ORIGINAL REPORT

Immune erythrocyte antibodies in adult patients with sickle cell disease and blood donors in Lagos, Nigeria: a comparative study

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Sickle cell disease (SCD) poses a major public health challenge in sub-Saharan Africa, including Nigeria. Blood transfusion is a mainstay in SCD treatment. Erythrocyte alloimmunization is known to complicate the transfusional care of patients with SCD. Immune alloantibodies are associated with hemolytic transfusion reactions and transfusion refractoriness. We aimed to determine the prevalence, specificities, and clinical associations/risk factors of immune erythrocyte alloantibodies among adult patients with SCD compared with healthy blood donors in Lagos, Nigeria, through a cross-sectional study. All participants were interviewed using a structured questionnaire to obtain details on bio-data, hemoglobin phenotype, blood transfusion history, and SCD history where relevant. Blood specimens obtained from each participant were subjected to antibody screening/identification using tube agglutination method. The mean age of the SCD participants and healthy blood donors was 27.92 and 29.04 years, respectively. The majority (72.5%) of the SCD participants had received at least 1 unit of red blood cell (RBC) transfusion in their lifetime, compared with only 7.5 percent of blood donors. Six SCD participants (7.5%) tested positive for atypical erythrocyte alloantibodies, with none among blood donors. Most of the antibodies (75%) belonged to the Rh blood group system. The most frequent antibody was anti-E, followed by anti-C and anti-D. Advancing age (30 years or more), recent transfusions (last 4 weeks), higher transfusion rates, and established renal disease were significantly associated with alloimmunization (p values of 0.026, 0.043, 0.002, and 0.043, respectively). This study suggests blood transfusion as a strong risk factor for RBC alloimmunization in SCD patients. Extended RBC phenotyping is recommended for all patients with SCD, especially those receiving regular transfusions. ***Immunohematology* 2021;37:131–137. DOI: 10.21307/immunohematology-2021-020.**

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Erythrocyte antibodies are either naturally occurring or acquired.[1,2] The naturally occurring antibodies are produced independent of antigenic exposure and include the ABO, H, Lewis, and P blood group systems.[1,3] The acquired antibodies develop after exposure to exogenous antigens through blood transfusion or pregnancy. Acquired antibodies are also termed as immune, unexpected, irregular, or atypical. Red blood cell (RBC) antibodies can also be allogeneic (alloantibodies) or autologous (autoantibodies). Immune antibodies that are frequently occurring and have capacity for *in vivo* hemolysis are described as clinically significant.[1,3]

Sickle cell disease (SCD) is a global public health challenge, with highest prevalence in West Africa, the Middle East, the Mediterranean basin, and far India.[4–6] In West Africa, Nigeria bears the greatest burden of SCD, being the most populous nation in that area. Estimates suggest SCD prevalence of 2–3 percent in Nigeria (population estimate of 170 million Nigerians).[7–9] This high burden of SCD in Nigeria is also coupled with considerable morbidity and mortality as a result of multiple factors, including low public health knowledge, delayed diagnosis, gross absence of dedicated sickle cell centers, poor access to specialized care/suboptimal care, and poverty.[10,11] Technically, the term "sickle cell disease" encompasses clinical syndromes characterized by the tendency of the intracorpuscular hemoglobin molecules to precipitate and deform the RBC to a sickle or crescent shape, resulting in chronic hemolysis and characteristic vaso-occlusive events.[12,13] SCD results from homozygous or compound heterozygous inheritance of the sickle beta hemoglobin gene (*HBB*). Homozygous disease is termed sickle cell anemia. Chronic hemolysis in SCD is associated with chronic anemia in most affected individuals, which may be interspersed by episodes of acute hemoglobin drop from several etiologies. Baseline (steady-state) hemoglobin level in SCD ranges from 6 to 9 g/dL.[13] Causes of worsening anemia or acute hemoglobin drop include hyper-hemolysis from infections or toxins, aplastic crisis, substrate (e.g., folate) deficiencies, renal impairment, and others.[14,15]

