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Evaluation of von Willebrand Factor Levels in Sickle Cell Patients Attending Babcock University Teaching Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOOD and EDE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AOOD and EA managed the analyses of the study. Authors EA and OC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: von Willebrand Factor (VWF) plays a vital role in platelet aggregation. The unusually large VWF multimers mediate the adhesion of sickle red blood cell (RBC) to endothelial cells and contribute to vaso-occlusive episodes.
Objective: The aim of this study was to determine if there is a correlation between VWF and disease severity in sickle cell Disease.

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Methods: The Babcock University Health Research Ethical committee approved the study. Fifty (50) subjects and 13 controls were recruited into this cross-sectional study. 22 patients presented during steady state while 28 patients presented during vaso-occulssive crisis. Five (5) ml of venous blood was collected. 2 ml was transferred into an Ethylene Diamine Tetra Acetic Acid (EDTA K3) bottle at a concentration of 1.2 mg of anhydrous salt per ml of blood. This was used for blood counts. Three ml of blood was added into 3.2% trisodium citrate in 5% HEPES at a concentration of 9 parts of blood to 1 part of the anticoagulant for the measurement of VWF: Ag level.
Results: The mean values of VWF in crisis state (143.8±28.00) and steady state (105.68±21.43) were higher than that of the control (78.30±28.10). There was a significant difference in platelet ($p<0.000$) count among the groups: crisis state has the highest mean value (370.64±128.78), followed by the steady state (333.04±120.47), and the control group (221.23±91.34).
Conclusion: Severity of sickle cell disease increases with elevation of VWF level in the plasma of patients in crisis state than those in the steady state. This is as a result of the contribution of VWF and the platelets in the formation of microthrombi.

Keywords: Von Willebrand factor; sickle cell; steady state; crisis state & vaso-occlusion.

1. INTRODUCTION

Sickle cell disease (SCD) is an inherited autosomal disorder of the beta-globin chain of haemoglobin, characterized by haemolytic anaemia, intermittent episodes of vascular occlusion that can cause both acute and chronic pain, an increased susceptibility to infections and some organ damage [1] Haemoglobin is a tetrameric protein which is composed of two pairs of globin chains. A normal adult red blood cell contains mainly adult haemoglobin ($\alpha_2\beta_2$), along with small amounts of A2 haemoglobin ($\alpha_2\delta_2$) and fetal haemoglobin ($\alpha_2\gamma_2$). Sickle cell anaemia is caused by substitution in the sixth position of beta-globin chain which leads to the replacement of glutamate with valine. The abnormal physiochemical properties of the resulting sickle haemoglobin are responsible for the disease. The underline pathology of much of these complications is the recurrent occurrence of vaso-occlusion due to micro-thrombi formation resulting in organ ischemia [2]. Although the clinical manifestations of the disease are quite heterogeneous, periodic vaso-occlusive crises, and chronic intravascular haemolysis are common in SCD patients [3].

Africa has 70% of the world's annual figure of 300,000 affected new births of SCD [4]. Nigeria has a population of 112 million with an annual growth rate of 3.2%. About 25% of adults throughout the country have the sickle cell trait (AS) while Hb C trait is largely confined to the south-western Nigeria, where it occurs in about 6% while the homozygous state is found in about 3% of the population [5] However, the prevalence of SCD in Ibadan, Oyo State, is 8.2% [4].

Von Willebrand factor (VWF) mediates initial platelet adhesion to the sub-endothelium after vascular injury.VWF is released in plasma due to the inflammatory process induced by the adhesion of sickled red blood cells to the endothelium. [6] Adhesion of platelets to the adherent sickled cells and their activation will release more VWF.

Von willebrand factor parameter assessment though not included by National guidelines for the control and management of sickle cell disease, plays a major role in monitoring conditions like thrombosis, occlusive crises, hemolysis and organ ischemia.

2. METHODS

2.1 Ethical Consideration and Informed Consent

Ethical approval was obtained from Babcock University Health Research Ethics Committee (BUHREC 172/17) and the patients gave their consent before sampling in accordance with the declarations of Helsinki.

2.2 Subjects

Sickle cell patients who attended the haematology out-patient clinic, those present at the emergency room of the hospital and those on admission at the medical ward of Babcock University Teaching Hospital were recruited into the study. Apparently healthy, age and sex-matched individuals with HbAA were recruited as control. Patients who received blood transfusion less than 6 weeks ago were excluded. Sixty-

three (63) were enrolled into the study. This was a cross sectional study. Participants were grouped as per crisis state and stable state. Structured questionnaire and laboratory data were used to enrol participants who meet the inclusion criteria. The sample size for the study was calculated using the following formula [7,8]:

$$n = Z^2 \times P \frac{(1-P)}{d^2}$$

Where *n* = minimum sample size, *Z* = standard normal variance = 1.96 at a 95% confidence interval,
d = absolute SE = 0.05, and *p* = prevalence of SCD = 8.2%.

