Original Article

The Use of Antithrombin as a Predictive Tool in Determining the Development of Stroke in Patients with Sickle Cell Anemia Based on Transcranial Doppler Ultrasound Risk Group

Olusola Olowoselu, Ebele Uche[1], Abosede Ogunlade, Olufemi Oyedeji, Obiefuna Ajie[2], Vincent Osunkalu, Akinsegun Akinbami[1], Jeremiah Oyedemi[3]

Departments of Haematology and Blood Transfusion, [2]Chemical Pathology and [3]Physiotherapy, College of Medicine, University of Lagos, [1]Department of Haematology and Blood Transfusion, Lagos State University College of Medicine, Lagos, Nigeria

Abstract

Background: Stroke affects up to 10% of individuals with sickle cell anemia (SCA), and its development has been linked to excessive intravascular hemolysis and arterial thrombosis Increased intracerebral blood flow (CBF) velocity as measured by the transcranial Doppler ultrasonography (TCD) identifies children with SCA with an increased risk of development of stroke. This study measured antithrombin (AT) levels among SCA patients as a predictor of TCD risk groups for the development of stroke. **Materials and Methods:** A total of 180 participants consisting of 135 SCA patients and 45 age-matched hemoglobin phenotype AA (HbAA) controls were enrolled into the study. CBF velocity was measured with TCD and results were used to classify the SCA group into standard risk, conditional risk, and high risk. AT functional activity, prothrombin time (PT), and activated partial thromboplastin time (APTT) of all participants were measured. Statistical tools including independent *t*-test, analysis of variance, Pearson's correlation, hierarchical multiple regression, and forward liner regression were used to analyze all continuous variables. $P < 0.05$ was considered statistically significant. **Results:** The AT levels were 83.01 ± 15.40% and 106.12 ± 14.79% in HbAA and SCA participants, respectively, with $t = -7.294$ and $P = 0.001$. The PT and APTT of the SCA and control groups were 15.51 ± 1.22 s, 13.78 ± 0.94 s, and 35.98 ± 3.24, 33.62 ± 2.49 s, respectively. Using ANOVA, there was a statistical difference ($P = 0.001$) in the AT levels of the standard-risk (89.07 ± 14.26%) and high-risk groups (73.10 ± 12.35%). Using Pearson's correlation, there was a significant negative correlation between AT levels and CBF ($r = -0.405$). With the use of multiple regression, AT showed the highest predictive value for CBF ($R^2 = 0.155$; $P \leq 0.001$; $F = 17.677$). **Conclusion:** AT functional activity levels were reduced in the SCA group compared with the HbAA-matched controls.

Keywords: Antithrombin, cerebral blood flow, sickle cell anemia, transcranial Doppler ultrasound

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Introduction

Sickle cell anemia (SCA) is one of the most common monogenetic disorders worldwide and is characterized by the presence of sickle hemoglobin (HbS).[1] The World Health Organization estimates that between 20 and 25 million people are living with SCA with about 15 million in sub-Saharan Africa; Nigeria bears the highest burden of the disease and accounts for 100,000 new births annually.[2]

SCA is characterized mainly by chronic hemolytic anemia and intermittent vaso-occlusion with acute exacerbations. These result in tissue ischemia, infarction, and ischemia reperfusion injury in different organs and tissues with resultant organ damage.[3]

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Cerebrovascular events (CVE) which consist of ischemic strokes and transient ischemic attacks are among the most severe sequelae of SCA. Stroke, which is a preventable life-changing debilitating complication of SCA is a significant cause of morbidity and mortality in children and young adults with SCA. They are thought to represent the culmination of large and small

Address for correspondence: Dr. Ebele Uche, Department of Haematology and Blood Transfusion, Lagos State University College of Medicine, Lagos, Nigeria.
E-mail: eifeyinwa2000@yahoo.com

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vessel disease and altered cerebral autoregulation, as well as the sequelae of chronic inflammation, hemolysis, and anemia.[4] Although CVE can occur at any age, the most vulnerable group as reported by Ohene-Frempong *et al.* is patients between 2 and 20 years (0.30–0.75 acute events/100 patients/year). Reportedly, one in ten children with SCA will experience stroke before the age of 20 years.[5]

