

## Original Research Article

# Assessment of Some Haemostatic Parameters in Sickle Cell Anaemia Subjects Resident in Rivers and Bayelsa States

### ABSTRACT

**Aim:** The aim of this study was to assess some haemostatic parameters in sickle cell anaemia subjects in Rivers and Bayelsa States.

**Study design:** This study is a cross-sectional observational study.

**Place and Duration of Study:** This study was carried out at the University of Port Harcourt Teaching Hospital, Rivers State, and the Federal Medical Centre, Yenagoa, Bayelsa State, between the months of February and August, 2020.

**Methodology:** A total of four hundred and fifty (450) subjects with age range of 1-50 years were randomly selected. There were about 200 registered patients (adults and children alike) at the sickle cell clinics of the University of Port Harcourt Teaching Hospital, and the Federal Medical Centre, Yenagoa, with an average of 4 new patients per month. The sample size was obtained using a prevalence of sickle cell anaemia of 2% and the sample size was calculated using Cochran sample size formula. Five milliliters (5ml) of venous blood sample was withdrawn from the peripheral vein in the upper limb of subjects using a standard venipuncture technique. The sample was rocked gently to mix and kept at room temperature and the haemostatic parameters (vWF, FVIII, D-dimer, L-arginine, fibrinogen, ADAMTS13) were assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions, while PT and APTT were analysed with Fortress reagent and Uniscope SM801A Laboratory using water bath. Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA) and p values less than .05 were considered statistically significant.

**Results:** The results showed the mean comparison of haemostatic parameters in sickle cell anaemia and control subjects. The comparison of haemostatic parameters showed significant ( $p < .05$ ) increases and decreases in **Vaso-Occlusive Crisis (VOC)** and steady state respectively compared with the control group. There was statistically significant reduction in the mean comparison of L-Arginine ( $p < .01$ ) in VOC condition than steady state in relation to the control group in our study population, while D-Dimer, ADAMTS13 were also significantly reduced statistically ( $p < .01$ ) in VOC condition than steady state compared with the control group. However, the mean FVIII inhibitor, Fibrinogen, PT (INR) and APTT were significantly higher ( $p < .01$ ) in VOC than steady state when compared to controls with normal haemoglobin (HbAA). Correlations of haemostatic parameters by sickle cell anaemia subjects' condition showed more significant positive correlations in VOC than steady state.

**Conclusion:** This study showed a heightened hypercoagulability in **Sickle Cell Anaemia (SCA)** subjects, and further pave way for better understanding particularly the diagnostic variables underlying SCA, specific to each subject condition (steady state and VOC). Subjects with SCA, particularly during VOC, undergo significant haemostatic alterations that increase their risk of developing coagulation activation-related complications. Thus, though selected markers of coagulation were significantly different between the subject conditions, they were often significantly higher in the SCA.

*Keywords: Haemostatic Parameters, Sickle Cell Anaemia, Rivers State, Bayelsa States*

## 1. INTRODUCTION

Sickle cell anaemia (SCA) is one of the most common severe monogenic disorders worldwide [1]. It is the most frequent variant (homozygous SS disease) and is caused by a single amino acid substitution at the sixth residue of the  $\beta$ -globin subunit gene on chromosome 11p15.5 [2], ( $\beta$ 6-Glu  $\rightarrow$ Val) which results in the production of the characteristic sickle haemoglobin [3]. In other words, the homozygous state is referred to as sickle cell anaemia (SS), where the red blood cells lacking normal adult haemoglobin are replaced by sickle cell haemoglobin [4]. This phenotype expresses severe haemolytic anaemia among other manifestations [4]. The sickle cell trait is a heterozygous state (AS), where the red blood cells contain both normal adult haemoglobin (HbA) and sickle cell haemoglobin, and rarely have a phenotypic expression of clinical significance [4]. There is also the double heterozygous state in an allele carrying HbS, and the other allele carrying other abnormal haemoglobins such as HbC, HbE or alpha or beta chain quantitative variant (thalassaemic gene Hb products) [4].

