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Original Article

The Effect of Alpha Thalassemia, HbF and HbC on Haematological Parameters of Sickle Cell Disease Patients in Ibadan, Nigeria

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Abstract. ***Background:*** **Sickle cell disease is a protean disease with limited data on Nigeria's phenotypic and genetic variants. This study was conducted to provide baseline data on these variants by characterising the existing forms of sickle cell disease and correlating these with basic haematological parameters.**
Methods: **Adult and paediatric patients with SCD were recruited from a tertiary health centre in Nigeria. Patients were age and sex-matched with healthy controls. Blood samples were obtained for Full Blood Count, phenotyping by High-Performance Liquid Chromatography, and genotyping for alpha thalassemia by multiplex Gap-polymerase chain reaction. Data analysis was done using IBM SPSS statistics version 23.**
Results: **A total of 130 patients with sickle cell disease and 117 controls were studied. Alpha thalassemia in the study population was due to a 3.7kb deletion in the alpha-globin gene cluster at a prevalence of 45.4% in the patients and 47% in the controls. The prevalence of the various existing forms of SCD genotype was: Homozygous S without alpha gene deletion (HbSS)- 39.2%; HbSC - 10.8%; HbSS$^{\alpha+1}$- 35.4%; HbSS$^{\alpha+2}$ - 6.9% and HbSF- 7.7%. In the control population, HbAA without alpha gene deletion had a prevalence of 42.7%, HbAA$^{\alpha+1}$ was 25.6%, HbAA $^{\alpha+2}$ was 6%, HbAS- 7.7%, HbAS $^{\alpha+1}$ – 11.1%, HbAS $^{\alpha+2}$ - 2.6%, HbAC – 2.6% and HbAC $^{\alpha+1}$ – 1.7%. HbA_2 was significantly elevated in HbSS individuals with two alpha gene deletions but reduced in normal controls (HbAA) with alpha gene deletions. HbF and HbA_2 were negatively correlated with each other (r= -0.587, p < 0.001). Individuals with the HbSC genotype followed by HbSSα^{+2} had the best haematological parameters.**
Conclusions: **Haematological parameters vary with haemoglobin genotype. The C haemoglobin and homozygous alpha-thalassemia deletion had a better ameliorating effect on SCD haematological parameters than the F haemoglobin in this population. The effect of alpha thalassemia on some haematological parameters in SCD patients are reversed in normal controls.**

Keywords: Sickle cell disease; Alpha thalassemia; Haemoglobin phenotype; Haemoglobin F; Haemoglobin C; Haemoglobin A_2.

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Introduction. Sickle cell disease (SCD) is a heterogeneous group of haemoglobin (Hb) disorders in which the pathology is attributed to the presence of sickle haemoglobin (HbS). It is highly prevalent in sub-Saharan Africa. The heterogeneity in phenotype-genotype has been described, including homozygosity for HbS represented as HbSS, the double heterozygous conditions: HbS with HbC disease (HbSC), HbSD Punjab, HbSO-Arab, HbSE and HbS with different genotypes of beta (β) thalassemia. All these haemoglobin types are results of mutations in the beta-globin gene. Other haemoglobins co-inherited with the sickle haemoglobin are fetal haemoglobin (HbF) and different genotypes of alpha thalassemia.

The major form of normal haemoglobin in an adult is haemoglobin (HbA), a tetramer of two alpha (α) globin and two beta (β) globin chains. Normal minor haemoglobins include HbA_2 (alpha and delta chains) and HbF (alpha and gamma chains).

In the normal adult, HbF is usually around 1% and below, while HbA_2 is usually about 2%.[1] However, in some SCD patients, the HbF level remains high, and the level of HbA_2 may be higher than expected due to the coinheritance of alpha or beta-thalassemia. Haemoglobin F and A_2 become diagnostically important when their amount exceeds their reference values in the blood.

The inheritance of alpha thalassemia and HbF with SCD partially explains the clinical and haematological heterogeneity of SCD.[2] The principal pathophysiology in SCD is HbS polymerisation in the deoxygenated state resulting in red cell sickling, vaso-occlusion and haemolysis. The course of disease in patients with SCD is ameliorated by the inheritance of HbF and alpha thalassemia, earning them the term genetic modifiers.[3] HbF molecules do not participate in the polymerisation between molecules of deoxygenated HbS, so elevated levels of HbF impair the polymerisation of deoxyHbS, resulting in a milder SCD. Concurrent inheritance of alpha-thalassemia with SCD reduces intra-erythrocyte HbS concentration, with a consequent reduction in the polymerisation of deoxyHbS, red cell sickling and haemolysis. This is particularly important in patients with HbSS, which is the most common and severe form of SCD[4]

A range of 0.9 to 16% has been reported for HbF level among our SCD patients using the alkali denaturation technique by Betke.[5,6] Sickle cell anaemia (HbSS) patients with HbF concentrations greater than or equal to 20% are compound heterozygotes for HbS and Hereditary Persistence of Foetal Haemoglobin which can be termed HbS-HPFH, and the phenotype is represented as HbSF.[7] Patients with HbS-HPFH have mild disease.[8] A study reported that HbF-increasing β globin gene locus haplotypes similar to Arab-Indian' and 'Senegal' haplotypes are lacking in Nigerian patients.[9] The haplotype described in Nigeria is the Benin haplotype.[4] Falusi *et al.* reported a high prevalence of alpha thalassemia, specifically −α3.7 deletion among Nigerian patients with SCD[10] These patients with alpha thalassemia also had the tendency to have elevated A_2. The degree to which both alpha thalassemia and HbF ameliorate our patients' SCD features is unknown.

