Original Article

Determination of the Level of Von Willebrand Factor and ADAMTS13 in Sickle Cell Anaemia Patients in Steady State

Akaba Kingsley, Essien Ofonime, Uboh Enobong[1], Ibanga Iquo

Department of Haematology and Blood Transfusion, University of Calabar Teaching Hospital, Calabar, Cross River State, [1]Department of Haematology and Blood Transfusion, University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria

Received: 02-Apr-2022;
Revision: 04-Jul-2022;
Accepted: 22-Jul-2022;
Published: 22-Sep-2022

Abstract

Background: Sickle cell anaemia (SCA) is a hypercoagulable state with alteration in the haemostatic parameters and may contribute to thrombosis in a steady state. The levels of von Willebrand factor (VWF) and ADAMTS13 antigen in the steady state as markers of thrombotic risk have not been fully investigated in our environment. **Aim:** Evaluation of the level of VWF and ADAMTS13 as a marker of thrombotic risk in SCA subjects in the steady state at UCTH, Calabar. **Subjects and Methods:** This is a comparative study carried out at UCTH, Calabar. Sixty SCA patients were evaluated in the steady state with apparently healthy controls matched for age and sex VWF.Ag, and ADAMTS13.Ag was evaluated using Assay Pro enzyme-linked immunosorbent assay kit. Data was analysed with IBM Statistical Package for Social Sciences Chicago Software version 26. **Results:** The median age of SCA and controls were 23 years and 20 years, respectively (P = 0.962). There were no significant differences in their sex distribution (P = 0.063). The mean ± standard deviation (SD) of VWF in the steady state and control were 1.34 ± 0.23 IU/mL and 1.41 ± 0.23 IU/mL with no significant difference in their mean (P = 0.864). The mean ± SD of ADAMTS13 in the steady state and control were 0.44 ± 0.06 µg/L and 0.62 ± 0.10 µg/L, respectively, with no significant difference in their mean (P = 0.171). **Conclusion:** There was no significant difference between VWF.Ag, ADAMTS13, and VWF.Ag: ADAMTS13 antigen ratio in SCA in the steady state and control. There is a need for further research to determine their functionality.

Keywords: *ADAMTS13, sickle cell anaemia, steady state, Von Willebrand factor antigen*

Introduction

Sickle cell disease (SCD) is a chronic autosomal recessive heterogeneous group of disorders, with a highly variable clinical spectrum.[1] The most prevalent form is sickle cell anaemia (SCA) Hemoglobin SS (HbSS), which is due to the inheritance of the sickle cell gene in a homozygous state. Other forms include the compound heterozygous forms in which the sickle beta-globin gene is co-inherited with another abnormal haemoglobin gene such as HbC in Hemoglobin SC (HbSC), β thalassaemia in HbSβ thalassaemia amongst others.[1,2] SCD is the most common genetic disorder in Sub-Saharan Africa. Nigeria bears a high disease burden with an estimated prevalence of 1–3% of its population being affected by the disease.[3] The disease burden in Nigeria (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) differs slightly from one geographical region to another. Akaba *et al*.[3] reported the prevalence rate of SCD to be 2.28% in Calabar, while Inyama *et al*.[4] reported a prevalence of 3.7% in a multi-centre study in Nigeria. SCD is a known hypercoagulable and pro-thrombotic disease state, in which there is a significant alteration of the haemostatic system characterised by increased levels

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_110_21

Address for correspondence: Dr. Akaba Kingsley, Department of Haematology and Blood Transfusion, University of Calabar Teaching Hospital, Nigeria. E-mail: akaba_kingsley@yahoo.com

How to cite this article: Kingsley A, Ofonime E, Enobong U, Iquo I. Determination of the level of von willebrand factor and ADAMTS13 in sickle cell anaemia patients in steady state. Niger J Clin Pract 2022;25:1393-7.