2.3 Statistical Analysis

The Statistical package for Social Science (SPSS) version 21.0 software was used for data analysis. Data obtained were presented using tables. One way analysis of variance (ANOVA) and Independent Student T-test were performed where appropriate.

2.4 Sample Collection

Five (5) ml of venous blood was collected. 2 ml was transferred into an Ethylene Diamine Tetra Acetic Acid (EDTA K3) bottle at a concentration of 1.2 mg of anhydrous salt per ml of blood. This was used for blood counts. Three ml of blood was added into 3.2% trisodium citrate in 5% HEPES at a concentration of 9 parts of blood to 1 part of the anticoagulant for the measurement of VWF: Ag level.

2.5 Full Blood Count

The full blood count was performed using an automated haematology analyzer Abacus 5, according to the manufactures instructions.

2.6 Von Willebrand Factor (VWF) Antigen

Von Willebrand's factor antigen, VWF: Ag level in plasma was measured using commercial ELISA kits from Diagnostic Automation according to the manufacturer's instructions.

3. RESULTS

Sixty three (63) subjects participated in the study, which included fifty (50) sickle cell patients HbSS and thirteen (13) apparently healthy individuals HbAA.

Table 1. The sociodemographic data of the test subjects

Variable	Frequency	Percent
Gender		
Female	26	52.0
Male	24	48.0
Age		
16-20	26	52.0
21-25	19	38.0
26-30	0	0
31-35	2	4.0
36 and above	3	6.0
Hb type		
sc	3	6.0
ss	47	94.0
Level of education		
Secondary	1	2.0
Bachelor's degree	49	98.0
Occupation		
Student	46	92.0
Civil servant	4	8.0
Marital status		
Single	47	94.0
Married	3	6.0

Out of the 50 test participants 42 attend the routine clinic, 48% attend routine clinic 1-2 times per year, with 98% on routine medication. The mostly used drug was folic acid with 88% followed by paludrine with 56% and the drug was taken daily by 96% of the subjects. 70% had painful crisis at least 1-3 times per year and 22% 4-6 times per year. 38% of the subjects handled their crisis by self-medication, 72% hospital and 18% through prayers. 45 subjects have been admitted in the last 1 year while 5 had never been admitted in the last one year.

The medical history in Table 2, shows that 60% of the subjects have been admitted about 1-2 times in the last 1 year and 26% had been admitted about 2-4 times. 46% have been transfused while 54% had not been transfused in the last 1 year, with 40% of the population transfused with about 1-2 units of blood. 16% 0f this population take supplement with 10% taking ciklavit and 10% taking discovite. All 50 subjects have never had leg ulcer or stroke. 62% of the subject knew their steady pcv, 56% of the population had a pcv within the range of 21-30%.

Table 2. Shows the medical history of the test subjects and their attitude towards routine clinics and routine medications

Variable	Frequency				Percent			
Routine clinic	**Yes 42**			**No 8**	**84.0**		**16.0**	
On routine medication	49			1	98		2	
Hospital admissions	45			5	90.0		10.0	
transfusions	23			27	46.0		54.0	
Steady PCV	31			19	62.0		38.0	
How often on medication	Daily /48			Weekly/ 1	96		2	
With Leg Ulcer	50				100			
With Stroke	50				100			
Clinic attendance	≤2x/yr	≤ 4x/yr	≤ 7x/	≥ 8x/yr				
	24	13	4	4	48.0	26.0	8.0	8.0
Admissions /yr	30	14	1	1	60.0	26.0	2.0	2.0
Painful crises	35	11	2	2	70.0	22.0	4.0	4.0
supplements	Ciklavit/5	Discovite/5	Others/50		10.0	10.0	100	
Routine Medications	Folic acid/44	Multivite/6	Paludrine/ 28	Others/8	88.0	12.0	56.0	16.01
PCV range	18-20%	21-23%	24-26%	≥27%				
	6	27	8	3	12.0	54.0	16	6.0
Management of Crisis	Self medication /19	Hospitalization /36	Prayer/9		38.0	72.0	18.0	

Table 3. Comparisons of mean values of analyzed parameters between crisis period, steady state and controls

Parameters	Steady state N=22	Crisis N=28	Control N=13	P-value
MCV(fl)	84.16±7.70	83.60±6.00	86.74±5.12	0.349
MCH(pg)	29.70±3.36	29.32±2.77	27.72±2.51	0.149
MCHC(g/l)	35.30±1.54	34.96±1.94	31.88±1.43	0.000*
PCV (%)	23.07±5.33	21.58±3.92	32.98±1.58	0.000*
HB(g/l)	8.09±1.74	7.54±1.23	10.48±0.48	0.000*
WBCx$10^{9/l}$	11.68±3.30	17.13±5.70	5.53±1.18	0.000*
PLATELETx$10^{9/l}$	333.04±120.47	370.64±128.73	221.23±91.34	0.002*
ANC	5.00±2.11	6.52±3.74	3.81±0.31	0.015*
ALC	13.06±17.59	14.80±16.84	2.56±0.60	0.060
RBCx$10^{12/l}$	2.77±0.77	2.61±0.59	2.62±0.92	0.718
VWF:Ag(%)	105.68±21.43	143.82±28.00	78.30±28.10	0.000*