SCD patients who inherit a high level of HbF, elevated A_2 or alpha thalassemia have distinct haematological features.[11] The distinct haematological parameter could be an inexpensive tool to suggest the coinheritance of other haemoglobins, the knowledge of which could be used to prognosticate patients. Despite the high burden of SCD in Nigeria, there is limited data on the different forms of the disease as influenced by the coinheritance of genetic modifiers. The genotype-phenotype profile of the disease in relation to haematological parameters is not well defined in many parts of the country. This limitation is because the diagnosis of SCD using electrophoresis with cellulose acetate at an alkaline pH as the principal method of routine screening in our setting has limited the detection of abnormal haemoglobin phenotypes to HbSS and HbSC and cannot directly quantify the haemoglobin fractions. The method cannot distinguish HbS from HbD, HbG and Hb Lepore. Similarly, it cannot distinguish HbC from HbA_2, HbE, HbO.[12,13] HbS and HbC being the most prevalent variant haemoglobins, all haemoglobins on the location of the band assigned to HbS and HbC are presumed to be such. The diagnoses of these haemoglobins are usually validated by the controls (A, S, C) and the presence of sickled erythrocytes and numerous target cells on peripheral films. High-performance liquid chromatography (HPLC), the gold standard for screening and detecting the coinheritance of these haemoglobins, is not easily accessible in many peripheral health centres, and haemoglobin genotyping is also not available. HPLC can quantify Hbs F, A_2 and S levels to decipher the heterogeneity of SCD further. Hence, this study aims to use HPLC to determine our SCD patients' haemoglobin phenotypes and the relationship of the haemoglobin phenotypes and alpha thalassemia status to the haematological parameters.

Materials and Methods. This is a cross-sectional study of patients diagnosed with SCD by haemoglobin electrophoresis at alkaline pH 8.6[14] and managed for SCD at the Pediatric Outpatient Clinic and Hematology Clinic of the University College Hospital, Ibadan. Age- and sex-matched controls, including school children, students of tertiary institutions and other individuals

from the community, were recruited. The sample size was 130 for patients and 117 for controls. All patients with SCD attending the clinic in a steady state and who had not received a blood transfusion in the last four months preceding the study were eligible and invited to participate. This exclusion was to avoid the analysis of haemoglobin fractions from transfused blood in the participants. The SCD patients were recruited into the study irrespective of whether they were on hydroxyurea therapy or not. Informed consent was obtained from both patients and controls: for children, informed consent was obtained from their parents, including assent from children aged 13-18 years. Questionnaires were administered to obtain information on demographic data. Venous blood was collected from each patient and control. Three milliliters was put in Ethylenediaminetetraacetic acid (EDTA) bottle for full blood count, high-performance liquid chromatography (HPLC) analysis and DNA extraction.

Samples were transported in a cold box from the collection site to the Genetics and Bioethics Research Unit laboratory, IAMRAT, College of Medicine, University of Ibadan, Nigeria. High-performance liquid chromatography (HPLC) by the Bio-Rad Variant II^{TM} HbA2/HbA1C Dual Program (Clinical Diagnostics, California, USA) was used as described in the instruction manual for the assay to identify and quantify HbA, HbS, HbC, HbF and HbA_2 in both SCD patients and controls.[15] The HPLC consists of 2 methods, of which the Beta-thal (HbA_2) method was used for analysis. Full blood count for SCD and controls was determined using the Swelab 3-part Haematology Analyser (Boule Diagnostics, Sweden). DNA extraction from whole blood was carried out at the Genetics and Bioethics Research Unit laboratory, IAMRAT, College of Medicine, University of Ibadan, Nigeria using Qiagen kit (QIAGEN Inc., MD, USA) according to manufacturer's instruction.[16] DNA analysis to determine deletions the alpha-globin gene cluster was carried out at the Haematology-Oncology Laboratory, Department of Medicine, University of Chicago. The deletion in the alpha globin gene cluster was determined by Multiplex gap-PCR.[17] The participants were identified as heterozygotes when there was only one gene deletion ($-\alpha^{3.7}/\alpha\alpha$) represented as α^{+1}, homozygotes are when there were two gene deletions ($-\alpha^{3.7}/-\alpha^{3.7}$), represented as α^{+2}. Ethical approval for this study was obtained from the University of Ibadan/University College Hospital, Ibadan Institutional Ethics Review Committee, College of Medicine, University of Ibadan and the Institutional Review Board at the University of Chicago

