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HAEMATOLOGICAL PROFILE AND BLOOD TRANSFUSION PATTERN OF PATIENTS WITH SICKLE CELL ANAEMIA VARY WITH SPLEEN SIZE

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ABSTRACT

Background: **The spleen serves critical haematological and immunological functions in the body. However it is also the first organ to be affected by the effects of sickling in sickle cell anaemia. While the splenic size has been evaluated in sickle cell anaemia, the spleen sizes of these patients has not been associated with any specific haematological pattern.**

Objectives: **To determine the haematological parameters of patients with sickle cell anaemia (SCA) in relation to spleen size.**

Methods: **The full blood count (FBC), the irreversibly sickled cells and blood transfusion use amongst SCA patients in steady state was evaluated. Abdominal ultrasound was also performed for all patients and HbAA control for splenic size categorization.**

Results: **Forty patients with SCA and 22 controls with HbAA were studied with mean age of 29.28 ± 8.10 years 28.23 ± 8.14 years respectively. The mean splenic longitudinal lengths in patients and controls were 6.3 ± 4.3cm and 8.9 ± 1.5 cm respectively (p-value < .05).**

The mean haematocrit and haemoglobin value were significantly lower in SCA cases than in controls (p < 0.001). Though the red cell indices were similar but the white blood cell and platelet count were significantly higher in patients than in controls.

Among SCA cases, the spleen size showed significant positive correlation with haematocrit (r = 0.371, p = 0.019) and the age at 1st transfusion (r = 0.447, p = 0.013) but significant negative correlation with MCV, MCH, MCHC and platelet count. Above 80% of patients with severe, moderate and mild autosplenectomy had been transfused compared to 25% of patients with splenomegaly.

Conclusions: **Similarities in red cell indices between patients and controls suggests an identical factor influencing the red cell indices which could be genetic such as thalassemia or environmental such as iron deficiency. The negative correlation of the spleen size with red cell indices, white cell count and platelet count and positive correlation with haematocrit suggest that spleen size can be used to determine clinical course of the disease. Earlier age at first transfusion, significantly higher frequency of blood transfusion and MCHC in patients with severe autosplenectomy suggest a more severe clinical course when compared with patients with splenomegaly, normal spleen, mild and moderate autosplenomegaly.**

Keywords: Sickle cell anaemia, Splenic size, Autosplenectomy, Haematological parameters

INTRODUCTION

Sickle cell anaemia is characterized by hemolytic anemia and vaso-occlusion leading to acute and chronic tissue ischemia, infarction, chronic organ damage and organ dysfunction. One of the earliest organs to be affected by recurrent infarction and progressive damage is the spleen[1]. The spleen serves both haematological and immunological functions. Variability of haemoglobin level with splenomegaly has been documented in both peadiatric and adult sickle cell anaemia patients [2,3] with few documentations on autosplenectomy. Haematological functions of the spleen includes: erythropoiesis, maturation of the reticulocyte, lymphopoiesis, reservoir of blood cells, pitting and culling function and destruction of old red cells [4]. The spleen presents a hypoxic, acidic and hypoglycemic environment for the erythrocytes resulting in sickled red cells due to the limited oxygen and glucose. The sickling leads to repeated infarctions of the spleen and subsequent reduction of both morphological size and function of the spleen. The splenic sizes in patients with sickle cell anaemia vary with the degree of insult it has received, thereby giving rise to a spectrum of sizes, from repeated infarction. One extreme is splenomegaly observed during infancy and early

childhood, consequent to splenic sequestration or during adulthood due to increased demand on the spleen to perform its functions[2,3]. The other extreme is autosplenectomy which appears as a small wrinkled firm, nodular remnant following progressive atrophy due to repeated episodes of vaso-occlusion and infarction[5].