The Cooperative Study of Sickle Cell Disease which is the largest US multicenter longitudinal study on complications of SCD reported an overall prevalence of stroke to be 3.75% in all patients with SCD. In childhood, the highest incidence (1.02/100 person-years) was found in patients between ages 2 and 5 years. The study also concluded that the rate of CVA vary between sickle cell genotypes. The age adjusted incidence of CVA was highest in SCA patients (0.61/100 person-years) compared with Haemoglobin C disease (HbSC) patients (0.15/100 person-years); the rates for HbSβ- or HbSβ0 were 0.09/100 person-years and 0.08/100 person-years, respectively.[5] In studying stroke prevalence among SCA patients in Nigeria, Madu *et al.*[6] reported a prevalence of 12.4/1000 SCA patients. Adams *et al.* in their study showed that patients at risk of developing cerebral vasculopathy can be detected with the use of the transcranial Doppler ultrasonography (TCD); elevated cerebral blood flow (CBF) velocity of >200 cm/s has been identified as a risk factor for stroke.[7]

The prevalence of abnormal time-averaged mean of the maximum velocity (TAMMV) has been reported to be 10.8% in Lagos State, Nigeria.[8] Studies have implicated the activation of coagulation pathway by increase in the plasma levels of markers of thrombin generation and antithrombin (AT) complexes. Low levels of natural anticoagulants such as Protein C, Protein S, and AT have also been documented in SCA patients in both steady state and during acute pain crises.[9,10] AT is one of the most important endogenous regulators of coagulation and provides 80% of inhibitory activity against thrombin by covalently binding to and inactivating it. The research has shown that the risk of thrombosis appears to be higher in patients with AT deficiency when compared with patients with Protein C and S deficiencies.[11] In a study by Adama *et al.*,[12] there was a statistically significant reduction of AT levels among Sickle cell anaemia (HbSS) patients when compared with age- and sex-matched apparently healthy Hb phenotype AA (HbAA) controls. Therefore, this study assessed the level of AT at different levels of CBF velocity (CBFV) in patients with SCA and compared with apparently healthy HbAA individuals. A second objective was to evaluate AT activity as a predictor of TAMMV risk group for cerebrovascular accident/stroke in SCA patients.

Materials and Methods

Study area and population

One hundred and eighty participants consisting of 135 SCA patients and 45 age-matched HbAA controls aged between 2 and 18 years were recruited into the study. All the SCA patients were already diagnosed using Hb electrophoresis and were registered patients of the sickle cell foundation. All SCA patients had a TCD done before being enrolled into the study group and then categorized based on CBF results. Participants' enrolment into the control group was done after the completion of the required sample size of the study group and were age and sex matched.

The patients were recruited at the Sickle Cell Foundation Center, Idi-Araba Lagos (which is a nongovernmental organization that manages SCA patients from all over Nigeria), in collaboration with the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria.

Study design

This was a comparative cross-sectional study involving SCA patients and apparently healthy age-matched HbAA controls.

Study period

The study was done between December 2018 and April 2019.

Sampling technique

Participants who met the inclusion criteria were recruited consecutively into the study until the required sample size was obtained.

Inclusion criteria

All SCA patients in the steady state whose parents/legal guardians gave informed consent.

Exclusion criteria

- SCA patients with a history of transfusion within the 3 months preceding the study
- Patients on anticoagulant medication, oral contraceptives or aspirin, and pregnant girls were also excluded from the study
- Individuals with a history of thrombosis or other coagulation disorders.

Participants informed consent

The parents/legal guardians of all participants were informed about the study as well as their rights and benefits. A written informed consent was obtained by means of voluntarily signed consent form. No parent or legal guardian was coerced in any way to participate in this study which was at no cost to them.