Statistical Analysis. Data were processed using the IBM SPSS statistics version 23. Variables were tested for normality using the Shapiro-Wilk test. Descriptive statistics of continuous variables were obtained using mean ± SD, median and interquartile range (IQR) and categorical variables reported in proportions. Student T-test was used to compare means between groups, and Mann-Whitney U test was used to compare median between groups. Age and hydroxyurea use were controlled for using ANCOVA in the comparison of haematological parameters in SCD patients. Pearson's correlation was used to test for a relationship between continuous variables. Statistical significance was set at $p<0.05$ (2-tailed). Sickle cell anaemia patients without alpha thalassemia and HbF lower than 20% were identified as HbSS, while patients who were not on hydroxyurea and had HbF ≥20% were categorised as having HPFH, thereby labelled as HbSF. HbA_2 values of 3.5% and above was considered elevated.[12]

Results.

Demography of Study Population. A total of 130 children and adults diagnosed with sickle cell disease (SCD) and 117 age and sex-matched controls without sickle cell disease were enrolled in the study. The median (interquartile range) age among SCD cases was 16 (9 - 29) years with a minimum age of 3 years and a maximum age of 63 years. Characteristics of the study participants are shown in **Table 1**. Twenty (15.4%) of the SCD patients in this study were on hydroxyurea.

Table 1. Characteristics of SCD patients and controls.

Variables	Cases	Controls
Number of participants	130	117
Number of Children	67	61
Number of Adults	63	56
Age of Children (years)	8.9±4.3	9.3±3.8
Age of Adult (years)	30.6±10.1	29.6±10.3
Male	70 (53.8%)	59 (50.4%)
Female	60 (46.2%)	58 (49.6%)

Distribution of Haemoglobin Phenotypes in the Study Population. Based on the results obtained from HPLC, 106/130 (81.5%) of the SCD patients were HbSS, 14/130 (10.8%) were HbSC, and 10/130 (7.7%) were HbSF. The HbSF comprised 7(10.4%) out of 67 children (<18 years) and 3 (4.8%) out of 63 adults (> or = 18 years). All patients who were not on hydroxyurea with HbF level > 20% were categorised as HbSF. For the control group, 87/117 (74.4%) were HbAA, 25 (21.4%) were AS, 5(4.3%) were HbAC.

Comparison of Haematological Parameters Between SCD Population and Controls. Participants without SCD had statistically significant higher HGB (Median = 12.60 g/dL), RBC (Median = 4.62 x 10^{12}/L), HCT (Median = 40.20%), and LYMF (Mean = 54.01 ± 11.42%) when compared with SCD patients: HGB (Median = 8.0 g/dL), RBC (Median = 2.79 x 10^{12}/L), HCT (Median = 25.0%),

Table 2. Relationship between haematological SCD population and controls.

Variable	SCD	CONTROL	P-value
	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)	
HGB (g/dL)	8.0 (7.28 - 9.15)	12.60 (11.80 - 13.60)	<0.001
RCC (x10^{12}/L)	2.79 (2.49 - 3.41)	4.62 (4.29 - 5.00)	<0.001
HCT (%)	25.0 (22.05 - 28.48)	40.20 (37.20 - 42.60)	<0.001
MCV (fl)	87.20 (80.70 - 94.38)	87.40 (83.00 - 91.40)	0.620
RDW (%)	22.55 (20.35 - 25.25)	15.95 (14.75 – 62.75)	<0.001
MCH (pg)	28.17 ± 3.48	27.26 ± 2.94	0.031
MCHC (g/dL)	32.60 (31.38 - 33.43)	31.70 (30.70 - 32.60)	<0.001
WBC (x10^9/L)	10.55 (4.7 – 14.80)	5.30 (4.40 – 6.80)	<0.001
LYMF (%)	46.07 ± 12.17	54.01 ± 11.42	<0.001
GRAN (%)	46.85 ± 13.00	39.25 ± 13.70	<0.001
PLT (x10^9/L)	331.0 (235.8 – 447.5)	233.0 (177.0 – 284.0)	<0.001
MPV (fl)	8.30 (7.73 – 8.98)	8.50 (7.90 – 9.20)	0.125
PCT (%)	0.28 (0.20 – 0.37)	0.20 (0.16 – 0.25)	<0.001
PDW (%)	10.75 (10.00 – 11.83)	11.05 (10.30 – 12.10)	0.128

Mann-Whitney U: median (IQR: interquartile range). T test: Mean ± SD. HGB: haemoglobin; RCC: red cell count; HCT: haematocrit; MCV: mean corpuscular volume; RDW: red cell distribution width; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell count; LYMF; lymphocyte count; GRAN; granulocyte count: PLT: platelet count. PCT: plateletcrit; MPV; mean platelet volume: PDW; platelet distribution width

and LYMF (Mean = 46.07±12.17%) respectively. The RDW (Median = 22.55%), MCH (Mean = 28.17± 3.48 pg), WBC (Median = 10.55 x 10^9/L) GRAN (Mean = 46.85±13.0%) and PCT (Median = 0.28%) were statistically significantly higher in SCD patients than among the control group: RDW (Median = 15.95%), MCH (Mean = 27.26±2.94 pg), WBC (Median = 5.30 x 10^9/L), GRAN (Mean = 39.25±13.70%) and PCT (Median = 0.20%) respectively (**Table 2**).

