Original Article

Evaluation of Von Willebrand Factor and other Coagulation Homeostasis Profile of Patients with Sickle Cell Anaemia attending a Tertiary Hospital at Enugu, Nigeria

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ABSTRACT

Background: Sickle cell anaemia (SCA)is a hypercoagulable state. However, there is a paucity of data that demonstrated these features in Nigerian Sickle cell anaemic patients.

Objective: This prospective study aimed to determine the plasma level of vWF, APTT, PT, platelet count and haematological parameters of SCA patients in steady state in comparison with apparently healthy individuals attending the University of Nigeria Teaching Hospital, Enugu, Nigeria.

Materials and Methods: A total of 85 participants were enrolled in this study. This comprised of 35 homozygous SCA patients in steady-state as test subjects (HbSS) and 50 apparently healthy individuals of Hb AA as the control group. Blood samples were collected from all participants; both PT and APTT were analysed using Quick and kaolin methods, respectively; vWF was assayed using Enzyme-Linked Immunosorbent Assay (ELISA). Haematological parameters were analysed through the use of an automated haematology analyser.

Results: There was significant elevation of mean±SD of vWF, APTT and platelet count among SCA patients (52.53 ± 1.45 iu/dl), (48.40 ± 4.99 s) and (287.68 ± 21.43 x10^9/l) when compared with the mean value in control group (40.16 ±1.50 iu/dl), (33.88 ± 1.34 s) and (219.67 ± 13.9 x10^9/l) ($p< 0.05$). However, there were significant decrease in PCV, haemoglobin concentration and red blood cell count among SCA patients, (24.00 ± 0.82 l/l), (7.89 ± 0.24 g/l) and (2.97 ± 0.8 x 10^{10}/l) when compared with control group (40.95 ± 0.73 l/l), (12.44 ± 0.26 g/l) and (4.81 ± 0.11 x ^{10}/l) ($p< 0.05$) respectively. In addition, significant elevation of mean ±SD MCH and MCHC with mean values of (26.63±0.93 pg) and (33.28 ± 0.52 g/l) among subjects when compared with control groups (25.68± 0.47 pg), (30.30 ± 0.18 g/l) ($p<0.005$). However, there were no significant differences in WBC, PT and MCV in both study groups.

Conclusion: Findings from this study demonstrated prolonged coagulation profile, thrombocytosis and leucocytosis among SCA patients in steady state.

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INTRODUCTION

Sickle cell anaemia is an inherited autosomal recessive disorder of the beta-globin gene characterised by clinical manifestations such as hemolytic anaemia and an episode of vascular occlusion.[1,2] Nevertheless, the clinical course of patients with sickle cell anaemia, a Mendelian trait, is highly variable. Haemoglobin F (HbF) concentration and the presence of alpha thalassemia are established modulators of the disease, but cannot account for all of its clinical heterogeneity.[3] As can be expected, individual differences in clinical presentations is also believed to be dependent on the environment, the extent of sickling, vascular endothelium, platelets, leucocytes, and the plasma proteins.[4]

The pathophysiology of sickle cell anaemia was established upon single base substitution, where adenine substitutes thymine, in the second position of codon 6, in the short arm of chromosome 11. Consequently, valine is synthesised in place of Glutamic acid in the globin chain (Glu6Val).[5] This gives rise to mutant haemoglobin tetramer which results in cellular stress and deformation. Recurrent deformation by polymer formation under low oxygen tension, give rise to sickle shaped red blood cells, which ultimately lead to sickle cell crises.[6,7,8]

High affinity of adherence of sickled red blood to vascular endothelium is now suggested as unique characteristics of these blood cells.[9,10] Nevertheless, pro-inflammatory environment was noted in the vasculature as a result of activated vascular endothelium, and Placenta growth factors (PLGF) synthesised from erythropoietin response to sickle-shaped red blood cell turn over.[11,12]