of von Willebrand factor (VWF), increased expression of P-selectins by platelets, increased platelet adhesion and aggregation, increased activation of coagulation proteins with attendant increase in thrombin as well as thrombin-antithrombin (TAT) generation, depletion of ADAMTS13, and protein C and S with impaired fibrinolytic activity.[5] All of these predispose to an increased risk of thrombosis. Hypercoagulability has also been reported to contribute to the pathogenesis of sickle cell crises and the acute complications in SCD including vaso-occlusive crisis.[5] ADAMTS13 and VWF antigen and their ratio are sensitive markers of intra-vascular coagulation and also have been described in connection with thromboembolic complications.[6] Since SCD is characterised by chronic haemolysis even in a steady state, free haemoglobin and myeloperoxidase activity released from cells during haemolytic episodes in SCD have been reported to impair the activity of ADAMTS13.[6] The implication of this is that the proteolytic action of this enzyme on high molecular weight VWF will be impaired predisposing to increased ultralarge von Willebrand factor (ULVWF) and hence increased procoagulant state. The latter may predispose to hypercoagulable events. Several observations have been reported on ADAMTS13 and VWF antigen level in SCD. Some studies have reported reduced a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity and ADAMTS13: VWF antigen ratio in sickle cell patients compared to normal controls.[7-9] Schnog *et al*.[10] reported that the ADAMTS13 level is normal in all states of SCA but its activity is reduced, and similarly, other studies have demonstrated elevated levels of ULVWF and VWF in an adult patient. There is inconsistency and paucity of studies on VWF antigen and ADAMTS13 antigen level in SCD subjects. Hence, this study seeks to evaluate the level of VWF antigen and ADAMTS13 activity in SCD patients in the steady state in Calabar.

The steady state is defined as the crisis-free period from at least 3 weeks since the last clinical event and 3 months from the last blood transfusion to at least 1 week before the start of a new clinical event.[11]

Research question

Is the level of VWF and ADAMTS13 antigen altered in the steady state amongst SCD subjects in Nigeria?

Null hypothesis

VWF and ADAMTS13 antigen level are not significantly altered in SCD in the steady state.

Alternate hypothesis

VWF and ADAMTS13 antigen level are significantly altered in SCD in the steady state.

Justification of study

SCD is a cause of significant disease burden in our environment; the disease is associated with the increase in sudden morbidity and mortality. Thromboembolic complication seem to be one of the major causes of mortality, therefore, understanding clearly that it is characterised by hypercoagulability; therefore, determination of VWF and ADAMTS13 antigen level in the steady state as a marker for thrombotic risk may provide a clue that will facilitate the design of the therapeutic intervention and thromboprophylaxis in the steady state.

Methodology

Study design

This is a comparative study.

Study area

This study was carried out in the haematology 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 (Ethics approval was received on 07-September, 2021).

The haematology unit has a workforce comprised of consultants, resident doctors, trained nurses, and allied staff. It receives referrals from neighbouring states such as Akwa Ibom, Bayelsa, Ebonyi, and Rivers. It also offers services such as automated red cell exchange for SCD patients and apheresis of blood products based on demand.

Subjects and sampling technique

The study population comprised two groups:

Group I: These were SCA (HbSS) patients of ages 16 years and above who gave their consent to participate. These patients were evaluated in the steady state. They were recruited consecutively from the outpatient clinic.

Group II: Healthy Hemoglobin AA (HbAA) individuals (confirmed by haemoglobin electrophoresis) matched for age and sex with subjects in group I were recruited. They were recruited consecutively from the blood donor clinic, hospital staff, and students.

Sample size calculation

The sample size was calculated using the formula for comparative study:[12]

$$N = (U+V)^2\ (\sigma_1^2+\sigma_2^2)\ /\ ((\mu_1-\mu_2)^2$$

where n = Minimum sample size,

V = Standard normal deviate at 5% significance (1.96)

U = Standard normal variate power at 80% (0.84)

Using mean and SD values of VWF in the SCD population and controls based on the study by Columnbatti *et al.*[8]

σ_1 = SD of VWF in HbSS—12.82%

σ_2 = SD of VWF in normal controls—6.2%

μ_1 = Population mean of VWF in HbSS—79.42%

μ_2 = Population mean of VWF in normal subjects—85.0%

$$n = (1.96 + 0.84)^2 \ (12.82^2 + 6.2^2) / (79.42 - 85.0)^2$$

$$n = (2.8)^2 \ (164.35 + 38.44)/(-5.58)$$

$$n = (7.84)(202.79)/33.64$$

$$n = 1589.87/33.64$$

$$n = 47.3$$

The working sample size for this research was 60 for each study group with 50 controls which allows for the accuracy and significance of the study.