Alpha Thalassemia in the Study Population. Alpha thalassemia in the study population was due to a 3.7kb α-globin gene deletion. Alpha thalassemia was present at a prevalence of 45.4% (59/130) in the patients and 47% (55/117) in the control group. Based on the combination of results from HPLC and analysis to detect alpha thalassemia, the SCD patients were divided into five groups. These included homozygous S without alpha gene deletion (HbSS), haemoglobin S with haemoglobin C disease (HbSC), haemoglobin S with coinheritance of alpha thalassemia with one gene deletion (heterozygous deletion) otherwise known as silent alpha thalassemia (HbSSα^{+1}), haemoglobin S with coinheritance of alpha thalassemia with two gene deletions (homozygous deletion) otherwise known as alpha thalassemia trait (HbSSα^{+2}) and haemoglobin S with hereditary persistence of foetal haemoglobin (HbSF). The prevalence of the various existing forms of SCD genotype was: Homozygous S without alpha gene deletion (HbSS)- 39.2%; HbSC - 10.8%; HbSSα^{+1}- 35.4%; HbSSα^{+2} – 6.9% and HbSF- 7.7%. Of the 59 patients with alpha thalassemia, 6 (10.2%) had HbA_2 ≥3.5%. Four of the SCD patients with α^{+1} deletion also had HbC and were categorised as HbSC for analysis. **Table 3** shows the distribution of sickle cell patients in each category. In the control population, HbAA without alpha gene deletion had a prevalence of 42.7%, HbAA$^{\alpha+1}$ was 25.6%, HbAA $^{\alpha+2}$ was 6%, HbAS- 7.7%, HbAS $^{\alpha+1}$ – 11.1%, HbAS $^{\alpha+2}$ - 2.6%, HbAC – 2.6% and HbAC $^{\alpha+1}$ – 1.7%.

Haematological Parameters for the Sickle Cell Genotype Categories. **Table 3** shows the haematological parameters for the different genotype categories, controlled for age and hydroxyurea use. There was marked variability of the haematological parameters among the various sickle cell disease genotypes. Patients with HbSC had the highest haemoglobin (HGB), haematocrit (HCT) and red cell count (RCC) and the lowest red cell distribution width (RDW). RDW was similar for HbSα^{+1}, HbSα^{+2} and HbSF. Homozygous S patients without alpha thalassemia and with HbF lower than 20% (HbSS) had the lowest HB, HCT, RCC and highest white blood cell count (WBC), platelet count (PLT) and RDW. The differences in WBC and PLT were significant only between SS and SC. The white cell count in HbSSα^{+2}, HbSSα^{+1} and HbSF were comparable. Platelet count was also significantly higher in SS than in SF. Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were lowest for HbSSα^{+2} followed by HbSSα^{+1}, and SC and highest for SS and SF ($p<0.001$). There was no significant difference in the MCV between HbSS without alpha thalassemia and HbSF individuals. There was no

Table 3. Comparison of the Haematological parameters in sickle cell disease patients with haemoglobin genotype and phenotype.

Variables	HbSS N = 51 1	HbSC* N = 14 2	HbSα^{+1} N = 46 3	HbSα^{+2} N= 9 4	HbSF N = 10 5	p	Post hoc (Sidak)
	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)		
HGB (g/dL)	7.75 ± 0.89	11.1± 1.73	8.00 ± 1.33	9.05 ± 1.82	8.23± 2.02	<0.001	2>1, 3, 4 & 5
RCC (x10^{12}/L)	2.63 ± 0.37	4.34± 0.89	2.98 ± 0.64	4.02 ± 1.04	2.72± 0.64	<0.001	2>1, 3, & 5 3>1 4>1, 3 & 5
HCT (%)	23.8 ± 2.94	34.3± 5.37	24.5 ± 4.07	29.3 ± 6.67	25.9± 5.58	<0.001	2>1, 3 & 5 4>1
MCV (fl)	91.2 ± 8.41	81.4± 6.97	85.1 ± 18.1	73.5 ± 5.73	95.8± 6.18	0.001	4<1 & 5 2<1 & 5
RDW (%)	24.1 (22.3 - 26.3	18.3 (17.8 - 18.6)	22.6 (21.2 - 24.8)	22.0 (20.4 - 23.5)	22.0 (19.7 - 22.6)	0.001	2<1 & 3
MCH (pg)	29.8 ± 3.13	27.1± 2.45	27.2 ± 2.85	22.9 ± 1.81	30.4± 2.91	<0.001	1>2, 3, & 4 3>4 5>3 & 4
MCHC (g/dL)	32.6 ± 1.34	33.2± 0.62	32.0 ± 2.19	31.1 ± 0.93	31.7± 1.52	0.071	
WBC (x10^{9}/L)	12.0 (9.65 - 14.8)	8.20 (4.7 - 9.40)	10.0 (8.18 – 12.5)	10.3 (7.05 - 14.8)	10.8 (7.10 - 12.1)	0.029	1>2
PLT (x10^{9}/L)	397.5 (310.8 - 485.5)	159.5 (145.5 - 222.5)	342.3 (255.3 - 402.0)	310.0 (212.5 - 450.8)	219.0 (181.5 - 270.5)	<0.001	1>2 & 5 3>2
HbA2(%)	1.52 ± 0.77	1.70± 0.34	2.02 ± 0.86	3.05 ± 0.83	0.33± 0.23	<0.001	4>1, 2, 3 & 5 3>5 2>5 1>5
HbF(%)$^{\#}$	6.80 (4.20 – 10.3)	1.10 (0.80 – 1.50)	6.10 (3.30 – 9.60)	8.55 (4.05 – 10.2)	25.3 (23.2 – 27.1)	<0.001	2<1, 3, 4 & 5 1<5 3<5
HbS (%)$^{\#}$	84.6 (80.2 – 86.3)	48.1 (46.8 – 48.6)	83.5 (79.0 – 87.2)	80.1 (78.8 – 83.2)	66.8 (65.9 – 68.8)	<0.001	2<1, 3, 4 & 5 5<1