Measurement of cerebral blood flow velocity

The CBFVs of the 135 HbSS participants were measured using Doppler machine (Doppler box × 1 7780) at the Sickle Cell Foundation, Lagos, Nigeria. The evaluations were performed by trained TCD technicians who were supervised by a consultant radiologist. CBFV were measured using a 2-MHz hand-held probe attached to a Doppler box according to the stroke prevention in sickle cell disease protocol (Nichols *et al.,* 2001). The velocities of blood flow in the middle cerebral artery, internal carotid artery, and anterior cerebral arteries were measured on both the left and right hemispheres of the brain. The highest velocity in each artery was recorded as the

TAMMV. TAMMV <170 cm/s was considered standard risk, values ≥170 cm/s but <200 cm/s were considered conditional risks, and velocity at least 200 cm/s was considered high risk.[13]

The results of the CBFV were used to classify the SCA patients into groups:

- Group I: HbSS patients with normal velocity (TAMMV <170 cm/s)
- Group II: HbSS patients with conditional risk (TAMMV 170–199 cm/s)
- Group III: HbSS patients with abnormal/increased risk (TAMMV ≥200 cm/s)
- The control group made up of HbAA patients was assigned to Group IV.

Blood sample collection

Seven and half (7.5) ml of venous blood was collected from all the participants from antecubital vein under aseptic conditions. Four and half (4.5) ml of blood was dispensed into plastic trisodium citrate bottle containing 0.5 ml of trisodium citrate (3.2 g/dl) anticoagulant (to make nine parts of blood to one part of anticoagulant) for prothrombin time (PT), activated partial thromboplastin time (APTT), and AT analysis. This was centrifuged at 3000g for 15 min to obtain platelet-poor plasma and aliquoted into cryovials. The PT and APTT were done immediately while the rest of the plasma was stored at −80°C and used for AT functional activity measurement. The remaining 3 ml of blood was dispensed into ethylenediaminetetraacetic acid anticoagulant bottles for full blood count and Hb electrophoresis (to determine the Hb phenotype of control participants). A semi-automated coagulometer (Genius CA 54) with agappe reagent for PT and APTT was used to measure the PT and APTT in the citrated plasma of study groups according to the manufacturer's instruction.[14] A chromogenic assay for the quantitative determination of the heparin cofactor activity of AT in human citrated plasma, using an anti-Xa method was employed for this study. Erba Chrome ATIII test kit (Cat no. EHL00008) from Czech Republic was used using an automated machine ECL 760 (Erba Mannheim, London. United Kingdom).

Statistical analysis

Data was analyzed by IBM SPSS (Statistical Package for Social Sciences, Inc.) statistics for windows version 20.0 Armonk, New York, USA. $P \leq 0.05$ was considered statistically significant. The results were summarized as means ± standard deviation for continuous variables and percentages for categorical variables. The mean difference between the two main groups (HbSS and HbAA) was analyzed using independent sample *t*-test. The mean difference across various groups was compared using ANOVA, with Bonferroni *post hoc* analysis for pair-wise comparison. The association between CBFV of SCA patients and AT functional activity was analyzed using Pearson's coefficient. Hierarchical multiple regression was done to predict CBFV from AT levels. Multiple regression scatter plot was done to graphically represent the correlation between the independent variables and CBFV.

Sample size calculation

The sample size for this study was calculated using the statistical formula that applies to comparative studies.[15]

Sample size $(n) = Z^2 \{P_1(100 - P_1) + P_2(100 - P_2)\}$

where

n = sample size

Z = 1.96 (at 95% confidence level)

P_2 = Reported prevalence in the general population = 0.012[16]

P_1 = Reported prevalence in the high-risk population = 1.24[6]

d = 5% (precision)

n = 1.962 ([1.24 (100 – 1.24] +0.012 [100 − 0.012])/25

n = 3.8416 (1.24 × 98.76) + (0.012 × 99.99)/25

n = 3.8416 × 123.659/25

n = 475/25

n = 19 for each group

However, 45 participants were enrolled for the control group HbAA, whereas 135 participants (45 each to three risk groups) were enrolled for the study HbSS group.

Ethical consideration

Ethical approval was obtained from the LUTH Health and Research Ethics Committee. (HREC No: ADM/DCST/HEC/APP/2660)

Results

HbSS patients were categorized into three groups based on the value of their TAMMV in this study into groups I–III. Individuals with HbAA formed the control group (Group IV). TAMMV value of <170 cm/s, 170–199 cm/s, and ≥200 cm/s represented the standard risk, conditional risk, and high risk among the HBSS patients, respectively.

