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Research Article

Plasma Level of Von Willebrand Factor: A Marker of Sickle Cell Anemia Vaso-Occlusive Crisis

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ABSTRACT

Sickle cell anemia (SCA) is a hypercoagulable and prothrombotic disease state characterized by hemostatic perturbations that may predispose to increased risk of vaso-occlusive crisis (VOC) and other vasculopathic events. Von Willebrand Factor (vWF) has been shown to play an important role in the pathophysiology of vaso-occlusion in SCA, enhancing adhesive interactions with blood cells and the vascular endothelium. However, the levels of vWF in patients with SCA in Nigeria and its role in the pathophysiology of SCA vaso-occlusion have not been fully evaluated. The aim was to evaluate the plasma concentration of vWF in SCA patients in steady state and VOC for the purpose of determining its clinical value as marker of sickle cell VOC. This was an observational study carried out at the University of Uyo Teaching Hospital, Uyo, Nigeria. Eighty patients and 40 healthy HbAA volunteers were recruited for this study. Forty patients presented during steady state and the same number of patients presented during VOC. Hematocrit (HCT), white blood cell count (WBC), Platelet (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), and plasma concentration of vWF were determined. The mean values of vWF in VOC (3.85+/-3.81 IU/ml) and steady state (2.69 1.89 lU/ml) were higher than that of the control (1.53+/-0.40 IU/ml). There were statistically significant differences in HCT, WBC, PLT, PT, and APTT between the cases and HbAA control ($p < 0.001$). Levels of vWF are elevated in SCA during VOC and steady state and thus may serve as a potential marker to identify patients at risk of developing VOC.

Keywords: Hypercoagulable state; vaso-occlusive crisis; steady state; von Willebrand factor; sickle cell anemia.

INTRODUCTION

The landmark work by Pauling *et al.*, in 1949, established sickle cell anemia (SCA) as the first molecular human disease [1]. Despite the astounding advances made in understanding the genetic basis of the disorder in the past couple of years, various aspects of its principal pathophysiology notably the mechanisms of sickling and vaso-occlusion are still poorly understood. While the sickling process is attributed to the recurrent polymerization of the hemoglobin S (HbS) molecule in the deoxygenated state, it remains unclear how this mechanistic phenomenon translates into the complications of painful crises, cardiomyopathy, acute chest syndrome, stroke, and other end-organ clinical sequelae of the disease [2]. SCA is a disorder of utmost public health significance that affects millions of people across the globe. Each year over 300,000 babies are born with major hemoglobin disorders worldwide, including more than 200,000 cases of SCA in Africa [3] and at least 150,000 cases in Nigeria alone annually. It is estimated that 1%–3% of the Nigerian population are affected by the disease, while 20%–30% are carriers of the sickle cell trait [4]. The disease burden in the country varies from one geographic region to another. Akaba *et al.* [5] in Calabar, South-South Nigeria, reported the prevalence rate of SCA to be 2.28%. A study in Lagos, South-West Nigeria, reported a prevalence of 2.4% [6].

Sickle cell disease (SCD) is considered to be a hypercoagulable and prothrombotic disease state, in which there is a significant disturbance of the hemostatic system marked by chronic activation of coagulation and an increased incidence of thrombotic events [7]. The pathogenesis of hemostatic defects in SCD is multifactorial, with contributions from hemolysis and nitric oxide deficiency,

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and increased sickle red blood cell phosphatidylserine expression, increased platelet activation, increased activation of coagulation proteins with an attendant raised levels of von Willebrand Factor (vWF), thrombin and tissue factor, depletion of protein C and S, and impaired fibrinolytic activity [8]. Thrombogenic events are heightened in patients with SCD both during the steady state and during crises [7, 8]. Thrombosis is a prominent component of the clinical spectrum of SCD, manifesting as stroke, chronic leg ulcer, avascular necrosis of long bones and large joints, retinopathy, pulmonary hypertension, and embolism [9].

Almost all aspects of hemostasis are abnormal in SCD, namely, platelet function, procoagulant, anticoagulant, and fibrinolytic systems [10, 11]. Sickle erythrocytes themselves are procoagulant, due to their altered phospholipid asymmetry with exposure of anionic phosphatidylserine on the outer membrane leaflet and release of microparticles [12]. Antibodies are often formed against the phospholipid moieties, particularly the phosphatidylserine, in patients with SCD [7].

vWF is a multimeric glycoprotein that is found in plasma, alpha granules of megakaryocytes and platelets, and subendothelial connective tissues. It mediates platelet adhesion and aggregation and also acts as a carrier molecule for Factor VIII (FVIII) protein in plasma, protecting it from proteolysis by activated protein C [13]. Systemic levels of vWF antigen constitute an index of blood coagulation and its plasma estimation may aid in the assessment of hemostatic function [9]. Several studies have reported that plasma vWF antigen values are elevated in a wide array of clinical conditions including SCA [10, 11, 14]. Raised plasma levels of vWF have been demonstrated in patients with SCD during the steady state compared with control subjects, although much higher figures occurred during painful crises [10, 11]. These changes that are predictive of frequency of crises and intervals between the SCD episodes further lend credence to the pivotal role of thrombogenic activity in the development of SCD crises. In addition, circulating levels of vWF have been shown to correlate with SCD severity, with severe forms of the disease having higher levels [10, 15].

