HAEMATOLOGIC AND BIOCHEMICAL CHANGES IN THE STEADY STATE SICKLE CELLS ANAEMIA CHILDREN AT STATE SPECIALIST HOSPITAL MAIDUGURI, BORNO STATE, NIGERIA

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ABSTRACT

Background: Sickle cell anaemia (SCA) is an inherited disorder of beta-globin chain synthesis that results in various physiological changes in hematologic and biochemical parameters.

Aim: The study aimed to determine hematologic and biochemical changes in sickle cell anemia children in steady state.

Methodology: This is a cross-sectional study that involved 80 sickle cell anaemia children in steady state and healthy controls that passed inclusion criteria and consented to participate in the study. A total of 6 ml of venous blood was collected aseptically by standard venepuncture. Automated analyzers were employed to determine both haematological and biochemical variables. The John's Macintosh Projects (JMP) version 11 was used for statistical analysis of the data.

Results: The mean Body mass index (BMI) (Kg/m^2) BMI was 15.48±3.79 and 19.12±4.32, and the mean weight was 29.06±10.39Kg and 39.71±19.49Kg for the subject and control groups, respectively. The mean haematologic values of subjects were low compared to controls except for reticulocytes% with: Rbc (2.58±0.81 and 4.79±0.47), Hb (6.87±1.29 and 13.03±1.45) and Retic% (10.89±5.38 and 0.96±0.25) all show a significant difference at $p<0.0001$ respectively. In differential leucocyte counts, leucocytosis was observed among sickle cell anaemia subjects compared with healthy controls. Biochemical parameters showed statistically significant difference except for serum calcium (2.23 ± 0.14 and 2.19 ± 0.18, P=0.2120). Total bilirubin level (mmol/l) 18.85±0.68 and 11.06±0.66, Total protein level (mmol/l) 69.5±1.31 and 59.15±1.34, Serum albumen level(mmol/l) 41.10±3.51 and 44.73±2.63 for subjects and controls, respectively.

Conclusion: The results of this study have demonstrated significant anaemia, leukocytosis (neutrophilia), proteinemia, and hyperbilirubinemia in the study subject, which is suggestive of chronic anemia, recurrent infections, and biochemical imbalance despite being in a steady state.

Keywords: Anaemia, Biochemical, Haematologic Sickle cell, Steady state,

INTRODUCTION

Sickle cell anaemia (SCA) is an inherited monogenetic disorder of hemoglobin that occurs worldwide with the highest frequency in Africa, resulting in a wide range of public health concerns (Antwi-Boasiaka *et al.*, 2018), as it appears to be one of the major hemoglobinopathies associated with morbidity and mortality in the continent and

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African ancestry in other parts of the globe (Akinbami *et al.*, 2012). It is inherited as homozygous "SS" or compound heterozygous "SC" mutations of the beta-globin gene (Inusa *et al.,* 2019). The disease prevalence is high in Africa, with Nigeria having the highest, with an approximate 1-3% of the population having an abnormal gene ''S'' (Kusfa *et al.,* 2018). The disease condition is associated with various complications affecting both hematological and biochemical equilibrium, which clinically present with episodes of pain and increased susceptibility to infections. There is no affordable cure for these diseases in these settings (Omoti, 2005), and most of the patients rely on routine management through evaluation of their hematological, coagulation, and biochemical profiles to remain in a steady state (Curtis *et al.,* 2015).

Steady state has been defined as a state or condition where SCD patients are free from all forms of painful complications such as crises, infections and changes due to treatments for a minimum period of three (3) months (Bookchin and Lew, 1996) and must pass all the criteria outlined by Ballas *et al.* (2010), which was adopted in this study. Such complications may occur due to qualitative changes, quantitative changes, or both in the normal physiology of the body as a result of the disease (Platt, 1982), affecting both cellular (hemolysis, leukocytes) and non-cellular components of the blood (proteinemia, bilirubinemia, and albuminemia), among others (Mombo *et al.,* 2019). Though the disease complications are mainly of red and white cell origin, which principally participate in vascular obstruction, alteration of biochemical substances such as bilirubin despite the availability of haptoglobin and hemopexin in circulations is the basic mechanism of vaso-occlusive crises among sicklers (Kato *et al.,* 2018).

Therefore, assessing the haematologic and biochemical profiles of these patients in steady state may be an important tool in predicting possible disease complications and severity in the current study.