It is estimated that the prevalence of live births with the disease is 4.4% in the world, where rates remain high on the main continents of Africa, Southeast Asia, and the Americas [5]. However, worrying estimates indicate that the number of newborns with SCA will increase from approximately 300,000 in 2010 to 400,000 in 2050 [6-7]. The African continent, which has 3.6 million new cases of sickle cell trait (HbAS) and 238,000 SCA, remains the largest cradle of SCA genetic inheritance [8-9] with a 3% and 2% prevalence of HbSS in Rivers and Bayelsa States respectively, both in South-Southern Nigeria [10-11]. Nigeria, and the Democratic Republic of Congo would urgently need to plan policies for prevention and management of SCA, so that implementations carried out in 2015 could save many lives by 2050 [11].

Studies have suggested that haemostatic factors relating to endothelial dysfunction were considered to be associated with SCA [12]. von Willebrand factor (vWF) is an important biomarker of endothelial dysfunction. It plays a crucial role in primary haemostasis and thrombus formation by mediating platelet adhesion and aggregation. Upon released from endothelial cells, ultra large vWF multimers which are the most thrombogenic are cleaved into the less active forms by a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13) [13]. Reduction in ADAMTS13 activity and elevated vWF antigen are associated with vascular complications [13] and other thrombotic diseases development such as ischemic stroke, [13] acute myocardial infarction, [14] and coronary artery disease [15]. Therefore, the aim of this study was to assess some haemostatic parameters in sickle cell anaemia subjects in Rivers and Bayelsa States.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

This study is a cross-sectional study. Four hundred and fifty (450) subjects were grouped into three as follows: Group 1 (150 sickle cell anaemia subjects at steady state that were clinically defined as free of infection, pain or any evidence of active disease at least 2 weeks prior to the next clinical visit and three months after blood transfusion) [16], Group 2 (150 sickle cell anaemia subjects with vaso-occlusive crises which were clinically defined as having pain in the bones, muscles, and joints not attributable to any other cause and requiring parenteral analgesic and hospitalization at the hospital for some hours) [16-17] and finally Group 3 (150 apparently healthy individuals with homozygous haemoglobin A genotype who served as the control subjects).

### 2.2 Study Area

The study was carried out in two Federal tertiary hospitals, the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt and Federal Medical Centre (FMC) Yenagoa, both in Nigeria. The two Federal hospitals were formerly located in the same state (old Rivers

State). They are referral centres for various health institutions in the respective states, Rivers and Bayelsa, Nigeria.

## 2.3 Study Population

A total of three hundred (300) subjects with age range of 1-50 years were randomly selected. There are about 200 registered patients (adults and children alike) at the sickle cell clinics of the University of Port Harcourt Teaching Hospital, and the Federal Medical Centre, Yenagoa, with an average of 4 new patients per month. The clinics run on Thursdays and Fridays respectively with a weekly attendance of between five (5) and ten (10) patients. It was runned by two(2) Consultants, two (2) Senior Registrars, and three (3) Registrars.

## 2.4 Eligibility Criteria

### 2.4.1 Inclusion Criteria

Subjects with homozygous haemoglobin S (HbSS) aged between 1 year and above who had been apparently well with no recent drop in the haemoglobin level and there was absence of infection, pain, acute complicating factors or acute clinical symptoms or crisis for a minimum of two (2) weeks before recruitment as established by a careful history and complete physical examination [18, 17]. Subjects with homozygous haemoglobin S (HbSS) aged one year and above who had crises, also referred to the episodes of acute illness attributable to the sickling phenomenon in which there was a sudden exacerbation of symptoms and signs of subjects who had hitherto been in stable condition. The pain was in the form of vaso-occlusive crisis, aplastic crisis, acute sequestration crisis, or haemolytic crises [18,17].

### 2.4.2 Exclusion Criteria

Subjects who non-homozygous genotype (Hb SS) were excluded from the study. Also, subjects with any type of infective illness (HIV, tuberculosis, SARS-COV 19) or has had recent blood transfusion during the preceding two weeks were excluded from the study. Furthermore, subjects who had recent intake of any myelosuppressive agent (e.g., Hydroxyurea) for the preceding two weeks were excluded.