Von Willebrand factor (vWF) is a glycoprotein best known for its critical role in haemostasis and its carrier function for coagulation factor VIII.[13,14] Dysfunction of vWF may be associated with a severe bleeding tendency known as Von Willebrand disease (vWD).[15] Von Willebrand factor (vWF) is synthesised in the endothelial cells (ECs), and megakaryocyte as vWF propeptide stored in Weibel Palade bodies and platelet alpha granules.[16]

It is believed that ultra large vWF polymers may be synthesised from the vascular endothelium following activation and inflammation of the vascular endothelium, polymeric vWF is in turn cleaved by a metalloprotease and ADAMTS-13 (vWF cleaving factor) into smaller multimeric and a monomeric form for homeostasis.[17] Persistent ultra-large VWF (ULvWF) molecule in circulation may be capable of spontaneously binding to platelet on sickled erythrocyte to promote cell adhesion to vascular endothelium.[18]

Recently, it was shown that the binding of extracellular haemoglobin (EcHb) to the A2 domain of vWF significantly block vWF cleavage by the metalloprotease and ADAMTS -13 in vitro. Therefore, it is believed that vWF multimer maintains their ultra-large structure in plasma if extracellular haemoglobin (EcHb) prevents their cleavage, but relevant information in respect to plasma level of vWF and extracellular haemoglobin effect on the fibrinolytic system in sickle cell anaemia is lacking.[19,20] The ultra-large vWF multimers are also known to mediate leukocyte rolling and adhesion to inflamed endothelium. Thus, vWF multimers contain all of the determinants necessary for red blood cells, platelets and leukocytes to stably adhere to the vascular endothelium.[20]

A study revealed significant disorders of white blood cells (WBC), red blood cells (RBC), platelets and prolonged coagulation parameters coupled with continuous activation and dysfunction of the vascular endothelium with hyperactivity of vWF in sickle cell anaemia, but detailed studies of the plasma levels of these vital parameters are lacking.[20] Therefore, there is a need to monitor plasma level of vWF, full blood count (FBC) and coagulation parameters (APTT and PT), to quantitate and compare their values with severity and variations in clinical presentations among patients with sickle cell anaemia. Hence, the aim of this prospective study was to determine the plasma level of vWF, APTT,

PT, platelet count and haematological parameters of SCA patients in steady state in comparison with apparently healthy individuals attending the University of Nigeria Teaching Hospital, Enugu, Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out in the sickle cell clinic of the University of Nigeria Teaching Hospital (UNTH) Ituku Ozalla Enugu State. UNTH is located 21 kilometres from Enugu Capital City along Enugu – Port Harcourt Express Way.

Ethical Consideration

Ethical approval was duly obtained from the ethical committee of the University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu State (UNTH/CSA/329/VOL.5). Written informed consent was also obtained from each subject. This study was conducted in accordance with the standards of Helsinki declaration (as revised in 2003). All data were analysed anonymously throughout the study.

Subjects Selection Criteria

Each presenting SCA patient was examined by the attending physician, coupled with laboratory evidence to ascertain the state of the disease. Patients in steady state were defined as those without any of the following clinical conditions 4 weeks prior to or at enrollment. These included painful bone crisis, severe anaemia, laboratory diagnosis of bacteremia, acute chest syndrome, aplastic anaemia, splenic sequestration, systemic inflammatory response syndrome (SIRS) and behaviors such as anxiety and hallucination. Patients with any one or a combination of these clinical and behavioral presentations were considered to be in an unsteady state or crisis. The following was the selection criteria:

a. Confirmed HbSS patients in steady state and not on hydoxyurea
b. Healthy subjects tested and confirmed positive for HbAA as the control group

Study Design

This was a case-control study that involved a total of eighty-five (85) participants were recruited for the study. Thirty-five (35) were sickle cell anaemic patients with haemoglobin phenotype (Hb SS) in steady-state of varying ages, sex, and socio-economic status, while fifty (50) apparent healthy subjects with haemoglobin phenotype (Hb AA) were used as the control group, which were all confirmed by haemoglobin electrophoresis.