There are limited studies on the levels of vWF in subjects with SCD and the correlation between vWF and occurrence of vaso-occlusive crises (VOC) in Nigeria. This study therefore aims to determine the plasma concentrations of vWF in SCA patients in steady state and in VOC in order to evaluate their clinical significance as markers of sickle cell VOC.

MATERIALS AND METHODS

Ethical approval was obtained from the Ethical Committee of University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria. A total of 40 (14 males and 26 females) HbSS subjects in VOC and 40 (17 males and 23 females) HbSS subjects in steady state were recruited from the Adult Haematology Out-patient clinic. Forty (16 males and 24 females) healthy HbAA volunteers matched for age and sex with subjects were used as controls in this study.

Subjects and controls were between 18 and 56 years. Diagnosis of SCA in steady state was made in patients who were crisis-free for a period of not less than 2 weeks, while SCA subjects in VOC were those with bone pains involving any sites of the skeleton or other pains lasting at least 12 hours at presentation.

Those included in the study were SCA patients and controls who signed informed consent. Excluded from the study were SCA patients who refused to give consent, those who had blood transfusion less than 3 weeks prior to date of recruitment, those who suffered from liver disease (confirmed by deranged liver function tests or positive viral hepatitis test results) as this could have increased the vWF levels, SCA patients who had sepsis, as this could also increase levels of vWF, pregnant women and SCA patients with a past history of thrombosis or on medications that are likely to interfere with hemostasis, e.g., aspirin, contraceptives, and anticoagulants, as well as HIV positive patients, as this is a hypercoagulable state associated with increased vWF production.

Specimen Collection and Preparation

Fifteen milliliters of free-flowing venous blood collected from each subject. Five milliliters of the blood was dispensed into and mixed in sodium ethylene diamine tetra-acetate (EDTA) sample bottles. This sample was used for full blood count and was analyzed within 2 hours of collection. The full blood count was carried out using the Mindray Haematology Analyzer. Another 5 ml aliquot of the sample was dispensed into and mixed in 0.109 mol/l trisodium citrate (3.2%) specimen bottles (1 volume of citrate to 9 volumes of blood). This was centrifuged within 30 minutes of collection at 2000 g for 15 minutes at room temperature. The plasma obtained was stored at -80°C until analysis was carried out. This was used for determination of prothrombin time test (PT) and activated partial thromboplastin time test (APTT). The PT and APTT tests of the steady state subjects and controls were done on the same day of collection and processing of specimens, while the tests of the VOC subjects involved pooling of the samples to obtain the required sample size before analysis, because the subjects presented at different times. The PT/APTT tests were determined using standard commercial PT/APTT reagents manufactured by Diagnostic Reagent LTD, Thames, Oxon, United Kingdom. Test procedures were carried out according to the manufacturer's instructions.

The remaining 5 ml of the blood was dispensed into 3.2% of trisodium citrate specimen bottles at a concentration of 9 parts of blood to 1 part of the anticoagulant for the measurement of vWF antigen (vWF:Ag) levels. These were immediately centrifuged at 2000 g for 15 minutes and later centrifuged at 4000 g for 15 minutes. The resulting platelet-poor plasma was transferred in aliquot using a polypropylene transfer pipette into plain cryotubes in aliquot and stored at -80°C, until analysis was done. Plasma concentration of vWF:Ag was measured using a commercial assay kit-assay max human vWF ELISA kit manufactured by Assay Pro. St. Charles, MO, USA.

DATA ANALYSIS

The data were collated, analyzed using Statistical Package for Social Sciences (SPSS) windows version 23.0 and presented in frequency tables. Statistical significance was determined with the paired Student t-test. Correlations were performed by Pearson's Correlation Method. P-values of less than 0.05 were considered to be statistically significant.

RESULTS

The results of the different tests are summarized in Tables 1–4. The mean ages of subjects in the different groups of the study were: 27.62 ± 6.21, 28.28 ± 8.06, and 28.25 ± 6.75 for VOC, steady state, and HbAA controls, respectively (Table 1). The mean hematocrit of 18.88 ± 1.34% for HbSS subjects in VOC and 22.25 ± 101% for HbSS subjects in steady state were significantly low compared to 41.75 ± 3.32% for HbAA controls ($p < 0.001$) (Table 2).