MATERIALS AND METHODS

The study was carried out at the sickle cell clinic of State Specialist Hospital Maiduguri, Borno State, Nigeria. The clinic runs on Tuesdays of every week for routine checkups. Patients who require special attention or an emergency are referred to the accident and emergency department for proper attention.

Study design

The study is cross-sectional and involved homozygous sickle cell anemia of school-aged children (Hb-SS) in steady state and homozygous haemoglobin AA (Hb-AA) apparently healthy individuals as controls. All were within the age range of 4–15 years who attend clinic at State Specialist Hospital Maiduguri, Borno State, North-Eastern Nigeria.

Study subjects

Subjects confirmed to be Hb-SS by Hb-electrophoresis in alkaline pH and in steady state**,** within the age range of 4–15 years, consented to participate in the study.

Exclusion criteria

Subjects who are heterozygotes for Hb-A, Homozygous for haemoglobin S, or those with other forms of haemoglobinopathies such as thalassemia and with chronic disease like HIV/AIDS, chronic kidney were excluded from the study

Criteria for selecting patients in steady state

The following criteria were adopted from Ballas *et al.* (2010):

1. No prior history of a severe, acute episode requiring hospitalization or emergency response (ER) care for a minimum of four weeks following a distressing crisis.
2. No prior history of hospital or emergency department admissions two to three days following the relevant period.

Previous research has demonstrated that 2-3 days prior to hospital admission in a crisis, the quantity of permanently sickled cells rises and RBC deformability falls. This alteration is consistent with the prodromal phase of the acute painful crisis, which is defined in children and adults who have experienced an acute painful episode that necessitated hospitalization or ER care for a minimum of four weeks following an earlier painful crisis.

3. There has never been a history of blood transfusions in the four months prior to this moment. Hb-electrophoresis is used. The blood bank that typically supplies the concerned institution may be able to provide a history of recent blood transfusions for these patients. Patients undergoing routine blood transfusions or exchange transfusions may not be subject to this criterion.
4. No previous experience of concomitant conditions like inflammation or infection occurred during the preceding four weeks.
5. No antibiotics or other drugs that could alter blood counts were administered over the preceding three weeks of treatment.
6. It is recommended to periodically determine the steady state values every two to three years because they may alter over time.

Sample size determination

The sample size for the study was determined using a standard formula for calculating the minimum sample size. Recent findings on the mean prevalence of sickle cell disease by Ekwere *et al*. (2013) in Uyo, Akwa Ibom State, Nigeria, were used to determine the sample size for this study.

$N=z^2 P(1-P)/d^2$

Where n=minimum sample size, Z= Desired level of significance at 95% (1.96), P= expected prevalence obtained in previous study 2% (0.02), d= precision corresponding to effect size 5%=0.05

N= 3.8416x 0.02(0.98)/0.0025

N=30.12, which was found to be 40. Equally, an equal number of apparently healthy matched controls were recruited for the study, making a total sample size of 80.

Ethical consideration and informed consent

Ethical clearance was obtained from the Ethics and Research Committee of Specialist Hospital Maiduguri with reference number SSH/GEN/641/VOL.1. Both written and verbal consents were obtained from all participants.

Data collection

A structured questionnaire was used to obtain demographic data, while that of the laboratory was obtained following sample collection and analysis.

Anthropometric data

The body weight was measured using a bathroom weighing scale with barefoot and light clothes. Height was measured in centimeters, which were converted to meters. The body mass index (BMI) was calculated from the weight and height obtained using a formula: BMI = weight in kg/height in M^2.

Sample collection

A convenient sampling method was employed for the study, where out of the 40 subjects recruited, 20 were males and 20 were females. A total of six milliliters (6 ml) of venous blood was aseptically collected using the standard venepuncture procedure as described by Decie and Lewis (2011) into a vacutainer container, three-point five milliliters (3.5 ml) into ethylene diamine tetra acetic acid (EDTA) for analysis of hematologic variables, and two-point five milliliters (2.5 ml) into a gel-type plain container for assessments of biochemical variables, respectively.

Laboratory analysis

Haematologic variables were analyzed by the automated haematology analyzer XN550 (Sysmex Middle East FZ-LLC Dubai Healthcare City, Dubai UAE), which works on the principle of impedance as described by Simmons *et al. (*1971).

