Original Research Article

Speed of capillary blood flow and d-dimer levels in sickle cell anaemia patients in Calabar, cross river state

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ABSTRACT

Background: The experience of painful episodes of ill health caused by sequelae of erythrocytes sickling, impaired blood flow, hypercoagulation and vaso-occlusion is one of the hallmarks of sickle cell disease. Preventing painful episodes and promoting the physical wellbeing of persons with sickle cell disease is usually a major objective in the management of the condition. The purpose of this study is to investigate capillary blood flow and D-Dimer activities in people with sickle cell anaemia.

Methods: A total of 90 subjects (27 males and 63 females) participated in the study, the study comprised of 34 HbSS patients, three were in crisis and thirty-one in steady state, 9 sickle cell carriers (HbAS) and 47 HbAA were control subjects. The haemoglobin phenotype was determined using haemoglobin electrophoresis at alkaline pH (8.6), speed of capillary blood flow was estimated using vascular Doppler ultrasonographic technique and D-Dimer was determined using ELISA method.

Results: Results obtained showed that there was a significant variation (p=0.042) in the mean levels D-Dimer among the groups. There was however no significant variations (p>0.05) in the other parameters among the groups. A post hoc analysis of mean D-Dimer in sickle cell patients, sickle cell carriers and controls showed that the sickle cell patients had significantly higher D-Dimer levels compared with controls.

Conclusions: Sickle cells patients in steady state had higher D-Dimer levels compared with controls and sickle cell patients in crisis had lower speed of capillary blood flow when compared to sickle cell patients in steady state and controls.

Keywords: Speed of capillary blood flow, D-dimer levels, Sickle cell anaemia

INTRODUCTION

During reproduction in humans, genes are transferred from the parents to their offspring. These genes code for various proteins that will be required by the child in the course of living. If any part of the parent genes changes in their usual structure, a condition which is termed mutation, any protein that they code for may also change in structure. In sickle cell diseases there is the transmission of a single point mutation in the genes that

code for haemoglobin from parents to their offspring leading to the production of mutant haemoglobin which functions abnormally in the offspring.[1,2]

The derangement of the haemoglobin function seen in the disease begins with the formation of a high-molecular-weight product by successive additions to or condensations of mutant haemoglobin which leads to diverse degrees of erythrocytic cell damage (including sickling and haemolysis) and consequently the occlusion of vasculature.[3] Besides haemolytic anaemia, erythrocytic sickling and vaso-occlusion, other secondary symptoms particularly of the complications of sickle cell disease such as acute pain episodes, acute chest syndrome, aplastic crisis, priapism, infection, liver complications, thrombosis, stroke, neurological complications, functional asplenia, avascular necrosis, leg ulcers, osteopenia and osteoporosis, pulmonary arterial hypertension, heart disease, nephropathy, gallstones/cholelithiasis may also arise.[4,5]

According to Adewoyin, the sickle cell disease ranks among the most common genetic disease globally and "its highest prevalence occurs in middle east, Mediterranean regions, southeast Asia and sub-Saharan Africa especially Nigeria.[6] A recent study of the prevalence of the condition in the city of Benin in South-Southern Nigeria by Nwogoh and colleagues suggests that one out of every fifty Nigerians has sickle cell disease.[7] Without proper management, the condition may be associated with poor health outcomes and even death.

Several research studies have suggested the monitoring of the biomarkers of fibrinolytic activities in individuals living with sickle cell disease in other to be able to predict episodes of sickle cell disease crisis for early intervention. For instance, a study of one hundred and one children, forty-one of which were known sickle cell patients in steady state showed that the level of D-Dimer, a biomarker of fibrinolytic activity are significantly elevated in children with sickle cell disease relative to children without the condition.[8] Similar studies in adults also showed that D-Dimer level are higher in sickle cell disease in a steady state and tend to be highly elevated in the presence of acute sickle cell disease crisis.[9-11]