Serious anemia in SCD patients often warrants blood transfusion therapy for correction of anemia. Other transfusion modalities in SCD include chronic blood transfusion and exchange blood transfusion with specific indications.[16,17] Available evidence suggests that chronic blood transfusion therapy and automated RBC exchange are poorly available for SCD care in Nigeria.[18,19] In Nigeria, local studies have suggested

that as many as 36.7–57 percent of pediatric SCD patients have had transfusion with at least 1 unit of allogeneic blood components.[20,21] Aside from iron overload, one of the most significant long-term complications of allogeneic transfusion in SCD is RBC alloimmunization. Clinical sequelae of erythrocyte alloimmunization include hemolytic transfusion reactions (HTRs), transfusion refractoriness, and possible hemolytic disease of the fetus and newborn in affected women.[22,23] The prevalence of delayed HTR has been observed to be as high as 11 percent in SCD patients.[24] Therefore, there is a need to continually evaluate SCD patients for their alloimmunization status.

In the Nigerian setting, however, alloantibody screening of at-risk individuals such as patients with SCD is not routine, unlike in developed nations such as the United States and the UK. Similarly, extended RBC phenotyping is grossly lacking in many underdeveloped settings like Nigeria.[19] It is pertinent to continually assess the burden of atypical RBC antibodies in SCD patients in places like Lagos, Nigeria. These data will help to evaluate the current local pattern of RBC alloimmunization, as well as design better strategies and appropriate interventions, and improve on current transfusion practices. A few cross-sectional studies have described the prevalence and pattern of erythrocyte alloimmunization among Nigerian patients with SCD. In Northern Nigeria, Kuliya-Gwarzo et al.[25] reported RBC alloantibody prevalence of 8.8 percent among multiply transfused patients with SCD. Ugwu et al.[26] reported RBC alloantibody prevalence of 9.3 percent among multiply transfused patients with SCD in Benin City. In Enugu, Nigeria, Kangiwa et al.[27] reported erythrocyte alloimmunization prevalence of 18.7 percent among previously transfused patients with SCD and 5 percent of all patients with SCD. The prevalence of alloimmunization in Ugandan patients with SCD is reported at 6.1 percent.[28] In Brazil, alloimmunization prevalence as high as 52 percent has been reported.[29] In the UK and Jamaica, the prevalence of RBC alloimmunization was observed to be 76 and 2.6 percent, respectively.[30] In Saudi Arabia, Bashawri reported RBC alloimmunization prevalence of 13.7 percent among patients with SCD.[31]

Existing data show that the prevalence of RBC alloantibodies in patients with SCD is highly variable in different parts of the world. Possible explanations include variations in disease severity, rates of blood transfusion, sources of allogeneic blood/racial mismatch, age at first transfusion, age of transfused RBCs, episodic transfusions, and other patient-related factors.[32–35] An understanding of the frequencies and specificities of clinically significant erythrocyte alloantibodies is crucial to improving SCD care in Nigeria. In this study, we aimed to determine the prevalence and specificities of immune antibodies, as well as identify possible risk factors/clinical association of erythrocyte alloimmunization, among study participants at the Lagos University Teaching Hospital (LUTH) in Lagos, Nigeria.

