STEADY STATE HAEMATOLOGICAL CHARACTERISTICS OF NIGERIANS WITH SICKLE CELL ANAEMIA AND THOSE WITH NORMAL ADULT HAEMOGLOBIN

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ABSTRACT

Background: *Sickle cell anaemia is a hereditary disorder associated with high morbidity and mortality.*

Objective: *To determine the haematological parameters among patients with sickle cell anaemia and to compare with those of haemoglobin AA individuals.*

Methods: *Cross-sectional comparative study was conducted among known sickle cell anaemia patients attending sickle cell clinic of Alex Ekwueme Federal University Teaching Hospital, Abakaliki and Haemoglobin AA individuals recruited from among blood donors, medical students and staff. About 2mls of blood was collected from each participant for haemoglobin electrophoresis and full blood count analysis using haematology autoanalyzer. Ethical approval was gotten from the Research and Ethics Committee of the institution and informed consent was gotten from the participants. Data was analysed using SPSS software, version 20.*

Results: *One hundred and eighty six participants were recruited, made up of 84 persons with sickle cell anaemia and 102 haemoglobin AA control. Sickle cell anaemia patients had a mean haematocrit, red blood cell count, haemoglobin concentration of 22.9%, 2.8 X 10^{12}/l and 7.3g/dlrespectively which were significantly lower than the haematocrit of 38.4% (P= 0.000), red blood*

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cell count of 4.7 X 1012/l (P= 0.000) and haemoglobin concentration of 12.5g/dl (P= 0.000) among haemoglobin AA control. On the other hand, patients with Sickle Cell Anaemia had a mean white blood cell count, platelet count, mean corpuscular haemoglobin concentration and red cell distribution width of 13.7 X10^9/l, 391 X10^9/l, 33.8 ±3.1g/dl and 64.4 ±15.4 respectively which were significantly higher than the white blood cell count of 5.6 X 10^9/l (P= 0.000), platelet count of 228 X 10^9/l (P= 0.000), mean corpuscular haemoglobin concentration of 32.9 ±1.7g/dl (P= 0.019) and red cell distribution width of 44.1 ±4.6 (P= 0.000) among haemoglobin AA control.

Conclusion: *Individuals with sickle cell anaemia have higher values for white blood cell, platelet count, red cell distribution width and mean corpuscular haemoglobin concentration but lower values for haemoglobin concentration, red blood cell count and haematocrit compared to haemoglobin AA individuals.*

Keywords: *Haematological parameters, sickle cell anaemia, Haemoglobin.*

INTRODUCTION

Sickle cell anaemia is a hereditary disorder of haemoglobin characterized by hemolysis and acute episodic clinical events called crises.[1] Vaso-occlusive (painful) crises is the most common and other forms of crisis include sequestration crisis, hyperhemolytic crisis and aplastic crises. Sickle cell anaemia is caused by a point mutation in which thymine (GTG)is substituted by adenine (GAG) nucleotide at the sixth codon of human globin gene on chromosome 11 leading to substitution of valine for glutamic acid at the sixth position of beta globin polypeptide chain.[2] The resultant effect is production of abnormal haemoglobin S (HbS). Because of the insolubility and instability of HbS under hypoxic condition, there is polymerization of deoxygenated HbS which distorts the shape of the red blood cell from normal biconcave to sickle shape.[3] This effect renders the red blood cells non-deformable to traverse the microvasculature. Consequently, affected individuals suffer repeated vaso-occlusive events characterized by ischaemic reperfusion injury and inflammation.[4] In as much as red cell sickling is more marked during crisis, continuous sickling does occur at a lower rate in steady state. Therefore, certain proportion of sickled red cells are always present in the circulation of individuals with sickle cell anaemia even in steady state.[5]

Sickle β globin gene is widely distributed throughout Africa, Middle East, Southeast Asia, the Mediterranean and by population movement to the North America, the Caribbean and Northern Europe[6]. The frequency of sickle carriers (HbAS) is up to 20% to 25% in West Africa, including Nigeria.[7]

Haemoglobin C carriers (HbAC) occur with a frequency of about 6% among Nigerians and are predominantly seen in the south western part of the country.[8] In Nigeria, HbSS and HbSC are the main forms of sickle cell disease, with the former being the most common affecting about 2 to 3% of the nation's

population.[9] Hence Nigeria, with a population of about 160 million and the most populous country in Africa has the largest concentration of patients with sickle cell anaemia in the whole world.[10]

