Original Article

Elevated Plasma D-dimer Levels In Children with Sickle Cell Anemia In Steady State Attending State Specialist Hospital Maiduguri, Borno State, Nigeria

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Abstract

Background: Sickle cell anemia (SCA) has been linked with altered plasma levels of D-dimer. However, routine assessment of D-dimer and other coagulation indices in SCA patients are rarely carried out. The aim of this study was to determine the mean plasma levels of D-dimer and other coagulation indices in children with SCA in a steady state and them with with that of healthy HbAA genotype controls. **Materials and Methods:** This was a case–control study involving 40 children with SCA in steady state and 40 healthy HbAA genotype controls. Plasma D-dimer, prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count were determined using appropriate laboratory techniques. Data analysis was by JMP software. Independent t-test was used to determine the difference in quantitative variables between SCA patients and controls. $P \leq 0.05$ was considered significant. **Results:** Mean ages of the SCA patients and controls were 13.98 ± 6.71 and 13.85 ± 7.45, respectively. The SCA patients had longer mean PT (20.48 ± 6.51 vs. 13.89 ± 2.13, $P = 0.0001$) and aPTT (40.91 ± 9.08 vs. 32.63 ± 4.44, $P = 0.0001$) compared to the controls. Similarly, the SCA patients had higher platelet count (449.70 ± 162.87 vs. $302.58 \pm 83.34 \times 10^9$/L, $P = 0.0001$) and plasma D-dimer (3.18 ± 2.72 mg/L vs. 0.29 ± 0.52 mg/L, $P = 0.0001$) compared to the controls. Equally, 98% of the SCA patients had high plasma D-dimer. **Conclusion:** The SCA patients had higher plasma D-dimer and platelet count and longer PT and aPTT compared to controls.

Keywords: D-dimer, Maiduguri, Nigeria, sickle cell anemia, steady state

INTRODUCTION

The global prevalence of sickle cell anemia (SCA) is high, with over 30 million people affected, and sub-Saharan Africa accounts for nearly 70% of the world's 300,000 newborn babies affected by SCA annually.[1] In Nigeria, about 25% of adults have the sickle cell trait (SCT), while the homozygous state (SS) is found in about 3% of the population.[2]

SCA is an inherited autosomal recessive disorder of the beta-globin chain of hemoglobin (Hb), characterized by hemolytic anemia, episodes of vaso-occlusion, and increased susceptibility to infections and sometimes organ damage.[3] The disorder is caused by a mutation leading to the replacement of glutamic acid with valine at the sixth position of the beta-globin chain.[4] The abnormal physiochemical properties of the resultant sickle red cell are responsible for the clinical manifestations of the disease. The underlying pathology

of most of these complications is the recurrent episodes of vaso-occlusion due to microthrombi formation resulting in organ ischemia.[5] Although the clinical manifestations of the disease are quite heterogeneous, periodic vaso-occlusive crises and chronic intravascular hemolysis are common in SCA patients.[6]

SCA is associated with a hypercoagulable state as a result of enhanced generation of thrombin, impaired anticoagulant activity, increased platelet activation, and abnormal activation

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of the fibrinolytic system.[7] The enhanced thrombin generation and altered the fibrinolytic system lead to fibrinogen proteolysis and the generation of fibrin degradation products, one of which is plasma D-dimer. Some studies have demonstrated an elevation in plasma D-dimer and alteration in coagulation indices in SCA patients, especially in steady state.[8-11] Plasma D-dimer has also been reported to be correlated with many clinical outcomes in the disease.[12,13]

However, while a number of previous studies have evaluated plasma D-dimer levels in SCA patients, the results are contradictory and inconsistent, thus suggesting the possible involvement of multiple pathophysiological mechanisms.[12,14,15] In addition, there is a paucity of information on the plasma level of D-dimer in SCA patients in this environment. Furthermore, despite its reported utility in predicting future clinical outcomes, plasma D-dimer is not routinely assessed in SCA patients. The aim of the study was to determine the mean plasma levels of D-dimer and other coagulation indices in children with SCA in a steady state and compare with that of healthy HbAA genotype controls.