- Sixty (60) subjects in the steady state.
- Fifty (50) control subjects.

Recruitment and eligibility

Inclusion criteria

1. Patients with SCD are in the steady state.

Exclusion Criteria

The following were excluded:

1. Participants with liver disease (confirmed by deranged liver function test) or proven viral hepatitis as they could have increased levels of VWF.
2. Critically ill patients with features of sepsis as they could have increased levels of VWF.
3. Subjects on hydroxyurea therapy to eliminate any confounder due to disease modification caused by the drug.
4. Subjects who were pregnant or receiving oral contraceptive therapy as these may increase VWF levels.
5. Patients on anticoagulants. Anticoagulants may alter the procoagulant characteristic of the disease and thus create a confounding effect.
6. Subjects with acute infections, inflammation, and trauma as these can increase VWF levels.
7. Human immunodeficiency virus (HIV) positive individuals. HIV infection is a hypercoagulable state associated with increased VWF production. Thus it is a potential confounder.

Data collection

Biodata and clinical information of study participants including the history of SCD were obtained directly from the subjects and their clinical records (case notes) using a questionnaire.

Blood sampling and storage

For each subject, the venepuncture site was carefully cleaned with 70% alcohol, and venous blood was collected from the ante-cubital vein with minimal stasis; under aseptic conditions.

The whole blood was dispensed into commercially prepared ethylene di-amine tetra-acetic acid bottles for basic haematological parameters, and the remaining were dispensed into commercial lithium heparin bottles for liver function tests (LFT). These samples were mixed gently but thoroughly to prevent cell lysis and ensure anticoagulation. The heparinised samples were centrifuged at 2,000*g* for 10 min to obtain the plasma for LFT. The remaining 4.5 mL of whole blood for VWF and ADAMTS13 analysis was dispensed into a plain sample bottle that contains 0.5 mL of 0.109 M sodium citrate (3.2%). This is to obtain blood: citrate ratio of 9:1. These were immediately centrifuged at 2,000 rpm for 15 min, after which the plasma supernatant was centrifuged again for 15 min at 4,000 rpm. Thereafter, the residue after removing the plasma (supernatant) was centrifuged at 4,000g for 15 min and stored at -80oC until further analysis.

Statistical analysis

The data were collected and analysed using the Statistical Package for Social Sciences version 26. Results were expressed as mean, SD, and frequencies as appropriate. Analysis of variance was used to test the differences in the mean of quantitative variables, while Pearson's and Spearman's correlation coefficients were used to correlate VWF and ADAMTS13 with numerical and categorical variables, respectively. *P* value was set at 0.05.

Result

The median age of SCA and controls were 23 years and 20 years, respectively, (P = 0.962). There were 23 (38.3%) males in the SCA group and 21 (42.0%) females in the controls. There were no significant

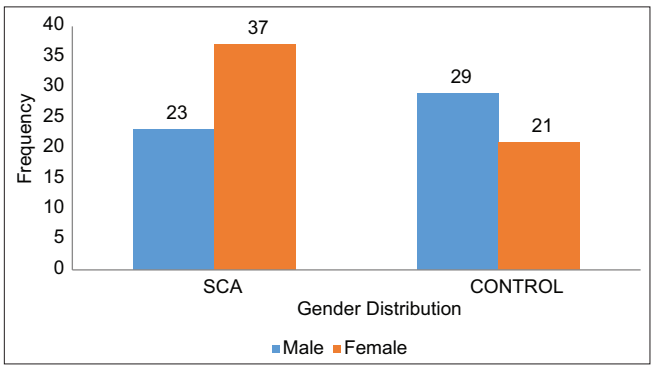

Figure 1: Showing the gender distribution of the participant

Table 1: Demographic Data

	SCA *n*=60 (%)	Controls *n*=50 (%)	*P*
Age group (years)			
<20	10 (16.7)	5 (10.0)	
21-25	23 (38.3)	20 (40.0)	
26-30	14 (23.3)	11 (22.0)	0.962
31-35	8 (13.3)	11 (22.0)	
36-40	4 (6.7)	3 (6.0)	
>40	1 (1.7)	0 (0.0)	
Marital status			
Single	52 (86.7)		
Married	8 (13.3)		
Educational level			
Secondary	16 (26.7)		
Tertiary	44 (73.3)		