Sociodemographic characteristics and mean cerebral blood flow

The mean age, gender distribution, and CBFV TAMMV of the participants are shown in Table 1. There was no statistically significant difference between the mean ages of both the SCD groups (7.58 ± 3.52 years, 6.47 ± 2.77 years, and 6.26 ± 3.52 years for standard-, conditional-, and high-risk groups, respectively) and HbAA control (7.13 ± 4.43 years) $P = 0.38$.

The proportion of females in all the four groups was more than males, and the control group had more females (62.22%) than any of the SCD groups. The findings also showed that there was a statistically significant difference in the mean CBFV across the various groups of SCD patients (144.77 ± 15.64 cm/s, 182.10 + 8.89 cm/s, and 225.03 + 23.87 cm/s for standard risk group, conditional risk group, and high risk group, respectively ($P = 0.001$).

Table 1: Sociodemographic and selected clinical characteristics of the study participants

Group characteristics	Standard risk ($n=45$)	Conditional risk ($n=45$)	High risk ($n=45$)	Controls ($n=45$)	*F*	*P*
Age (year)	7.58±3.52	6.47±2.77	6.26±3.52	7.13±4.43	1.029	0.380
TAMMV (cm/s)	144.77±15.64	182.10±8.89	225.03±23.87	133.99±27.32	166.49	0.001
Gender	**Standard, *F* (%)**	**Conditional, *F* (%)**	**High, *F* (%)**	**Control, *F* (%)**		**Total, *F* (%)**
Male	21 (46.67)	18 (40.00)	19 (42.22)	17 (37.78)		75 (41.66)
Female	24 (53.33)	27 (60.00)	26 (57.78)	28 (62.22)		105 (58.33)

TAMMV: Time-averaged mean of the maximum velocity

Table 2: Comparison between coagulation parameters of HBSS group and the controls

Parameters	HBSS group ($n=135$), X±SD	Controls ($n=45$), X±SD	*t*	*P*
AT (%)	83.01±15.40	106.12±14.79	−06.12	0.001
PT (sec)	15.51±1.22	13.78±0.94	7.243	0.001
APTT (sec)	35.98±3.24	33.62±2.49	3.691	0.001

AT: Antithrombin, PT: Prothrombin time, APTT: Activated partial thromboplastin time, SD: Standard deviation

Comparison of the antithrombin functional activity, prothrombin time, and activated partial thromboplastin time between the study and the control group

The mean differences between AT levels, PT, and APTT of the study and control groups are presented in Table 2. AT activity was significantly lower ($P < 0.001$) in the study group when compared with the control group. Even though the PT and PTTK values of both the study and control groups were within the normal limits, the difference between the two groups was statistically significant with $P < 0.001$.

Comparison of antithrombin functional activity, prothrombin time, and activated partial thromboplastin time between the various sickle cell anemia groups and control

ANOVA and Bonferroni post hoc analysis were used to summarize the comparison between the AT functional activity, PT, and APTT of each SCA groups and the control in Table 3.

The *P* values for the posthoc analysis are seen in Table 4.

Correlation between cerebral blood flow velocity time-averaged mean of the maximum velocity and antithrombin functional activity, prothrombin time, and activated partial thromboplastin time in sickle cell anemia patients

Pearson correlation showed that AT correlated negatively with TAMMV ($r = -0.405$, $P < 0.05$), both PT and APTT showed weak positive correlations with TAMMV ($r = 0.210$, $P < 0.05$; $r = 0.193$, $P \leq 0.01$), respectively, in Table 5.

Summary of hierarchical regression analysis for antithrombin, prothrombin time, and activated partial thromboplastin time in predicting cerebral blood flow velocity (time-averaged mean of the maximum velocity) among the SCA groups ($n = 135$)

Hierarchical multiple regression was used to predict TAMMV using AT activity, PT, and APTT. In the first model, it was observed that a unit change in AT can significantly predict a 15.5% of variance in TAMMV ($R^2 = 0.155$; $F = 17.677$; $\beta < 0.01$). In model 2, a nonsignificant increase in the prediction was obtained with the addition of PT ($R^2 = 0.159$; $F = 1.453$; $\beta = 0.231$) to the first model. Adding APTT to the third model increased the overall prediction of the model by 0.4% ($R^2 = 0.163$; $F = 1.387$; $\beta = 0.242$). The addition of both APTT and PT showed no significant difference to the overall prediction of model 3 in Table 6.