MATERIALS AND METHODS

Study area
The study was carried out at the sickle cell clinic of the State Specialist Hospital Maiduguri, Borno State, North-East Nigeria. The clinic runs weekly, mainly on Tuesdays with an inflow of ten to twenty patients per visit.

Study population and design
This case–control study involves SCA children in steady state and healthy HbAA genotype controls. Steady state was defined as the absence of acute painful crisis or any changes due to therapy for at least four consecutive weeks after a previous painful crisis.[13] A simple random sampling technique using a random number table was used to recruit the patients and an equal number of age- and sex-matched healthy HbAA controls were recruited.

Inclusion criteria
Participants are known to be SCA patients based on Hb electrophoresis results showing SS, who:
i.   Fall within the age range of 4–15 years
ii. Were in steady state
iii. Not on oral anticoagulant therapy.

Exclusion criteria
i.   Those with other hemoglobinopathies (based on history and clinical examination)
ii. HIV-positive patients
iii. Patients known to be hepatitis B positive or those with chronic liver disease from any cause.

Sample size determination
The sample size for the study was determined using G*Power computer software version 3.1.9.4.[16] Mean ± standard deviation (SD) plasma D-dimer in HbSS patients in steady state and HbAA subjects of 51.72 ± 34.12 and 37.25 ± 34.85, respectively, from a previous study by Ekwere *et al*.[12] was used which gave an effect size (d) of 0.4196. Using this effect size, α level of significance of 0.05, and the statistical power of 0.8, we obtained a sample size of 78.

Ethical clearance
Ethical clearance was obtained from the Ethics and Research Committee of the State Specialist Hospital, Maiduguri, with reference number: SSH/GEN/641/VOL. 1. Since the participants were minors, written informed consent was obtained from their respective parents/guardians as appropriate.

Data collection
Interviewer-administered data capture form was used to obtain sociodemographic, anthropometric, and laboratory data of the participants.

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

Laboratory procedures
A total of 8 ml of venous blood was drawn using a vacuum container (vacutainer) needle and emptied into two separate containers: 4.5 ml into trisodium citrate containing 3.2% sodium citrate for coagulation assays and 3.5 ml into ethylenediaminetetraacetic acid for hematological analysis. D-dimer was determined using enzyme-linked immunosorbent assay fluorescence immune assay kit (Guangzhou Wondfo Biotech Co., Ltd., Guangzhou, China) according to the manufacturer's instructions. The test is based on fluorescence immunoassay techniques of antigen-antibodies reaction. When the sample is added into the sample well of the test cartridge, the flourescence-labelled detector D-dimer antibodies on the sample pad bind to D-dimer antigens in the plasma and form immune complexes. These complexes are then detected by fluorescence immunoassay.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined using HumaClot Junior, a semi-automated coagulation machine with reference number 18,680 SN-HC-6281 (Human Gesellschaft für Biochemica und Diagnostica mbH Max-Planck-Ring; Wiesbaden, Germany) according to the manufacturer's instructions using PT reagent (Chemelex, S. A. Barcelona, Spain).

Platelet count was determined by direct current impedance method with hydrodynamic focusing using Sysmex Automated Hematology Analyzer XN550 (Sysmex Middle East FZ-LLC, Dubai Healthcare City, Dubai, UAE) as described by Simmons *et al*.[17]

Data analysis
Data were analyzed using JMP statistical software version 11 (SAS Institute Inc., NC, USA). Independent *t*-test

was used to compare the mean values between the two groups, and Pearson's correlation coefficient was used to determine the linear relationship between D-dimer and other coagulation indices among SCA patients. The results were presented as mean ± SD and $P \leq 0.05$ was considered statistically significant.

Results

A total of 80 participants consisting of 40 SCA patients in a stable state and 40 healthy HbAA children were recruited. The mean age of the SCA patients and the healthy controls was 13.98 ± 6.71 and 13.85 ± 7.45 ($t = 0.126$, $P = 0.9374$), respectively.

