Original Article

FACTORS ASSOCIATED WITH STEADY STATE IN SICKLE CELL ANEMIA PATIENTS: INSIGHTS FROM A TERTIARY HOSPITAL IN NORTHWESTERN NIGERIA

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ABSTRACT

Context:

Sickle Cell Anaemia *(*SCA) is a highly variable disease in which the steady state is periodically interrupted by crisis. Familiarity with factors associated with steady state can aid in early diagnosis, monitoring and treatment of SCA patients, especially during crisis in resource-poor settings.

Aims:

To identify haematological and biochemical factors associated with steady state in SCA patients.

Settings and Design:

A descriptive cross-sectional study involving adult SCA patients in steady state at Aminu Kano Teaching Hospital (AKTH).

Materials and Methods:

Structured questionnaires and case files were used to obtain clinical information. Haematological (haematocrit, white blood cell count, platelet count and foetal haemoglobin) and biochemical (total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase and alanine

aminotransferase) parameters were evaluated.

Statistical Analysis used:

Data were analyzed using SPSS version 20, P < 0.05 was considered statistically significant.

Results:

One hundred SCA patients in steady state aged 18-43 years were studied, with 78 females and male to female ratio of 1:3.5. The mean age at diagnosis was 45months. Mean haematological and biochemical values obtained were haematocrit 23.4±4.5%, white blood cell count (WBC) 12.1±3.6 x 10^9/l, platelet count 366.1±131 x 10^9/l, foetal haemoglobin (HbF) 7.5±4.3%, total bilirubin 23.7 ± 18.7 µmol/l, direct bilirubin 9.7±11.3 µmol/l, indirect bilirubin 14.0±12.2 µmol/l, aspartate aminotransferase (AST) 44.9±19.0 U/l and alanine aminotransferase (ALT) 28.8±11.6 U/l. Only HbF showed significant gender differences (p=0.003).

Conclusion:

The study identified moderate anaemia, elevated WBC/platelet counts, raised total bilirubin, AST, ALT and elevated HbF as factors associated with steady state in SCA patients. Going forward, clinicians should consider these findings while attending to SCA patients.

Keywords: Sickle cell anaemia, Steady state, Foetal haemoglobin and Nigeria

INTRODUCTION

Sickle cell disease is an inherited chronic haemolytic disorder and the most common haemoglobinopathy in the world. Sickle Cell Anaemia (SCA), the homozygous (HbSS) state of the disease, is the most severe form.[1] Although sickle cell disease is found globally, 70% of the patients live in sub-Saharan Africa.[2] Nigeria bears the highest burden of the disease with about three per cent of the population having HbSS and one in every four people (25%) carrying the sickle cell gene.[3]

The abnormal sickled haemoglobin (HbS) results from substitution of glutamic acid for valine at position six of the β-globin chain of the adult haemoglobin (HbA).[4] Sickled red cells are mainly responsible for vaso-occlusive crisis (VOC) and haemolytic anaemia associated with the disease which affects virtually all organs of the body and causing complications such as stroke, acute chest syndrome, pulmonary hypertension,

kidney injury, priapism, avascular necrosis of the bone as well as chronic leg ulcers.[5] Predicting which SCA patient will later develop a crisis is almost impossible due to the influence of both genetic and environmental factors such as climatic conditions, nutrition and access to health care services.[6]

Steady state is defined as the period when there is no evidence of infection, absence of sickle cell crises in the last one month (30 days) and the absence of blood transfusion in the last three months (120 days).[7] However, defining steady state as the period when the patient is clinically asymptomatic may not be entirely accurate, as SCA is a chronic inflammatory condition characterized by ongoing subclinical haemolysis.[8] This can affect the levels of various haematological (haematocrit, white blood cell count, platelet count and foetal haemoglobin) and biochemical (total bilirubin, direct bilirubin, indirect bilirubin, lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase) parameters in SCA patients, differing from those of individuals with normal haemoglobin (HbAA).[9–11] Thus, resulting in varying degrees of anaemia, raised white cell counts, serum bilirubin and lactate dehydrogenase levels among others. These laboratory parameters have been found to be variable among SCA patients in different regions and ethnic groups,[6,11] emphasizing the need for local studies to enhance our understanding of the disease process and its relationship with haematological and biochemical parameters.

