.3%. No antibody was detected in the controls. Advancing age (30 years and above) and ABO blood group were statistically associated with alloimmunization (P values of 0.043 and 0.013, respectively). ***Conclusion:*** Repeated blood transfusion is associated with the development of alloantibodies. Immunohaematologic tests in transfusion care of SCA patients should be improved to include extended red cell phenotyping and routine alloantibody screening and identification.

Keywords: Immune red cell alloantibodies, antibody screening and identification, sickle cell anaemia, blood donors, Uyo, South-South Nigeria.

INTRODUCTION

Sickle cell disease (SCD) is a global public health problem and the most common genetic disease that affects mankind [1], with highest prevalence in Equatorial Africa, Sicily in Southern Italy, the Middle East and Central India [2]. In West Africa, Nigeria bears the greatest burden of SCD given that it is the most populous nation in the region [2, 3]. The World Health Organization (WHO) estimates the prevalence of SCD to be 2-3 percent in Nigeria (population estimate of 170 million Nigerians) [4-6]. The high burden is also bedevilled by increased morbidity and mortality owing to a myriad of factors, including, low public health awareness, delayed /wrong diagnosis, inaccessibility to specialized care/suboptimal care, poverty, pertinacious traditional and cultural beliefs and practices [5, 7]. Technically, the term "Sickle Cell Disease" refers to a group of inherited haemoglobin disorders characterized by the tendency of the intracorpuscular haemoglobin molecules to precipitate and deform the red blood cells (RBCs) into a sickle or crescent shape, resulting in chronic haemolysis and vaso-occlusive events [8, 9]. SCD arises from homozygous or compound heterozygous inheritance of the sickle beta globin gene. The homozygous state (HbSS or sickle cell anaemia) is the commonest and most severe form of the disease. The less common and milder forms result from double heterozygosity involving the HbS gene and thalassaemia (HbS4/β thal) and other haemoglobin variants such as C (HbSC), D(HbSD), O(HbSO) and E(HbSE) [9, 10], all of which

share a similar basic pathophysiologic mechanism marked by red cell sickling, chronic haemolysis, leucocytosis, thrombocytosis and vasculopathies [11].

Chronic haemolysis in sickle cell anaemia (SCA) is associated with chronic anaemia of varying severity in a vast majority of patients. Baseline (steady – state) haemoglobin level in SCD usually ranges from 6 to 9 gldl [8, 10]. Causes of protracted anaemia or acute anaemia episodes include hyper-hemolysis from infections or toxins, aplastic crisis, sequestration crisis, substrate (e.g. folate) deficiencies, renal impairment and others [7, 8, 11]. Severe anaemia in SCD patients often necessitates blood transfusion therapy for its correction. Transfusion modalities employed in the management of patients with sickle cell anaemia include top-up transfusion, chronic blood transfusion regimen and exchange blood transfusion with specific indications [10]. Existing data suggest that chronic blood transfusion program and automated red blood cell exchange are scarcely available for SCA care in Nigeria [4, 12]. In Nigeria, some studies have shown that as many as 36.2 – 59.3 percent of SCA patients have had transfusion with at least a unit of allogeneic blood products [4, 13]. Aside from iron overload and transfusion transmissible infections such as HIV and hepatitis, one of the prominent long-term complications of allogeneic blood transfusion in SCA is RBC alloimmunization [4, 10].

Red blood cell alloimmunization commonly occurs following transfusion of allogeneic blood components or in pregnancy, when red cells that express antigens absent in the individual's own blood enter the circulation. Generally, erythrocyte antibodies are classified as naturally occurring or acquired [14]. The naturally occurring antibodies are elaborated irrespective of antigenic stimulation and include the ABO, H, Lewis and P blood group systems [14, 15]. The acquired antibodies are formed after exposure to foreign antigens through blood transfusion or pregnancy. Acquired antibodies are also known as immune, unexpected, irregular, or atypical antibodies. Red cell antibodies can be allogeneic (alloantibodies) or autologous (autoantibodies). Immune antibodies that are frequently occurring and have the tendency to cause haemolysis in vivo are considered to be clinically significant [4, 14, 15]. These antibodies can cause acute and delayed haemolytic transfusion reactions, transfusion refractoriness, and haemolytic disease of the foetus and newborn in affected women [16, 17]. Different studies have reported varying prevalence of these transfusions –related morbidities in SCA patients [4, 5, 7, 13]. Therefore, there is a compelling need to constantly evaluate SCA patients for their alloimmunization status.