## 2.5 Sample Size

Purposive sampling and randomized method were used in the selection of subjects, with due consideration of the total number of patients admitted in the clinic/ward in the University of Port Harcourt Teaching Hospital, Port Harcourt and the Federal Medical Centre, Yenagoa. The sample size was obtained using a prevalence of sickle cell anaemia of 2% [10] and the sample size was calculated using Cochran sample size formula [19].

$$N = \frac{Z^2 pq}{d^2}$$

Where N = the desired sample size

Z = the Standard Normal deviate usually set at 1.96 corresponding to the 95% Confidence level

p = the prevalence of target population

q = 1- p

d = degree of accuracy desired set at 0.05

Therefore N =  $\frac{(1.96)^2 \times 0.02 \times (1-0.02)}{(0.05)^2}$

p = % or 0.02

N = 30

By adding 10% of non-respondent, the sample size will be 33.

## 2.6 Sample Collection and Handling

Five milliliters (5ml) of venous blood sample was withdrawn from the peripheral vein in the upper limb of subjects using a standard venipuncture technique with minimum stasis under aseptic conditions from the dorsum of the hand or ante-cubital vein as the case may be as described by Ian in 2017 [20]. This was done by fastening a soft tourniquet to help locate and define peripheral veins to achieve successful and safe venipuncture, not more than 2 minutes to enable the index finger feel a suitable vein. The punctured site was then cleaned with 70%

alcohol (methylated spirit) before collection of samples with 21G dry sterile hypodermic needle with a 5ml syringe, thereafter, 2ml of the whole blood was transferred into EDTA bottle.

The sample was rocked gently to mix and kept at room temperature and the haemostatic parameters were analyzed within 4 hours of samples collection. The separated plasma was stored at -20°C prior to assay. Assay was carried out on the plasma sample thawed only once. The sample bottles were then assigned a study code with a non-water-soluble ink with date, sex and time of collection and logged on to a paper log after dispensing the blood sample into the sample bottles.

## **2.7 Laboratory Methods of Analysis for Specific Objectives**

### **2.7.1 Prothrombin Time (PT)**

Prothrombin time was analyzed using Fortress PT with calcium reagent which is based on a modified PT method [21]. Uniscope SM801A Laboratory Water Bath for précised temperature control throughout the range [21]. The calcium in whole blood is bound by sodium citrate, thus preventing coagulation. Tissue Thromboplastin, to which calcium has been added, is mixed with the plasma, and the clotting time is noted.

### **2.7.2 Activated Partial Thromboplastin Time (APTT)**

Activated Partial Thromboplastin Time was analyzed using Fortress APTT reagent which is based on a modified PT method [21]. Uniscope SM801A Laboratory Water Bath for précised temperature control throughout the range [21].

### **2.7.3 ADAMTS13 Activity Assay**

ADAMTS13 activity was assayed quantitatively with Bioassay **TECHNOZYM® ADAMTS13 Activity ELISA cat 5450701 (Technoclone GmbH, Vienna, Austria)** kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions [22-25].

### **2.7.4 von Willibrand Factor Activity Assay**

von Willibrand Factor (vWF) Activity was assayed quantitatively with Bioassay **TECHNOZYM® vWF:Ag ELISA Kit cat 5450201 (Technoclone GmbH, Vienna, Austria)** **measures quantity instead of VWF activity** kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions [22].

### **2.7.5 FVIII Activity Assay**

Factor VIII (FVIII) activity was assayed quantitatively with Bioassay **TECHNOZYM** kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions [22-25].

### **2.7.6 Fibrinogen Activity Assay**

Fibrinogen activity was assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions [23-25].

### **2.7.7 D-dimer Activity Assay**

The D-dimer levels was assayed quantitatively with Bioassay **TECHNOZYM** kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions [22-25].

### 2.7.8 L-Arginine

L-Arginine activity was assayed quantitatively with Bioassay **TECHNOZYM** kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an ISO 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions [22-25].