In Figure 1, multiple regression scatter plot was used to depict the correlation between unstructured predictive factor of AT, PT, and APTT against TAMMV, and a prediction of 19% variance in TAMMV/unit change in AT, PT, and APTT was obtained ($R^2 = 0.190$).

Discussion

This was a comparative cross-sectional study, in which AT activity and its association with CBFV was assessed in SCA patients. Our study found significantly lower AT levels among the SCA patients compared with the control group. This is in keeping with results of other studies which showed lower AT levels among HbSS patients in steady state when compared with healthy HbAA individuals.[12,17-20] However, some other studies documented higher levels of AT among HbSS patients compared with normal controls,[21] whereas a few others documented normal AT levels among SCA patients.[22,23]

These seemingly conflicting data may stem from the differences in methodology, whereas some researchers used thrombin-based assay method, our study used the factor-Xa method. Assays based on thrombin lead to overestimation of AT levels because of the interference of thrombin with heparin cofactor II (HCII). HCII inhibits thrombin, leaving most of the AT untreated and resulting in its overestimation.[24]

In a study by Liesner *et al.*,[21] AT levels in patients with SCA having normal and abnormal cerebral vasculature were normal. Adama *et al.*[12] also reported no differences in the AT levels of patients with SCA in steady state and during vaso-occlusive crisis. These are in contrast to the finding from our study which showed a reduced level of AT in conditional-risk patients (, and was much more significantly reduced in high-risk patients compared to those of standard-risk patients. The significantly reduced levels of AT may suggest a relationship between AT and increased CBF in SCA patients and may be due to increased consumption as a consequence of the ongoing thrombin

Table 3: Comparison of coagulation parameters in each HBSS groups and the control

Parameters	HBSS groups			Control	*F*	*P*	*Post hoc*
	Standard risk (*n*=45), X±SD	Conditional risk (*n*=45), X±SD	High risk (*n*=45), X±SD	Control (*n*=45) X±SD			
AT (%)	89.07±14.26	87.00±14.68	73.10±12.35	106.12±14.79	28.78	<0.001	a*c, a*d, b*d, c*d
PT (sec)	15.05±1.02	15.48±1.21	16.00±1.25	13.78±0.94	22.58	<0.001	a*c, a*d, b*d, c*d
APTT (sec)	35.01±2.85	35.98±2.55	36.96±3.94	33.63±2.49	6.87	<0.001	c*d

*Significant at *P*<0.05 level. Values are mean (X)±SD. A: Standard risk, b: Conditional risk, c: High risk, d: Hemoglobin AA control. SD: Standard deviation, AT: Antithrombin, PT: Prothrombin time, APTT: Activated partial thromboplastin time

Table 4: *Post hoc* analysis of the coagulation parameters of individual HBSS group and the control

	AT	PT	APTT
A*B	1.00	0.84	1.00
A*C	00.01*	0.01*	0.73
A*D	<0.01*	<0.01*	0.45
B*C	0.01	0.41	1.00
B*D	<0.01*	<0.01*	0.17
C*D	<0.01*	<0.01*	<0.01*

*Significant at *P*<0.05 level. A: Standard risk, B: Conditional risk, C: High risk, D: Hemoglobin AA control, AT: Antithrombin, PT: Prothrombin time, APTT: Activated partial thromboplastin time

Table 5: Correlations between cerebral blood flow velocity (time-averaged mean of the maximum velocity) and coagulation variables

	AT	PT	APTT	TAMMV
AT	1			
PT	-0.240*	1		
APTT	-0.174	0.136	1	
TAMMV	-0.405*	0.210*	0.193**	1

*Correlation is significant at the 0.05 level (two-tailed). TAMMV: Time-averaged mean of the maximum velocity, AT: Antithrombin, PT: Prothrombin time, APTT: Activated partial thromboplastin time