The SCA patients had significantly higher plasma D-dimer (3.18 ± 2.72 vs. 0.29 ± 0.52, $t = 6.59$, $P = 0.001$) and platelet count (449.70 ± 162.87 vs. 302.58 ± 83.34, $t = 5.08$, $P = 0.0001$) compared to healthy controls, respectively. Similarly, the SCA patients had prolonged PT (20.48 ± 6.51 vs. 13.89 ± 2.13, $t = 6.08$, $P = 0.0001$) and aPTT (40.91 ± 9.08 vs. 32.63 ± 4.44, $t = 5.28$, $P = 0.0001$) compared to the healthy controls, respectively. The results of coagulation parameters of the subjects are shown in Table 1.

Using a reference value of plasma D-dimer of 0.4–0.5 mg/L, about 98% of the SCA patients had higher plasma D-dimer to the control's 8% ($\chi^2 = 65.66$, $P = 0.0001$) [Table 2].

D-dimer did not correlate with any other coagulation index among SCA patients [Table 3].

Discussion

This study investigated plasma levels of D-dimer and other coagulation indices among children with SCA in a steady state. Coagulation profile including platelet count, PT, aPTT, and plasma D-dimer levels were assessed for both SCA patients and controls. We found a significantly higher mean platelet count among the SCA patients in steady state compared to the controls. Several studies have linked SCA with increased platelet activation.[13,18,19] The higher platelet count observed in the SCA patients is believed to be due to increased release of markers of platelet activation, associated functional asplenia, and failure or lack of splenic sequestration of platelets.[7,20,21]

Our study demonstrated longer PT and aPPT among SCA patients compared to the controls. This is similar to what was reported by Chinawa *et al.*[22] among SCA patients in Nigeria. The prolongation in PT and aPTT in SCA patients observed in this study could be due to elevated platelet and thrombin levels, which activate the intrinsic clotting pathway even under normal conditions, thus leading to consumption of a number of clotting factors.[7] Liver injury and Vitamin K deficiency could also impair the former's ability to synthesize and release new clotting factors as rapidly as they are consumed, thus leading to relative deficiency.[23,24] Indeed, PT tests the integrity of Vitamin K-dependent factors such as II, V, VII, and X, while aPTT tests that of factors such as VIII, IX, and XI.[25] Most of these factors play significant roles in the intrinsic coagulation pathway.

Table 1: Mean coagulation parameters of the participants

Variable	Mean±SD ($n=40$)		Test statistic	P
	Hb-SS	Hb-AA		
PT (s)	20.48±6.51	13.89±2.13	6.08	0.0001*
aPTT (s)	40.91±9.08	32.63±4.44	5.28	0.001*
Platelet (×10⁹/mL)	449.70±162.87	302.58±83.34	5.08	0.0001*
D-dimer (mg/L)	3.18±2.72	0.29±0.52	6.59	0.0001*

*Statistically significant variable. Statistically significant variable, PT: Prothrombin time, aPTT: Activated partial prothrombin time, SD: Standard deviation, Hb: Hemoglobin

Table 2: Association between plasma D-dimer values and sickle cell status

Variable	HbSS, n (%)	HbAA, n (%)	χ^2	P
Normal	1 (2.5)	37 (92.5)	65.66	0.0001
High	39 (97.5)	3 (7.5)		

Hb: Hemoglobin

Table 3: Correlation between D-dimer and other coagulation parameters among sickle cell anemia patients

Variable	R	P
PT	0.0424	0.7949
aPTT	−0.0586	0.7193
Platelet	−0.1353	0.4053

PT: Prothrombin time, aPTT: Activated partial prothrombin time

The mean D-dimer level in the SCA patients in steady state was found to be significantly higher compared to the controls. This indicates a hyperactive coagulation system with activation of fibrinolytic activity leading to generation of fibrin degradation products like D-dimer. Various studies involving SCA in steady state have reported higher plasma D-dimer levels.[13,26,27] However, Philips *et al.*[28] have documented lower plasma D-dimer levels, while Ekwere *et al.*[12] found no significant difference in the mean plasma D-dimer values of SCA patients compared to healthy controls. The differences observed between our results and others may be due to differences in research methodologies and the sample size employed. We found no correlation between D-dimer and other coagulation indices among SCA patients.