The clinical and laboratory profile of patients with sickle cell anaemia are continuously being studied to understand the biology of the disease[6,7]. The haematological parameters are the most commonly requested investigation to determine the line of management, adjust therapy and predict outcome. The various types of SCD manifest considerable differences in haematological parameters[8]. These differences are further complicated by both physiological factors and acute or chronic complications. A change in the size of the spleen is a chronic complication which is postulated to affect haematological parameters and could also be associated with a unique clinical course. Paediatric patients with splenomegaly have lower mean haematocrit; retrospective analysis of their case files showed that these patients had predominantly anaemic crises while those without splenomegaly had had predominantly vaso-occlusive crises[9,10]. An enlarged spleen is rare in adults with sickle cell anaemia.[11] Functional changes of the spleen is related to anatomic regression of the spleen[11]. The slower progression to atrophy is the result of a reduced tendency to sickling in vivo therefore adult sickle cell patients who retain their spleen are judged to have less severe clinical disease. However an inverse relationship between severity and autosplectomy has been reported when blood transfusion was used to determine clinical severity [12]. A study by Awotua-Efebo *et al* showed that splenomegaly is associated with poorer haematological indices whilst autosplenectomy is associated with better indices.[10] The transitory anatomic size of spleen between normal and autosplenectomy are not well recognized and studied. The patients with SCA require regular monitoring and evaluation for various reasons, therefore the knowledge of changes in haematological indices of patients induced by variation in splenic size may be useful in the management of patient. Most of the local literatures relating the spleen size to the haematological parameters have been in paediatric sickle cell anaemia patients and were not related to blood transfusion requirements[13,14]. The spleen in sickle patients in sub Saharan Africa is believed to be different from that of sickle cell patients outside sub Saharan Africa[15]. Therefore there is a need to generate local data to better understand the pathogeneses of the disease in our environment. This study aims to identify the link between haematological parameters, blood transfusion history and variations in spleen size of patients with sickle cell anaemia.

METHODS

This was a cross sectional study conducted amongst Sickle cell anaemia patients in steady state attending Haematology Clinic of the University College Hospital Ibadan, Nigeria. The diagnoses of patients and controls were confirmed using haemoglobin electrophoresis at alkaline pH. The controls were haemoglobin AA apparently healthy age and sex matched individuals. Ethical approval was obtained from the joint UI/UCH Ethics committee. Written and verbal consents were obtained from each participant. Consecutive patients with SCA who were in steady state were recruited into the study. The exclusion criteria were; history of blood transfusion in the last 3 months, other sickle haemoglobin phenotypes, patients on hydroxyurea and previous history of surgery. Interviewer administered questionnaire was used to collect the following information from all participant: demographic characteristics, past medical history, drugs and blood transfusion history. The medical history included clinical problem of first presentation and age at diagnosis, numbers of painful crises and hospital admissions in the last one year, number of units of blood transfused in the patient's lifetime. None of the patients was on hydroxyurea.

Three milliliters of blood was collected from both patients and controls into ethylenediamine- tetraacetic acid (EDTA) bottle for full blood count (FBC). The sample for full blood count was analyzed immediately using sysmex autoanalyzer Kobe, Japan. The following parameters were determined; haemoglobin concentration (Hb), haematocrit (Hct), red blood cell count (RCC), mean corpuscular haemoglobin (MCH), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white cell count (WCC) and differentials, and platelet count (plt ct). The irreversibly sickled cells (ISC) were manually estimated as percentages of the total red cells enumerated from blood films made with the Leishman stain and examined under high power microscope (X100) field as described by Dacie and Lewis[16]. Patients were sent for abdominal ultrasound immediately samples for FBC were collected.

Ultrasonographic evaluation

All SCA cases and controls were scanned using an Ultrasonix SP ultrasound machine with a 3 – 5 MHz curvilinear transducer. In the posterior axillary line in the area of the 10th rib was located and scanned through the intercostal space to have the entire the longitudinal view of the spleen with the hilus. In this position, maximum length were measured between

the most superomedial and the most inferolateral points of the spleen.