Methods

The study was a hospital-based, analytic, cross-sectional study conducted at LUTH. LUTH is a federal tertiary health institution situated in Idi Araba, Lagos. The hospital renders inpatient and outpatient specialty services to clients and patients within Lagos and surrounding states and provides care for people with SCD through routine sickle cell clinics. The two study groups included adult patients with SCD attending the outpatient sickle cell clinics and healthy (voluntary) blood donors attending the donor clinic. A minimum sample size of 79 was calculated using the formula for comparative cross-sectional study [$N = (Z_\alpha + Z_1 - \beta)^2[(P_1(1 - P_1) + P_2(1 - P_2)]/(P_1 - P_2)^2]$, where statistical power is set at 80 percent (z of 1.96), with 9.3 percent prevalence of erythrocyte alloantibodies in individuals with SCD[26] and 0.09 percent prevalence of alloantibodies among healthy blood donors.[36] Eighty consecutive adults with SCD and 80 healthy blood donors were recruited into the study using a convenient, non-random sampling method. Adult patients with SCD with a history of stem cell transplant (irrespective of outcome) were excluded. Also, any patient with SCD with passive anti-D immunization in the last 3 months was excluded. Ethics approval was obtained from the LUTH Research and Ethics Committee with study protocol number ADM/DCST/HREC/APP/1035. Written informed consent was obtained from each study participant after detailed explanation of the study protocol.

Each participant was interviewed with a structured, interviewer-administered questionnaire to obtain and document relevant bio-data and clinical data. Thereafter, antecubital venous samples were collected for alloantibody screening/identification plus an autocontrol, and a direct antiglobulin test (DAT), if the autocontrol was positive. All laboratory testing was conducted according to standard operating procedures.[37,38] The reagent RBCs (screening and identification) were designed to identify alloantibodies in the Rh, Kell, Duffy, Kidd, Lewis, MNS, P1, and Lutheran blood group systems. Reagents (MaxiScreen 3, Identicells, and antihuman globulin [AHG] elite) were commercially sourced from Lorne Laboratories (Earley, UK). Testing was conducted in three phases. The

first phase was performed in saline at room temperature. The second phase was incubation at 37°C after enhancement with bovine serum albumin and then a third phase with AHG. Low-ionic-strength saline, polyethylene glycol, or proteolytic enzymes were not used.

Screening cells (2–5% RBC suspension of blood group O single-donor screening cells) displayed homozygous expression of the major blood group antigens. Negative AHG tests were controlled with check cells. Check cells were immunoglobulin G–sensitized (DAT+) RBCs. Any negative indirect antiglobulin test that did not show a positive result after addition of check cells was considered invalid and repeated. All centrifugations were carried out at 1000*g* for 10 seconds or for a suitable alternate *g* force and time. Before reading each test after centrifugation, the tube was shaken gently to dislodge the RBC button from the bottom of the tube. All test results were read and interpreted immediately after centrifugation because delay might cause dissociation of antigen–antibody complexes, resulting in weak-positive or false-negative reactions. Reagent RBCs were stored at 2–8°C when not in use. Control reagent RBCs were used on each analytical run to ensure optimal sensitivity, specificity, and speed of the reagents used. Optimal reacting conditions for antigen–antibody interaction were ensured. The temperature of the water bath was quality controlled with an external thermometer.

Data were obtained from questionnaires and results of sample analysis. Data were inputted and analyzed using SPSS for Windows, version 16.0 (IBM, Armonk, NY). All descriptive data were analyzed and are presented in frequency tables and charts. The proportion of alloimmunization between the SCD population and the general patient population was compared using Fisher's exact test. Possible clinical associations or risk factors of RBC alloimmunization were tested using χ^2 analysis, and the strength of association was expressed as odds ratio. Probability score <5 percent ($p < 0.05$) was considered statistically significant.

Results

The mean age of the adult patients with SCD and healthy blood donors was 27.92 and 29.04 years, respectively (Table 1). The predominant hemoglobin phenotype in the SCD cohort was SS (87.5%), whereas most (85%) of the blood donors were hemoglobin phenotype AA (normal adult hemoglobin phenotype). SCD participants had a significantly higher proportion of blood transfusions (Table 2). Fifty-eight (72.5%) of the SCD participants had a positive transfusion history compared with six (7.5%) of the healthy blood donors. Five (6.25%) of the SCD participants had an established renal disease.

Six (7.5%) of the adult patients with SCD had immune antibodies compared with none among the healthy blood donors (Table 3). Rh alloantibodies (particularly anti-E) were the most common, accounting for 75 percent of the positive antibody screening results (Table 4). Among the adult SCD participants, age greater than 30 years and renal disease were significantly associated with alloimmunization (Table 5).