## 2.8 Statistical Analysis

Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA). The mean/standard deviation and Analysis of Variance (ANOVA) with interaction effects where significant differences existed in the subject states or interaction of the states with any of the independent factors were determined. Post Hoc Tests was conducted using the Tukey's Honestly Significant Difference (HSD) procedure to compare all possible pairs of the parameters means. Pearson Correlation analysis was used to determine the direction of relationships within and between the haemotological parameters by the sickle cell anaemia subjects' state.

## 3. RESULTS AND DISCUSSION

The study showed that there was highly significant difference ( $p < .05$ ) in the haemostatic parameters across the subject condition studied compared with the control (Tables 1 and 2). The mean comparison of D-Dimer was significantly increased statistically ( $p < .01$ ) in VOC condition than steady state compared with the control group (Table 1). Several studies provide evidence of a relationship between D-dimer and sickle cell anaemia. The level of D-dimer was significantly higher in 71% of the subjects; and significant elevation was observed in subjects with VOC when compared with steady state, this is in keeping with many reports of elevated D-dimer in SCA as reported by Mohamed and colleagues [26]. Elevated plasma D-dimer levels indicate increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increase thrombin activity triggers the formation of densely-packed fibrin clots composed of thin fibrin fibers compared to normal clots [27]

**Table 1: Comparison of Haemostatic Parameters in Sickle Cell Anaemia and Control Subjects**

Parameter	Steady State (n=150)	Subject State Vaso-Occlusive Crisis (VOC) (n=150)	Control (n=150)	Test Statistics
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	P value
D-Dimer (mg/mL)	102.97 $\pm$ 11.516 <sup>a</sup>	444.85 $\pm$ 80.66 <sup>b</sup>	47.99 $\pm$ 2.131 <sup>c</sup>	<0.05*
ADAMTS13 (ng/mL)	4.54 $\pm$ 0.205 <sup>a</sup>	0.82 $\pm$ 0.033 <sup>a</sup>	19.80 $\pm$ 2.163 <sup>b</sup>	<0.05*
FVIII (ng/mL)	5.84 $\pm$ 0.799 <sup>a</sup>	54.27 $\pm$ 3.790 <sup>b</sup>	0.93 $\pm$ 0.037 <sup>a</sup>	<0.05*
vWF (ng/mL)	40.56 $\pm$ 2.237 <sup>a</sup>	161.90 $\pm$ 10.490 <sup>b</sup>	0.85 $\pm$ 0.041 <sup>c</sup>	<0.05*

Abbreviations: SD: Standard Deviation; ADAMTS13: A Disintegrin and Metalloproteinase with a Thrombospondin Type 1 motif, member 13; FVIII: Factor VIII Inhibitor; vWF: von Willebrand Factor. Within parameters, means  $\pm$  SD across subjects' states with different superscripts (a, b, c) are significantly different at  $p < .05$ . Significance Level: \* = significant ( $p < .05$ ), ns = not significant ( $p > .05$ ).

The mean comparison of ADAMTS13 was significantly reduced statistically ( $p < .01$ ) in VOC condition than steady state compared with the control group (Table 1). This is supported by the findings of Hermandand colleagues [28] and Chenand colleagues[29] that summarized three pathways which induce a variable deficiency of ADAMTS13 activity and an increased release of vWF from the endothelium together with a decreased susceptibility of vWF to cleavage by ADAMTS13, thereby promoting the blood accumulation of ultra-large hyper adhesive and hyperactive forms of vWF. The pathways are involved to imbalance the vWF/ADAMTS13 couple. First, chronic inflammation is associated with the release of cytokines that bind to ADAMTS13 and may inhibit its catalytic site [30-31] and induce a functional deficiency of ADAMTS13; cytokines also stimulate the release of vWF from endothelial cells. Second, cell-free haemoglobin (Hb) released during

intravascular haemolysis can bind to both ADAMTS13 (thus inhibiting its catalytic activity) and the vWF-A2 domain (thus blocking ADAMTS13 access to the vWF cleaving site, a specific peptide bond located within its A2 domain)[32-33].