Splenic sizes were determined by ultrasonographically measured craniocaudal lengths of the spleen in our subjects. In this study, we categorized splenic sizes into three groups; Splenomegaly, normal size and autosplenectomy. A splenic length of 7cm – 13 cm was regarded as normal in this study, according to the generally accepted values [17, 18]. Autosplenectomy were splenic lengths ≤ 6cm according to the report of Ojo *et al.*[15] Autopslenectomy was further categorized in this study as; severe-absence of a spleen or splenic tissue less than 1cm in the absence of any history of splenectomy [5, 13]. Moderate form was defined as the long axis of the spleen measured < 1cm but less than 5.0cm (regarded as shrunken by some researchers) while Splenomegaly was defined when the long axis of the organ longer than 13.0cm [17,18]. Furthermore, we classified splenic lengths >5.0cm but ≤ 6.9cm as mild splenectomy.

Statistical analysis

Data were analyzed using SPSS version 23 (Statistical Package for Social Sciences, Inc., Chicago, Ill). The descriptive data were given as means ± standard deviation (SD) for continuous variable and frequency and percentages for categorical data. ANOVA was used to determine the significance of differences in more than 2 variables. Spearman correlation was used to determine the relationship between the splenic sizes and haematological parameters. Chi square was used to determine significance among proportional variables. Logistic regression was used to determine the degree of association. The differences were considered to be statistically significant when the p value obtained was < 0.05.

RESULTS

Demographic and clinical characteristic of patients

A total of 40 patients with SCA and 22 apparently healthy HbA controls were recruited. The mean age of patients was 29.3 ± 8.1 years while the mean age of the controls was 28.2 ± 8.1 years. The male: female ratio for patients and controls were 1: 1.3 and 1.1:1 respectively.

Sickle cell anaemia was diagnosed in most of the cases (52.5%) at childhood, while only one case (2.5%) was diagnosed at adulthood in the study population. The commonest first clinical presentation was dactylitis in 62.5% of the patients while splenomegaly was the least first clinical presentation in 5% of cases. In the preceding one year of study, 90% of the patients presented at the hospital with vaso-occlusive crises. Aplastic crises were the least documented presentation in only 2.5%. Ninety percent of the patients have been admitted for hospital management while 77.5% had received blood transfusion.

Splenic size evaluation on Ultrasonography and haematological profile of patients and controls

The spleen size was smaller than normal in most patients with SCA (70%) while about 20% and 10% had severe autosplenectomy and splenomegaly respectively (Figure 1). Among the controls (90.9%) had normal splenic size and 9.1% had mild reduction in size. None of the controls had splenic size that was classified as splenomegaly, moderate or severe autosplenectomy.

There was statistically significant difference between the mean spleen length of cases (6.3 ± 4.3cm) and controls (8.9 ± 1.5cm) (p = 0.001). The mean Hct and Hb value for patients were significantly lower than the value for controls (p < 0.001) while the WCC and platelet count were significantly higher in cases than in controls (Table 1). There was no difference in the red cell indices between the 2 groups.

Haematological parameters of patients with spleen size

There was a significant difference in the Hct, Hb, MCV and MCH values among SCA cases with different splenic sizes [p =0.006, 0.013, 0.038 and 0.008 respectively]. Post-hoc test (Tukey's HSD; Honest Significance difference test) showed that MCV and

Table 1: Haematological Parameters of Patients with SCA and Controls

Variables	SCA Cases	HbAA Controls	P-value
	Patients with SA Cases N, Mean ± SD	Controls N, Mean ± SD	
Age(years)	29.3 ± 8.1	28.2 ± 8.1	0.628
Splenic Size(cm)	6.3 ± 4.3	8.9 ± 1.5	0.001
Haematocrit (%)	25.5 ± 5.3	40.0 ± 4.5	<0.001
Haemoglobin (g/l)	76.6 ± 15.9	119.2 ± 13.6	<0.001
MCV (fl)	89.2 ± 9.5	87.1 ± 5.8	0.353
MCH(pg)	26.8 ± 3.3	25.9 ± 2.2	0.285
MCHC (g/dl)	300.1 ± 9.6	297.4 ± 7.5	0.255
Platelet Count 10^9 /L	360.8 ± 138.3	256.6 ± 60.5	<0.001
Total WBC Count 10^9/L	10.3 ± 3.3	5.2 ± 1.1	<0.001

Table 2: Variation in haematological parameters of patients with sickle cell anaemia according to splenic length