Patients with SCA exhibit increased plasma levels of prothrombin fragment and thrombin–antithrombin complexes, which are markers of thrombin generation.[7] Plasma from patients with sickle cell disease exhibits higher thrombin peak height, thrombin generation rates, and endogenous thrombin potential compared to age-matched controls.[29,30] Recent studies have suggested that the increased thrombin generation observed in SCA not only leads to thrombotic complications but also contributes to vascular inflammation and multiple organ damage in mouse models of the disease.[20,31] Indeed, it has been reported that red blood cell-mediated

coagulation hyperactivation and endothelial dysfunction leads to inflammation, vascular leakage, and thrombosis.[32-35]

Limitations

Our study is limited by our inability to assess other coagulation indices such as fibrinogen, Proteins C and S, and specific factors. We were also unable to assess other fibrin degradation products. Despite this shortcoming, we believe that the coagulation indices assessed in the study can give a good reflection of coagulation activity.

CONCLUSION

The SCA patients had significantly higher plasma D-dimer and platelet count and longer PT and aPTT compared to controls. There was no significant relationship between plasma D-dimer and other coagulation indices among the SCA patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Akodu S, Diaku-Akinwumi I, Njokanma O. Age at diagnosis of sickle cell anaemia in lagos, Nigeria. Mediterr J Hematol Infect Dis 2013;5:e2013001.
2. Akinyanju O. Guest editorial: Issues in management and control of sickle cells disorder. Achiev Ib Med 2001;2:37-41.
3. Mohamed AO, Nawal EO, Maria MH. Correlation between the plasma level of von willebrand factor and the severity of sickle cell disease in Sudanese patients. FASEB J 2007;21:A671.
4. Emechebe GO, Onyire NB, Orji ML, Archigbu KI. Sickle cell disease in Nigeria: A review. IOSR JDMS 2017;16:87-94.
5. Robbin K, Cotran M. Von Willebrand facto. In: Robbin K, Cotran M, editors. Text Book of Basic Pathology. 6th ed.. Philadelphia: Elsvier Saunders; 2005. p. 159-65.
6. Zhou Z. Haemoglobin blocks von willebrand factor proteolysis by ADAMTS-13: A mechanism associated with sickle cells disease. Thromb Haemost 2009;9:1070-7.
7. Ataga KI, Key NS. Hypercoagulability in sickle cell disease: New approaches to an old problem. Hematology Am Soc Hematol Educ Program 2007;2007:1:91-6.
8. Furie B, Furie BC. The molecular basis of blood coagulation. Cell 1988;53:505-18.
9. Nsiri B, Gritli N, Mazigh C, Ghazouani E, Fattoum S, Machghoul S. Fibrinolytic response to venous occlusion in patients with homozygous sickle cell disease. Hematol Cell Ther 1997;39:229-32.
10. Hagger D, Wolff S, Owen J, Samson D. Changes in coagulation and fibrinolysis in patients with sickle cell disease compared with healthy black controls. Blood Coagul Fibrinolysis 1995;6:93-9.
11. Nsiri B, Gritli N, Bayoudh F, Messaoud T, Fattoum S, Machghoul S. Abnormalities of coagulation and fibrinolysis in homozygous sickle cell disease. Hematol Cell Ther 1996;38:279-84.
12. Ekwere T, Ogunro SP, Akanmu AS. Assessment of some fibrinolytic proteins in sickle cell anemia patients in steady state and in vaso-occlusive crises. J Appl Hematol 2013;4:131-6.
13. Kusfa IU, Mamman AI, Babadoko AA, Yahaya G, Ibrahim IN, Awwalu S, *et al*. A comparative study of D-dimer levels in adult patients with sickle cell anemia at Ahmadu Bello University Teaching Hospital Zaria, Nigeria. Blood 2017;14:92-5.