Therefore, understanding steady state haematological and biochemical parameters of SCA patients will be vital in detecting any deviation from the steady state which may be an early warning sign of crisis. However, because of paucity of local data on steady state, this study aimed at finding the haematological and biochemical factors associated with steady state in a cohort of SCA patients at Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria.

MATERIALS AND METHODS

Study area: The study was conducted at the Sickle Cell Clinic of Aminu Kano Teaching Hospital (AKTH), Kano. AKTH provides tertiary health care and medical training to the people of Kano and the country in general. Kano state is the most populous state in Nigeria according to the National Bureau of Statistics 2022 Bulletin.[12] It is situated in

the northwest region of Nigeria in the Sudan Savannah between latitude 12.05º north and longitude 8.51º, and covers an area of 20,131 square kilometers.

Study design: This is a descriptive cross-sectional study.

Study population: One hundred steady state SCA patients aged between 18 and 45 years were consecutively enrolled as they came for follow up at the adult Sickle Cell Clinic of AKTH Kano between January 2016 and December 2016.

Inclusion criteria: Steady state SCA patients who consented to be part of the study and were at least eighteen (18) years of age were recruited. The steady state was defined as; absence of evidence of hyperhemolytic and vaso-occlusive crises in the last one month (30 days), absence of history of blood transfusion in the last 120 days and absence of active infection at the time of patient recruitment.[7]

Exclusion criteria: Patient on hydroxyurea because of the effect of the drug on haematological parameters,[7] and pregnant women were all excluded.

Sample size determination: One hundred (100) patients were recruited into the study based on scientific calculation using Fischer's formula.

Bio demographic data: Patient bio data was obtained from an interviewer administered questionnaire. Questionnaires were administered by two trained resident doctors of Haematology department during each clinic visit which lasted for one year. Information on selected haematological and biochemical parameters was retrieved after laboratory analysis.

Study procedure: Seven milliliters of blood was collected from each patient, 3ml was dispensed into EDTA bottle and 4ml into a gel separator bottle for some selected haematological (Haematocrit, white cell count, platelet count, fetal haemoglobin level) and biochemical (total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase levels) analysis respectively. For haematological parameters; Full blood count (FBC) was analyzed with Swelab Alfa 3-part automated haematology Analyzer (Swelab haematology systems, Sweden), Fetal Haemoglobin (HbF) with BIO-RAD D-10 fully automated High Performance Liquid Chromatography

machine (Bio-Rad Laboratories, France) and reticulocyte count was estimated manually as percentage on new methylene blue stained blood films. Biochemical analysis including serum bilirubin was measured using colorimetric method described by *Donmas et al*[13] while Aspartate aminotransferase and Alanine aminotransferase levels were measured by quantitative in-vitro determination as described by *Reitman et al*.[14] Haematologic and Biochemical analysis were done by experienced scientists at haematology and biochemistry laboratories of AKTH. The normal reference range for adult HbF is 0.5-2.5%.[15] Normal reference for adult haematocrit is 40-54% in men and 36-46% in women,[15] while for white blood cell and platelet counts it is 2.6-10.2 x 10^9/l and 100-300 x 10^9/l respectively.[16] For the Biochemical parameters, the normal reference range for total bilirubin is 2-12 µmol/l, direct bilirubin 0-7 µmol/l, indirect bilirubin 0-12 µmol/l, aspartate aminotransferase 0-12 U/l and alanine aminotransferase 5-35 U/l.[17]

Ethical considerations*:* Ethical approval was obtained from AKTH Institutional Review Board (AKTH/MAC/SUB/12A/P-3/VI/1190).

Data analysis: The data was analyzed using the Statistical Package for Social Sciences software (SPSS version 20, IBM corporation, New York, USA). Descriptive statistics were summarized using means and standard deviation for continuous data and percentages for categorical data. Independent Sample T-test was used to compare means and p-value of <0.05 was considered as statistically significant.