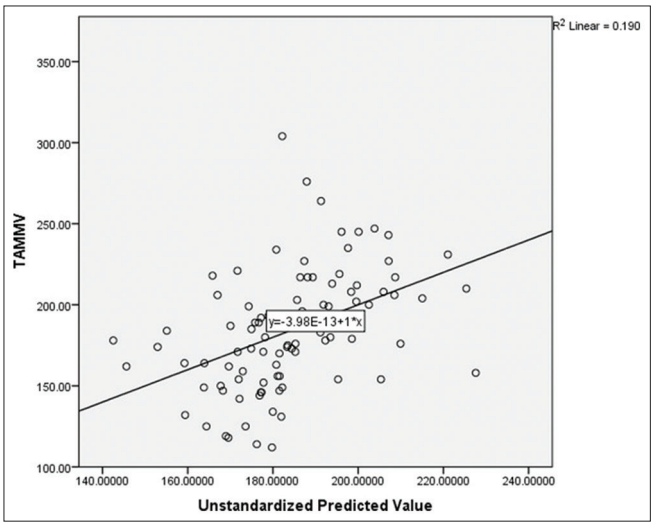

Figure 1: Multiple regression scatter plots describing the correlation between time-averaged mean of the maximum velocity and unstandardized predictive factor of coagulation variables (antithrombin, prothrombin time, and activated partial thromboplastin time)

generation and formation of thrombin antithrombin complex. Other possibilities for this include liver dysfunction and/or chronic inflammation.[25,26]

There was no statistical difference between the PT and APTT levels of the SCA patients when compared with the HbAA control group even though the values were higher. In contrast, prolongation of PT and APTT values has been reported by Chinwawa *et al.*, Awoda *et al.*, and Adama *et al*.[27,28] The mechanism behind the prolongation of PT in SCA is not yet understood. It is suggested that impaired liver function[29] and depletion of coagulation factors VII and V due to continuous activation may play a role in the prolongation process.[30]

There was a statistically significant negative correlation between AT activity and CBFV. No significant correlation was found between PT/APTT and CBFV.

Based on the result of multiple hierarchical regression analysis in this study, it was observed that AT remained the only significant correlate for TAMMV. Lagunju *et al.*[31] reported age and hematocrit remain the only significant predictor for TAMMV in HBSS patients while platelet count did not show any significant predictive value for TAMMV in their multiple regression analysis.

Furthermore, David *et al.*[32] who determined the association of TAMMV with biochemical parameters documented a significant correlation of TAMMV with age ($P = 0.008$), Hb ($P < 0.001$), lactate dehydrogenase ($P = 0.048$), aspartate transaminase (AST) ($P = 0.005$), white blood cell count ($P = 0.021$), and creatinine level ($P = 0.004$). However, only Hb ($P = 0.001$) and AST ($P = 0.025$) maintained significance in multiple regression. Interestingly, Deane *et al.*[33] in their 2008 study documented TAMMV to insignificantly correlate with Haemoglobin C disease (HBC), neutrophil, platelet count, lactate dehydrogenase, age, and percentage foetal Haemoglobin (HBF) while only platelet count was reported to show significant relationship with TAMMV ($r = 0.339$; $P = 0.020$) in HbSC patients using multivariate regression. However, none of these researchers measured AT activity in their research.

AT deficiency has previously been linked to thrombosis and cerebral infarction. In 1993, while reviewing ten stroke patients, Martinez *et al.*[34] attributed the presence of acquired

Table 6: Summary of hierarchical regression analysis for coagulation variables predicting cerebral blood flow velocity (time-averaged mean of the maximum velocity) among the sickle cell disease groups (n=135)

Variable	Model 1			Model 2			Model 3		
	B	SE (*B*)	β	*B*	SE (*B*)	β	*B*	SE (*B*)	β
AT	−T (1	−T (1	0.000	−.000	−.000	0.000	−.000	−.000	0.001
PT				3.667	3.042	0.231	3.312	0.108	0.280
APTT							1.327	0.115	0.242
R^2		0.155			0.159			0.163	
F		17.677*			1.453			1.387	

*P<0.05. B: Beta coefficient, SE (*B*): Standard error of beta coefficient, β: Beta value for multiple regression; R^2: Proportion of the variance in the dependent variable that is predictable from the independent variable, *F*: F-statistics. AT: Antithrombin, PT: Prothrombin time, APTT: Activated partial thromboplastin time

AT deficiency to the development of stroke in five out of the ten patients reviewed. There has also been documented cerebral arterial thrombosis due to AT deficiency in a number of cases.[35]

These reports alongside findings of low AT activity observed in high-risk patients in our study suggest that AT deficiency may be a risk factor for the development of stroke and may be used as a predictor for increased CBFV in patients with SCA. Using forward regression analysis, it was observed that AT showed higher prediction for CBF than PT and APTT. However, further research with a larger sample size and assay of more coagulation factors is required to validate this finding.