Sample Collection and Processing

Five (5) ml of venous blood was collected from each participant with a sterile 10mls syringe and then 2.5ml was immediately delivered into a sodium citrate tube containing 250µl of anticoagulant, spin at 1500g for 10 minutes to obtain platelet-poor plasma. Coagulation parameters; Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and von Willebrand Factor (vWF) were all estimated from the plasma. The remaining 2.5ml of blood was delivered into a container containing di-potassium Ethylene Diamine Tetraacetic Acid (K_2EDTA) this was used to obtain blood for Full Blood Count (FBC) by Hematology autoanalyser (Mindray, 2300, China).

Laboratory Analytical Protocol

Von Willebrand Factor (vWF)

Plasma Von Willebrand Factor concentration was determined using human highly sensitive vWF Sandwich ELISA kit (AssayMax ™, AssayPro ®, Missouri, USA). The assay was done, and results interpreted as described by the kit's manufacture instructions.

Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and Hemoglobin electrophoresis

APTT, and PT were analysed using Quick and kaolin method respectively, while haemoglobin

electrophoresis was analysed using standard cellulose acetate paper technique as described by Dacie and Lewis [21].

Full Blood Counts

Subjects' blood counts were done using an automated haematology analyser by Mindray 2300 (Shenzhen, China). Daily quality control was done on the equipment and tests to ensure the accuracy of the test results.

Statistical Analysis

Statistical analysis was performed with Graph Pad Prism (California, USA) version 6.0. Student's t-test was used to determine significant difference in the mean values of all continuous variables between the groups. P values <0.05 were considered to be significant in all comparisons.

RESULTS

There was significant elevation of mean±SD plasma vWF, APTT and platelet count among SCA patients (52.53 ± 1.45 iu/dl), (48.40 ± 4.99 s) and (287.68 ± 21.43 x10^9/l) when compared with the mean value in control group (40.16 ±1.50 iu/dl), (33.88 ± 1.34 s) and (219.67 ± 13.9 x10^9/l) ($p< 0.05$) (Table 1). However, there were significant decrease in PCV, haemoglobin concentration and red blood cell count among SCA patients, (24.00 ± 0.82 l/l), (7.89 ± 0.24 g/l) and (2.97 ± 0.8 x 10^{10}/l) when compared with control group (40.95 ± 0.73 l/l), (12.44 ± 0.26 g/l) and (4.81 ± 0.11 x ^{10}/l) ($p< 0.05$) respectively. In addition, Furthermore, significant elevation of mean ±SD MCH and MCHC with mean values of (26.63±0.93 pg) and (33.28 ± 0.52 g/l) among subjects when compared with control groups (25.68± 0.47 pg), (30.30 ± 0.18 g/l) ($p<0.005$). However, there were no significant differences in WBC, PT and MCV in both study groups (Table 2).

Table 1: Comparison of Coagulation Variables of Sickle Cell Anaemia Subjects and Control Group

Variable	SCA (Hb SS) (N=35)	Control Group (Hb AA) (N=50)	*p* value
Vwf (Iu/Dl)	52.53 ±45	40.16 ±1.50	0.0001
APTT(Secs)	48.40 ±4.99	33.88 ±134	0.016
PT(Secs)	33.70 ±9.13	18.56 ±0.68	0.15
PLTS (X10^9/L)	287.68 ±21.43	219.67 ±13.19	0.027

Key:
Values were expressed as mean ± SEM, P-values ≤ 0.05 were considered statistically significant, vWF (iu/dl) = von Willebrand Factor, APTT(s) = activated partial thromboplastin time, PT(s) = Prothrombin time

Table 2: Comparison of Full Blood Counts of Sickle Cell Anaemia Subjects and Control Group