ANCOVA: controlled for age and hydroxyurea use. HGB: haemoglobin; RCC: red cell count; HCT: haematocrit; MCV: mean corpuscular volume; RDW: red cell distribution width; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell count; PLT: platelet count. ***With and without alpha thalassemia.**

significant difference in the Mean Corpuscular Haemoglobin Concentration (MCHC) among the different SCD genotypes. Sickle cell disease patients who were on hydroxyurea had significantly higher MCV (Median = 92.30, IQR = 88.05; 99.03) than SCD patients who were not on hydroxyurea (Median = 86.90, IQR = 79.83; 93.28) p=0.014. Similarly, there was a statistically significant difference in MCH between SCD patients on hydroxyurea (29.9 ± 3.26) and SCD patients not on hydroxyurea (28.0 ± 3.44) p= 0.040.

The mean HbS percentage was significantly lower in SF than in the HbSS, HbSSα^{+1} and HbSSα^{+2} (**Table 3**). The HbA_2 level was lowest (0.33 ± 0.23%) in HbSF and highest (3.05 ± 0.83%) in HbSSα^{+2} (p<0.001). Among the groups of patients, HbF level was lowest in HbSC and comparably low in HbSS, HbSSα^{+1} and HbSSα^{+2} (**Table 3**).

Haematological Parameters for the Control Group. In the control population, the HGB and HCT concentration reduced with decreasing number of alpha chains, while RCC increased. These, however, did not reach statistical significance (**Table 4**). The difference in the MCV among HbAA controls (89.8 ± 6.27 fl), HbAS controls (84.8 ± 6.92 fl), HbAAα^{+1} patients (83.6 ± 11.3 fl), HbAAα^{+2} patients (75.8 ± 4.29 fl), and HbAC controls (81.3 ± 5.08 fl), were statistically significant, p <0 .001. For adequacy of statistical analysis, the HbAS and HbAC controls could not be separated into those with and without alpha thalassemia. Thirteen HbAS (11.1%) individuals had single deletion, and three (2.6%) had double deletion. Two of the five HbAC individuals had single alpha deletion. MCV in both HbAAα^{+1} and HbAAα^{+2} controls were statistically significantly lower than in HbAA (p=0.008 and p<0.001), respectively. However, the mean MCV for AS and AC did not differ significantly from other groups, respectively. MCH level in HbAAα^{+2} controls was significantly lower than in AAα^{+1} (p=0.049), HbAS (p=0.006) and AA (p<0.001) respectively. The result also showed that the MCH level in HbAAα^{+1} controls was significantly lower than in AA controls (p=0.001).

Haemoglobins F and A_2 in the Study Population. The mean HbF level in the patients that were not on hydroxyurea was 8.3 ± 7.3% (0.2 - 30.6%), while the mean HbF level of patients on hydroxyurea was 9.9±7.3% (1.8-25.1%). There was a negative correlation

Table 4. Haematological parameters in controls with haemoglobin genotype and phenotype.