Biochemical parameters were analyzed by an automated chemistry analyzer (PKL 125 Paramedical SRS SNC84098 Pontecagnano Faiano, Italy, version 05/2016), which works on the principle of photometry as described by Clarada *et al. (*1991)

Statistical analysis

The data obtained was entered into an Excel sheet and analyzed using John's Macintosh Projects (JMP) statistical software version 11 (SAS Institute Inc., NC, USA). An independent t-test was used to compare the mean values between the groups. The results were presented as mean ± SD, and a p-value ($P \leq 0.05$) was considered statistically significant in all comparisons.

RESULTS

The study involved eighty participants, comprising 40 sickle cell anaemia subjects in steady state and 40 apparently healthy controls.

Table 1 shows the mean anthropometric parameters of study subjects and controls. The mean±SD of ages among study subjects and controls were 13.98 ± 6.71 years and 13.85 ± 7.45 years, respectively; the mean height of study subjects and controls were 1.36 ± 0.26m and 1.39 ± 0.31 m, respectively; the mean body weight of study subjects and controls were 29.06 ± 10.39 kg and 39.71 ± 19.49 kg, respectively; and the mean body mass indexes of study subjects and controls were 15.48 ± 3.79 kg/m^2 and 19.12 ± 4.32 kg/m2, respectively. Comparing the mean values of anthropometric parameters between the study subjects and controls, the mean ages and heights showed no significant difference with P-values of 0.9374 and 0.6162, respectively. On the other hand, there is a significant difference with P-values of 0.0031 and 0.0001, respectively, when the mean values of body weight and BMI were compared.

Table 1: Anthropometric parameters of study Participants

Parameters	Hb-SS	Hb-AA	P-Values
Age (years)	13.98 ± 6.71	13.85 ± 7.45	0.9374
Height (M)	1.36 ± 0.26	1.39 ± 0.31	0.6162
Weight (Kg)	29.06 ± 10.39	39.71 ± 19.49	0.0031*
BMI (Kg/m^2)	15.48 ± 3.79	19.12 ± 4.32	0.0001*

Legend: *P<0.05 indicates a statistically significant difference; all values are expressed as mean ± SD; BMI = body mass index; Hb-SS = sickle cell; Hb-AA= normal control subject.

Table 2. shows the mean ± SD of haematological parameters of study subjects and controls. The mean RBC of subject and controls were 2.58 ± 0.81X10^{12}cell/l and 4.79 ± 0.47 X10^{12}cell/l respectively, the mean White blood cell (WBC) of subjects and control were 16.44 ± 6.39 X10^9cell/l and 7.54 ± 1.89 X10^9cell/l respectively, the mean Packed cell volume (PCV) of subjects and controls were 20.12 ± 4.08 l/l and 40.08 ± 4.06 l/l respectively, the mean value of haemoglobin (Hb) of subjects and control were 6.87 ± 1.29g/dl and 13.03 ±1.45 g/dl, the mean value of red cell distribution width (RDW) subjects and controls was 22.69 ± 2.80 SD and13.05 ± 0.88 SD, the mean reticulocytes of subjects and controls was 10.89 ± 5.38 % and 0.96 ± 0.25 %, the mean MCV (fl) of subjects and controls was 80.73 ± 10.63 and 83.82 ± 5.19, the Mean value of MCH (Pg) of subjects and controls was 27.72 ± 4.22 and 27.25 ± 1.88, the mean value of MCHC (%) of subjects and control was 34.02 ± 2.36 and 32.51 ± 1.41 respectively. Comparing the mean values of the subjects and controls, the mean RBC, WBC, PCV, Hb, RDW, and Retics showed a significant difference at P = 0.0001. On the other hand, MCV and MCH showed no significant difference at P values of 0.1034 and 0.5177, while those of MCHC showed a significant difference at P = 0.0009, respectively.