The mean total white cell count of 17.52 ± 1.30 × 10^9/l for subjects in VOC was significantly increased compared to 5.53 ± 1.46 × 10^9/l for the HbAA control subjects ($p < 0.001$), and 12.03 ± 0.58 × 10^9/l for HbSS subjects in steady state was also significantly increased compared to the controls ($p < 0.001$). The mean platelet count of 352.80 ± 50.54 × 10^9/l for subjects in VOC was significantly increased compared to 174.60 ± 24.30 × 10^9/l for the control subjects ($p < 0.001$); similarly, the mean platelet count of 363.63 ± 34.90 × 10^9/l for subjects in steady state was significantly increased when compared to controls ($p < 0.001$). There was no statistically significant difference between the mean value of platelet count for steady state subjects and that of the HbSS subjects in VOC ($p > 0.05$) (Table 2).

The mean PT test of 13.17 ± 1.01 seconds for subjects in VOC was statistically shortened compared to 15.37 ± 0.64 seconds for HbSS in steady state ($p < 0.001$); similarly, the mean PT test of 12.95 ± 1.16 seconds for controls was significantly shortened when compared to that of the subjects in steady state ($p < 0.001$). No significant difference was observed when the mean PT test results of HbSS in VOC and controls were compared ($p > 0.05$). However, the mean APTT was significantly prolonged among subjects in VOC (49.47 ± 4.35 seconds) $p < 0.001$ and in steady state (46.22 ± 7.29 seconds) $p < 0.001$ when both were compared with the controls (35.51 ± 2.15 seconds) (Table 3).

The mean plasma vWF antigen concentration of 3.85 ± 3.81 IU/ml for subjects in VOC was not significantly different from that of the steady state subjects (2.69 ± 1.89 IU/ml) $p > 0.05$. However, the mean plasma vWF concentration for subjects in VOC and those in steady state was significantly increased compared to 1.54 ± 0.41 IU/ml for controls ($p < 0.05$) (Table 4).

DISCUSSION

Increased plasma levels in SCA patients in steady state and during the course of VOC have been reported in several studies [16–18]. In the present study, the plasma concentration of vWF among HbSS subjects in VOC and those in steady state were significantly increased compared to the concentration of vWF in the HbAA subjects. This finding is similar to those observed in other studies [11, 15, 16, 18]. In the study by Sins *et al.* [16], 24 subjects were recruited. Of these, 16 were homozygous for HbS, 6 had HbS ^{o} thalassemia, and 2 had HbSC disease and Hb S^{+} thalassemia. The patients were either asymptomatic (steady state), hospitalized for VOC, or had acute chest syndrome (ACS). In the study, the authors reported that the patients hospitalized for VOC and ACS

Table 1: Age and sex distribution of subjects and controls according to clinical states.

	HbAA control		HbSS in steady state		HbSS in VOC	
Age (yr)	Male	Female	Male	Female	Male	Female
≤ 20	0	2	2	3	2	1
21–25	5	8	5	7	4	11
26–30	5	7	5	5	5	6
31–35	5	5	4	4	2	5
36–40	0	2	0	3	0	2
41–45	0	0	0	0	0	1
46–50	0	0	0	1	1	0
≥ 51	1	0	1	0	0	0
Total	**16**	**24**	**17**	**23**	**14**	**26**
Mean	**28.25 ± 6.75**		**28.28 ± 8.06**		**27.62 ± 6.21**	

Table 2: Comparison of mean hematological parameters of subjects and controls.

Hematological indices	HbAA control (n = 40)	p^1 value	HbSS steady state (n = 40)	p^2 value	HbSS VOC (n = 40)	p^3 value
HCT	41.75 ± 3.32	0.000***	22.25 ± 1.01	0.000***	18.88 ± 1.34	0.000***
WBC	5.53 ± 1.46	0.000***	12.03 ± 0.58	0.000***	17.52 ± 1.30	0.000***
PLT	174.60 ± 24.30	0.000***	363.63 ± 34.90	0.000***	352.80 ± 50.54	0.272

p^1 value for steady state vs HbAA control
p^2 value for VOC vs HbAA control
p^3 value for VOC vs steady state
Significant level – $p < 0.001$***

Table 3: Comparison of mean prothrombin time (PT) and activated partial thromboplastin time (APTT).

Blood coagulation parameters	HbAA control (n = 40)	p^1 value	HbSS steady state (n = 40)	p^2 value	HbSS VOC (n = 40)	p^3 value
PT in seconds	12.95 ± 1.16	0.000***	15.37 ± 0.64	0.391	13.17 ± 1.01	0.000***
APTT in seconds	35.51± 2.15	0.000***	46.22 ± 7.29	0.000***	49.47 ± 4.35	0.028*

p^1 value for steady state vs HbAA control
p^2 value for VOC vs HbAA control
p^3 value for VOC vs steady state
Significant levels – $p < 0.05$* and $p < 0.001$***

Table 4: Comparison of mean vWF:Ag concentration among subjects and control.

