Correlation between coagulation profile and haemoglobin concentration among children with sickle cell anaemia in steady state and crisis.

Chinawa JM1, Emodi I2, Ikefuna A, Ocheni S , Uwaezuoke SN

Department of Haematology & Immunology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria

Abstract

Sickle cell anaemia (SCA) may be associated with alterations in coagulation profile which may be associated with derangement of haemostatic mechanisms that may impart a thrombogenic tendency. This coagulation activation in SCA triggers haemolysis thus affecting haemoglobin concentration. The aim of this study is to determine the correlation if any between haemoglobin concentration and coagulation profile among children with SCA in steady state and crisis and those with normal HbAA genotype. To compare the mean values of haemoglobin concentration in steady state and crisis with those with normal haemoglobin genotype. This is a prospective observational study involving 50 children with SCA in steady state, 50 with vaso-occlusive crisis and 50 with HbAA genotype carried out from June 2009 through October 2009. All the values were matched for age and sex. Among sickle cell patients in steady state, haemoglobin concentration has a significant negative correlation with activated partial thromboplastin time r= (-0.33 for steady state) and (r=-0.26 for crises) and shows a negative correlation with prothrombin time, bleeding time and thrombin time. Haemoglobin concentration however shows a positive correlation with platelet count. Among sickle cell anaemia patients in vaso-occlusive crisis, haemoglobin concentration shows a significant positive correlation with platelet count (r=0.38), activated partial thromboplastin time(r=-0.26) and a significant negative correlation with prothrombin time(r=-0.29). Among normal HbAA patients, haemoglobin concentration shows no significant correlation with coagulation variables. The mean haemoglobin concentration(g/dl) of children with sickle cell anaemia in steady state (7.2$\pm$1.2) and crisis (6.8$\pm$1.7), were significantly lower than those with normal haemoglobin genotype (10.8 $\pm$1.2); P < 0.000. Haemoglobin concentration correlates positively with platelet and negatively with other coagulation profile among SCA in steady state and crises but not affected in normal HBAA genotype.

Introduction

Sickle cell anaemia (SCA) is a genetic haematological 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 disorder affects millions of people worldwide and is particularly common among people whose descents are from Africa [3]. In Nigeria it occurs in about 3% of the popula-

tion while the trait is about 18-25% [4]. Furthermore, in America, approximately 0.10% of the populations (most of whose ancestors are from Africa) are affected [5]. The disease occurs in about 1 in every 500 African births, as against 1 in every 1000 - 1400 Hispanic American births.

Sickle cell anaemia is associated with a hypercoagulable state that may contribute to certain morbidities such as vaso-occlusion and cerebrovascular accidents [6]. It is also worthy to note that decreased levels of natural anti-coagulant proteins (protein C and S) are observed in sickle cell anaemia and even more so in vaso-occlusive crisis [7,8].These reduced levels may be the consequence of chronic consumption of coagulation factors arising from increased thrombin generation and haemolysis which occur in the vascular endothelium [9].The correlations observed between haemoglobin concentration and plasma measures of coagulation activation suggest that the activated blood coagulation present in SCD is due, at least in part, to hemolysis with resultant scavenging of nitric oxide by cell-free plasma hemoglobin. These results are consistent with a recent report that hemolysis and decreased nitric oxide bioavailability appear to contribute to platelet activation in SCD-associated PHT [10].

This study therefore aims at determining the correlation, if any, and levels of some coagulation profiles among patients with sickle cell disorder in this environment when compared with subjects with normal haemoglobin 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 especially during crisis.

Methodology

The study was carried out at the children emergency room (CHER), children's outpatient (CHOP) and consultants' clinics of the Paediatrics Department of the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla, Enugu, Nigeria. The subjects were children with sickle cell anaemia in steady state aged 6months to 18yrs attending the sickle cell clinic. There are about seven hundred 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 to 20 patients. It is run by 3 Consultants, 2 Senior Registrars, and 4 Registrars.