Table 2: Distributions of hematological parameters of study participants

PARAMETERS	Hb-SS	Hb-AA	P-Values
RBC ($\times 10^{12}$ cells/l)	2.58 ± 0.81	4.79 ± 0.47	0.0001*
WBC($\times 10^{9}$cells/l)	16.44 ± 6.39	7.54 ± 1.89	0.0001*
PCV(l/l)	20.12 ± 4.08	40.08 ± 4.06	0.0001*
Hb (g/dl)	6.87 ± 1.29	13.03 ±1.45	0.0001*
RDW(%)	22.69 ± 2.80	13.05 ± 0.88	0.0001*
RETICS (%)	10.89 ± 5.38	0.96 ± 0.25	0.0001*
MCV(FL)	80.73 ± 10.63	83.82 ± 5.19	0.1034
MCH(pg.)	27.72 ± 4.22	27.25 ± 1.88	0.5177
PLT($\times 10^{9}$cells/l)	449.70 ± 162.87	302.58 ± 83.34	0.0001*

Legend: *P<0.05 indicates a statistically significant difference; all values are expressed as mean ± SD; RBC: red blood cell; WBC: white blood cell. PCV: packed cell volume; Hb: hemoglobin concentrations; RDW: red cell distribution width. RETICS: Reticulocytes, MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentrations; Hb-SS = sickle cell anemia subjects; Hb-AA = normal control subjects.

Table 3 shows the mean± standard deviation of differential leucocytes of the study subjects and controls, with neutrophils having 45.3±12.06 and 37.8± 9.51 at P<0.0001, lymphocytes having 39.12±11.53 and 47.6±7.78 at P<0.0001, the mean monocytes are 9.45±3.35 and 8.83±2.72, the mean eosinophil count was 5.0±3.85 and 4.43±2.72 while that of basophils was 0.96±0.51 and 0.65±0.31 respectively. Comparing the mean value between subject and controls, neutrophils show a significant difference with the subject having a higher value than the controls (P= 0.0001), and lymphocytes show a significant difference with the subject having a lower value than the control (P= 0.0001). Similarly, the mean basophil and monocyte count show a significant difference with the study subject having a higher value than the control. On the contrary, the mean eosinophil (P= 0.073) count shows no significant difference between subjects and controls, respectively.

Table 3: **Leucocytes differential counts of study participants.**

Variables (%)	Hb-SS Mean± SD	Hb-AA Mean± SD	P Value
Neutrophils (%)	45.3±12.06	37.8± 9.51	0.0001*
Lymphocytes (%)	39.12±11.53	47.6±7.78	0.0001*
Monocytes (%)	9.45±3.35	8.83±2.72	0.032*
Eosinophils (%)	8.83±2.72	4.43±2.72	0.073
Basophils (%)	0.96±0.51	0.65±0.31	0.0013*

Legend: *P<0.05 indicates statistically significant difference, all values are expressed as mean ± SD, %=percentage, Hb-SS= Sickle cell anaemia subjects, Hb-AA= Normal control subject.

Table 4 shows the mean biochemical parameters of sickle cell subjects and those of healthy matched controls. The mean serum calcium was.23 ± 0.14 and 2.19 ± 0.18; the mean albumen was 41.10± 3.51 and 44.73 ± 2.63; the mean total protein was 69.5±1.31 and 59.15±1.34; and finally, the mean total bilirubin was 18.85±0.68 and 11.06±0.46 respectively. Comparing the mean between sickle cell subjects in steady state and those of apparently healthy matched controls, serum albumen levels were significantly lower in sickle cell subjects than those of healthy controls (p 0.0001), and serum protein and albumen levels were significantly higher among sickle cell subjects than those of healthy controls (p 0.0001). On the other hand, the mean serum calcium shows no significant difference between the study subjects and the controls (p = 0.2120).

Table 4. Shows biochemical Profile of the study Participants .

PARAMETERS	Hb-SS	Hb-AA	P-Values
Calcium (mmol/l)	2.23 ± 0.14	2.19 ± 0.18	0.2120
Albumen (g/l)	41.10± 3.51	44.73± 2.63	0.0001*
Total protein(g/dl)	69.5 ±1.31	59.15 ±1.34	0.0001*
Total bilirubin(g/dl)	18.85 ±0.68	11.06 ±0.46	0.0001*

Legend : *P<0.05 indicates statistically significant difference, all values are expressed as mean ± SD, %=percentage, Hb-SS= Sickle cell anaemia subjects, Hb-AA= Normal control subject, mmol/l= millimolperlitre, g/l= gramper liter, g/dl=gramper decaliter.