Coagulation protein	HbAA control (n = 40)	p^1 value	HbSS steady state (n = 40)	p^2 value	HbSS VOC (n = 40)	p^3 value
vWF:Ag IU/ml	1.53 ± 0.40	0.000***	2.69 ± 1.89	0.001**	3.85 ± 3.81	0.096

p^1 value for steady state vs HbAA control
p^2 value for VOC vs HbAA control
p^3 value for VOC vs steady state
Significant levels – $p < 0.01$** and $p < 0.001$***

had much higher levels of vWF compared to the symptomatic SCA subjects. The study inferred that the complications of SCA, including VOC, result in marked production vWF and its detection may be used as a marker for the presence of the complications. In a similar study conducted by Dickson *et al.* [11], 50 subjects were studied, of which 22 were in steady state while 28 were in painful crisis, all the subjects in the study were homozygous for HbS; they reported significantly elevated levels of vWF in steady state as well as painful crisis compared to the control subjects ($p < 0.001$). In like manner, Al-Awadhi *et al.* [18] reported raised plasma levels of vWF in both pediatric and adult SCD patients in contrast to normal controls. On the contrary, in another study by Nawal *et al.* [19], the workers did not observe any significant difference in the mean vWF concentration in both steady state and VOC ($p = 0.479$). However, their study was able to establish a positive correlation between vWF and the severity of the disease, while allotting scores to identify such severe conditions.

The pathophysiology of VOC has been shown to be intimately linked to adhesion of the blood cells, including sickle red cells, leucocytes, and platelets to the vascular endothelium [2]. In SCD, the endothelium is in a constant state of activation by inflammatory cytokines resulting in increased production and secretion of vWF [18]. The clinical importance of the raised levels of vWF in SCD is related to the degree of adhesion of the blood cells to the vascular endothelium, thereby playing a pivotal role in the pathogenesis of VOC and a legion of thrombotic complications, which negatively affect the severity of SCD [2, 10]. The release of the vWF from the endothelial cells leads to adhesion of more blood cells to the endothelium with attendant narrowing of the vascular lumen especially under conditions of high shear stress [2, 17, 20].

In the current study, a significant increase in white cell and platelet counts was observed among sickle cell subjects during VOC, and in the steady state, when compared with the controls ($p < 0.001$). This finding is similar to those observed in several other studies [8, 11, 15]. Also, the differential associations of hematocrit, PT, and APTT in cases and controls are consistent with findings of previous studies [15, 16, 21, 22]. It is believed that the observations in these subjects primarily mirror the integrative influence of vascular endothelium and blood cells in the development of vaso-occlusive events and other complications of SCD.

Data from our series have confirmed that SCA is a hypercoagulable state. Continuous endothelial stimulation is widely recognized as a prominent feature of the disease, and vWF is one of the markers of endothelial dysfunction [7, 13, 15]. High levels of vWF mediate the adhesion of blood cells to the vessel wall and contribute to the occurrence and propagation of vaso-occlusive events. Taking the foregoing into cognizance, it is possible that modulating this activity might mitigate symptoms.

Our study has some strengths and limitations. It was sufficiently powered to detect any difference in the measured variable in the study population. Another strength of this study is that our sample size was larger than those of comparable studies. In addition, the cases were a homogenous population of HbSS subjects excluding cofounders due to other SCD phenotypes. As regards limitations, the descriptive study design did not allow us to determine whether the high vWF levels during VOC is a true causal event in the pathophysiology of VOC in SCD, or rather a result of the VOC. However, in vitro studies have demonstrated that ultra-large vWF can impede blood flow, by binding platelets, leukocytes, and erythrocytes as well as by adhering to the vascular endothelium [13]. Evaluation of this large molecule was beyond the scope of this study owing to financial concerns.

CONCLUSION

This study, like earlier work in other populations, has confirmed that vWF antigen levels are significantly elevated in VOC. Interventions targeting vWF interactions with the vascular endothelium and blood cells may therefore be explored as veritable means to modulate VOC in SCA patients. We hereby recommend that further studies be carried out to elucidate whether vWF is merely a marker of or an actual contributor to the pathophysiology of VOC in SCA.

AUTHORS' CONTRIBUTIONS

This work was carried out by both authors. Author ISA contributed substantially to the conception and design, acquisition of data, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content, final approval of the version to be published. Author CBN contributed to acquisition of data, drafting the article, revising it critically for important intellectual content, final approval of the version to be published.

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