Variables	HbAA N = 50 1	HbAS* N = 25 2	HbAα^{+1} N = 30 3	HbAα^{+2} N= 7 4	HbAC* N= 5 5	p	Post hoc (Sidak)
	Mean ± SD /Median (IQR)	Mean±SD /Median (IQR)	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)	Mean ± SD /Median (IQR)		
HGB (g/dL)	13.1 ± 1.77	13.0 ± 1.30	12.2 ± 2.21	11.5 ± 1.51	12.6 ± 1.45	0.070	
RCC (X10^{12}/L)	4.60 ± 0.54	4.80 ± 0.50	4.70 ± 0.93	4.98 ± 0.68	4.57 ± 0.19	0.539	
HCT (%)	41.4 ± 5.35	40.7 ± 5.10	39.8 ± 7.44	37.7 ± 4.66	37.1 ± 2.50	0.306	
MCV (fl)	89.8 ± 6.27	84.8 ± 6.92	83.6 ± 11.3	75.8 ± 4.29	81.3 ± 5.08	<0.001	1>3& 4
RDW (%)	15.1 ± 1.36	15.3 ± 1.13	15.7 ± 1.53	15.5 ± 0.72	15.4 ± 0.08	0.518	
MCH (pg)	28.0 ± 2.90	27.1 ± 2.46	26.1 ± 2.39	23.2 ± 1.64	27.3 ± 2.94	<0.001	4<1,2,& 5 3<1
MCHC (g/dL)	32.0 ± 2.46	32.0 ± 2.04	30.7 ± 2.36	30.7 ± 2.71	33.8 ± 2.02	0.022	5>3&4
WBC (x10^{9}/L)	5.59 ± 1.74	4.90 ± 1.29	5.54 ± 1.89	5.81 ± 2.02	7.04 ± 0.84	0.119	
PLT (x10^{9}/L)	238.8 ± 97.8	229.2 ± 77.6	222.4 ± 111.1	261.9 ± 84.3	305.0 ± 91.5	0.441	
HbA2 (%)	2.88 ± 0.55	3.45 ± 0.87	3.00 ± 0.41	2.50 ± 0.32	3.38 ± 0.33	<0.001	2>1,3,4
HbF (%)	0.65 (0.50 – 1.00)	0.50 (0.10 – 1.20)	0.55 (0.40 – 0.100)	0.90 (0.50 – 1.80)	0.90 (0.80 – 1.80)	0.412	
HbA0 (%)	85.5 ± 2.03	55.5 ± 8.51	83.9 ± 9.90	85.6 ± 1.71	58.0 ± 15.8	<0.001	2<1, 3& 4 5<1, 3& 4

***With and without alpha thalassemia.**

between HbF level and age in patients not on hydroxyurea (r= -0.260, p= 0.009). All the different categories of SCD have varying levels of HbF irrespective of the alpha thalassemia status. Excluding patients on hydroxyurea, 29/110 (26%) patients had HbF level >10%. The mean HbA_2 level in the SCD patients was 1.71 ± 0.95% with a range of 0.1 - 4.0%, while the mean HbA_2 level in controls was 3.03 ± 0.63% with a range of 0.9 - 4.42%. The level of HbA_2 (%) in HbAS controls was significantly higher than in HbAA (p=0.002), HbAAα^{+1} (p=0.029) and HbAAα^{+2} (p=0.005) respectively (**Table 4**). Six (10.7%) of the SCD patients with alpha thalassemia had HbA_2 ≥3.5%, while 22 (40.0%) controls with alpha thalassemia had HbA_2 ≥3.5%. Very low HbA_2 were observed in some patients with HbSF, HbSC, HbSS without alpha thalassemia.

Discussion. This study shows the heterogeneity of sickle cell disease among our patients and attempts to relate it to haematological indices. Homozygous S (HbSS) patients constituted 81.5% of our patients, which is lower than 90% reported among SCD in the Northern part of the country.[18] and then 92.1% of a previous study among children in our centre.[19] The disparity in the prevalence of SS between this study and the previous study in our centre might be attributed to the separation of HbSF from HbSS in the current study, unlike in the previous study. The dissimilarity with this study conducted in South Western Nigeria from the study in Northern Nigeria may reflect the lower prevalence of HbSC (4%) in Northern Nigeria[18] compared to South Western Nigeria with higher HbSC mutation (10.8%) as reflected in our study. This lower prevalence in the Northern part of the country has also been documented by Akinyanju et al.[20] The prevalence of SC in our study is consistent with Brown et al.[21]. The documentation on the prevalence of HbSF in Nigerian patients with SCD is scanty. A prevalence of 4.8% among our adult patients is higher than 2% reported for adult patients in Lagos by Akinbami et al.,[22] but this disparity may not be significant considering the sample size of the adult patients in both studies. A prevalence of 3.2% in 180 pediatric patients in Lagos reported by Adeyemo et al.[23] is much lower than the 10.4% of the 67 paediatric patients of this study. Factors influencing the behavior and decision of patients to go to the hospital can contribute to variation in prevalence rate in hospital-based studies. In line with those mentioned above, most studies on the prevalence of SF have been hospital-based, leaving out SCD patients with a mild clinical course and less likely to be followed up in the Haematology clinic. If the patients were eventually registered in the clinic, they might visit the hospital infrequently depending on how conducive and convenient the hospital environment is. This behavior may cause variability in reports from hospital-based studies. Since the health system in Nigeria is pay out of pocket, SCD phenotypes with less severe disease manifestations are also less likely to be encountered in a hospital-based study, except they are financially buoyant. HbSF was not detected in a survey of 10,001 infants and children from the Northern part of the country.[19] This finding underscores the variation in the geographical distribution of Hb phenotype within the same country and the high variability of HbF level in the Benin haplotype seen in the West African population where Nigeria belongs.[22] The possibility of the spread of the Bantu haplotype with the least HbF level to Northern Nigeria being close to the Cameroon region should be considered since the haplotype in the study participants

was not investigated.