	Severe auto splenectomy	Moderate autosplenectomy	Mild autosplenectomy	Normal	Splenomegaly	P-value	Turkey HSD
	[illegible] $\bar{x} \pm SD$	[illegible] $\bar{x} \pm SD$	[illegible] $\bar{x} \pm SD$	[illegible] $\bar{x} \pm SD$	[illegible] $\bar{x} \pm SD$		
Hct (%)	25.6±5.5	22.2±5.3	22.4±3.6	29.4±4.8	28.2±3.6	0.006	4>2 & 3
Hb (g/l)	78.8±17.5	67.0±15.1	67.8±11.3	88.2±14.7	79.3±10.4	0.013	4>3
MCV(fl)	93.2±7.7	87.4±5.8	91.2±7.9	89.5±10.8	76.3±9.0	0.038	5<1 & 3
MCH(pg)	28.6±2.7	26.2±1.7	27.5±2.6	26.7±3.5	21.7±3.4	0.008	5<1, 3 & 4
MCHC(g/l)	307.0±7.3	301.6±8.4	301.3±6.8	299.0±7.8	284.0±10.7	0.001	5<1, 2, 3 & 4
Platelet Count(10^9/L)	388.6±135	376.6±204	408.8±104	342.6±126	191.5±84.6	0.082	
WBC(10^9/L)	9.10±2.0	11.7±3.8	9.93±2.1	11.4±4.4	8.45±4.1	0.333	
ISC(HPF)*	5 (3 - 5.5)	14.0(4.0 – 14.0)	4.0(3.0 – 8.50)	3.0(2.0– 10.0)	5.5(3.0 – 7.00)	0.538	

**Kruskal-Wallis H test: Median (IQR)*

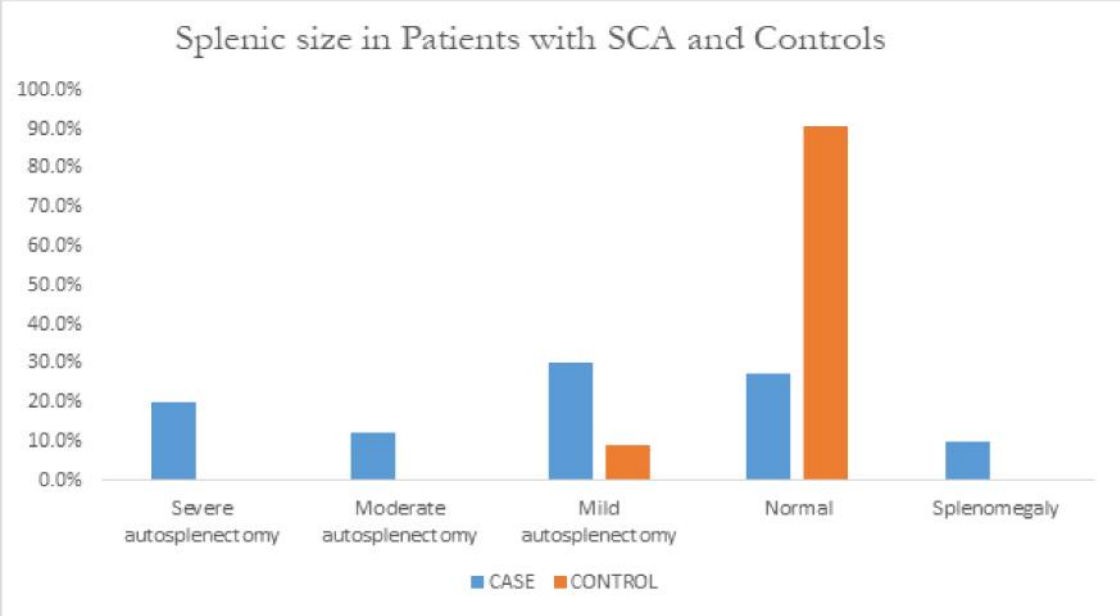

Fig. 1: Splenic size in patients with sickle cell anaemia and controls

Table 3: Correlation between haematological parameters and splenic length in sickle cell anaemia patients