DISCUSSION

The mean red cell indices in the present study are within reference value; the MCV and MCH in both subjects and controls revealed no significant differences. while the mean MCHC between the study subjects and controls showed a significant difference despite being within the normal reference value, with a higher value in study subjects than controls. This finding is similar to those reported by Rumaney *et al.* (2014) on MCV and MCH, but contrary to that of Akodu *et al.* (2015), who reported higher values of MCV and MCH and a lower value of MCHC among sickle cell anaemia patients in their studies, which was attributed to iron deficiency anaemia, probably due to iron chelating agents to minimize the risk of hemochromatosis on the cause of repeated transfusion among sickle cell disease subjects.

The mean RDW of the study subjects was significantly higher than that of the controls, indicating haemodilutions associated with sickle cell anaemia and also suggesting the presence of fragmented red cells in circulation, sickled red cells, infections, and agglutinations. Similar findings have been reported by Fasola and Adekanmi, (2019). The mean WBC count in the subjects was significantly high compared to the controls. This is due to repeated infections commonly associated with sickle cell disease. A similar increase in WBC was reported by Obeagu *et al.* (2014), Fasola and Adekanmi (2019), who attributed their findings to oxidative stress and the generation of a covert inflammatory response leading to the release of cytokine mediators, one of whose main functions is increased neutrophil production by the bone marrow.

The differential leukocyte count (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) in the present study was significantly higher in sickle cell anaemia subjects compared with apparently healthy age and sex-matched controls. Elevated leucocyte counts (leucocytosis) are associated with impaired immunity, recurrent infections, and inflammatory conditions associated with the disease condition. These findings are in agreement with a report by Erik *et al. (*2019.

The mean platelet count was significantly higher in the study subjects than the controls, indicating a higher tendency to thrombotic disorders. The finding agrees with that of Chinawa *et al.* (2013), Shome *et al.* (2018), and Aliu *et al.* (2020), respectively, who reported normal to elevated platelet counts and the associated thrombosis in SCA due to increased polymerization of erythrocytes as a result of repeated sickling accompanied by adhesion of sickled erythrocytes to the vascular endothelium. Also, platelets are significantly activated even in a steady state and provide a link between thrombosis and inflammation (Vogel and Thein, 2018). On the contrary, Gardon *et al.* (1974) reported low platelet counts in sickle cell anemia; these variations might be due to the state of the patients in whose report's subjects are in a crisis state.

The mean serum calcium levels in the present study are within the normal reference value, and there is no significant difference in the mean serum calcium levels between the subjects and the controls.

These results are in agreement with the report of Fey *et al.* (1997), who documented normal calcium levels in sickle cell anemia subjects in the steady state and normal controls, but they are dissimilar from the reports of Oladipo *et al.* (2005), Antwi-Baffour *et al.* (2019), and Charles *et al.* (2019), who recorded hypocalcemia in sickle cell disease compared to normal control subjects, which was associated with increased calcium magnesium ATPase activity, decreased absorption from Gastrointestinal tracts (GIT), and impaired vitamin D synthesis. The serum albumen in the current study was significantly lower compared to that of apparently healthy matched controls. The low albumen levels might be attributed to the lack of appetite observed in sickle cell patients, low protein intake, and increased body demand and/or utilization. These findings agree with a report by Ugonabo *et al. (*2006). On the other hand, the serum protein level in the present study was significantly higher in the study subjects compared to that of the controls; these findings are in agreement with a report by Isichei (1979. On the contrary, Ugonabo *et al.* reported that no significant differences exist between the sicklers and normal controls, which was attributed to age differences, where they used adolescents and adults aged 15–30 years rather than children aged 1–15 years, respectively. The total bilirubin level was significantly higher in sickle cell subjects compared to that of the controls; elevated serum bilirubin level is associated with chronic hemolytic anemia, deflation of hemopexin, and haptoglobin in circulation. This finding agrees with a report by Shah *et al. (2017),* respectively.

CONCLUSION

Based on the results of the present study, the authors concluded that SCA patients in the steady state have low red cells with reticulocytosis, leukocytosis, and thrombocytosis and elevated serum protein levels, albumen, and total bilirubin levels despite being in the steady state, suggesting anemia with marrow hyperplasia, a risk of infection, and thrombosis. Therefore, these hematological and biochemical changes, when observed in routine investigations of sickle cell anaemia subjects, do not necessitate emergency treatment, as these markers will serve as guides in determining the response to treatment of possible changes that may be predisposed to crises and other forms of complications.

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Conflicts of interest

There are no conflicts of interest to declare.

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