RESULTS

Socio-demographic distribution of the patients:

Majority of the patients were females 78 (78%) with males accounting for only 22 (22%) and Male to Female ratio of 1: 3.5. Their mean age was 22.7 ± 4.9 years with a range of between 18 and 43years. Females were younger (22.1 ± 4.9 years) than males (24.9 ± 4.8 years); p =0.028. Table: 1 shows the age and sex distribution of the steady state SCA patients. The approximate mean age at diagnosis of the patients was 45 months with males (40months) diagnosed earlier than females (47months) (Table: 2).

Steady state hematological and biochemical parameters of sickle cell anaemia patients:

Table 3 shows the steady state haematological and biochemical variables of the patients. The mean haematocrit was 23.4 ± 4.5 %; WBC 12.1 ± 3.6 x 10^9/l; Platelets 366.1 ± 131.2 x 10^9/l; and HbF 7.5 ± 4.3 % and Retics 5.2 ± 3.2%. The mean Total bilirubin was 24.9 ± 20.3 µmol/l; Direct bilirubin 10.5 ± 11.9 µmol/l; Indirect bilirubin 14.4 ± 12.6 µmol/l, AST 45.9 ± 19.7 U/l and ALT 28.8 ± 11.6 U/l.

The results showed that the mean haematological and biochemical parameters in males and females were similar except for HbF which was significantly higher in females (8.0%) than males (4.9%) with p-0.003 (Table:4).

Table 1: Age and Sex distribution of the steady state sickle cell anemia patients

			Age (years)		
	Frequency	**Percentage (%)**	**Minimum**	**Maximum**	**Mean (SD)**
Male	22	22%	18	34	24.9 (±4.8)
Female	78	78%	18	43	22.1 (±4.9)
Total	100	100%	18	43	22.7 (±4.9)

P=0.028

Table 2: Age at diagnosis of the sickle cell

		Minimum	Maximum	Mean (SD)
Age at	**Male**	4	192	39.5(±43.3)
diagnosis	**Female**	4	288	46.4(±64.7)
(months)	**Total**	4	288	44.9(±60.5)

anemia patients in steady state

Table 3: Mean steady state Hematological and Biochemical parameters of the steady state sickle cell anemia patients

HbF=fetal hemoglobin, HCT=hematocrit, WBC=white blood cell count, PLT=platelet count, RETICS=reticulocyte count, T.BIL=total bilirubin, DIR.BIL=direct bilirubin, IND.BIL=indirect bilirubin, AST=aspartate aminotransferase, ALT=alanine aminotransferase.

Table 4: Mean Hematological and Biochemical parameters of the Male and Female steady state sickle cell anemia patients

	Mean		
Variable	**Male (n=22)**	**Female (n=78)**	**p value**
HbF (%)	4.9	8.0	0.003*
HCT (%)	24.2	23.1	>0.05
WBC ($x10^9$/l)	12.1	12.1	>0.05
PLT ($x10^9$/l)	342.1	373.0	>0.05
RETICS (%)	4.9	5.5	>0.05
T. BIL (µmol/l)	30.6	21.7	>0.05
DIR. BIL (µmol/l)	13.1	8.8	>0.05
IND. BIL (µmol/l)	17.5	13.0	>0.05
AST (U/I)	45.6	44.7	>0.05
ALT (U/l)	28.2	28.9	>0.05

HbF=fetal hemoglobin, HCT=hematocrit, WBC=white blood cell count, PLT=platelet count, RETICS=reticulocyte count, T.BIL=total bilirubin, DIR.BIL=direct bilirubin, IND.BIL=indirect bilirubin, AST=aspartate aminotransferase, ALT=alanine aminotransferase. *Statistically significant

DISCUSSION

This study highlighted the haematological and biochemical parameters associated with steady state in SCA patients in north-western Nigeria. It notably includes a higher number of steady state patients compared to most previous studies and evaluates biochemical parameters in addition to the commonly haematological ones.