	Splenic size	
	Correlation coefficient	**P-value**
Age (years)	-0.144	0.377
Haematocrit (%)	0.371*	0.019
Haemoglobin (g/L)	0.265	0.099
MCV(fl)	-0.340*	0.032
MCH (pg)	-0.418	0.007
MCHC (g/dl)	-0.527**	<0.001
Platelet count (10^9/L)	-0.353*	0.026
Total WBC count(10^9/L)	-0.039	0.810
ISC per high power field	-.107	0.512

MCH of SCA patients with splenomegaly were significantly lower than that of SCA patients with severe and mild autosplenectomy (Table 2). Sickle cell anaemia patients with splenomegaly also had statistically significant lower MCHC (28.4.0 ± 10.7) compared to HbSS patients with severe autosplenectomy (30.7.0 ± 7.3), moderate autosplenectomy (30.1.6 ± 8.4), mild autosplenectomy (30.1.6 ± 6.93) and normal spleen (29.7.8 ± 7.55) respectively [F (4,35) = 6.338, p = 0 .001].

Haemoglobin and haematocrit (Hct) were significantly lower in patients with splenic size smaller than the normal size (Table 2). The variation in WCC, platelet count and number of ISC among the patients were not significant even though platelet count and WCC were lowest among patients with splenomegaly.

Spearman's correlation of the haematological profile with splenic sizes showed that Hct values of SCA patients had a statistically significant positive correlation with splenic size (r = 0.371, p = 0.019) (Table 3). There was a statistically significant negative correlation between MCV (r = -0.340, p = 0.032), MCH (r = -

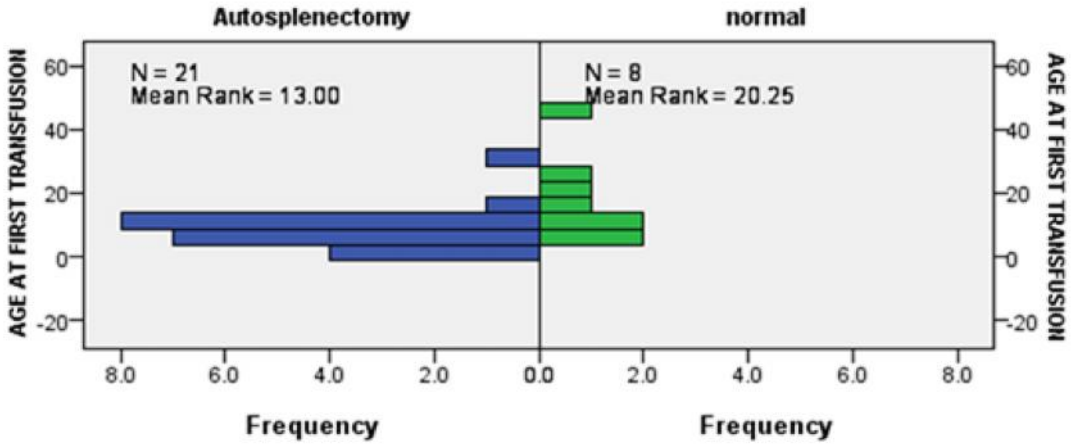

Figure 2: Comparison of age at first transfusion between sickle cell anaemia patients with autosplenectomy and normal spleen size

