ORIGINAL ARTICLE

Coagulation profile of children with sickle cell anemia in steady state and crisis attending the university of Nigeria teaching hospital, Ituku-Ozalla, Enugu

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Abstract

Background: Sickle cell anemia is associated with a hypercoagulable state that may lead to alterations in a coagulation profile. Measurements of coagulation factors are known to have some predictive value for clinical outcome.
Objectives: To determine the coagulation profile of children with SCA in steady state and crisis and compare it with those with normal HbAA genotype.
Materials and Methods: This is a prospective observational study involving 50 children with SCA in steady state, 50 in crises, and 50 with HbAA genotype, carried out from June to October 2009. All the values of coagulation profile were matched for age and sex.
Results: The mean Prothrombin time (PT) (12.5 ± 1.2 secs), Activated partial thromboplastin time (aPTT) (41.6 ± 1.0 secs), and Thrombin time (TT) (12.3 ± 1.2 secs) of subjects with SCA in steady state as well as those during crises [PT (12.6 ± 1.8 secs), aPTT (45.6 ± 1.3 secs), TT (12.5 ± 1.7 secs)] were significantly prolonged compared to those of subjects with HbAA genotype. The mean bleeding time (BT) of 3.4 ± 1.0 mins was significantly shorter in children with SCA in steady state compared to those with HbAA genotype (3.7 ± 1.1 mins), ($P < 0.038$).
Conclusion: Coagulation profile of patients with SCA is prolonged both in steady state and during crisis and when compared to those with HbAA genotype, though all values are within normal range.

Key words: Children, coagulation profile, sickle cell anemia

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Introduction

Sickle cell anemia (SCA) is a genetic hematological disorder characterized by red blood cells that assume an abnormal rigid, sickle shape.[1] This hereditary disorder contributes the equivalent of 3.4% mortality in children aged under 5 worldwide or 6.4% in Africa.[2]

Sickle cell disorders were originally found in the tropics and subtropics but are now common worldwide due to migration of people from the tropical to temperate zone.[2] The prevalence of SCA in Nigeria ranges from 0.4% to 3% affecting about 20 per thousand new borns.[3] About 85% of sickle cell disorders and over 70% of all affected births occur in Africa.[4] It is noted that at least 5.2% of the world population carry a significant trait.[5]

SCA is associated with a hypercoagulable state that may

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contribute to certain morbidities such as vaso-occlusion and cerebrovascular accidents.[5] It is noted that decreased levels of natural anticoagulant proteins are observed in SCA and even more so in vaso-occlusive crisis.[6,7] These reduced levels may be a consequence of chronic consumption arising from increased thrombin generation which occurs in the vascular endothelium.[8]

Patients with this disease suffer from a variety of clinical events associated with small and large vessel occlusion, including vaso-occlusive painful episodes, strokes, and acute chest syndrome.[9] Nduka *et al*.[5] noted 25% of cases of vaso-occlusion among patients with SCA while Westerman and colleague[6] observed pulmonary infarction in one out of every six patients with this disorder. Such events may be associated with derangements of plasma and cellular haemostatic mechanisms that may impart a thrombogenic tendency to this disorder.[9] Reported changes include increases in thrombin generation, platelet activation, decreased levels of circulating anticoagulants, and contact factors.[10] These changes may be due to shortened platelet survival, increased plasma levels of adenosine diphosphate and epinephrine, and decreased levels of circulating anticoagulants such as protein C and S.[10] These proteins are serine proteases whose activity are modulated by naturally occurring plasma inhibitors. They are key inhibitors of the blood coagulation system.[10]

Measurements of such coagulation factors are known to have some predictive value for clinical outcome. For instance, an increase in fibrinolytic activity has been reported to correlate with frequency of painful episodes.[11] This increased fibrinolytic activity may be due to inhibition of the binding of protein S to β-2 glycoprotein 1 by antiphospholipid antibodies. Besides, higher levels of thrombin and prothrombin generation have also been reported in patients with strokes and vaso-occlusive crisis.[11]

This study therefore aims at determining the actual value of some coagulation profiles (PT, APTT, TT, Platelet count, Bleeding time) among patients with SCA in steady state and crises in this environment and compare with subjects with normal hemoglobin genotype. The findings from this study may add to the increasing knowledge of this challenging disease and may help to improve management of children with this disorder since there has not been much study among children in this locality. In addition, this study would also help in establishing baseline values of coagulation indices among SCA patients in steady state.

Materials and Methods

Study area

The study was carried out at the Children emergency room (CHER), Children's outpatient (CHOP), and Consultants Paediatric clinics (CPC) of the Paediatrics Department of the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla, Enugu, Nigeria. UNTH has a total bed space of 800 and provides specialized services in the major fields of medicine. It is a referral center for various health centers in Enugu state and environs. The Paediatrics Department comprises the children's outpatient clinic (CHOP), the children emergency room (CHER), the general ward, and the new born special care unit (NBSCU).