**Table 2: Comparison of Haemostatic Parameters in Sickle Cell Anaemia and Control Subjects**

Parameter	Steady State (n=150)	Subject State Vaso-Occlusive Crisis (VOC) (n=150)	Control (n=150)	Test Statistics
	Mean ± SD	Mean ± SD	Mean ± SD	P value
Fibrinogen (mg/mL)	2.02±0.168 <sup>a</sup>	9.88±0.656 <sup>b</sup>	0.67±0.057 <sup>c</sup>	<0.05*
L-Arginine (µg/mL)	20.27±0.837 <sup>a</sup>	2.80±0.201 <sup>b</sup>	67.82±1.477 <sup>c</sup>	<0.05*
PT (sec)	20.41±0.383 <sup>a</sup>	30.17±0.564 <sup>b</sup>	12.63±0.144 <sup>c</sup>	<0.05*
INR	1.85±0.035 <sup>a</sup>	2.74±0.052 <sup>b</sup>	1.11±0.014 <sup>c</sup>	<0.05*
APTT (sec)	35.56±0.494 <sup>a</sup>	43.67±0.603 <sup>b</sup>	25.13±0.324 <sup>c</sup>	<0.05*

Abbreviations: SD: Standard Deviation; PT: Prothrombin Time; INR: International Normalized Ratio; APTT: Activated Partial Thromboplastin Time. Within parameters, means ± SD across subjects' states with different superscripts (a, b, c) are significantly different at  $p < .05$ . Significance Level: \* = significant ( $p < .05$ ), ns = not significant ( $p > .05$ ).

The mean comparison of FVIII inhibitor was significantly higher statistically ( $p < .01$ ) in VOC condition than steady compared with the control group (Table 1). Our study agrees with previous reports [34-35], that factor VIII in SCA subjects have an isolated elevation, and it may explain the shortened activated partial thromboplastin time (aPTT) [36]. This finding is further supported by the fact that deficits at variable rates in physiological coagulation inhibitors and the increased activity of factor VIII are abnormalities of hemostasis that reflect the existence of a pro thrombotic state during vaso-occlusive state of SCA [26]. This significant elevation of factor VIII therefore reflects the existence of a pro thrombotic state in sickle cell VOC that can explain the increase of incidence of thrombosis in SCA. Factor VIII clotting consistently high in SCA may well be a prime therapeutic target in the treatment of thrombotic manifestations of this disease [38].

The mean comparison of von Willebrand factor (vWF) was significantly increased statistically ( $p < .01$ ) in VOC condition than steady state compared with the control group (Table 1). This finding disagrees with [38] in his study whereby increased vWF levels was observed in subjects with SCA during steady state. The finding therefore corroborates with that carried out by Sins and colleagues [39] which was observed that the vWF reactivity was higher during VOC and was associated with inflammation and neutrophil activation. Hyper-adhesive vWF may promote VOC in sickle cell anaemia.

There was statistically significant increase in the mean comparison of Fibrinogen ( $p < .01$ ) in VOC condition than steady state compared with the control group of our subjects (Table 2). The fibrinogen concentration (FC) in our subjects showed within high level was in contrast with the finding of [40] in 67% of the subjects and the difference between the VOC and steady state groups was statistically insignificant. This is in agreement to earlier studies by Mohamed and colleagues [37] in Sudan which had reported elevated levels of fibrinogen in SCA subjects in steady state, with a further elevation during crises. This may result to increased fibrinogen-mediated platelet aggregation may play a role by raising blood viscosity during pain crisis in SCA subjects [41].