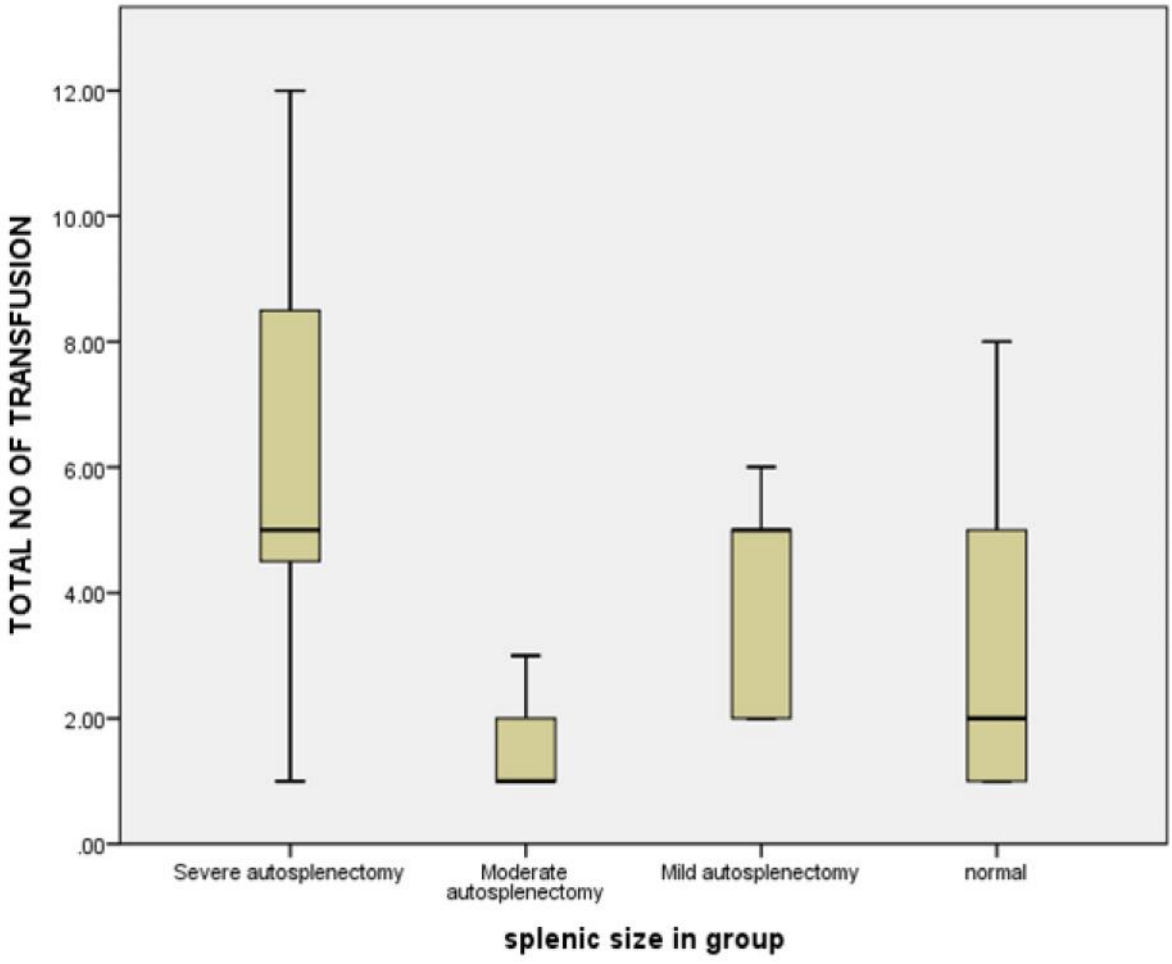

Fig 3: Frequency of Blood Transfusion in Relation To Spleen Size in Patients with Sickle Cell Anaemia

0.418, p = 0.007) MCHC (r = -0.527, p <0.001).and the splenic size of patients.

Blood transfusion history of patients with SCA and spleen sizes

With regards to blood transfusion pattern, 7/8 (88%) of HbSS patients with severe autosplenectomy had ever been transfused. Likewise, 5/5 (100%) of patients with moderate autosplenectomy and 10/12 (83%) of patients with mild autosplenectomy in this study had ever been transfused. In contrast, 8/11 (73%) and 1/4 (25%) of patients with normal spleen and splenomegaly had ever been transfused (p=0.069). The median age at first transfusion for patients with severe,

moderate and mild autosplenectomy were 6 years (IQR= 4.5 to 8.5 years), 5 years (IQR= 3 to 10 years) and 12 years (IQR= 8 to 13 years) old respectively. Patients with normal spleen size had higher median age at first transfusion 13.5years (IQR= 8.50 to 23.5 years). Further analysis showed that there was a significant difference in age at first transfusion between autosplenectomy cases and normal spleen (p=0.041) (Fig. 2).

Patients with severe autosplenectomy had the highest frequency of blood transfusion with a median of 5 (IQR = 4.5 to 8.5) times. While patients with moderate and mild autosplenectomy had relatively lower number of blood transfusion with median of 1 (IQR = 1.0 to 2.0) and 5 (IQR = 2.00; 5.00) times respectively (Fig. 3).