Alpha thalassemia reduces the concentration of HbS in the red cell of the patients; however, the consequence of the reduction varies in different populations. In the Democratic Republic of Congo, among pregnant women, morbidity related to sickle cell complications in the mother and foetus were less frequent in the HbSS/alpha-thal than in HbSS.[24] The protective role of α-thalassemia against the risk of abnormal TCD in Nigerian children with SCA has been described.[25] The coinheritance of alpha thalassemia and SCA has also been associated with lower consultation rates and increased survival.[26] However, a frequent and severe vaso-occlusive crisis has been associated with alpha thalassemia in children.[27] A significant proportion (45.4%) of our SCD patients had alpha-thalassemia due to 3.7kb α-globin gene deletion, suggesting heterogeneity in the sickle cell genotype and supporting the report of the previous study carried out by Falusi et al. in the same centre[10] and in blacks.[28,29] Of all the SCD patients with alpha thalassemia, the ameliorating effect may be expected in the 15.3% with homozygous gene deletion because the single gene deletion has minimal effect on the pathophysiology of sickle cell disease.[30] The haematological parameters in our patients varied with the haemoglobin phenotypes and genotypes, further underscoring the significance of haematological parameters in categorising the patients. Patients with HbSC had the highest HGB level, RCC and HCT, followed by patients with homozygous alpha-thalassemia and then HbSF. Red cell count was significantly higher in homozygous alpha-thalassemia than in heterozygous alpha-thalassemia; the higher HGB and HCT was not significant. Higher HGB and RCC in patients with alpha thalassemia is due to a reduction in the rate of hemolysis[31] and increased red cell precursor mitosis, smaller red cells (low MCV) and relative erythrocytosis for the degree of anaemia.[32] Our finding corroborates the previous report about high haemoglobin in patients with α-thalassemia, which is related to the number of alpha deletions.[33]

White blood cell count (WBC) and platelet count (PCT) have been documented to be higher in patients with SCD compared with their healthy counterparts.[34] This is consistent with the findings in our study with mean WBC and platelet count of $10.5x10^9$/L and 331.0 x 10^9/L respectively in SCD compared to $5.30x10^9$/L and $233x10^9$/L in the non-SCD controls. There are multiple causes for leukocytosis in SCD. The most important reason is the marked inflammation that characterises the disease. In addition to inflammation contributing to a higher platelet count in SCD than in controls, auto splenectomy could also contribute to the elevated platelet count. In agreement with reports from other studies,[31,35] the mean HGB, HCT, and RCC counts of the patients showed gradual increment from no alpha deletion, through one alpha deletion to 2 alpha deletions; while MCV, MCH, RDW, MCHC, WBC, PLT and HbS level showed a gradual decrement in the same direction. This shows a reversal of the expected high WBC and PLT in patients with SCD. Also, significant gradual decrement is observed in controls' MCH and MCV values with the number of alpha deletions. Haemoglobin concentration and haematocrit increased with the number of alpha genes deleted in the patients, unlike in the control group, where the reverse was the case. The degree of anaemia, leukocytosis and platelet count due to alpha thalassemia in the patients may be reduced because the severity of the disease is ameliorated by reducing the amount of sickled RBC, the intracellular HbS level and inflammatory process. All these lower cell damage and improve the hemolysis profile. The inheritance of 2 alpha deletions together with HbSS in our study population had a more identifiable haematologic phenotype than the inheritance of one alpha deletion with HbSS. In this population of patients, homozygous alpha thalassemia has a better ameliorating effect on haematological parameters than fetal haemoglobin. An explanation for the lower levels of haemoglobin, hematocrit and red cell count in HbSF is that HbF level per F cell (HbF/F-cell) is the critical predictor of the impact of HbF on the manifestation of disease and not the absolute level of HbF in the blood.[36] The effect of the Benin haplotype on HbF distribution within the cells is not known. The distribution of HbF among F cells determines its ability to inhibit HbS polymerisation. Patients with high HbF can have a severe disease if HbF is unevenly distributed among F-cells. The uneven distribution of HbF may result in some cells having insufficient concentrations to inhibit HbS polymerisation.[36,37] This suggests that certain patients may not benefit from the use of HbF inducing agents such as hydroxyurea despite attaining a "threshold" HbF value of >20%, which is the protection level for sickle cell-related morbidity.[37]