Study population

There are about 700 children registered at the sickle cell clinic of UNTH, Enugu, with an average of 5 new patients a month. The clinic runs on Mondays with a weekly attendance of between 15 and 20 patients. It is run by 3 Consultants, 2 Senior Registrars, and 4 Registrars.

The subjects studied included known SCA patients aged between 6 months and 18 years considered clinically to be in bone pain crisis as defined by Quirolo *et al*. as bone or joint pains in a single or multiple sites needing analgesics or hospitalization.[1] The stable patients were those with HbSS who had been apparently well for a minimum of 4 weeks before recruitment.[1] Patients excluded include those with any type of infective illness and any patient with recent blood transfusion during the preceding 3 months.[12,13]

The control population were children who were apparently healthy with normal hemoglobin genotype (HbAA) confirmed by hemoglobin electrophoresis coming for follow up after recovery from an acute illness like malaria and upper airway disease.

Children with SCA who were attending the sickle cell clinic or presented at the children emergency ward who fulfilled the inclusion criteria were consecutively recruited into the study. For the control group, the same method was used to recruit apparently healthy children (with HbAA genotype confirmed by hemoglobin electrophoresis) coming for follow up.

Ethical clearance for the study was obtained from the Research and Ethical Committee of the University of Nigeria Teaching Hospital. A written consent was obtained from the parents/caregivers of the subjects and controls after explaining to them, in detail, the objectives of the study as well as the method of specimen collection.

Determination of PT, APTT, TT, platelet count, and bleeding time

Prothrombin time was ascertained by delivering 0.1 ml of plasma into a glass tube placed in a water bath with 0.1 ml of thromboplastin and calcium (a saline brain extract containing tissue factor and a lipoprotein). Activated partial thromboplastin time in kaolin was done by mixing equal volumes of the phospholipids reagent and the kaolin

suspension. Thrombin time was done by adding 0.1 ml thrombin solution to 0.2 ml of control plasma in a glass tube at 37°C, while the activity was timed with the stopwatch.[12]

Platelet count and hemoglobin concentration were analyzed using automated Sysmex KX-21N model.[12] Bleeding time was estimated using the Ivy's method.[14]

All the laboratory tests were also done on the control samples that had been matched with age and sex with the subjects.

Data analysis

Data was analyzed by SPSS version 13. The mean, ranges, and standard deviation of the coagulation values in steady state were compared with values of normal hemoglobin genotype using the ANOVA test. Correlation between hemoglobin concentration, coagulation variables, and bleeding time were compared using the Pearson correlation variable. Hemoglobin concentration of subjects was compared with controls using the Student *t* test (T). The relationship between age, sex, and coagulation profile was calculated using the chi-square test. The level of significance was set at $P \leq 0.05$.

Results

Demographics

A total of 150 children aged 6 months to 18 years were recruited into the study; 100 were confirmed to have hemoglobin SS genotype: 50 of whom were in steady state while the remaining 50 were in bone pain crises. The control consisted of 50 children within the same age group who had hemoglobin AA genotype.

As illustrated in Table 1, the mean ages of subjects in steady state was 8.41 yrs ± 4.80, in crises 8.56 yrs ± 5.2, and for control 8.39 yrs ± 5.38. The subjects (children with SCA) and controls were also well matched for sex; males (56%), females (44%) in steady state; males (52%) and females (48.0%) in bone pain crises ($P = 0.983$); males (44.0%) and females (56%) in controls ($x^2 = 0.213$, $P = 0.644$).

Table 1: Demographic characteristics of SCA subjects and control

Age (years)	Steady state, *n* (%)	Crisis state, *n* (%)	Control, *n* (%)
<3	5 (10)	4 (8)	6
3–5	6 (12)	6 (12)	7
6–8	9 (18)	11 (22)	10
9–11	9 (18)	7 (14)	8
12–14	13 (26)	12	10
>15	8 (16)	10 (20)	9 (18)

$P = 0.983$, f = 0.018

Coagulation variables of SCA in steady state and control

Table 2 shows that prothrombin time, APTT, and thrombin time were prolonged in the sickle cell steady state patients compared to those of the normal HbAA patients. Platelet count was higher in the sickle cell steady state patients compared to those of the normal HbAA patients. While the bleeding time was of shorter duration in the sickle cell steady state patients.

Coagulation variables of SCA subjects in crises and steady state

In Table 3, the mean values of the activated partial thromboplastin time differed significantly between sickle cell patients in steady state (41.6 ± 1.0 secs) and those in crises (45.6 ± 1.3 secs). $P = 0.036$. Other coagulation values were within the reference range for both states.

The table shows the normal reference values

N/B Though coagulation parameters among children with SCA are prolonged when compared to those with normal hemoglobin genotype, it still fell within normal reference range.