DISCUSSION

The study aims to determine the relationship between haematological parameters, blood transfusion history and variations in spleen size of patients with sickle cell anaemia. The essential splenic changes in SCA are splenomegaly and subsequent shrinkage in size of spleen (autosplenectomy)[18,19]. Severe autosplenectomy in this study is far less than 55.4% reported among Northern Nigerian patients with SCA and 33.3% of patients in Turkey but more than the 6.6–15.5% reported among Eastern Saudi Arabian patients [18,20,21]. Splenomegaly in SCA is unusual after the first decade of life, however, in this current study, 10% of patients had splenomegaly which is lower the 35.3% documented in paediatric patients[22]. This may be due to relatively older population in our study. Moderate autosplenectomy observed in 15% of our patients was also less than 31% of patients reported in another study from Nigeria[18]. The variability of the spleen sizes is often linked to co-existence of another heamoglobinopathy. However, changes could occur in the sonographic appearance of the spleen in patients with SCA in a region with improvement in supportive care and the use of acute and chronic transfusion therapy which reduces the occurrence of autosplenctomy [23].

The importance of some haematological values of patients in steady state in predicting clinical severity and management of SCD has been documented [19]. Comparing haematological parameters in patients with SCA and normal HbA controls, the Hb and Hct of patients are significantly less than that of non SCA while the white cells and platelet counts are higher. This findings corroborates previous studies [13, 22].

In comparison, the reports on red cell indices are rather inconsistent. While Akinbami [24] and Omoti [25] both from different parts of Nigeria reported a lower MHC, MCV and MCHC in patient than in the controls, these indices were comparable for both patients and controls in our study as well as some other studies from Nigeria by Iheanacheo[22]. The explanation for the lower values in patients compared to controls in the studies were anaemia of chronic disease, infections and haemolysis. Adeyemo *et al* in a related study attributed the low MCV and MCH, to a high frequency of haemoglobin variants (such as beta thalassaemia trait) co-existing with HbS[26]. The reason for similarities in both patients and controls might be due to similar environmental and biological factors that determine these indices while patient are in steady state and are regular with intake of their routine drugs. The biological factor is likely to be a co-inheritance of alpha or beta-thalassaemia as suggested by Adeyemo *et al.* which has similar frequencies in Nigerians with or without homozygous sickle-cell disease (SS, AA, AS and AC genotypes)[26, 27].

The changes observed in the haematological values of patients with SCA have been generally attributed to autosplenectomy in a blanket assumption, irrespective of the degree of reduction in splenic size[28]. This study shows that haematological profiles of patients with sickle cell anaemia vary in steady state with splenic size of the patient. Low Hb, Hct, WCC and platelet count are established features of splenomegaly. In contrast to report by previous worker of lower haemoglobin and haematocrit in sickle cell anaemia patients with splenomegaly[2,9], the patients with splenomegaly in this study had higher haemoglobin and haematocrit than patients with autosplenectomy. There was a significant positive correlation between spleen size and haematocrit as well as haemoglobin suggesting that autosplenectomy may be an indicator of disease severity. The presence of modifiers of the disease may reduce incidence of autosplenectmy[27]. Other forms of sickle cell disease associated with splenomegaly could not be detected using haemaoglobin electrophoresis at alkaline pH [28,29], the method of SCA diagnosis in our patients. This study contradicts the report by Awotua-Efebo *et al.* which did not study patients with autosplenectomy and did not observe significant differences in haematocrit value between patients with splenomegaly and those without[3]. The significant positive correlation between the splenic size and haematocrit of the patients suggests that the degree of autosplenectomy is a function of severity of the disease. It may also suggest that malaria may not be the only key player in the development of splenomegaly in adult patients with sickle cell anaemia particularly as there was less demand for blood transfusion in these patients. The higher haematocrit in

patients with normal splenic size and splenomegaly suggests that haemolytic process may not necessarily be accelerated in the presence of a spleen, particularly in the presence of genetic modifiers [30].