However, the discomfort associated with blood draws as well as the use of sophisticated equipment required for laboratory determination of D-Dimer level in the blood sample makes daily monitoring of D-Dimer levels for persons with sickle cell disease unfeasible. This study therefore seeks to extend previous research works by finding the link between D-Dimer levels and blood flow speed in sickle cell disease, measured with an affordable, non-invasive, portable device (BF-560 Ultrasound Vascular Doppler) which can be used for domestic monitoring of daily blood flow speed as a surrogate of D-Dimer level for predicting episode of sickle cell disease crisis. The Doppler effect provides a unique capability for ultrasound to measure blood flow.[12] The findings of this study will show whether or not reduced capillary blood flow is associated with increased levels of D-Dimer - a fibrin degradation product (FDP) whose increase may be caused by resultant hypercoagulability state of slow capillary blood flow in sickle cell disease.

METHODS

This study was carried out between June 2018 and February 2019. A cross-sectional research design was used to investigate the speed of capillary blood flow and D-Dimer activities in people with Sickle cell disease and to find out if there are any relationships between them and episodes of vaso-occlusive crises. This one-facility, hospital-based study was conducted in University of Calabar Teaching Hospital (UCTH) which is located in the capital city (Calabar) of Cross River State, South-Southern Nigeria. Participants of this study comprised of patients seeking clinical care at the University of Calabar Teaching Hospitals outpatient (they comprised of apparently healthy Intern, Medical Laboratory Scientists, Pharmacists, Physiotherapists, Radiographers) that came for Medical examination and sickle cell anaemia patients attending clinic at Haematology Day Care Unit. The sample that was drawn for this study was comprised of 90 participants (both controls and cases). These were systematically selected among patients seeking clinical care at the University of Calabar Teaching Hospital's Outpatient and sickle cell disease clinics. Demographic data was compiled by face-to-face interview and use of a structured questionnaire. The duration of period since last vaso-occlusive crisis were calculated in days.

Sickle cell anemia patients (who were not in crises) were recruited in steady clinical conditions (that is no acute illness or crises for at least 2 weeks and no blood transfusion in the 3 months prior to recruitment).[13]

Ethical approval

Ethical clearance was sought and obtained from the Health Research Ethics Committee of University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State.

Inclusion criteria

Only patients attending the University of Calabar Teaching Hospitals outpatient and sickle cell disease clinics that voluntarily consented to participating in the study was included.

Exclusion criteria

Severely ill patients and those who have recently (within the last one week) received or are currently receiving blood transfusion or large fluid infusion related treatments as well as any medication containing anticoagulant and antiplatelet agents was excluded from the study.

Data collection methods

Both laboratory and non-laboratory methods was used to garner data on participants demographic characteristics (age and sex) and last episode of vaso-occlusive crisis, hemoglobin genotype, speed of capillary blood flow and D-Dimer levels.

Laboratory methods

Blood sample collection and preparation

Using minimal constriction and without stasis, five milliliters (5 mls) of blood was septically collected from the vein around the cubital fossa of each participants and dispensed into a clean properly labeled plain bottle for D-Dimer and haemoglobin genotype determinations. The blood sample in the labeled plain bottle was kept for 30 minutes at room temperature for it to clot. The clotted blood sample in the labeled plain bottle was then spun in a centrifuge at 3000 rpm (resolution per minutes) for 10 minutes. The serum was decanted into another clean properly labeled plain bottle for D-Dimer determination. If not analyzed immediately it was stored at -800C without repeated freeze-thrawing until the analysis was done. The red blood cells sediment was used for the haemoglobin genotype determination. The red cells was washed 3 times in low ionic strength medium. Drabkins solution was added to the washed red cell at the ratio of 2:1 and mixed thoroughly to lyse the cells. The mixture was centrifuged at 3000 rpm for 5 minutes. The supernatant obtained thereafter was the haemolysate to be used for the haemoglobin genotype determination. All samples were processed in batches of 21 samples per day. Where storage is inevitable, this was done at a temperature of 4oC and for not more than 24 hours.

The D-Dimer was done using ELISA (Enzyme Linked Immunosorbent Assay) method.