Conclusion

This study suggests that AT activity is reduced in patients with SCA and much more reduced in those with high CBFV. Based on these results, baseline measurements of AT activity may help in identifying SCA patients who have the highest risk of having stroke and this will assist in prioritizing them for possible preventive measures and comprehensive healthcare. However, these results need to be validated with a larger sample size.

Study limitations

Markers of thrombin generation (e.g. thrombin AT complex) were not assayed due to financial constraints. This could have provided more insight in the interplay that leads to higher CBFV.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Weatherall DJ. The challenge of haemoglobinopathies in resource-poor countries. Br J Haematol 2011;154:736-44.
2. Fleming AF, Storey J, Molineaux L, Iroko EA, Attai ED. Abnormal haemoglobins in the Sudan savanna of Nigeria. I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. Ann Trop Med Parasitol 1979;73:161-72.
3. Serjeant GR, Serjeant BE, Fraser RA, Hambleton IR, Higgs DR, Kulozik AE, *et al*. Hb S-β-thalassemia: Molecular, hematological and clinical comparisons. Hemoglobin 2011;35:1-2.
4. Webb J, Kwiatkowski JL. Stroke in patients with sickle cell disease. Expert Rev Hematol 2013;6:301-16.
5. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, *et al*. Cerebrovascular accidents in sickle cell disease: Rates and risk factors. Blood 1998;91:288-94.
6. Madu AJ, Galandaci NA, Nalodo AM, Garba KU, Fowodu OF, Hassan A, *et al*. Stroke prevalence among sickle cell disease patients in Nigeria: A multicentre study. Afr Health Sci 2014;14:446-52.
7. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, *et al*. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med 1998;339:5-11.
8. Adekunle MO, Animasahun AB, Diaku-Akinwumi IN, Njokanma OF. Pattern of cerebral blood flow velocity using transcranial doppler ultrasonography in children with sickle cell disorder in Lagos state, Nigeria. Mediterr J Hematol Infect Dis 2017;9:e2017050.
9. Westerman MP, Green D, Gilman-Sachs A, Beaman K, Freels S, Boggio L, *et al*. Antiphospholipid antibodies, proteins C and S, and coagulation changes in sickle cell disease. J Lab Clin Med 1999;134:352-62.
10. Tomer A, Harker LA, Kasey S, Eckman JR. Thrombogenesis in sickle cell disease. J Lab Clin Med 2001;137:398-407.
11. Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency: Epidemiology, pathogenesis and treatment options. Drugs 2007;67:1429-40.
12. Adama IL, Usman AM, Modu BK, Abdullahi AB. A study of antithrombin 111 in sickle cell anaemia patients in steady state and during vaso-0cclusive crisis in North-Eastern Nigeria. Am J Sci Ind Res 2013;4:161-6.
13. Nichols FT, Jones AM, Adams RJ. Stroke prevention in sickle cell disease (STOP) study guidelines for transcranial Doppler testing. J Neuroimaging 2001;11:354-62.
14. Laffan M, Manning R. Investigation of haemostasis. In: Lewis SM, Bain BJ, Bates I, editors. Practical Haematology. Philadelphia USA: Churchill Livingstone; 2006. p. 398-400.
15. Goyal RC. Research Methodology for Health Professionals Including Proposal, Thesis and Article Writing. 1st ed. London: Jaypee Brothers Medical Publishers; 2013.
16. Daniel ST, Jonathan HV. Pediatric stroke: A review. Emerg Med Int 2011; 2011. p. 10:Article ID 734506.
17. Onyemelukwe GC, Jibril HB. Anti-thrombin III deficiency in Nigerian children with sickle cell disease. Possible role in the cerebral syndrome. Trop Geogr Med 1992;44:37-41.
18. Bashawri LA, Al-Mulhim AA, Ahmed MA, Bahnassi AA. Platelet aggregation and physiological anticoagulants in sickle-cell disease. East Mediterr Health J 2007;13:266-72.
19. Sorour, MA, Dabbous SA, Afifi RA. A Possible role of Hemoglobin S in implicating hemostatic and inflammatory reactions: Study on Saudi Arabian population. J Appl Hematol 2015;6:64-9.
20. Kusfa IU, Mamman AI, Aminu SM, Hassan A, Muktar HM. Protein C and antithrombin levels in patients with sickle cell anemia in Ahmadu Bello University Teaching Hospital Zaria, Nigeria. Niger J Clin Pract 2017;20:998-1001.