Variable	SCA (Hb SS) (n=35)	Control Group(Hb AA) (n=50)	*p* value
PCV(l/l)	24.00 ±0.82	40.95 ±0.73	0.0001
Hb(g/l)	7.89 ±0.24	12.44 ±0.26	0.001
RBC(x10^{12}/l)	2.97 ±0.1	4.81 ±0.11	0.025
MCV(fl)	88.30 ±1.98	84.81 ±1.25	0.216
MCH(pg)	29.64 ±0.93	25.68 ±0.47	0.003
MCHC(g/l)	33.28 ±0.52	30.30 ±0.18	0.0026
WBC(x10^9/l)	14.78 ±9.09	10.33 ±0.81	0.523

Key: Values were expressed as mean ± SEM, P-values ≤ 0.05 were considered statistically significant, PCV (l/l) = Pack Cell Volume, Hb (g/l) = Haemoglobin, RBC (x10^{12}/l) =Red Blood Cell, MCV (fl) = Mean Corpuscular Volume, MCH (pg) = Mean Cell Haemoglobin, MCHC (g/l) = Mean Cell Haemoglobin concentration, WBC (x10^9/l) = White Blood Cell.

DISCUSSION

Sickle cell disease (SCD) is one of the commonly reported genetic structural disorders of haemoglobin affecting around 30 million people worldwide. It results from a single point mutation at position 6 of β-globin chain, leading to the substitution of glutamic acid by valine, resulting in abnormal haemoglobin (HbS). The homozygous HbS is responsible for sickle cell anaemia in addition to some other documented complications.[22] This case-control study was carried out among sickle cell anaemic patients attending sickle cell clinic, University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla, Enugu State, South Eastern, Nigeria. It consists of 35 Sickle cell anaemia (Hb SS) patients across all ages and 50 apparently healthy (Hb AA) control. The study evaluates some hemostatic and haematological parameters of sickle cell anaemic patients and control group.

In this study, statistically significant higher levels of von Willebrand Factor was observed among sickle cell anaemic patients compared to controls. It agreed with a study carried out among adult's sickle cell patients and age and sex-matched controls at Ilishan-Remo, Ogun State, Nigeria, by Dickson *et al.*[23] This may be related to hypercoagulability activity of sickle cell anaemic state. Likewise, Colombatti *et al* reported similar higher levels of von Willebrand factor antigen (vWF: Ag) among children with sickle cell disease and age and blood group matched controls Italy.[24]

The prolongation of PT and aPTT in patients with SCA could be due to hepatocyte dysfunction and hepatic injury that significantly reduce the biosynthesis of platelet factors, synthesis of abnormal clotting factors or exhaustion of coagulation factors. Similar findings were reported by Saud *et al.*[22] Additionally, sickle cell anaemic patients had prolonged Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and higher platelet counts which is in agreement with a report in a previous study among children (2-16 years) in steady-state and controls at Basra, Iraq.[22] It was supported by another study carried out among sickle cell children in steady and in crises aged 6 months to 18 years when compared with healthy controls in the same University of Nigeria Teaching Hospital, Enugu, South East, Nigeria.[25] Our findings are in keeping with a study conducted in Ghana, which reported prolonged APTT, PT and higher mean platelet count among adults with sickle cell disease compared to normal controls.[26] However, in a different study shorter APTT and PT were reported among adults' sickle anaemia in Zaria, Nigeria, but higher mean platelet count in the same study and in a different similar one.[24,27] All these variations in the mean values of haemostatic parameters may be attributed to impaired liver function and auto-splenectomy/ or non-splenic function associated with sickle cell anaemia.