**statistically significant p<0.05 using one-way ANOVA. RBC: Red Blood Cell MCH: Mean cell haemoglobin MCV: Mean cell volume MCHC: Mean cell haemoglobin concentration ANC: Absolute neutrophil count ALC: Absolute lymphocyte count PCV: Packed cell volume VWF: Ag: von Willebrand Factor Antigen*

4. DISCUSSION

Patients with SCD had higher levels of VWF when compared to the controls, with highest mean value in the crisis state (143.82±28.00) and steady state (105.68±21.43). This was in agreement with results reported by [9,1,10]. In the process of adhesion of sickle cells to the endothelium there is activation of the endothelium and such activated endothelium will become a source of VWF [11]. This mechanism has also been correlated to the degree of SSRBCs adhesion to the endothelium [12]. The release of these VWF from the endothelia cells, leads to adhesion of more sickled erythrocytes to the endothelium. with the eventual reduction of the lumen of the vessels high shear rate at this site are creating [13]. On the contrary, [9] did not observe any significant difference in the mean VWF of both states (P=0.479). Their study was also able to establish a significant positive correlation between VWF and the severity of the

Table 4. Comparisons of mean values of analyzed parameters between the crisis state and steady state

Parameters	Steady N=22	Crisis N=28	P-value
MCV(fl)	84.16±7.70	83.60±6.00	0.777
MCH(pg)	29.70±3.36	29.32±2.77	0.671
MCHC(g/l)	35.30±1.54	34.96±1.94	0.488
PCV (%)	23.07±5.33	21.58±3.92	0.282
HB(g/l)	8.09±1.74	7.54±1.23	0.219
WBCx10$^{9/l}$	11.68±3.30	17.13±5.70	0.000*
PLATELETx10$^{9/l}$	333.04±120.47	370.64±128.73	0.293
ANC	5.00±2.11	6.52±3.74	0.078
ALC	13.06±17.59	14.80±16.84	0.726
RBCx10$^{12/l}$	2.77±0.77	2.61±0.59	0.430
VWF: Ag (%)	105.68±21.43	143.82±28.00	0.000*

**statistically significant p<0.05, using t-test RBC: Red Blood Cell MCH: Mean cell haemoglobin MCV: Mean cell volume MCHC: Mean cell haemoglobin concentration ANC: Absolute neutrophil count ALC: Absolute lymphocyte count PCV: Packed cell volume VWF: Ag: von Willebrand Factor Antigen*

disease, while assigning scores to mark such severe conditions.

Table 5. Pearson's correlation between von Willebrand Ag and haematological parameters

Parameters	R-value	P-value
MCV(fl)	0.021	0.871
MCH(pg)	0.244	0.054
MCHC(g/l)	0.345**	0.006
PCV(%)	-0.592**	0.000
HB(g/l)	-0.583**	0.000
WBCx10$^{9/l}$	0.541**	0.000
PLATELETx10$^{9/l}$	0.303*	0.016
ANC	0.215	0.090
ALC	0.144	0.259
RBCx10$^{12/l}$	-0.234	0.065

**Statistically Significant at P <0.05. RBC: Red Blood Cell MCH: Mean cell haemoglobin MCV: Mean cell volume MCHC: Mean cell haemoglobin concentration ANC: Absolute neutrophil count ALC: Absolute lymphocyte count PCV: Packed cell volume*

Furthermore, the mean value for platelet level was observed to be significantly higher in sickle cell patients (crisis (370.64±128.78) and steady state (333.04±120.47)) when compare to control (221.23±91.34) which was in agreement with the result of [9]. The increase in platelet will also aid in the adhesion process. This adhesion of platelets to the adherent sickle cells and their activation will release more VWF [14].

High levels of VWF mediate the adhesion of sickled RBC to endothelial cells and contributes to vaso-occlusive episodes. These vasoocclussive crises may occur when endothelial cells are greatly stimulated to release the content of their Weibel-Palade bodies [15].

Sickle red cells, in contrast to normal red blood cells, may have receptors for VWF on their surface because they are relatively younger cells, or because of alterations in their membrane structure induced by cycles of HbS polymerization-depolymerization in conjunction with shear forces in the blood [16].

In SCD VWF antigen levels often increases in times of crises, suggesting that endothelial cell stimulation or damage occurred [17]. We therefore propose that this mechanism of endothelial cell damage will lead to the exposure of the sub endothelia structure, with eventual activation of platelets, and the formation of micro thrombi.

5. CONCLUSION

Severity of sickle cell disease increases with elevation of VWF level in crisis state than those in the steady state, this contributes to the vasoocculsive episode.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee

has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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