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 ± 83.34 × $10^9$/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, $\alpha$ 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].

**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 ($\times 10^9$/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, $n$ (%) | $\chi^2$ | $P$ |
|---|---|---|---|---|
| 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

## 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.

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.

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