The similarity of red cell indices between controls and patients and significant variation in the red cell indices of patients with changes in splenic size suggest a pathophysiological link between the spleen size and haematological profile of the patients. In the SCA patients, the red cell indices showed significant negative correlation to the spleen size which was more marked with MCHC. The patients with normal spleen and splenomegaly had consistently lower MCV and MCHC compared to patients with severe autosplenectomy. Variability in red cell indices with age particularly the consistent trend for lower values of MCHC occurring in older children that stabilizes as patients get older has been linked to and well documented in alpha thalassemia [31]. Concurrent alpha thalassemia in patients with splenomegaly might be responsible for the significant lower MCV, MCH and MCHC. Mean cell haemoglobin concentration (MCHC) is a determinant of disease severity[32]. The low intraerythrocytic concentration of haemoglobin S as suggested by low red cells indices retards polymerization of the HbS and reduces sickling of the cells resulting in longer red cell survival. This might account for the higher haematocrit and low frequency of blood transfusion in patients with normal size spleen and splenomegaly. The inability to test for the thalassemia status of these patients is a limitation to this study.

The number of ISCs did not significantly vary with splenic size corroborating the report by Serjeant [33], though the same author documented low ISC in sickle cell anaemia patients with splenomegaly[34]. The combination of splenic hostile environment and higher MCHC favour the sickling process in patients with moderate autosplenectomy. This might be responsible for higher number of ISCs in patients with moderate autosplenectomy compared to the number in patients with splenomegaly and autosplenectomy. As MCHC increases, blood oxygen (O2) affinity decreases with increased tendency for red cell to sickle[33]. This is not the case in patients with autosplenectomy in whom the hostile environment of the spleen is absent. The low intra-erythrocytic haemogblobin S as suggested by reduced MCH and MCHC in patient with splenomegaly diminishes intravascular sickling and favour low level of irreversibly sickled cell count. Among the suggested haematological determinant of variable clinical severity of SCA, WBC, platelet count and ISC showed negative correlation with the spleen size corroborating the suggestion by Gale *et al* that autosplenectomy may suggest a severe clinical course[35].

The implication of the variation in haematological parameters with splenic size is reflected in the blood transfusion history of the patients. The blood transfusion requirements of the patients are strongly associated with the morphological insult the spleen received as reflected by the splenic size. Patients with history of splenic sequestration had significantly smaller spleens than others[35]. The blood transfusion history of the patients with evidence of the consequences of anatomic splenic injury such as mild, moderate and severe autosplenectomy suggests a more severe clinical course than patients whose splenic size did not show evidence of significant anatomic splenic injury. More than 80% of patients with mild, moderate and severe autosplenectomy have had blood transfusion. In contrast, about 63% and 25% of patients with normal spleen and splenomegaly had ever been transfused. (P=.047). The mean frequency of blood transfusion was significantly higher among patient with smaller than normal spleen size with the highest frequency being among patients with severe autosplenectomy. The higher mean rank age at first transfusion in patients with normal spleen compared with patients with severe autosplenectomy and moderate autosplenectomy is in agreement with the observation by McCarville *et al.* [36] However, our finding does not agree with another study from Nigeria which reported that transfusion needs for SCA patients with and without splenomegaly is similar and blood transfusion requirement had a non-significant negative correlation with splenic size[3]. Our study does not also support the finding by Helvaci *et al.*[11] that autosplenectomy was associated with less transfusion in patient's life time. The younger age at first transfusion and higher demand for blood transfusion in patients with severe autosplenectomy suggest the degree of autosplenectomy may be used to determine severity of clinical course and to identify patients that may benefit from disease ameliorating therapy.

CONCLUSION

This work demonstrates a pathophysiological link between haematological profile and spleen size in patients with SCA. There were significant variations in the red cell indices with splenic sizes of the patients which was more marked with the MCHC. Autosplenectomy in patients is associated with lower haematocrit compared to normal spleen size and spleneomegaly. In view of the younger age at first transfusion and higher demand for blood transfusion in patients with severe autosplenectomy, the degree of autosplenectomy may be used to determine severity of clinical course of patients.

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