Principle

D-Dimer quantitative test using ELISA (Enzyme Linked Immunosorbent Assay) utilize microliters wells coated with an antibody with a high affinity for D-Dimer. Incubation with plasma results in the binding of any D-Dimer present. A labeled anti-body is then added and the amount of bound labeled substance is determined by a colourmetric reaction.[14]

Haemglobin phenotype was done using cellulose acetate electrophoresis method to confirm the genotype of each of the subjects.

Non-laboratory methods

The researcher asked participants about their sex and date of last episode of vaso-occlusive crisis. Their response was recorded in a register maintained for this research purpose only. Ultrasonographic technique was used to measure the speed of capillary blood flow of participants using BF-560 ultrasound vascular doppler.

Data analysis

The data collected in this study was analyzed using Statistical Package for Social Science (SPSS) version 20.0. Chi square and t-test was used to test for differences while regression analysis was used to test for relationships or associations between variables. Statistical significance was set at $p \leq 0.05$.

RESULTS

The demographic characteristics of the study participants are shown in table 1. A total of 90 persons (27 males and 63 females) participated in the study comprised of 34 sickle cell patients, 9 sickle cell carriers and 47 controls. Majority of the participants were on vitamins as preventive medication.

Table 1: Demographic characteristics of the study participants.

Characteristics		Sickle cell patients n=34	Sickle cell carriers n=9	Control n=47
Sex	Male	12 (35.3)	2 (22.2)	13 (27.7)
	Female	22 (64.7)	7 (77.8)	34 (72.3)
Genotype		SS	AS	AA
Medication currently on n (%)	Nil	18 (52.9)	4 (44.4)	26 (55.3)
	Vitamins	9 (26.5)	2 (22.2)	13 (27.7)
	Analgesics	3(8.8)		2 (4.3)
	Analgesics and antimalarials	0 (0)	0 (0)	1 (2.1)
	Haematinics	2 (5.9)	3 (33.3)	4 (8.5)
	Antibiotics	1 (2.9)	0 (0)	1 (2.1)
	Vitamins, antibiotics and hydroxyurea	1 (2.9)	0 (0)	0 (0)
Preventive medication always taken n (%)	Nil	8 (23.5)	4 (4.44)	25 (53.2)
	Vitamins	12 (35.3)	3 (33.3)	21 (44.7)

Continued.

Characteristics		Sickle cell patients n=34	Sickle cell carriers n=9	Control n=47
	Analgesics	5 (14.7)	0 (0)	0 (0)
	Analgesics and antimalarials		0 (0)	0 (0)
	Haematinics	4 (11.8)	2 (22.2)	1 (2.1)
	Antibiotics	0 (0)		0 (0)
	Vitamins and antimalarials	3 (8.8)		0 (0)
	Hydroxyurea	2 (5.9)		0 (0)

Table 2: Speed of Capillary blood flow and D-Dimer (ng/ml) in sickle cell patients, sickle cell carriers and normal controls.

	Speed of capillary blood flow	D-Dimer
Sickle cell patients (n=34)	91.0±21-74	309.4±547.85
Sickle cell carriers (n=9)	85.6±15.34	86.3±54.1
Controls	92.1±16.87	106.8±208.25
F(P) value	0.458 (0.634)	3.29 (0.042)*
Results expressed as Mean ± SD, *Significant at p<0.05, Post hoc analysis for D-Dimer value		
Sickle cell patients versus Sickle cell carriers p value		0.111
Sickle cell patients versus control p value		0.017
Sickle cell carriers versus controls p value		0.875

Key F (P) value = comparison of mean value of D-Dimer and speed of capillary blood flow among sickle cell patients, sickle cell carriers and controls using ANOVA. Sickle cell patients versus sickle cell carriers P-value = comparison of mean value D-Dimer between sickle cell patients and sickle cell carriers using T-Test. Sickle cells patients vs controls P-value = comparison of mean value D-Dimer between sickle cell patients and controls using T-Test.