Patients with sickle cell anaemia present with wide spectrum of clinical manifestations. Qualitative and quantitative changes in red blood cells have been reported in sickle cell anaemia.[11] Hemolysis could be intravascular or extravascular. Intravascular hemolysis results from complement-sensitive red cells while extravascular hemolysis occurs by phagocytosis of red cells that have undergone sickling and physical entrapment of rheologically compromised red cells.[12] Certain factors correlate with the degree of hemolysis in sickle cell anaemia such as percentage of irreversible sickle cells, relative proportion of haemoglobin fractions as well as the degree of haemoglobin polymer formation, calculated from the mean corpuscular haemoglobin concentration.[13]

Although sickle cell anaemia is primarily a disease of the red blood cells, leucocytes and platelets are also affected. High absolute neutrophil count is associated with clinical severity of sickle cell anaemia and many complications of sickle cell anaemia are associated with leucocytosis.[14] In addition, thrombocytosis has also been associated with increased risk of developing stroke and other complications in sickle cell anaemia.[15]

Previous studies have determined the haematological parameters among patients with sickle cell anaemia in steady state in different parts of the country but to our knowledge, no published study on that subject exist in our environment. The aim of this study was to provide the baseline haematological profile of patients with sickle cell anaemia in steady state and to compare it with that of haemoglobin phenotype AA individuals.

MATERIALS AND METHODS

Cross-sectional comparative study was conducted among known sickle cell anaemia patients in steady state attending sickle cell clinic of Alex Ekwueme Federal University Teaching Hospital, Abakaliki as well as Haemoglobin AA individuals recruited from among blood donors, medical students and staff from October 2016 to February 2017.

Information on socio-demographic profile of the participants, history of previous blood transfusion, date of last crisis, use of hydroxyurea and other drugs and co-morbidities were obtained using pretested semi-structured questionnaire. Inclusion criteria were patients with haemoglobin phenotype SS aged 18 years and above, no history of crises in the past three months established by a careful history taking and complete physical examination, no history of blood transfusion in the past three months, patients not on hydroxyurea, those without other co-morbidities such as HIV infection, malignancies and so on. Exclusion criteria were history of blood transfusion in the past three months, those on hydroxyurea, patients with haemoglobin SC phenotype, pregnant women, patients with other co-morbidities and those less than 18 years. Control group were individuals with haemoglobin AA phenotype confirmed by

haemoglobin electrophoresis, who also completed questionnaires containing information on socio-demographic characteristics, history of blood transfusion, co-morbid condition(s) and drug intake.

About 2mls of venous blood was collected by clean venipuncture from each participant via the ante-cubital vein, using a plastic syringe with minimum stasis, into Ethylene Di-amine Tetraacetic Acid (EDTA) bottles. Complete blood counts (CBC) were analyzed using Mindray haematologyautoanalyzer BC-5300 (manufactured by Shenzhen Mindray Bio-Medical Electronic Co. Ltd, Germany), a five-part auto-analyser able to run 23 parameters per sample including haematocrit, haemoglobin concentration, red blood cell concentration, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell count, platelet count and so on. Well mixed blood sample was aspirated by letting the equipment sampling probe into the blood sample and then pressing the start button. Approximately, 20ul of blood was aspirated by the haematology auto-analyser. After approximately one minute, result of analysis was displayed on the screen. A printout copy of result was released on the thermal printing paper. Haemoglobin phenotypes of all patients and controls were confirmed using cellulose acetate haemoglobin electrophoresis at pH 8.6. About 50ul of blood samples were lysed with equal volume of water. Lysed samples and known AA, AS, SC and SS controls were placed on cellulose acetate paper in batches using an applicator. The paper was placed in electrophoretic tank with tris-buffer at pH 8.6 and electric current was applied. All samples and known controls migrated from negative to positive pole at 350 volts. Haemoglobin separation occurred within 20minutes and haemoglobin bands were compared with that of the known controls.

Data was analyzed using SPSS software, version 20. Descriptive statistics were used to compute percentages, proportions, means and standard deviation. Student t-test was used to compare means. Results were presented in tables and expressed as frequencies, percentages, mean and standard deviation. P- value < 0.05 was considered significant.

Ethical approval for this study was gotten from the Research and Ethics Committee of the institution. Informed written consent was gotten from each participant before being included in the study.

RESULTS

One hundred and eighty six participants were recruited, made up of 84 persons with sickle cell anaemia and 102 haemoglobin AA control with mean age of 29± 8.9 years. Participants with sickle cell anaemia were made up of 48 (57.1%) females, mostly students and single while Haemoglobin AA control group were made up of 34 (33.3%) females, majority were also students and single (Table 1).