14. Famodu AA, Reid HL. Plasma fibrinogen levels in sickle cell disease. Trop Geogr Med 1987;39:36-8.
15. Buseri FI, Shokunbi WA, Jeremiah ZA. Plasma fibrinogen levels in Nigerian homozygous (Hb SS) sickle cell patients. Hemoglobin 2007;31:89-92.
16. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav Res Methods 2009;41:1149-60.
17. Simmons A, Schwabbauer ML, Earhart CA. Automatic platelet counting with the autoanalyzer. J Lab Clin Med 1971;77:656-60.
18. Francis RB. Large-vessel occlusion in sickle cell disease: Pathogenesis, clinical consequences, and therapeutic implications. Med Hypotheses 1991;35:88-95.
19. Kenny MW, George AJ, Stuart J. Platelet hyperactivity in sickle-cell disease: A consequence of hyposplenism. J Clin Pathol 1980;33:622-5.
20. Helms CC, Marvel M, Zhao W, Stahle M, Vest R, Kato GJ, *et al*. Mechanisms of hemolysis-associated platelet activation. J Thromb Haemost 2013;11:2148-54.
21. Solovey A, Gui L, Key NS, Hebbel RP. Tissue factor expression by endothelial cells in sickle cell anemia. J Clin Invest 1998;101:1899-904.
22. Chinawa JM, Emodi IJ, Ikefuna AN, Ocheni S. Coagulation profile of children with sickle cell anemia in steady state and crisis attending the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. Niger J Clin Pract 2013;16:159-63.
23. Mills LR, Mwakyusa D, Milner PF. Histopathologic features of liver biopsy specimens in sickle cell disease. Arch Pathol Lab Med 1988;112:290-4.
24. Charlotte F, Bachir D, Nénert M, Mavier P, Galactéros F, Dhumeaux D, *et al*. Vascular lesions of the liver in sickle cell disease. A clinicopathological study in 26 living patients. Arch Pathol Lab Med 1995;119:46-52.
25. Katz D, Beilin Y. Disorders of coagulation in pregnancy. Br J Anaesth 2015;115:75-88.
26. Francis RB Jr. Elevated fibrin D-dimer fragment in sickle cell anemia: Evidence for activation of coagulation during the steady state as well as in painful crisis. Haemostasis 1989;19:105-11.
27. Fakunle EE, Eteng KI, Shokunbi WA. D-dimer levels in patients with sickle cell disease during bone pain crises and in the steady state. Pathol Lab Med Int 2012;4:1-5.
28. Philips G, Hartman J, Keller VA, Santiago MA, Pizzo S. Regulation of tissue plasminogen activator in sickle cell anemia. Am J Hematol 1990;35:167-70.
29. Noubouossie DF, Lê PQ, Corazza F, Debaugnies F, Rozen L, Ferster A, *et al*. Thrombin generation reveals high procoagulant potential in the plasma of sickle cell disease children. Am J Hematol 2012;87:145-9.
30. Noubouossie DC, Le PQ, Rozen L, Debaugnies F, Ferster A, Demulder A. Evaluation of the procoagulant activity of endogenous phospholipids in the platelet-free plasma of children with sickle cell disease using functional assays. Thromb Res 2012;130:259-64.
31. Sparkenbaugh EM, Chantrathammachart P, Mickelson J, van Ryn J, Hebbel RP, Monroe DM, *et al*. Differential contribution of FXa and thrombin to vascular inflammation in a mouse model of sickle cell disease. Blood 2014;123:1747-56.
32. Platt OS. Sickle cell anemia as an inflammatory disease. J Clin Invest 2000;106:337-8.
33. Arumugam PI, Mullins ES, Shanmukhappa SK, Monia BP, Loberg A, Shaw MA, *et al*. Genetic diminution of circulating prothrombin ameliorates multiorgan pathologies in sickle cell disease mice. Blood 2015;126:1844-55.
34. Sparkenbaugh E, Pawlinski R. Interplay between coagulation and vascular inflammation in sickle cell disease. Br J Haematol 2013;162:3-14.
35. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood 2016;127:801-9.