There was a strong association between previous transfusion and positive alloantibody screen with odds of 5.57,

Table 1. Bio-data of study participants

Characteristics		Patients with sickle cell disease	Blood donors
Age, years	<20	16	4
	20–29	34	42
	30–39	23	24
	40–49	4	10
	≥50	3	0
	Mean ± SD	27.92 ± 8.82	29.04 ± 7.59
Gender	Male	41	59
	Female	39	21

N = 160.

Table 2. Clinical characteristics of study participants

Characteristics		Patients with sickle cell disease	Blood donors
Hemoglobin phenotypes	SS	70	—
	SC	10	—
	AA	—	68
	AS	—	12
Previous transfusion	Yes	58	6
	No	22	74
Last episode of transfusion	Last week	3	—
	Last 4 weeks	4	—
	Last 12 weeks	6	—
	Last 12 months	9	—
	More than 1 year	36	6
Renal disease	Yes	5	—
	No	75	80

N = 160.

Table 3. Antibody screening results of the study participants

Antibody detected	Patients with sickle cell disease, *n* (%)	Blood donors, *n* (%)
Alloantibody	6 (7.5)	0 (0)
No antibody	74 (92.5)	80 (100)

N = 160, χ^2 = 6.234; *p* = 0.014.

Table 4. Antibodies identified in participants with positive antibody screens

Antibody specificity	Frequency (*n*)	Percentage (%)
Anti-E	3	37.5
Anti-C	2	25
Anti-D	1	12.5
Anti-Le[a]	1	12.5
Unidentified	1	12.5

Six participants with sickle cell disease (7.5%) had positive antibody screens; 7 alloantibodies were identified; 75% of the identified alloantibodies belong to the Rh blood group system. One of the participants had a panreactive antibody—no specificity identified.

Table 5. Association between alloimmunization in sickle cell disease participants and other variables

		Antibody screen		Odds ratio; *p* value
		Positive	Negative	
Age, years	15–29	1	49	9.80; 0.026
	≥30	5	25	
Gender	Male	4	37	2.00; 0.362
	Female	2	37	
Hemoglobin phenotype	SS	6	64	1.857*; 0.436
	SC	0	10	
Renal disease	Yes	2	3	11.83; 0.043
	No	4	71	
ABO blood group	O	4	35	0.906†; 0.824
	B	1	19	
	A	1	18	
	AB	0	2	
Rh(D) blood type	D+	5	69	0.36; 0.375
	D–	1	5	

N = 80.
*Adjusted odds ratio.
†χ^2 test.

although this result was not statistically significant (Table 6). No association was observed between age at first transfusion and alloantibody formation. Individuals who had at least one transfusion episode in the last 4 weeks preceding the study were more likely to be alloimmunized (Table 6). Participants with higher transfusion rates in their lifetime were more likely to be alloimmunized.

Table 6. Association between alloimmunization in sickle cell disease participants and blood transfusion

		Antibody screen		χ^2; *p* value
		Positive	Negative	
Previous transfusion	Yes	6	52	5.57*; 0.135
	No	0	22	
Age at first transfusion, years	No data	2	39	4.873; 0.181
	<1	0	4	
	1–15	1	20	
	≥16	3	11	
Last transfusion episode	No data	0	22	6.272; 0.043
	Last 4 weeks	2	5	
	>4 weeks	4	47	
Last transfusion episode	No data	0	22	3.074; 0.215
	Last 12 weeks	2	11	
	>12 weeks	4	41	
Total lifetime transfusions	No data	1	34	15.197; 0.002
	0–5	1	31	
	6–15	2	7	
	≥16	2	2	

N = 80.
*Adjusted odds ratio.