Table 1: Socio-demographic characteristics of the participants

Parameters	Hb SS		Hb AA	
	Males	Females	Males	Females
Frequency (%)	36 (42.9%)	48 (57.1%)	68 (66.7%)	34 (33.3%)
Occupation				
Civil servant	6 (16.7%)	5 (10.4%)	19 (27.9%)	4 (11.7%)
Student	17 (47.2%)	23 (47.9%)	27(39.7%)	16 (47.1%)
Artisan	4 (11.1%)	8 (16.7%)	10 (14.7%)	5 (14.7%)
Trader	9 (25.0%)	12 (25.0%)	12 (17.7%)	9 (26.5%)
Total	36 (100%)	48 (100%)	68 (100%)	34 (100%)
Marital status				
Single	30 (79%)	41 (85.4%)	48 (70.6%)	27 (79.4%)
Married	6 (21%)	7 (14.6%)	20 (29.4%)	7 (20.6%)
Total	36 (100%)	48 (100%)	68 (100%)	34 (100%)

Overall, Sickle cell anaemia (SCA) patients had a mean packed cell volume of 22.9% which was significantly lower than the packed cell volume of 38.4% (P =0.000) in haemoglobin AA control. Likewise SCA patients had a mean haemoglobin concentration of 7.3g/dl and red cell count of 2.8 X 10^{12}/l which were significantly lower than haemoglobin concentration of 12.5g/dl (P = 0.000) and red cell count of 4.7 X 10^{12}/l ±0.5 (P = 0.000)in Haemoglobin AA control respectively. On the other hand, patients with SCA had mean white blood cell count, platelet count, mean corpuscular haemoglobin concentration and red cell distribution width which were significantly higher than that of haemoglobin AA control (Table 2).

Table 2: Mean values of some blood parameters among patients with SCA and Hb AA controls.

Parameter	Hb SS	Hb AA	P-value
Haemoglobin concentration (g/dl)		12.5 ±1.7	0.000
Packed Cell Volume (%)	22.9 ±9.4	38.4 ±4.0	0.000
Red Blood Count X 10^{12}/l	2.8 ±0.7	4.7 ± 0.5	0.000
Mean Cell Volume (fl)	80.1 ±11.2	82.0 ±7.5	0.183
Mean Cell Haemoglobin (pg)	27.1 ±3.2	27.0 ±3.0	0.833
Mean Cell Haemoglobin Concentration (g/dl)	33.8 ±3.1	32.9 ±1.8	0.019
Red cell distribution width	64.4 ±15.4	44.1 ±4.6	0.000
White Blood Cell X 10^9/l	13.7 ±6.0	5.6 ±1.4	0.000
Platelet X 10^9/l	391.9 ±148.2	228.5 ±67.0	0.000

The mean haemoglobin concentration of male cases was 7.6g/dl ±1.6, packed cell volume 24.2% ±5.0, red blood cell count of 2.8 X 10^{12}/l ±0.8 which were significantly lower than that of male haemoglobin AA individuals. On the other hand, white blood cell count and platelet count of male HbSS individuals were 15.1 X 10^9/l ±5.5 and 397 X 10^9/l±180 respectively which were significantly higher than that of HbAA individuals. For the female cases, the mean haemoglobin concentration was 7.0g/dl ±1.2, packed cell volume 21.2% ±1.4 and red blood cell count of 2.7 X 10^{12}/l ±0.7. which were significantly lower than that for female HbAA control while the white cell count and platelet count were significantly higher among female HbSS compared to female HbAA control (Table 3).

Table 3: Comparison of some mean haematological parameters for female cases and female controls as well as male cases and male controls

Parameters	Female HbSS	Female HbAA	P-value	Male HbSS	Male HbAA	P-value
Hb (g/dl)	7.0 ±1.2	11.8 ±1.0	0.000	7.6 ±1.6	12.9 ±1.8	0.000
PCV (%)	21.2 ±1.4	35.7 ±3.2	0.000	24.2 ±5.0	39.8 ±3.7	0.000
RBC X 10^{12}/l	2.7 ±0.7	4.4 ±0.4	0.000	2.8 ±0.8	4.9 ±0.6	0.000
WBC X10^9/l	12.7 ±6.2	5.8 ±1.4	0.000	15.1 ±5.5	5.5 ±1.4	0.000
Platelet X 10^9/l	336 ±114	238 ±86	0.000	397 ±180	224 ±54	0.000

Hb – haemoglobin, PCV – Packed cell volume, RBC – Red Blood Cell, WBC – White blood Cell.

However, the parameters were not significantly different, except for platelet count, when compared between male and female HbSS as well as male and female HbAA individuals (Table 4).