Table 2: Mean and Ranges of ADAMTS13

	SCA steady state		Controls		*P*
	Mean±SD	Range	Mean±SD	Range	
VWF (IU/mL)	1.34±0.23	0.04-8.96	1.41±0.23	0.02-5.89	0.864
ADAMTS13 (µg/mL)	0.44±0.06	0.01-1.88	0.62±0.10	0.01-2.72	0.171
ADAMTSI3: VWF (IU/µg)	0.54±0.07	0.02-3.30	0.67±0.02	0.18-0.72	0.355

differences in their sex distribution (P = 0.063). The mean ± SD of VWF in the steady state and control were 1.34 ± 0.23 IU/mL and 1.41 ± 0.23 IU/mL with no significant difference in their mean (P = 0.864). The mean ± SD of ADAMTS13 in the steady state and control were 0.44 ± 0.06 µg/L and 0.62 ± 0.10 µg/L, respectively, with no significant difference in their mean (P = 0.171).

Discussion

SCA is a severe form of haemolytic anaemia characterised by chronic haemolysis and is associated with increased thrombotic risk.[13] SCA is a hypercoagulable state which is characterised by alteration of the haemostatic system thereby increasing the level of VWF, P-Selectin, thrombin generation as well as TAT generation with depletion of natural anti-coagulant, ADAMTS13, and impaired fibrinolytic activity. All these predispose to an increased risk of thrombosis.[14] Elevated VWF levels in SCD have been attributed to increased secretion and impaired processing by its cleaving protease ADAMTS13.

The index study shows a female preponderance with a male: female ratio of 1:1.6 which matches with age and sex of the control [Figure 1]. Also, the age range of our participants was between 16 years and 42 years, with a median age of 23 years [Table 1]. All of these can be attributed to the health-seeking behaviour and low pain threshold of females. Also, the age range did not differ significantly between the steady state and control. However, the life expectancy was <40 years compare to the life expectancy of SCA in developed climes.[14] This can be attributed to poverty, malaria, increase in the prevalence of communicable disease, illiteracy, and lack of policy on routine newborn screening and limited access to health care and disease-modifying drugs. The participants were mostly single with tertiary education, and this can be attributed to the social stigmatisation of individuals with the SS gene and the proximity of the hospital to a tertiary institution, respectively.

In the index study, the level of VWF was significantly higher in control than in the steady state, this can be attributed to the continuous effect of ADAMTS13 on VWF [Table 2], due to the stimulatory effect of chronic inflammation. This was similar to the finding of Schong and Sin *et al*.,[10,15] respectively. However, this is at variance with the study by Ladeira *et al*.[16] which reported an increase level of VWF in the steady state. Julien *et al*.[17] in France reported an elevated level of VWF. Furthermore, another study conducted by Olayanju[18] in Nigeria reported no difference. The inconsistency and variations in these studies might be due to the difference in study design, their study comprises a heterogeneous group of SCD.

The level of ADAMTS13 was said to be lower in the steady state than in the control, and this could be biologically plausible as a compensatory effect of continuous production of its substrate VWF, as a result of background inflammation in SCA. This was similar to the findings by Schong *et al*.[9] and attributed to the continuous consumption of ADAMTS13 even at the steady state, since SCA is characterised by chronic inflammation, also, due to the hypercoagulable state of SCA patients there is increased production of thrombin, plasmin, and other pro-inflammatory cytokines which inactivate ADAMTS13. Furthermore, SCA is also characterised by chronic haemolysis with continuous release of free haemoglobin which directly inhibits ADAMTS13.

Conclusion

This study has shown that there is no change in the level of VWF.Ag and ADAMTS13 in the steady state; therefore, they cannot suffice as markers for thrombotic risk in SCA.

Recommendation

There is a need to further investigate the functionality of ADAMTS13 and VWF.Ag in SCA in the steady state in our environment. Similarly, this study can be replicated in other SCD phenotypes to evaluate the impact of other sickle cell phenotypes on VWF and ADAMTS13 levels.

Authors contribution

Akaba Kingsley, Akaba Edakabasi, and Patrick Osho wrote the research protocol and first draft of the manuscript, and Essien Ofonime and Uboh Enobong managed the literature, while Iquo Ibanga edited the manuscript. All the authors were involved in proofreading the final draft.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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