Table 2 shows the mean±SD comparison of speed of capillary blood flow and D-Dimer (ng/ml) in sickle cell patients, sickle cell carriers and normal controls using ANOVA. There was a significant variation (p=0.042) in the mean levels D-Dimer among the groups. There were however no significant variations (p>0.05) in the other parameters among the groups. A post hoc analysis of mean D-Dimer in sickle cell patients, sickle cell carriers and controls showed that the sickle cell patients had significantly higher D-Dimer levels compared with controls (Table 2).

Mean ± SD value of speed of capillary blood flow and D-Dimer ng/ml compared among sickle cell patients in steady state, sickle cell patients in crisis and normal controls using ANOVA shown in Table 3. There were no significant variations (p>0.05) in any of the parameters among the groups.

Table 3: Mean ± SD value of speed of capillary blood flow and D-Dimer (ng/ml) compared among sickle cell patients in steady state, sickle cell patients in crisis and normal controls using (ANOVA).

	Speed of capillary blood flow	D-Dimer
Sickle cell patients in steady state	93.1±21.43	295.6±548.19
Sickle cell patients in crisis	68.3±7.64	452.9±640.98
Controls	92.1±16.87	106.8±208.25
F(P) value	3.101 (0.091)	2.893 (0.061)
Results expressed as Mean ± SD, *Significant at P< 0.05. A Post hoc analysis of mean speed of capillary blood flow, D-Dimer in sickle cell patients in crisis and controls		
Sickle cell patients in steady state versus Sickle cell patients in crisis p value	0.030*	0.506
Sickle cell patients in steady state versus controls p value	0.797	0.039*
Sickle cell patients in crisis versus controls p value	0.035*	0.140

However, a post hoc analysis of mean speed of capillary blood flow and D-Dimer in sickle cell patients in steady state, sickle cell patients in crisis and controls. (Table 3) showed that the sickle cell patient in steady state had significantly higher (p=0.039) D-Dimer levels compared with controls. On the other hand, sickle cell patients in crisis had significantly lower mean speed of capillary blood flow when compared to sickle cell patients in steady state (p=0.035) and controls (p=0.030).

DISCUSSION

Both microvascular and macrovascular thrombosis is thought to be a significant factor in the pathophysiology of complications in sickle cell disease.[15] The mechanism of hypercoagulability in SCD is multifactorial, including activation of coagulation at the surface of sickled cells, endothelial dysfunction, and inflammation.[15,16] Due to the high morbidity and mortality associated with this disease

a lot of research into less invasive methods of predicting the vaso-occlusive events are being carried out. This has led to the introduction of the use of transcranial Doppler test for the risk of stroke and ocular doppler velocimetry for measuring orbital vascular resistance.[17-19] This study therefore assessed the possible use of capillary blood flow as a tool in predicting these events.

The sickle cell patients in this study had significantly higher D-Dimer levels compared with controls. This signifies that there is an increased activation of coagulation in sickle cell disease. The fibrin D-Dimer degradation fragment is produced by plasmin degradation of cross-linked fibrin. Elevated plasma D-Dimer levels indicate increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation.[20] Elevated D-Dimer levels have been shown to correlate to stroke, retinopathy, and vaso-occlusive crises.[16] This agrees with the work of Kusfa who reported highly elevated levels of D-Dimer in sickle cell patients in their study.[11]

The sickle cell carriers in this study had D-Dimer levels comparable to that of the controls signifying normal activation of coagulation and fibrinolysis in sickle cell carriers. The mechanism responsible for higher D-Dimer in sickle cell mutation carriers is not clear. It may have been due to the small sample size of the sickle cell carriers in the study. It could also be that the sickle cell carriers in this study may not have been exposed to conditions which may trigger erythrocyte sickling and endothelial activation which consequently leads to higher D-Dimer levels. This however contrasts with the findings by Naik et al who reported that Median levels of D-Dimer were higher among sickle cell trait carriers compared with non-carriers.[16] Also studies by Westerman et al, Lawrie et al, and Amin et al, have also associated higher D-Dimer levels with the sickle cell trait.[21-23] Raffield et al suggested that relatively low partial pressure of oxygen and dehydration in certain tissues may lead to erythrocyte sickling and endothelial activation, which may result in tissue damage, exposure of extracellular and intracellular proteins, which ultimately trigger activation of the blood coagulation system in the sickle cell carriers.[24]