Nonetheless, in sickle cell anaemic patients; there was lower mean Packed Cell Volume (PCV) haemoglobin (Hb) and red blood cells (RBC) count, but higher mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and white blood cell (WBC) count. Lower mean haemoglobin, higher mean cell volume (MCV) and white blood cells (WBC) was reported in a different study, this agreed with the findings in our study.[24] Dickson *et al* reported a slightly lower mean cell volume (MCV), lower mean PCV and haemoglobin; but higher mean platelet count, RBC and WBC.[23] Saud *et al* reported lower mean haemoglobin and higher mean WBC among sickle cell patients compared to controls.[22] Antwi-Baffour *et al* documented lower haemoglobin levels among the higher percentage of sickle cell anaemic patients compared to controls.[26] Despite the paucity of recent data on the haematological picture of patients with SCA, the lower mean haematological parameters reported among patients with SCA in this study may be due bone marrow aplasia and continues destruction by

reticuloendothelial system as a result of haemoglobinopathies. Hence, this study was limited by the inability to determine reticulocyte counts and other platelet factors such as fibrinogen. This will have provided better inference for the full haemostasis and haematological status of our study participants.

Although, patients in this study were not on hydoxyurea, it has been shown to positively modify SCD pathogenesis and its has been utilized in the treatment of SCD complications.[28] Essentially, hydroxyurea has significantly reducd SCD crisis, chronic organ damage and stroke.[28] In a recent study, hydroxyurea was reported to be effective in reducing the incidence of vaso-occlusive crises, acute chest syndrome, need for blood transfusions and prolonged hospital stay in patients with SCD.[28]

CONCLUSION

Findings from this study demonstrated prolonged coagulation profile, thrombocytosis and leukocytosis among SCA patients in steady state. This demonstrated the need for thorough routine coagulometric evaluation of SCA patients to promptly detect any haemostasis disorder for accurate management and better their life span.

Conflict of Interest

None declared

REFERENCES

1. Scheiermann, C., Kunisaki, Y. and Lucas, D. Adrenergic nerves govern circadian leukocytes recruitment to tissues. *Immunity*. 37(2) 2012: 290-301.
2. Roberts, S., Kenneth, A. and Henry, M. Measurement of coagulation Factors in sickle cell anaemia, 4th edition, London McGraw-hill Medical Publishers. 2005:329-30.
3. Ataga, KI, Moore CG. Hillary CA. Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. *Haematologica*. 2008:20-24.
4. Schaer DJ. Beuhler PW. Alayash AL. Belcher JD. Haemolysis And free haemoglobin revisited: exploring haemoglobin and hemin Scavengers as a novel class of therapeutic proteins. *Blood*. 121(8) 2013:1276-676.
5. Dhamet N. Ensing G. Minniti C. Prospective echocardiography assessment of pulmonary hypertension and its potential aetiologies in children with sickle cell disease. *American Journal Cardiology.* 2009:104: 713-20.
6. McGann, PT. Ware RE. Hydroxyurea for sickle cell anaemia what have we learned and what questions still remain< *Curr Opin Heamatol* 2011;. 18 (3): 158-175.
7. Gutsaeva, DR. Parker JB. Yerigenanlly SD. Inhibition of cell adhesion by anti-P-selectin aptamer; a new potential therapeutic agent for sickle cell disease. *Blood.* 117(20) 2011:727-735.
8. Scheinman, JI. Sickle cell disease and the kidney. National Clinical Practise. *Nephrol*. 5. 2009:78-88.
9. Wun T. Soulieres D. Frelinger AL. A double-blind, randomised, Multicentric phase 2 study of prasugrel versus placebo in adult patients With sickle cell disease. *J Haematol Oncol*. 2013. 6:17.
10. Cheng H. Yan R. Li S. Yaun H. Lui J. Raun C. Dai K. Shear-Induced interaction of platelets with von Willebrand factor results in glycol-protein shedding. *Am J Physiol*. 2009. 297:2128-2135.
11. Machado, R. F. and Gladwin, M. T. Pulmonary complications of Haematological disease. Textbook of respiratory Medicine. 5th edition Philadelphia. 178-210.
12. Person DA. Hematopoietic stem cell gene transfer for the treatment of Haemoglobin disorders. *Am J of Haematol* 2009: 690-97.