Table 4: Comparison of some mean haematological parameters for male and female cases as well as male and female controls

Parameters	Female HbSS	Male HbSS	P-value	Female HbAA	Male HbAA	P-value
Hb (g/dl)	7.0 ±1.2	7.6 ±1.6	0.164	11.8 ±1.0	12.9 ±1.8	0.110
PCV (%)	21.2 ±1.4	24.2 ±5.0	0.559	35.7 ±3.2	39.8 ±3.7	0.743
RBC X 10^{12}/l	2.7 ±0.7	2.8 ±0.8	0.377	4.4 ±0.4	4.9 ±0.6	0.152
WBC X10^9/l	12.7 ±6.2	15.1 ±5.5	0.851	5.8 ±1.4	5.5 ±1.4	0.435
Platelet X 10^9/l	397 ±180	336 ±114	0.029	238 ±86	224 ±54	0.001

Hb – haemoglobin, PCV – Packed cell volume, RBC – Red Blood Cell, WBC – White blood Cell.

Most of the cases (81/84, 96.4%) had packed cell volume less than 30%.All the controls (102/102, 100%) had packed cell volume greater than 30%.

Over half (58/84; 69%) of patients with SCA had white blood cell count greater than 11 X 10^9/l, while the remaining 24 (28.6%) had white blood cell count between 4 - 11 X 10^9/l and only 2 (2.4%)had white cell count less than 4 X 10^9/l. Among HbAA control group, 87.3% (89/102) had white blood cell count between 4 - 11 X 10^9/l, and none (0%) had white cell count greater than 11 X 10^9/l (Table 5). Many patient with SCA (33/84; 39.3%) had platelet count greater than 400 X 10^9/l and none had platelet count less than 100 X 10^9/l. Most HbAA control group, 98% (100/102) had platelet count between 100 to 400 X 10^9/l (Table 5).

Table 5: Proportion of case and control groups with different levels of blood parameters.

Parameters	HbSS N (%)	HbAA N (%)
PCV		
<30%	81 (96.4%)	0 (0%)
≥	3 (3.6%)	102 (100%)
Total	84 (100%)	102 (100%)
Hb		
<10g/dl	80 (96.4%)	1 (0.9%)
⩾10g/dl	4 (3.6%)	101 (99.1%)
Total	84 (100%)	102 (100%)
WBC		
<4 X 10^9/l	2 (2.4%)	13 (12.7%)
4 – 11 X 10^9/l	24 (28.6%)	89 (87.3%)
>11 X 10^9/l	58 (69%)	0 (0%)
Total	84 (100%)	102 (100%)
Platelet		
<100 X 10^9/l	0 (0%)	1 (0.9%)
100 – 400 X 10^9/l	51 (60.7%)	100 (98.2%)
>400 X 10^9/l	33 (39.3%)	1 (0.9%)
Total	84 (100%)	102 (100%)

Hb – haemoglobin, WBC – White blood cell, PCV – Packed cell volume

DISCUSSION

There is marked variation in clinical manifestation of sickle cell anaemia despite identical genetic mutation. This has been attributed to certain factors including derangement in haematological parameters. This study found that overall, the haemoglobin level, packed cell volume and red cell count were significantly lower in patients of sickle cell anaemia (SCA) compared to hemoglobin AA individuals, with most (96.4%) patient with SCA having packed cell volume less than 30% while none of haemoglobin AA control had PCV less than 30%.Previous studies have also shown similar findings.[16-18]The same results was also seen when the parameters were compared among same gender in both HbSS and HbAA groups. Both male and female HbAA control groups were found to have significantly higher haemoglobin level, red cell count and packed cell volume but lower white cell and platelet count compared to male and female individuals with SCA (HbSS) respectively. These results were not surprising considering the fact that patients with sickle cell anaemia suffer from continuous hemolysis, with a short survival rate of the red blood cells.[19] Hence, the haemoglobin values, packed cell volume and red blood cell count are usually lower than normal healthy individuals. Additionally, sickle nephropathy impair erythropoietin production and response to anaemia.[20]Most patients have adapted to low haemoglobin levels and so there is no clinical benefit in treating anaemia in sickle cell anaemia routinely with blood transfusion unless in symptomatic cases or special cases that require prophylactic chronic transfusion such as chronic transfusion to prevent stroke when there is high risk as evidenced by elevated transcranial doppler values. Moreover, blood transfusion is associated with other complications.[21] It has been shown that

haemoglobin S releases oxygen more easily to the tissues compared to haemoglobin A and that may explain the reason why individuals with sickle cell anaemia are usually stable at low haemoglobin concentrations compared to those with haemoglobin AA.[22] On the other hand, raising the packed cell volume to over 30% could increase blood viscosity with resultant risk of vaso-occlusive crisis and other adverse consequences.