**Table 3: Pairwise Correlations of Haemostatic Parameters by Sickle Cell Anaemia Subjects' State**



Parameter	By Parameter	Subjects' State					
		Steady State (n=150)		Vaso-Occlusive Crisis (VOC) (n=150)		Control (n=150)	
		R value	P-Value	R value	P-Value	R value	P-Value
ADAMTS13	D-Dimer	-0.143	0.0806	-0.062	0.4552	0.107	0.1950
FVIII	D-Dimer	-0.051	0.5320	0.562	<.0001*	0.049	0.5550
FVIII	ADAMTS13	-0.063	0.4419	0.007	0.9309	0.001	0.9878
vWF	D-Dimer	-0.060	0.4688	0.055	0.5074	-0.099	0.2288
vWF	ADAMTS13	0.094	0.2508	0.137	0.0938	0.068	0.4107
vWF	FVIII	-0.013	0.8778	0.004	0.9608	0.012	0.8863
Fibrinogen	D-Dimer	-0.100	0.2215	-0.072	0.3812	0.029	0.7251
Fibrinogen	ADAMTS13	-0.053	0.5213	-0.036	0.6591	-0.050	0.5414
Fibrinogen	FVIII	-0.025	0.7632	-0.002	0.9852	-0.146	0.0751
Fibrinogen	vWF	0.002	0.9804	-0.127	0.1218	0.057	0.4853
Arginine	D-Dimer	-0.098	0.2306	-0.048	0.5601	0.009	0.9134
Arginine	ADAMTS13	0.207	0.0110*	-0.057	0.4899	0.047	0.5718
Arginine	FVIII	0.026	0.7519	0.003	0.9676	-0.079	0.3383
Arginine	vWF	-0.008	0.9208	-0.213	0.0089*	-0.094	0.2519
Arginine	Fibrinogen	-0.089	0.2788	0.018	0.8306	-0.124	0.1294

Abbreviations: SD: Standard Deviation; ADAMTS13: A Disintegrin and Metalloproteinase with a Thrombospondin Type 1 motif, member 13; FVIII: Factor VIII Inhibitor; vWF: von Willebrand Factor.

Significance Level: \*= $p < 0.05$

There was statistically significant reduction in the mean comparison of L-Arginine ( $p < .01$ ) in VOC condition than steady state in relation to the control group in our study population (Table 2). Recent studies suggest that subjects with SCA suffer from decreased nitric oxide reserves [42]. Blood plasma levels of nitric oxide precursors have also been found to be depressed in patients with SCA, particularly during vaso-occlusive crisis and the acute chest syndrome, and these levels vary inversely with pain symptoms [42]. Furthermore, nitric oxide-dependent blood flow is impaired in subjects with sickle cell anaemia [42]. Adult subjects with sickle cell anaemia have been reported to have low plasma L-arginine levels, with subsequent reduction in the bioavailability of nitric oxide, even in steady state [42]. L-arginine is important in the formation of nitric oxide.

With coagulopathy, our study found that subjects with SCA had a significant statistically prolonged mean PT (INR) and APTT in VOC than steady state when compared to controls with normal haemoglobin (HbAA).

**Table 4: Pairwise Correlations of Haemostatic Parameters by Sickle Cell Anaemia Subjects' State**

Parameter	By Parameter	Subjects' State					
		Steady State (n=150)		Vaso-Occlusive Crisis (VOC) (n=150)		Control (n=150)	
		R value	P-Value	R value	P-Value	R value	P-Value
PT	D-Dimer (mg/mL)	-0.020	0.8056	-0.134	0.1043	0.040	0.6280
PT	ADAMTS13	-0.133	0.1038	0.008	0.9257	0.037	0.6488
PT	FVIII	-0.122	0.1379	-0.081	0.3247	0.092	0.2628

