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BRIEF REPORT

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Determination of von Willebrand factor level in patient with sickle cell diseasein vaso-occlusive crisis

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Abstract

Background: Sickle cell anemia (SCA) is a hypercoagulable state characterized by a significant alteration in hemostatic parameters that may predispose to increased risk of vaso-occlusive crisis (VOC). The role of von Willebrand factor (VWF) in the pathogenesis of VOC has not been fully investigated in Nigeria.

Objective: The objective of this study was to evaluate the level of VWF in subjects with sickle cell disease (SCD) in Calabar, Nigeria, to determine its role in the pathogenesis of VOC.

Methods: This was a comparative study carried out at the University of Calabar Teaching Hospital, Calabar, Nigeria. Sixty patients with SCA in VOC and 50 healthy controls were included. VWF levels were measured using Assaypro enzyme-linked immunosorbent assay kits.

Results: The mean age of patients with SCA in VOC and controls was 23.5 ± 7.2 years and 26.5 ± 5.6 years, respectively. The means (standard deviations) of VWF in patients in VOC and controls were 2.52 ± 0.34 IU/mL and 1.41 ± 0.23 IU/mL, respectively. There was no correlation of hematocrit and VWF in VOC (r = −0.034; P = .80), while there was a modest inverse correlation in controls.

Conclusions: Levels of VWFare elevated in a VOC state and thus may be implicated in the pathogenesis of VOC.

KEYWORDS

sickle cell anemia, vaso-occlusive crisis, von Willebrand factor

Essentials

- Sickle cell disease is characterized by alteration of the hemostatic system.
- The study was carried out in the hematology department at the University of Calabar Teaching Hospital.
- Hematocrit and VWF were positively correlated in vaso-occlusive crisis (VOC) and inversely correlated in controls.
- Elevated VWF may be implicated in the pathogenesis of VOC.

1 | INTRODUCTION

Sickle cell disease (SCD) is a heterogeneous group of autosomal recessive disorders with a variable clinical spectrum.[1] The most prevalent type of SCD is sickle cell anemia (hemoglobin [Hb] SS), which is linked to the inheritance of the sickle cell gene in a homozygous state. There are additional types of SCD such as the compound heterozygous form, in which the sickle β-globin gene is coinherited with another abnormal hemoglobin gene such as HbC in HbSC, β-thalassemia in HbSβ thalassemia among others.[1,2] SCD is the most common genetic disorder in sub-Saharan Africa. Nigeria is reported to bear a high disease burden, with an estimated prevalence of 1%-3% of its population being affected by the disease.[3] It is also estimated that 20%-30% of the Nigerian population carry the sickle cell gene with a normal hemoglobin gene (sickle cell trait). The disease burden in Nigeria varies from one geographic region to another. Akaba et al[2] reported the prevalence rate of SCD to be 2.28% in Calabar. A study by Nwogoh et al[4] reported a prevalence of 2.4% in Benin city.

SCD is known as a hypercoagulable and prothrombotic disease state, in which there is a significant alteration of the hemostatic system characterized by an increased level of von Willebrand factor (VWF), increased expression of P-selectins by platelets, increased platelet adhesion and aggregation, increased activation of coagulation proteins with an attendant increase in thrombin as well as thrombin-antithrombin generation, depletion of natural anticoagulants, and impaired fibrinolytic activity. All these predispose to increased thrombosis. Hypercoagulability has been reported to be actively involved in the pathogenesis of sickle cell crises and the acute complications in SCD including vaso-occlusive crisis (VOC).[5] Generally, VWF is a marker of endothelial dysfunction. It has been shown that the level of VWF antigen is a marker of intravascular coagulation and has also been linked to thromboembolic complications. Several studies have demonstrated elevated levels of ultra-large VWF and VWF in adult patients with SCD.[6] Their levels have been reported to correlate with disease severity, with severe genotypes of the disease having higher levels and thus implicated in contributing to VOC. There is a paucity of studies, however, on the levels of VWF in subjects with SCD and their contribution to VOC in Nigeria. Hence, this study aimed to evaluate VWF levels in patients with SCD in VOC in Calabar, Nigeria.

2 | METHODS

This study was carried out in the hematology outpatient clinic, medical wards, and pathology research laboratory at the University of Calabar Teaching Hospital (UCTH). It is a federal government–owned tertiary institution, situated in Calabar Municipality LGA, Calabar, Cross River State. The hematology unit was operated by consultants, resident doctors, trained nurses, and allied staff.

The study population includedcases and controls. Cases were patients with SCA (HbSS) in VOC, 16 years old and older, who gave their consent to participate. They were recruited consecutively from the outpatient clinic and medical emergency unit at UCTH. Controls were healthy individuals with HbAA (confirmed by hemoglobin electrophoresis) recruited consecutively from the blood donor clinic, hospital staff, and students.

A sample size of 60 patients in VOC and 50 controls was determined for comparison of means to achieve 80% power at α .05 to detect an approximately 5% difference in VWF between study groups.

Participants were excluded if they (i) suffered from liver disease (confirmed by abnormal liver function test) or proven viral hepatitis as they could have increased levels of VWF; (ii) were critically ill with features of sepsis, as they could have increased levels of VWF; (iii) were on hydroxyurea therapy to eliminate any confounder due to disease modification caused by the drug; (iv) were pregnant or receiving oral contraceptive therapy, as these may increase VWF levels; (v) were on anticoagulants, which might alter the procoagulant state creating confounding; (vi) had acute infections, inflammation, or trauma, as these can increase VWF levels; and (vii) were HIV positive, as this is a hypercoagulable state associated with increased VWF production.