21. Richardson SG, Matthews KB, Stuart J, Geddes AM, Wilcox RM. Serial changes in coagulation and viscosity during sickle-cell crisis. Br J Haematol 1979;41:95-103.
22. Porter JB, Young L, Mackie IJ, Marshall L, Machin SJ. Sickle cell disorders and chronic intravascular haemolysis are associated with low plasma heparin cofactor II. Br J Haematol 1993;83:459-65.
23. Liesner R, Mackie I, Cookson J, Chitloe SA, Donohoe S, Evans J, *et al*. Prothrombotic changes in children with Sickle cell disease: Relationship to cerebro-vascular disease and transfusion. Br J Haematol 1998;103:1037-44.
24. Odegård OR, Abildgaard U. Antithrombin III: Critical review of assay methods. Significance of variations in health and disease. Haemostasis 1978;7:127-34.
25. Bayazit AK, Kilinç Y. Natural coagulation inhibitors (protein C, protein S, antithrombin) in patients with sickle cell anemia in a steady state. Pediatr Int 2001;43:592-6.
26. Levis M. Der-Poll TV. Inflammation and coagulation. Crit Care Med 2010;38:26-34.
27. Chinawa JM, Emodi IJ, Ikefuna AN, Ocheni S. Coagulation profile of children with sickle cell anemia in steady state and crisis attending the university of Nigeria teaching hospital, Ituku-Ozalla, Enugu. Niger J Clin Pract 2013;16:159-63.
28. Awoda S, Daak AA, Husain NE, Ghebremeskel K, Elbashir MI. Coagulation profile of Sudanese children with homozygous sickle cell disease and the effect of treatment with omega-3 fatty acid on the coagulation parameters. BMC Hematol 2017;17:18.
29. Raffini LJ, Niebanck AE, Hrusovsky J, Stevens A, Blackwood-Chirchir A, Ohene-Frempong K, *et al*. Prolongation of the prothrombin time and activated partial thromboplastin time in children with sickle cell disease. Pediatr Blood Cancer 2006;47:589-93.
30. Platt OS. Preventing stroke in sickle cell anemia. N Engl J Med 2005;353:2743-5.
31. Lagunju I, Olugbemiro S, Paul T. Prevalence of transcranial Doppler abnormalities in Nigerian children with sickle cell disease Published online 15 February 2012 Am J Haem 2012;544-7.
32. David CR, Moira CD, Sue EH, Sandra O, Keith RE, David E, *et al*. A simple index using age, hemoglobin, and aspartate transaminase predicts increased intracerebral blood velocity as measured by transcranial Doppler scanning in Children with Sickle Cell. Anemia Pediat 2008;121:E1628-32.
33. Deane CR, Goss D, O'Driscoll S, Mellor S, Pohl KR, Dick MC, *et al*. Transcranial Doppler scanning and the assessment of stroke risk in children with HbSC [corrected] disease. Arch Dis Child 2008;93:138-41.
34. Martinez RA, Rangel-Guerra HR, Marfil LJ. Ischaemic stroke due to deficiency of coagulation inhibitors. Report of 10 young adults. Stroke 1993;24:19-25.
35. Hossmann V, Heiss WD, Bewermeyer H. Antithrombin III deficiency in ischaemic stroke. Klin Wochenschr 1983;61:617-20.