The mean total white blood cell count (WBC) among patients with sickle cell anaemia was significantly higher than that of haemoglobin AA control, with 69% of patients with SCA having leucocytosis while none among haemoglobin AA individuals had leucocytosis. This corroborates the findings from previous studies which reported rise in total white cell count in individuals with sickle cell anaemia.[16,23]This may be due to redistribution of white blood cells between the marginal and circulating pools, which has been reported to be associated with factors such as pain, anxiety, inflammation in the absence of infection.[24]Leucocytosis in sickle cell anaemia may also be due to autosplenectomy resulting from recurrent splenic vessel occlusion.[25]In addition, leucocytosis could be as a result of generation of a covert inflammatory response leading to the release of cytokine mediators, one of whose main function is increased neutrophils production by the bone marrow.[14] Studies have shown that leucocytosis is associated with poor prognosis and risk of premature death in patients with sickle cell anaemia.[26] The benefit of hydroxyurea therapy in the management of sickle cell anaemia includes reducing the leucocyte count in addition to other advantages.[27]

This study also found that the overall mean platelet count in patients of SCA was significantly higher than that of haemoglobin AA control, with 39.4% of SCA patient having thrombocytosis while only 1% had thrombocytosis among Hb AA individuals. This corroborates the findings from previous studies.[23,28]Loss of splenic platelet pool function in adult sickle cell patients consequent upon autosplenectomy may have contributed to higher mean platelet count in sickle cell anaemia compared to HbAA controls. Another reason for the thrombocytosis observed in sickle cell anaemia may be due to negative feedback effect on erythropoietin production as a result of anaemia. Erythropoietin has structural homology with thrombopoietin. Though thrombopoietin is larger than erythropoietin, about half of thrombopoietin structure has similarity to erythropoietin at the N-termnial region. It is therefore well recognized that thrombocytosis is associated with anaemia of chronic disease and some other types of anaemia.[29]

Though the haemoglobin level, packed cell volume and red blood cell count were lower in patients with sickle cell anaemia compared with to haemoglobin AA individuals generally, males were noted to have higher values than females in both sickle cell anaemia group and haemoglobin AA control group, though not statistically significant. This may be due to enhanced erythropoiesis caused by androgens in males. In addition,

blood loss during menstruation in females may be responsible for lower values of haemoglobin, packed cell volume and red blood cell count in females.[30] Platelet count was however, found to be significantly higher in females than males for both HbSS and HbAA groups. Previous studies have reported similar findings,[31,32] the cause of which has been attributed to compensatory mechanism associated with menstrual blood loss.

This study also found that mean corpuscular haemoglobin concentration (MCHC) was significantly higher in patients with sickle cell anaemia compared to HbAA control group. Studies conducted by Antwi-Boasiako *et al*[28] and Mombo *et al*[33] also reported similar findings. MCHC is the amount of haemoglobin present per unit volume of packed red cells. It has been observed that high MCHC is associated with increased red cell sickling, rigidity and less deformability[13].This phenomenon may be due to the fact that high MCHC levels encourages polymerization of sickle haemoglobin[34].In contrast, there was no significant difference in the MCV and MCH values among patients with SCA and HbAA control group, as also reported by Iheanacho *et al.*[23] This may be due to the fact that the patients with sickle cell anaemia in this study were in steady state without increased hemolysis and reticulocytosis.

Red cell distribution width (RDW) was also found to be significantly higher in patients with sickle cell anaemia compared to Hb AA controls. This is in agreement with previous studies.[28,35] This finding suggests that SCA is associated with marked anisocytosis which may be because of more rapid erythropoiesis consequent upon hemolysis, in which, cells at different stages of maturation, with different sizes, are present in the peripheral blood.

CONCLUSION

Individuals with sickle cell anaemia have lower haemoglobin levels, packed cell volume, red cell count but higher values for MCHC, RDW as well as white blood cell and platelet counts compared to haemoglobin AA individuals.

Limitations of the study

Individuals with sickle cell anaemia were all diagnosed by only haemoglobin electrophoresis in alkaline medium. This might have resulted in missing some of them who might have thalassaemia trait such as Sβthal which could impact on the haemoglobin concentration.

Inability to do corrected white blood cell count could have resulted to the value for WBC being falsely higher due to the presence of nucleated red blood cells which are sometimes present in the peripheral blood, and will be recognized and counted as white blood cell by haematology autoanalyser.

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