Biodata and clinical information, including a history of crises and chronic complications of SCD, were obtained directly from the subjects and their clinical records (case notes) using a questionnaire. Blood was collected with standard methods and minimal stasis into an ethylenediaminetetraacetic acid tube for hematologic parameters, a commercial lithium heparin tube for liver function tests, and into 0.5 mL of 0.109 mol/L sodium citrate (3.2%) for VWF analysis. These were immediately centrifuged at 2000 *g* for 15 minutes, after which the plasma was centrifuged again for 15 minutes at 4000 *g*. Resulting platelet-poor citrated plasma was stored at −80°C until further analysis. An enzyme-linked immunoassay kit (ASSAYPRO LLC) was used to measure VWF antigen level according to the manufacturer's instructions. Data were analyzed with SPSS version 21 software (IBM, Armonk, NY, USA).

3 | RESULTS AND DISCUSSION

Table 1 shows participant characteristics and Tables 2 and 3 show levels of VWF and hematologic parameters. VWF antigen was significantly elevated in the VOC state compared to controls. Table 4 shows no correlation of VWF with hematocrit in cases while there was a modest inverse association in controls.

The elevated level of VWF in VOC is likely due to widespread endothelial activation, which stimulates the production and secretion of VWF by the activated endothelium. This is similar to the observations of Schnog et al,[7] who reported markedly elevated levels of VWF in VOC state in a case-control study. Sins et al,[8] in a longitudinal study, also demonstrated a significant change in VWF antigen levels in a VOC state compared to controls. In like manner, Al-Awadhi et al[9]reported elevated levels of VWF antigen in both pediatric and adult patients with SCD than in the healthy controls. The differential

TABLE 1 Sociodemographic characteristics and disease status of the participants

	SCA n = 60 (%)	Controls n = 50 (%)	*P* value
Age group, y			
<20	10 (16.7)	5 (10.0)	
21-25	23 (38.3)	20 (40.0)	
26-30	14 (23.3)	11 (22.0)	.96
31-35	8 (13.3)	11 (22.0)	
36-40	4 (6.7)	3 (6.0)	
>40	1 (1.7)	0 (0.0)	
Sex			
Male	23 (38.3)	29 (58.0)	.06
Female	37 (61.7)	21 (42.0)	
Marital status			
Single	52 (86.7)		
Married	8 (13.3)		
Educational level			
Secondary	16 (26.7)		
Tertiary	44 (73.3)		
Disease status			
VOCs per year			
0	4 (6.7)		
1-2	42 (70.0)		
≥3	14 (23.3)		
Hospital admissions per year			
0	29 (48.3)		
1-2	27 (45.0)		
≥3	4 (6.7)		

TABLE 2 VWF in cases and controls

VWF	Mean ± SD (IU/mL)	Range (IU/mL)	*P* value
Cases	2.52 ± 0.34	0.06-10.77	.006
Controls	1.41 ± 0.23	0.02-5.89	

association of VWF with hematocrit in cases and controls is similar to the findings of Chen et al[10] and Sin et al.[7]

We confirm that SCA is a hypercoagulable state. Chronic endothelial activation characterizes the disease, and VWF is one of the markers of endothelial injury.[5] Increased production of VWF may predispose to increased adhesion of the sickle cell to the vessel wall and thus provoke and propagate vaso-occlusive events. In this regard, it is possible that interfering with this activity might ameliorate symptoms.

The current study had some strengths as well as limitations. The study was adequately powered to detect any difference in the measured analyte in the study population. The sample size was larger compared to other studies. The study population was a homogeneous population of patients with HbSS, reducing confounding due to other SCD phenotypes. In terms of limitations, ultra-large VWF was not evaluated because the appropriate technology for its determination was not readily available locally.

TABLE 3 Hematologic parameters of cases

Hematologic parameters	SCA VOC	
	Mean ± SD	Range
WBC ($\times 10^{11}$/μL)	15.1 ± 5.2	6.7-33.6
GRA ($\times 10^{11}$/μL)	9.2 ± 4.0	1.8-21.3
LYM ($\times 10^{11}$/μL)	5.6 ± 3.1	2.0-17.6
Hematocrit (%)	20.8 ± 4.7	13.0-36.9
MCV (FL)	82.3 ± 6.9	67-102.0
MCH (μg)	24.5 ± 2.7	17.8-32.3
MCHC (mg/dL)	31.8 ± 2.0	24.2-36.0
PLT ($\times 10^{11}$/μL)	387 ± 119	116-615

GRA, granulocyte count; LYM, lymphocyte count; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean cell volume; PLT, platelet count; SCA, sickle cell anemia; VOC, vaso-occlusive crisis; WBC, white blood cell count.

TABLE 4 Association between VWF and hematocrit in VOC and control

	VOC		Control	
	r	*P* value	*r*	*P* value
	−0.034	.80	−0.39	.005
Mean hematocrit(%)	20.8 ± 4.7		43.5 ± 2.9	

Note: No correlation was observed between hematocrit and VWF in VOC ($r = -0.034$, $P = .80$). However, a modest inverse association was observed between VWF and hematocrit in controls.

In conclusion, similar to previous work in other populations, this study confirms that VWF antigen levels are significantly elevated in VOC. Therefore, the use of agents that target VWF interaction with endothelium and blood cells could be explored as potential targets to modulate VOC in SCD subjects.

RELATIONSHIP DISCLOSURE

The authors report nothing to disclose.

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