In the Nigerian setting however, antibody screening of at-risk individuals such as patients with SCA is not common practice, in contrast to what is obtainable in advanced climes like the United States and the UK. Similarly, extended RBC phenotyping is not routinely done in many developing nations like Nigeria [12]. Despite the aforementioned scenarios, it is interesting to note that a good number of workers have described the prevalence and pattern of RBC alloimmunization among Nigerian SCA patients. In Southwestern Nigeria, Adewoyin *et al.,* [14] reported erythrocyte alloantibody prevalence of 7.5 percent among multiply transfused patients with SCD. Ugwu *et al.,* [13], in their study documented RBC antibody prevalence of 9.3 percent among similar cohort of SCA patients in Benin City, South-South Nigeria. In Enugu, Southeastern Nigeria, Kangiwa *et al.,* [18] reported RBC alloimmunization prevalence of 18.7 percent among previously transfused patients with SCD. In Egyptian patients with SCD, Elalfy *et al.,* [19] reported a prevalence of 16%. In Brazil and the UK, much higher RBC alloimmunization frequencies of 52 and 76 percent have been reported, respectively [20, 21].

Available evidence shows that the prevalence of RBC alloimmunization in patients with SCD is highly variable across the globe. Plausible explanations for these observations include variation in disease severity, number of units of blood transfused, sources of allogeneic blood/racial admixture and mismatch, age at first transfusion, status of transfused RBCs, episodic transfusions, antigenic differences between the donor and recipient population and other patient-related factors [22-24]. An assessment of the frequencies and specificities of clinically relevant RBC alloantibodies is critical to designing robust and appropriate intervention strategies, and improving the overall SCD care in Nigeria. In this study, we sought to determine the prevalence and specificities of immune antibodies among study participants at University of Uyo Teaching Hospital, Uyo, South-South Nigeria.

MATERIALS AND METHODS

This was a hospital-based, analytical, cross-sectional study conducted at University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, South-South Nigeria between January 2021 and December 2021. The hospital renders specialty services to clients and patients within Akwa Ibom State and neighbouring states and also provides care for people with SCD through routine sickle cell clinics. The study population was made up of adult SCA patients aged 18years and above and healthy HbAA (Voluntary) blood donors attending the donor clinic. A minimum sample size of 79 was calculated using the formula for comparative cross-sectional study [$n_1 = (Z_1 - \sigma/2 + Z_1 - \beta)^2 [\frac{P_1(1-P_1)+P_2(1-P_2)}{(P_1-P_2)^2}]$ where statistical power is set at 80 percent (2 of 1.96), with 9.3 percent prevalence of erythrocyte alloantibodies in individuals with SCD [13] and 0.09 percent prevalence of alloantibodies among

healthy blood donors [25]. Eighty consecutive adults with SCA and 80 healthy HbAA blood donors were recruited into the study using a convenient, non-random sampling method. Adult patients with SCA with a history of stem cell transplant or passive anti – D immunization in the last 3 months were excluded. Approval for the study was obtained from the research and Ethics committee of University of Uyo Teaching Hospital. Written informed consent was obtained from each study participant after an elaborate description of the study protocol.

A structured, interviewer – administered questionnaire of personal characteristics including history of blood transfusion, the total number of units of blood received and demographic variables was completed for each participant. Thereafter, 5ml of antecubital venous samples were collected from each participant; 3ml of the blood were dispensed into plain bottles for antibody screening and identification while the remaining 2ml were dispensed into ethylene diamine tetracetic acid bottles for autocontrol and a direct antiglobulin test (DAT), if the autocontrol was positive. All laboratory tests were conducted in accordance to standard operating procedures [26].

The reagents were designed to identify alloantibodies of the Rh, kell, Duffy, Kidd, Lewis, MNS, P1 and Lutheran blood group systems. Reagents (Maxi-screen 3, identicells and antihuman globulim [AHG]) were commercially procured from Lorne Laboratories (Earley, Berkshire, United Kingdom). The tests were carried out in three phases. The first phase was done in saline at room temperature. The second phase was at 37^0C following enhancement with bovine serum albumin and the third phase with AHG. Other enhancing agents were not used.

The major blood group antigens were displayed by the screening cells (2 – 5% RBC suspension of blood 0 single – donor screening cells). Negative AHG tests were controlled with immunoglobulin G-sensitized (DAT+) RBCs. Negative indirect antiglobulin tests that did not become positive after addition of immunoglobulin G-sensitized RBCs were adjudged invalid and repeated. Samples were spun at 1000g for 10 seconds. Before taking the readings of each test after centrifugation, the tubes were tapped gently to dislodge the RBC button from the bottom of the tubes. All test results were read and interpreted immediately after centrifugation to avoid delay which is likely to cause dissociation of antigen – antibody complexes, resulting in weak – positive or false – negative reactions. Reagent RBCs not in use were stored at 2 – 8^0C. Control reagent RBCs were part of each analytical run to ensure optimal sensitivity, specificity and speed of the reagents used. An external thermometer was used to quality control the water bath.