There were more females than males in this study, reflecting a pattern previously reported both within the country and beyond.[18,19] This trend may suggest that females are more consistent with follow-up appointments,

likely due to their higher health-seeking behaviour compared to males, as documented in earlier studies.[20] It could also mean that there may be more surviving female patients with SCA than males as was previously reported from the southern part of the country.[11,20] However, this must be interpreted with caution because males are typically more preoccupied with responsibilities in the ethnicity of our study participants (Hausa-Fulani setting). Therefore, male patients may likely present to the hospital only when they are in crisis. Furthermore, the age at diagnosis which is a marker of disease severity in SCA patients was found to be lower in males compared to females in this study. Our results also showed that male SCA patients in the study were more likely to be diagnosed seven months earlier than female patients. However, the value of age at diagnosis in disease severity has been disputed by some studies including one from Nigeria.[21] This may be due to the fact that it is affected by other factors such as availability of newborn screening program which is not readily available in most parts of the country, socioeconomic status and history of sickle cell disease in the family.

The mean steady state haematological parameters of SCA patients in this study showed moderate anaemia with elevated WBC and platelet counts. This pattern is similar to what has been reported earlier in the literature.[9,10,22] The low haemoglobin in SCA patient is due to the continuous haemolysis of sickled RBCs which takes place even in steady state when the patient has no crisis.[8] This is supported by the reticulocytosis observed in this study. Other factors that may contribute to the anaemia include blunted response to erythropoietin secretion, resulting in haemoglobin rise that is not proportional to the degree of anaemia and high levels of inflammatory cytokines which affects iron utilization in these patients.[23] The mean steady state haematocrit obtained in this study can serve as a yardstick for monitoring patients in our clinics, thereby reducing unnecessary blood transfusions in our facilities and in neighboring states with similar sociodemographic characteristics. The elevated mean WBC reported in this research may be attributed to redistribution of white blood cells from the marginated to the circulating pool and, to some extent, to the accelerated release from the bone marrow due to raised cytokine levels.[24] High WBC

count in SCA patients is associated with increased risk of early death and SCA complications such as stroke, priapism and acute chest syndrome.[25] The elevated mean platelet count observed in this study was similar to what has been documented in the past.[9,11] A rise in platelet count in SCA patients may be the result of the loss of splenic platelet pool as a consequence of auto splenectomy and also as a result of increased erythropoietin drive due to anaemia from chronic hemolysis.[26] Increased platelet count in SCA has been found to increase the risk of thrombosis and vaso-occlusive crisis.[5] Even though sickle cell disease is a pro coagulant condition, those with platelet count above steady state levels may be at greater risk of thrombosis associated complications such as stroke, myocardial infarction and deep venous thrombosis. Therefore, it is important to identify these patients so that they can benefit from prophylaxis using anticoagulation or antiplatelet therapy.

In the present study, increased levels of biochemical parameters were observed in the steady state. This pattern of elevated biochemical parameters among steady state SCA patients was reported in the past.[27] The elevated mean AST and ALT observed in this study were in line with previous reports from the country and beyond.[18,28,29] Elevated serum AST in patients with SCA is attributed to red cell lysis.[5,7] Elevated ALT observed in SCA patients may be attributed to microinfarcts in the liver, as a result of hepatic crisis or cholelithiasis.[7] While AST is a marker of haemolysis in SCA, ALT on the other hand, is more specific to liver damage.[5,27] Increased mean total bilirubin, direct bilirubin and indirect bilirubin obtained in this study were consistent with that reported in the literature.[30,31] The elevated bilirubin levels may be attributed to red cell breakdown and microinfarcts in the liver.[32] The findings of reticulocytosis in the presence of low mean haematocrit and elevated mean serum levels of AST and bilirubin, suggest an ongoing haemolysis in the steady state study population.