The red cell distribution width (RDW) was high for all Hb phenotypes. It was highest in HbSS without alpha deletion and least in HbSC. High RDW is associated with impaired erythropoiesis and abnormal red blood cell survival.[38] This RDW may reflect the order of severity of the disease as an increased RDW has a high negative predictive value for diagnosing a variety of disorders and also gives information as to prognosis.[39] Low red cell count with high RDW and high normal platelets may suggest an iron deficiency in the HbSS without alpha deletion, but the normal MCV, MCH and MCHC make the diagnosis unlikely even though iron studies were not carried out on the participants. Mean corpuscular haemoglobin concentration (MCHC) is assumed to be a determinant of disease severity;[40] however, it might not play this role among our patients as it does not show significant difference among the various Hb phenotypes of our patients. Despite the non-significant difference among the various Hb phenotypes, HbSα^{+2} had the

lowest MCHC. However, our finding was consistent with a report by Velasco-Rodríguez et al.,[40] that MCH and MCV stood out as the most efficient red cell indices to predict the deletion of two alpha genes. Also, our findings corroborated with documentation by Old[41] that the MCHC is normal in heterozygous coinheritance with thalassemia while the MCV and MCH are significantly lower than in SCD without alpha thalassemia. Since there was no significant difference in the MCHC for all the Hb phenotypes in this group of patients, MCHC may not be useful to differentiate the various Hb phenotypes; rather, MCV and MCH could be used.

Interestingly, in the control population, MCHC is significantly higher in HbAC individuals than those with homozygous and heterozygous alpha thalassemia. The explanation lies in the fact that the rigidity and dehydration of this abnormal haemoglobin cause microcytosis and compacted normal amounts of haemoglobin. Though MCV and MCH were lower in SCD with homozygous alpha-thalassemia than in heterozygous alpha thalassemia, only MCH showed a significant difference between the two disorders, suggesting a low MCH is a stronger indicator of the severity of the thalassemia than low MCV. Mean corpuscular haemoglobin concentration is traditionally calculated by dividing the haemoglobin quantity by the hematocrit. The advent of automated haematology analysers for routine haematology investigation has caused a shift of emphasis from MCHC, which is affected by the methodology used to derive value.[42] Therefore, MCHC is mostly normal in microcytic anaemias while the value of MCH closely parallels the value of MCV.[43]

This study shows that HbS patients with alpha thalassemia have varying levels of HbF. Iron deficiency anaemia and inheritance of thalassemia could affect the correlation between HbF levels and red cell indices.[43] Higher MCH and MCV levels in HbSS without alpha thalassemia and HbSF individuals than SCD individuals with heterozygous and homozygous alpha-thalassemia are expected. This is due to the lower amount of alpha chains in the red blood corpuscles of patients with alpha thalassemia. The higher MCH and MCV in non-thalassemic individuals relative to alpha thalassemia presence is also observed in normal controls. The contribution of folate deficiency to the MCV value could not be ruled out in these patients whose compliance to the prescribed routine folic acid was not investigated, but the MCV of less than 100fl in the patients makes it unlikely.

The observation of a higher HbA_2 level in patients with alpha thalassemia than in those without corroborates the findings of previous studies by other authors.[11,27] However, considering the range of the HbA_2 values in our thalassemic patients, it can also be inferred that the absence of elevated A_2 does not necessarily rule out alpha-thalassemia in patients with SCD, even if elevated HbA_2 may be suggestive of alpha thalassemia in SCD patients. Abnormally low HbA_2 were seen in some SCD patients without alpha thalassemia gene deletion. Certain structural Hb variants may also reduce the level of HbA_2 at varying degrees.[44] The patients with abnormally low HbA_2 had higher levels of some minor components in areas designated for HbA1a, and HbA1b, HbA1c, which are post-translational modifications of the major haemoglobin component A_0. Since HbSS individuals do not have HbA_0, these may represent unidentified modified variants of HbS which compete with HbA_2 for the alpha chain. The elevated HbA_2 in SCD and HbAS individuals can be attributed to the combination of alpha chain preferably with available normal delta chain over abnormal beta S chain,[12] especially in the presence of limiting alpha chains as is the case in alpha thalassemia. The reduced HbA_2 level observed in HbAA individuals with coinheritance of 2 alpha deletion is due to the underproduction of α globin chains that could combine with delta chains to form HbA_2. Our findings corroborate previous reports that a reduction in HbA_2 level is sometimes indicative of α-thalassaemia trait in individuals with normal Hb genotype,[45] unlike in the SCD population. These HbAA individuals with alpha thalassemia have ineffective erythropoiesis and excess beta-globin chains, which precipitate to cause haemolysis and reduced haemoglobin level. This may contribute to the lower HGB and HCT observed (though not statistically significant) in our thalassemic control population. HbF level ≥20% (HbSF) was associated with a significantly lower HbA_2 level. The inverse relationship between HbF and HbA_2 may be explained by the fact that the more the gamma chains are available to combine with the alpha chains to form HbF, the fewer are the alpha chains available to combine with the delta chains to form HbA_2 and vice versa.

The sample size of patients in some of the genotype groups such as $HbSS\alpha^{+2}$, HbSC and SF is small, and a validation of the above findings in a larger cohort of patients with a higher number of individuals with these less common SCD genotypes is therefore desirable.

Conclusions. Our results have shed more light on the heterogeneity of SCD among our patients. The study also demonstrated that C haemoglobin and homozygous alpha-thalassemia tend to ameliorate the haematological parameters more than the HbF level in our SCD population. Thus, the haematological effects of alpha thalassemia on HbA_2, HGB and HCT in SCD patients may be reversed in normal HbAA individuals.

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