Data were analyzed using SPSS for Windows Version 23. All results were presented in frequency tables. Description statistics was used to compute percentages and averages. Chi-square test was used to test for associations between variables. Level of significance was set at 0.05.

RESULTS

A total of 160 participants were recruited for the study. They comprised 80 SCA patients and 80 healthy HbAA blood donors. The SCA subjects consisted of 33(41.3%) males and 47(58.8%) females, the control subjects were made up of 36(45.0%) males and 44(55.0%) females (Table 1).

Table 1: Age and sex distribution of the participants

Characteristics	Patients with SCA		Blood donors
Age years	< 20	1	4
	20 – 29	47	47
	30 – 39	27	23
	40 – 49	4	4
	≥ 50	1	2
	Mean ± SD	29 ± 5	28 ± 7
Gender	Male	33	36
	Female	47	44

n = 160

SCA subjects had a significantly higher proportion of blood transfusion. Seventy-one (88.8%) of the SCA participants had a positive history of transfusion compared with four (5.0%) of the healthy HbAA blood donors (Table 2).

Table 2: Transfusion history of study participants

Characteristics	Patients with SCA		Blood donors
Previous	Yes	71	4
Transfusion	No	9	76
Age at first transfusion	No transfusion	9	76
	< 1	4	0
	1 – 15	41	2
	> 15	26	2
Last episode of transfusion	Last week	3	-
	last 4 weeks	4	-
	Last 12 weeks	12	-
	Last 12 months	23	-
	More than 1year	29	4
ABO group	A	21	28
	B	13	4
	AB	1	1
	O	45	42
Rh (D) blood group	D^+	72	70
	D^-	8	10

Nine (11.3%) of the patients with SCA showed positive results during antibody screening. All the antibodies were alloantibodies. None of the subjects in the control group had antibodies. Rh alloantibodies (anti-E and anti-C) were the most common (Table 3).

Table 3: Antibody screening results of the subjects

Antibody detected	Patients with SCA n(%)	Blood donors n(%)
Alloantibody	9	0
No antibody	71	80
Antibody specificity	Frequency (n)	Percentage (%)
Anti – E	2	22.2
Anti – C	2	22.2
Anti – D	1	11.1
Anti – e	1	11.1
Anti – K	1	11.1
Anti - Kp^a	1	11.1
Anti- Lu^b	1	11.1

There was an association between Age and ABO blood groups and alloantibody formation. However, development of alloantibodies in relation to sex, previous transfusion, age at first transfusion, last transfusion episode, total number of units of blood received and Rh(D) blood was not statistically significant (table 4).

Table 4: Association between alloimmunization in sickle cell disease subjects and some variables

		Antibody Screen		
		Positive	Negative	P value
Age, years	< 20	0	1	0.043
	20 – 29	2	45	
	30 – 39	5	22	
	40 – 49	2	3	
Gender	Male	4	29	0.836
	Female	5	42	
Previous transfusion	No	0	9	0.257
	Yes	9	62	
Age at first transfusion, years	< 1	1	3	0.586
	1 – 15	5	36	
	> 15	3	23	
Last transfusion episode	< 4 weeks	2	5	0.215
	4 – 12 weeks	3	9	

		Antibody Screen		
		Positive	Negative	P value
	> 12 weeks	4	48	
Total lifetime transfusions	0 1 – 5 6 – 15 > 16	0 3 3 3	9 31 19 12	0.455
ABO blood group	A B AB O	2 3 1 3	19 10 0 42	0.013
Rh (D) blood type	D^+ D^-	9 0	63 8	0.288

DISCUSSION

Red cell transfusion is an invaluable component of therapy in the management of sickle cell anaemia. It improves blood and tissue oxygenation, reduces the propensity for sickling by diluting the host cells and suppresses endogenous sickle cell production [27]. In spite of the salutary effects of transfusion therapy in sickle cell anaemia, there are still deleterious effects associated with transfusion that can result in severe complications including transmission of infections, transfusion hemodiserosis, alloimmunization among others [7].