When the mean haematological and biochemical parameters of male and female patients were compared only HbF showed significant difference between the two groups. The result showed female have significantly higher HbF levels than their male counterparts which is consistent with previous findings.[33] On the contrary, a study

from the southern part of the country by Kotila *et al* reported higher HbF levels in male than female SCA patients.[34] However, it is important to note that the difference in the contradictory report was not significant, and secondly our study used a newer and better method (High Performance Liquid Chromatography machine) to measure HbF levels. SCA patients with increased levels of HbF tend to have a relatively mild clinical disease because HbF does not participate in the sickling process and thus delays HbS polymerization.[1] Though, the level of HbF needed to prevent vaso-occlusive crisis or complications has been a controversial topic for a long time.[35] Nonetheless, any increase in HbF level in individuals with SCA has been shown to be beneficial.[36] Interestingly, the finding of a significantly higher steady state HbF level among the female SCA patients in this study may explain the late mean age at diagnosis among the female patients and the higher number of female patients in the study. Therefore, our study also support the long held belief by earlier researchers that female SCA patients may have increased survival advantage over their male counter parts.[20,37]

CONCLUSION

This study identified moderate anaemia, slightly elevated WBC and platelet counts, moderately raised total bilirubin and aspartate aminotransferase levels, and markedly elevated HbF level as key haematological and biochemical factors associated with the steady state among the study population. HbF was the only parameter that showed a statistically significant difference between male and female SCA patients in the steady state (p=0.003). However, larger-scale studies are necessary to validate these findings, as the present study may have been limited by its small sample size.

Reference

1. Lal A VE. Sickle cell disease. In: Hoffbrand A, Catovsky D TE, editor. Postgraduate Haematology. USA: Blackwell publishers; 2016. page 104–18.

2. Global, regional, and national prevalence and mortality burden of sickle cell disease, 2000-2021: a systematic analysis from the Global Burden of Disease Study 2021. Lancet Haematol 2023;10(8):e585–99.

3. Kassebaum NJ, Noyes TS, McHugh TA, Thomson AM, Hay SI, Murray CJL.

Global burden of sickle cell disease in 2021 - Authors' reply. Lancet. Haematol.2023;10(10):e792–3.

4. Pauling L, Itano H, Singer S, Wells K. Sickle cell anaemia, a molecular disease. Science (80-) 1949;110:543–8.

5. Piccin A, Murphy C, Eakins E, Rondinelli MB, Daves M, Vecchiato C, et al. Insight into the complex pathophysiology of sickle cell anaemia and possible treatment. Eur J Haematol 2019;102(4):319–30.

6. Steinberg MH. Fetal hemoglobin in sickle cell anemia. Blood 2020;136(21):2392–400.

7. Kato G, Gladwin M, Steinberg M. Deconstructing Sickle cell disease: Reappraisal of the role of haemolysis in the development of Clinical Subphenotypes. Blood 2007;21:37–47.

8. Akinola N, Stevens SME, Franklin IM, Nash GB, Stuart J. Subclinical ischaemic episodes during the steady state of sickle cell anaemia. J Clin Pathol 1992;45:902–6.

9. Abjah UA, Medugu JT, Bulama HA, Nasir IA, Wakbe JK, Peyou GAB. Comparative Haematological Evaluation of Sickle Cell Anaemic Patients in Steady State and During Vaso-occlusive Crisis at Maiduguri, Nigeria. Int J Clin Med Res 2017;4(5):51–5.

10. Akinbami A, Osunmu A, Oshinaike O, Adebola P, Arogundede O. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. BMC Res Note 2012;5:396–400.

11. Omoti CE. Haematological values in sickle cell anaemia in steady state and during vaso-occlusive in Benin city, Nigeria. Ann Afri Med 2005;4:62–7.

12. Demographic Statistical Bulletin 2022. Natl Bur Stat :Accessed;20/12/2024.

13. Perry B, Donmas B, Bayse D, Butler T, Cohen A, Fellows N, et al. A candidate reference method for determination of bilirubin in serum. Test for transferability. Clin Chem 1983;29:297–301.

14. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J clin pathol 1957;28:56–

63.

15. Cheesbrough M. Haematological tests. In: District Laboratory Practice in Tropical Countries. Hong Kong: Cambridge University Press; 2006. page 296–334.