Parameter	By Parameter	Subjects' State					
		Steady State (n=150)		Vaso-Occlusive Crisis (VOC) (n=150)		Control (n=150)	
		R value	P-Value	R value	P-Value	R value	P-Value
PT	vWF	-0.016	0.8479	-0.211	0.0097*	0.011	0.8917
PT	Fibrinogen	-0.067	0.4134	-0.002	0.9796	-0.142	0.0833
PT	Arginine	-0.228	0.0049*	-0.003	0.9754	-0.101	0.2170
INR	D-Dimer	-0.020	0.8124	-0.129	0.1181	-0.003	0.9720
INR	ADAMTS13	-0.133	0.1053	0.010	0.9030	0.006	0.9449
INR	FVIII	-0.122	0.1382	-0.084	0.3068	0.120	0.1436
INR	vWF	-0.016	0.8451	-0.218	0.0074*	-0.010	0.9030
INR	Fibrinogen	-0.067	0.4159	-0.008	0.9240	-0.073	0.3739
INR	Arginine	-0.228	0.0050*	-0.003	0.9674	-0.136	0.0982
INR	PT	1.000	<.0001*	0.992	<.0001*	0.609	<.0001*
APTT	D-Dimer	0.002	0.9776	0.018	0.8288	0.028	0.7378
APTT	ADAMTS13	0.023	0.7789	0.112	0.1708	0.097	0.2374
APTT	FVIII	0.158	0.0541	-0.069	0.4016	0.005	0.9546
APTT	vWF	-0.043	0.6045	-0.094	0.2505	-0.005	0.9490
APTT	Fibrinogen	0.035	0.6736	-0.071	0.3851	-0.053	0.5188
APTT	Arginine	0.019	0.8221	-0.024	0.7682	0.092	0.2628
APTT	PT	0.043	0.6030	0.365	<.0001*	-0.012	0.8801
APTT	INR	0.043	0.6009	0.361	<.0001*	0.058	0.4777

Abbreviations: SD: Standard Deviation; PT: Prothrombin Time; INR: International Normalized Ratio; APTT: Activated Partial Thromboplastin Time.  
Significance Level: \*= $p < .05$

The finding of prolonged PT and APTT among the SCA participants (Table 2), could be as a result of reported decrease in the plasma levels of factor V, total factor VII, and factor VII zymogen in the pain episode group than the steady state in SCA subjects [44]. This was in line with a study by Buseri and colleagues [45] that found the mean PT in HbSS patients to be significantly higher than the mean PT value in HbAA control subjects [45]. This reduction will produce a normal or slightly prolonged PT instead of the expected shortening, which might predispose them to bleeding and thrombocytosis [44].

The pairwise correlations of haemostatic parameters by sickle cell anaemia subjects' state showed statistically significant positive correlation observed in the steady state subjects (n=150) between Arginine and ADAMTS13 ( $r = 0.207$ ;  $p < .01$ ), and between INR and PT ( $r = 1.000$ ;  $p < .01$ ) (Tables 3 and 4). On the other hand, statistically significant negative correlation was observed between Fibrinogen and Haemoglobin F ( $r = -0.194$ ;  $p < .05$ ), between PT and Arginine ( $r = -0.228$ ;  $p < .05$ ), and between INR and Arginine ( $r = -0.228$ ;  $p < .05$ ). Statistically significant positive correlation was observed in the vaso-occlusive crisis subjects (n=150) between FVIII and D-dimer ( $r = 0.562$ ;  $p < .01$ ), between INR and PT ( $r = 0.992$ ;  $p < .01$ ), between APTT and PT ( $r = 0.365$ ;  $p < .01$ ), and between APTT and INR ( $r = 0.361$ ;  $p < 0.0001$ ). Contrarily, statistically significant negative correlation was observed between Arginine and vWF ( $r = -0.213$ ;  $p < .01$ ), between PT and vWF ( $r = -0.211$ ;  $p < .05$ ), and between INR and vWF ( $r = -0.218$ ;  $p < .01$ ). Statistical significant positive correlation was observed in the control subjects (n=150) between INR and PT ( $r = 0.609$ ;  $p < 0.0001$ ), and between APTT and Haemoglobin F ( $r = 0.0468$ ).

#### 4. CONCLUSION

This study showed a heightened hypercoagulability in SCA subjects, and further pave way for better understanding particularly the diagnostic variables underlying SCA, specific to each subject condition (steady state and VOC). Subjects with SCA, particularly during VOC, undergo significant haemostatic alterations that increase their risk of developing coagulation activation-related complications. Thus, though selected markers of coagulation were significantly different between the subject conditions, they were often significantly higher in the SCA.



## CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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