Discussion

The findings of this study provide further evidence of coagulation abnormalities among children with SCA. The study shows that children with SCA in steady state have a prolonged PT and APTT, when compared with children with normal hemoglobin genotype and these are further prolonged during crises. This is in keeping with the findings of Richardson[15] *et al*, who noted prolonged PT and APTT in subjects with SCA when compared with normal hemoglobin genotype individuals. On the other hand, the significantly prolonged aPTT of subjects in crises when compared to steady state may be due to the production of cytokines and anti-lupus factor which are elaborated during infections which in turn activate the intrinsic factors leading to their consumption.[6]

However, Babiker[16] and colleagues noted no difference between these coagulation variables among subjects and control, but later found prolonged levels among children with crises after the 4th day of crises. He thus concluded that the changes of coagulation levels noted, rather than being primarily responsible for the onset of crises, can only be secondary changes arising from the etiological factors of crises, i.e. stasis and acute phase proteins.

There is also a prolonged thrombin time of subjects when compared with control. This is in tandem with the study of Ataga *et al*.[17] in North Carolina who noted high level of thrombin generation and depletion of anticoagulant proteins. He thus concluded that treatment that could

Table 2: Comparison of mean values of coagulation variable for steady state sickle cell patients and normal hemoglobin AA patients

Coagulation variables	Mean value steady state patients	Mean value normal Hb AA patients	T	*P*
Prothrombin time (seconds)	12.5±1.2 (*n*=50)	11.2±0.8 (*n*=50)	6.55*	0.000
APTT (seconds)	41.6±1.0 (*n*=50)	38.8±7.7 (*n*=50)	1.55	0.062
Thrombin time (seconds)	12.3±1.2 (*n*=50)	9.9±1.5 (*n*=50)	8.62*	0.000
Bleeding time (minutes)	3.4±1.0 (*n*=50)	3.7±1.1 (*n*=50)	–1.37*	0.038
Platelet Count ($\times 10^3$)	301.5±173.2 (*n*=50)	213.7±81.4 (*n*=50)	3.24*	0.001

APTT = Activated partial thromboplastin time. *Difference is significant ($P \leq 0.05$)

Table 3: Comparison of mean values of coagulation variables of patients with sickle cell anaemia in steady state and in crises

Coagulation variables	Mean values in steady state	Mean salues in crises	T	*P*	Normal values
Prothrombin time (seconds)	12.5±1.2 (*n*=50)	12.6±1.8 (*n*=50)	–0.36	0.359	11-14 s
aPTT (seconds)	41.6±1.0 (*n*=50)	45.6±1.3 (*n*=50)	–1.82*	0.036*	26-36 s
Thrombin time (seconds)	12.3±1.2 (*n*=50)	12.5±1.7 (*n*=50)	–0.61	0.271	8-14 s
Bleeding time (minutes)	3.4±1.0 (*n*=50)	3.2±0.7 (*n*=50)	1.26	0.105	2-9 min
Platelet Count ($\times 10^3$)	301.5±173.2 (*n*=50)	273.2±150.5 (*n*=50)	0.87	0.194	140-450$\times 10^3/\mu L$

*Difference is significant ($P<0.05$)

decrease thrombin generation or platelet activation may be beneficial in both the treatment of sickle cell disease and prevention of complications that characterize the disorder.

It is important to note here that Nsiri *et al*.[18] noted a shortened thrombin time among 28 children with SCA which is contrary to all the findings above. He noted that TT was constantly shortened in the steady state and therefore concluded that the shortened value could be due to the presence of circulating activated clotting factors in SCA and also an elaborated tissue plasminogen activator (t-PA) inhibitor levels which create an imbalance of the profibrinolytic and antifibrolytic systems. The results of Nsiri may be inconclusive due to the small sample size used.

Bleeding time among both SCA in steady state was found in this study to be shorter than those of normal hemoglobin genotype. This shortened bleeding time among SCA children could be due to platelet hyperaggregation. This was similar to a study by Maina[19] *et al*, in Kenya, who noted an increased platelet aggregation time among patients in steady state when compared to control. The prolonged coagulation profile and shortened bleeding time is similar to that of Ataga[17] *et al*, who noted a curious paradox between these variables and traced it to nature's way of arresting bleeding in sickle cell patients.

Children with SCA have higher platelet count when compared with those with normal hemoglobin genotype. This is in keeping with the findings of Kenny[20] *et al*, who detected a significant increase in platelet number among 16 sickle cell adults when compared with controls. He noted similar changes among 12 splenectomized patients without SCA. The reason for this rise is traceable to the increased circulating population of young metabolically active platelets resulting from autosplenectomy. Although the platelet count among SCA children is higher than those with normal hemoglobin genotype in this study, they still fall within normal reference ranges.

The study confirms that though children with SCA in steady state have prolonged coagulation indices when compared with those with normal hemoglobin genotype, their coagulation values still fall within the reference ranges. It is also recommended that children with this disorder be screened for coagulation profile especially when in vaso-occlusive crises.

In addition, one should have a high index of suspicion of possible bleeding or thromboembolic episodes among these children especially when they are prepared for surgical procedures; this makes it expedient for screening for such coagulation variables.

Conclusion

The study confirms that though children with sickle cell anaemia in steady state have prolonged coagulation indices when compared with those with normal haemoglobin genotype, coagulation values still fall within the reference ranges.

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