16. Okpala I. Normal haemopoiesis and Bone marrow failure. In: Okpala I, Johnson C, editors. Synopsis of Haematology including blood diseases in the tropics. Nigeria: Createspace publishers; 2010. page 1–15.

17. Cheesbrough M. Clinical chemistry tests. 2nd editio. USA: Cambridge University Press; 2006.

18. Kotila T, Adedapo K, Adedapo A, Oluwasola O, Fakunle E, Brown B. Liver dysfunction in steady state sickle cell disease. Ann Hepatol 2005;4:261–3.

19. Thein SL, Howard J. How I treat the older adult with sickle cell disease. Blood 2018;132(17):1750–60.

20. Kotila T, Shokunbi W. Survival advantage in female patients with sickle cell anaemia. East Afri Med J 2001;78:373–5.

21. Adegoke SA, Adeodu OO, Adekile AD. Sickle cell disease clinical phenotypes in children from South-Western , Nigeria. Niger J Clin Pr 2015;8:95–101.

22. Okocha E, Onwubuya E, Osuji C, Ahaneku G, Okonkwo U, Ibeh N, et al. Blood Disorders & Transfusion Disease Severity Scores and Haemogram Parameters in Nigerian Sickle Cell Disease Patients. 2015;6(6).

23. Pulte ED, McKenzie SE, Caro J, Ballas SK. Erythropoietin levels in patients with sickle cell disease do not correlate with known inducers of erythropoietin. Hemoglobin 2014;38(6):385–9.

24. Russell E Ware, Mariane de Montalembert, Leon Tshilolo MRA. Sickle Cell Disease. Lancet Haematol 2017;15(30(10091)):311–23.

25. Miller ST, Sleeper LA, Pegelow CH et al. Prediction of adverse outcomes in children with sickle cell diseaseitle. N Engl J Med [Internet] 2000;342:83–9. Available from: https://europepmc.org/articles/PMC2200853

26. Sundd P, Gladwin MT, Novelli EM. Pathophysiology of Sickle Cell Disease. Annu Rev Pathol 2019;14:263–92.

27. Akuyam SA, Abubakar A, Lawal N,

Yusuf R, Aminu SM, Hassan A, et al. Assessment of biochemical liver function tests in relation to age among steady state sickle cell anemia patients. Niger J Clin Pract 2017;20(11).

28. Rasheed Y, Abdulaziz H, Aliyu A, Philip O. Biochemical and ultrasound assessment of the liver in sickle cell anaemia patients in Zaria, Nigeria. Ann Niger Med 2014;8:69–72.

29. Tripathi P, Tripathi M. Biochemical assessment of liver in sickle cell disease patients at a tertiary care hospital of north India. Int J Res Med Sci 2016;4:57–60.

30. Hebel R. Reconstructing sickle cell disease: a data base analysis of the 'hyperhaemolysis paradigm' for pulmonary hypertension from the perspective of evidence-based Medicine. Am J haematol 2011;86:123–54.

31. Ballas K, Marcolina M. Hyperhaemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anaemia. Transfusion 2006;46:105–10.

32. Shah R, Taborda C, Chawla S. Acute and chronic hepatobiliary manifestations of sickle cell disease: A review. World J Gastrointest Pathophysiol 2017;8(3):108–16.

33. Ballas SK, Pulte DE, Lobo C, Riddick-Burden G. Letters to Blood To the editor : Case series of octogenarians with sickle cell disease. Blood 2016;128(19):2367–70.

34. Kotila T, Fawole O, Shokunbi W. Haemoglobin F and clinical severity of sickle cell anaemia among Nigerian adults. Afr J Med Sci 2000;29:229–31.

35. Steinberg MH. Predicting clinical severity in sickle cell anaemia. Br J Haematol 2005;129:465–81.

36. Keikhaei B, Yousefi H, Bahadoram M. Hydroxyurea: Clinical and Hematological Effects in Patients With Sickle Cell Anemia. Glob J Health Sci 2015;8(3):252–6.

37. Morris J., Dunn D., Bekford M., Grandson Y. MK and HD. The haematology of haemozygous sickle cell disease after the age of 40years. Br J Haematol 1991;77:382–5.