This study showed that the prevalence of red cell alloimmunization among adult patients with SCA is 11.3%. This finding further confirms that blood transfusion is associated with the development of alloantibodies. None of the subjects in the control group had alloantibodies; as was also reported by other workers [13, 28]. The prevalence of 11.3% observed in this study is higher than prevalence in other studies, 7.5% in Lagos [4] and 9.3% in Benin City [13] but lower than 18.7% reported in Enugu [18].

Red cell alloimmunization has been widely documented in patients with SCA in developed economies, with a varying frequency ranging from 8% to 50% [13]. The marked disparities may be attributed to the sensitivity (automated gel technology) of the method used in most international studies as opposed to manual visual method used in our study and several other Nigerian studies. The use of Coombs reagents with enzymes such as papain, bromelin or ficin in most foreign studies enhances reactivity of some red cell antibodies, explaining the higher detection rate of the antibodies as compared with our local studies.

The specificities of alloantibodies recorded in this study were in the Rh, Kell and Lutheran blood group systems. Rh antibodies were the predominant antibodies accounting for 66.7%. Anti-E (22.2%) and anti-C (22.2%) were the most frequent alloantibodies. Findings from this study are in keeping with those of previous studies [4, 13]. The high proportion of Rh antibodies is somewhat due to the high immunogenicity of Rh antigens [14]. In contrast to ABO antibodies that are naturally occurring, Rh antibodies are immune antibodies and only occur when an individual who lacks the antigen encounters it through blood transfusion or pregnancy. Rh antibodies are mainly 1g G type and react optimally at 37^0C and therefore play a pivotal role in the pathogenesis of haemolytic transfusion reactions and haemolytic disease of the newborn (HDN). Anti-D, anti-C, anti-E and anti-e which were all detected in our SCA subjects, have been reported to be involved in haemolytic transfusion reactions, especially delayed reactions [14, 15]. It is important to determine the prevalence of these clinically significant antibodies, in every donor-recipient population in order to better assess the risk of antibody formation after blood transfusions. In addition, routine blood typing for Rh D status in both blood donors and transfusion recipients has reduced the incidence of transfusion reactions caused by sensitization to D antigen as well as other Rh antigens which can pose a challenge in transfusion medicine, particularly in multi-transfused patients [16].

Studies have shown that the Kell antigens are the third most potent, after ABO ad Rh antigens at triggering an immunologic reaction [13, 17, 21]. Like Rh antibodies, antibodies elaborated against Kell antigens are usually 1gG type and rarely, if ever, bind complements and therefore haemolysis is primarily extravascular in nature [14]. Anti-Kell antibodies have been implicated in the aetiology of haemolytic transfusion reactions and HDN. Unlike ABO and Rh sensitization, HDN due to Kell sensitization is as a result of suppression of fetal production of red blood cells by maternal anti-Kell antibodies [13]. Anti-Kell antibodies have also been reported to promote the destruction of early erythroid progenitor cells resulting in anaemia which may be severe [13, 22]. Lutheran alloantibodies are rare antibodies that have been observed to cause delayed haemolytic transfusion reaction (DHTR) [13, 14]. Anti-Lu^b antibodies found in our study have been reported in Lu (a-b-) individuals following blood transfusion or pregnancy [13].

The likelihood of developing alloimmunization is determined by many factors

namely age of the patient, number of units of blood received, status of transfused RBCs and antigenic differences between the donor and recipient population [22-24]. The tendency to react to alloantigens varies from person to person. Some individuals may not be immunized despite recurrent transfusions (non-responders) whereas others may become immunized when transfused with any of the antigens they do not possess (responders) [22]. This is consistent with our work and a similar study which revealed that development of alloantibodies is generally not influenced by the number of units of blood received. Proclivity to elaborate alloantibodies after blood transfusion as in responders has been reported to be affected by patient- related factors, dose and route of administration and immunogenicity of the antigen [23].

In the present study, advancing age appeared to be significantly associated with the risk of red cell alloimmunizaiton. This observation could be explained by the fact that increasing survival age in SCA portends more likelihood of numerous blood transfusions. Earlier studies have documented that the frequency of development of alloantibodies is higher in women than men [29, 30]. However, development of alloantibodies in relation to gender was not statistically significant in the index study (P = 0.836).

Although the prevalence of alloimmunization in SCA is lower compared with other studies, particularly those done in developed countries; there is still a clinically significant burden of red cell alloimmunization among our SCA patients. Therefore, there is a need for continuous evaluation and formulation of local strategies to mitigate its occurrence. Serological tests in transfusion care of Nigerians with SCA should be expanded to include routine antibody screening. Extended phenotyping and matching of all SCA blood recipients and donor units is recommended to prevent or reduce red cell alloimmunization in sickle cell anaemia population.

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