Sickle cell patients in steady state had significantly higher D-Dimer than the controls. Although the sickle cell patients in crises had higher D-Dimer than the controls this was not significant. Chronic activation of coagulation is commonly observed in patients with sickle cell patients in steady state compared to healthy control subjects with normal hemoglobin. This is evidenced by increased plasma levels of in vivo markers of thrombin and fibrin generation, including thrombin-antithrombin complexes, prothrombin fragment, fibrinopeptide A, D-dimers and plasmin-antiplasmin complexes.[25] Our observation agrees with findings by Akinola et al., and Fakunle et al., who both reported that significantly high D-Dimer levels in

patients with SCA in steady state compared to controls.[10,13] They stated these changes were consistent with minor episodes of microvascular stasis but the episodes were insufficient to cause overt vaso-occlusive crisis.

Among sickle cell patients in crises, this study showed no significant increase in D-Dimer levels. This result contrasts those of previous studies which documented elevated D-dimers concentration in sickle cell patients in crises.[9,26] This observation may probably be due to the small sample size of subjects used in this study. Another reason could be due to the fact that some medications given to ameliorate vaso-occlusive crises in sickle cell patients have properties that may result in reduction of D-Dimer levels. A reduction in plasma D-Dimer level in patients treated with hydroxyurea has been reported.[21] Hydroxyurea increases plasma concentrations of microparticles and reduces coagulation activation and fibrinolysis in patients with sickle cell anemia.[27] Additionally, hydroxyurea has major anti-inflammatory properties, which may be due to both the downstream effects of the inhibition of HbS polymerization and due to direct anti-inflammatory effects. Hydroxyurea therapy significantly reduces leukocyte counts in patients with sickle cell disease, potentially reducing the amplitude of inflammatory responses in the disease. In addition, hydroxyurea lowers the expression and activity of adhesion molecules on the surface of RBCs, leukocytes and the endothelium in SCD sickle cell disease.[28,29] Low dose anticoagulation treatment with warfarin has also been associated with a significant reduction in the D-Dimer levels.[30] Some studies have reported a decreased fibrinolysis in sickle cell patients both in steady state and in crises others have reported enhanced fibrinolysis in this disorder.[31-33]

There was no significant correlation between D-Dimer and speed of capillary blood flow the reason for this and the underlying mechanism is unclear. There was also no correlation between either D-Dimer or speed of capillary blood flow and the Number of days since last painful crisis in the sickle cell patients. The implication of this is that the speed of capillary blood flow may not be useful as a noninvasive marker for increased thrombin activity and fibrin formation. Also, the lack of correlation between D-Dimer or speed of capillary blood flow and the Number of days since last painful crisis in the sickle cell patients suggest that both D-Dimer and speed of capillary blood flow may not be useful in the prediction of vaso-occlusive events. This agrees with the findings of Francis who reported that no correlation was observed between levels of D-Dimer in sickle cell patients and the time since the last painful crisis.[20]

However, the speed of capillary blood flow in the sickle cell patients in crises was significantly lower compared to those in steady state and controls. This may be attributed to vaso-occlusive crises. It would be interesting to see if

there is any relationship between speed of capillary blood flow and the severity of pain during the crises.

Limitations of the study

The number of Haemoglobin AS (9) recruited as control in the study was lower than the Haemoglobin AA control subjects (47). The number of Haemoglobin SS subjects recruited were not up to original sample size proposed (34 versus 36). We only had three sickle cell anaemic subjects in crises and this is too small to make any significant conclusion.

CONCLUSION

Sickle cell patients in steady state had higher D-Dimer levels compared with controls and sickle cell patients in crisis had lower speed of capillary blood flow when compared to sickle cell patients in steady state and controls. Speed of capillary blood flow may not be useful in predicting vaso-occlusive events. D-Dimer is a more suitable marker.

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