13. Frelinger A L. Jakuboski JA. Brooks JK. Platelets Activation and Inhibition in sickle cell disease (PAINS) Study. *Platelets.* 2013. Doi: 10 3109/09537104.
14. Western M. Pizzey A. Hirschman J. Microvesicles in haemoglobinopaties offer insights into mechanisms or hypercoagulability, haemolysis and the effects of therapy. *Br J Haematol* 2008. 142:126-35.
15. Hidalgo A. Chang J. Jang JE. Peired AJ. Ciang EY. Frente P S. Heteretypic interactions enabled by polarised neutrophil microdomiamediate thromboinflammatory injury. *Nat Med J* 2009. 15(4):384-391.
16. Smith ER. McClain CD. Heeney M. Scott RM. Pail synangiosis patients with mayoama syndrome and sickle cell anaemia perioperative management and surgical outcome. *Neurosurg Focus.* 2009. 26:1-8.
17. Boonefoy A. Romijn RA. Vandervoort, P. A. H. Van Rompaey I. Vermeer J. Hoylaerts MF. von Willebrand factor A1 domain can adequately substitute for A3 domain in recruitment of flowing platelets to collagen. *J Thromb Haemost* 2006. 4: 2151-2161.
18 Zhang X. Halverson K. Zhang CZ. Mechanoenzymatic cleavage of the ultra large vascular protein von Willebrand factors. *Sci Haem* 2009:1330-4.
19. Zhou Z. Han H. Crush MA. Haemoglobin regulation of von Willebrand factor proteolysis by ADAMTS 13, a mechanism associated with sickle cell disease. *Thront Hermost* 2009. 101:1070-7.
20. Jang JE. Hidalgo A. Frenette PS. Intravenous Immunoglobins modulate neutrophil activation and vascular injury through Fegamma RIII and SHP-1. *Circulatory Res*. 2012. 110(8): 1057-3748.
21. Dacie JV. Lewis ML. Practical haematology, 11th Edition, Elsevier Churchill Livingstone, London. 2012. 36-49.
22. Saud WH, Hassan MK, Al-Salait SK. Coagulation activation in patients with sickle cell disease in Basra, Iraq. *J Appl Hematol* 2017;8:54-60.
23. Dickson OAO, Ezigbo ED, Erukpeme A, Okeke C. Evaluation of von Willebrand Factor Levels in Sickle Cell Patients Attending Babcock University Teaching Hospital. *AHRJ* 2018; 1(2): 1-6.
24. Colombatti R, De Bon E, Bertomoro A, Casonato A, Pontara E, Omenetto E, et al. Coagulation Activation in Children with Sickle Cell Disease Is Associated with Cerebral Small Vessel Vasculopathy. 2013. *PLoS ONE* 8(10): e 7 8 8 0 1 . https://doi.org/10.1371/journal.pone.0078801
25. Chinawa J M, Emodi IJ, Ikefuna A N, Ocheni S. Coagulation profile of children with sickle cell anaemia in steady state and crisis attending the university of Nigeria teaching hospital, Ituku-Ozalla, Enugu. *Niger J Clin Pract* 2013;16:159-63
26. Antwi-Baffour S, Kyeremeh R, Annison L. Severity of Anaemia Has Corresponding Effects on Coagulation Parameters of Sickle Cell Disease Patients. *Dis* 2019, *7*(4), 59; https://doi.org/10.3390/ diseases7040059
27. Kusfa IU, Aminu SM, Mamman AI, Hassan A, Babadoko AA, Mohammed MH, *et al*. Basic hemostatic parameters in adults with sickle cell anaemia at Ahmadu Bello University Teaching Hospital, Zaria Nigeria. *Sahel Med J* 2018;21:157-61.
28. Ofakunrin AOD, Oguche S, Adekola K, Okpe ES, Afolaranmi TO, Diaku-Akinwumi IN, Zoakah AI, Sagay AS. Effectiveness and Safety of Hydroxyurea in the Treatment of Sickle Cell Anaemia Children in Jos, North Central Nigeria. *J Trop Pediat* 2020, 66, 290–298 doi: 10.1093/tropej/fmz070