Discussion

The proportion of irregular alloantibodies among adult patients with SCD who participated in this study was 7.5 percent. This observation agrees with findings by Ugwu et al.,[26] who determined the prevalence of erythrocyte alloantibodies in Benin City among adult patients with SCD to be 9.3 percent. In a study conducted by Kangiwa et al.,[27] 5 percent of all people affected with SCD had irregular alloantibodies, and 18.7 percent of individuals who had received blood transfusion in the last 4 weeks were alloimmunized. Similar to observations from both studies, the predominant alloantibodies formed were Rh alloantibodies. A longitudinal study will give a better description of the actual burden and rate of alloimmunization in SCD because it has been reported that up to 25 percent of alloantibodies disappear from serum within a median 10 months of follow-up.[39]

It is important to accurately define the prevalence of those blood group antigens, known to produce clinically significant antibodies, in every donor–recipient population to be able to

assess the risk of antibody formation after blood transfusions. Additionally, routine alloantibody screening tests in at-risk blood transfusion recipients will provide necessary information on the distribution of specific alloantibodies implicated. Based on the nature/pattern of alloimmunization in the United States and the UK, extended RBC typing for C, E, and K is an established protocol for transfusion of non-immunized patients with SCD.[40] In Uganda, Natukunda et al.[41] suggested limited RBC typing for C, E, and S based on the local pattern of erythrocyte immunization observed in that population. Eighty percent of observed alloantibodies among transfused patients with SCD in Uganda were in the Rh and MNS blood group systems—E (33.3%), D (23.3%), C (6.7%), S (13.3%), and M (3.3%).[41] An understanding of the local pattern of antibodies is crucial to immunohematologic testing for pretransfusion compatibility checks in SCD. In an index study, though a small cohort, the predominant alloantibodies (accounting for 75%) belonged to the Rh blood group system. Anti-E (37.5%) and anti-C (25%) were the most frequent alloantibodies. A case of anti-D alloimmunization was observed in a D– SCD male participant (among six D– SCD participants). These findings suggest serious deficiencies in our local transfusion service. There is a need for stricter guidance/regulation on routine ABO blood grouping and compatibility testing, particularly in transfusion care of patients with SCD.

Advancing age appeared to be significantly associated with the risk of alloantibody formation. This finding is perhaps explained by the fact that increasing survival age in SCD portends more chances of blood transfusions. However, SCD with renal disease also appears to be a strong risk factor for alloantibody formation. As such, there may be a need for a well-designed case control study to describe the burden and strength of association of alloimmunization in patients with SCD and with renal disease.

This study also investigated the details of transfusion history including age at first transfusion, interval of last transfusion episode through study participation, and total lifetime transfusions as risk factors for alloantibody development. Individuals who had blood transfusion in the last 4 weeks had the highest prevalence of alloantibodies. As noted earlier, up to 25 percent of alloantibodies will disappear after a median follow-up of 10 months. Hence, the farther the last transfusion episode, the lower the chances of a positive antibody screen result. Additionally, SCD participants with more transfusion episodes (16 or more) had the highest alloimmunization burden in the study cohort. This finding is understandable because a greater antigenic exposure will be associated with a higher total lifetime transfusion. Specificity of one of the implicating alloantibodies in the positive antibody screens could not be identified because of pan-reactivity/agglutination. An additional panel of reagent RBCs and possible use of enzyme potentiators were not available to resolve the specificity of the unidentified alloantibodies.

Although the prevalence of alloimmunization in SCD is lower compared with other parts of the world, such as the United States and UK, there is still a clinically significant burden of erythrocyte alloantibodies among affected individuals in Lagos. As such, there is a need for continual evaluation and design of local strategies to reduce its incidence. Immunohematologic testing in transfusion care of Nigerians with SCD should be improved to include routine alloantibody screening. Phenotypic matching of all SCD blood recipients and donor units should be extended beyond ABO/D to include at least the five major Rh antigens. Regional facilities should be developed to produce indigenous RBC panels for screening and identification of atypical antibodies. There is a need for a reference (central) transfusion laboratory where antibody screens and extended phenotyping can be performed.

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