The control population were children who were apparently healthy with normal heamoglobin genotype (HbAA) confirmed by haemoglobin electrophoresis. UNTH has a total bed space of 480 and provides specialized services in the major fields of medicine. It is a referral centre for various health centers in Enugu state and environs. The Paediatrics department comprises the children outpatient clinic (CHOP), the children emergency room (CHER), the general ward, and the new born special care unit

(NBSCU). The children outpatient clinic runs every weekday and a total of 840 patients are seen monthly.

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.

The subjects studied included known sickle cell anaemia patients aged between 6 months to 18 years considered clinically to be in bone pain crisis 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. Patients excluded include those with any type of infective illness and any patient with recent blood transfusion during the preceding 3 months.

The controls were patients with normal Haemoglobin AA genotype matched for age and sex. Children with sickle cell anaemia 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 haemoglobin electrophoresis) coming for follow up after recovery from an acute ill health like malaria and upper airway disease malaria at the children out patient department and the consultant's clinic

Prothrombin time was ascertained by delivering 0.1ml of plasma into a glass tube placed in a water bath with 0.1ml 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 0.1ml thrombin solution to 0.2ml of control plasma in a glass tube at 37^{oc} while the activity was timed with the stopwatch [11].

Platelet count and haemoglobin concentration were analyzed using automated Sysmex KX-21N model [11]. Bleeding Time was estimated using the Ivy's method [12].All the laboratory tests were also done on the control samples that had been matched with age and sex with the subjects.

Data was analyzed by SPSS version 13. An initial frequency count of all variables was done. The mean, ranges and standard deviation of the coagulation values in steady state were compared with values of normal haemoglobin genotype using ANOVA test. Correlation between Haemoglobin concentration, coagulation variables and bleeding time were compared using the Pearson correlation

variable. Haemoglobin concentration of subjects was compared with controls using student T test. The relationship between age, sex and coagulation profile were calculated using chi-square. The Level of significance was set at P ≤ 0.05.

Results

A total of 150 children aged 6 months to 18 years were recruited into the study; 100 were confirmed to have haemoglobin 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 haemoglobin AA genotype.

As illustrated in Table 1, the mean ages of subjects in steady state (8.41yrs±4.80), in crises (8.56yrs±5.29) and control (8.39yrs±5.38) were comparable; (p= 0.983).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). The sex distribution in the groups of patients was examined with the Chi-square test and the gross differences were not statistically significant (p= 0.644). This indicates that the groups were matched by age and sex.

Table 1 also shows that children with normal haemogobin AA had a significantly higher mean haemoglobin concentration (10.8±1.2); P=0.000, than sickle cell anaemia patients who were either in steady state or in crisis. However there was no significant difference between the haemoglobin concentration of sickle cell patients in steady state and those in crisis.

Table I. Demographic characteristics of subjects and control AND Comparison of haemoglobin concentration(g/dl) among children with sickle cell anaemia

	Steady state n=50 n (%)	Crises state n=50 n (%)	Normal Hb n=50 n (%)		
Age(in yrs)					
<5	11(22)	10(20)	13 (26)		
5-9	9(18)	11(22)	10(20)		
10-189	30 (60)	29(58)	2(4)		
	P= 0.018	f=0.983			
Gender					
Male	28(56)	26(52)	22(44)		
Female	22(44)	24(48)	28(56)		
	X^2=0.213	p=0.026			
Haemoglobin conc (g/dl)	**Steady state patients (n = 50)**	**Crisis state patients (n = 50)**	**Normal Hb AA patients (n = 50)**	**f**	**p**
Range (g/dl)	5.0-10.1	2.3-10.0	8.2-14		
Mean Hb (g/dl)	7.2 ± 1.2	6.8 ± 1.7	10.8 ± 1.2	121.4	0.000

The overall analysis (ANOVA) is significant (p =0.000)

Table II: Correlation between haemoglobin concentration and mean values of coagulation variables among subjects (HBSS in steady state and in crisis) and control (HBAA)

Hb Conc.	Mean PT	Mean APTT	Mean TT	Mean PC	Mean BT
Steady state					
4.0 – 5.9	13.2 ± 1.3	53.7 ± 13.8	12.5 ± 1.4	217.1 ± 153.6	3.8 ± 1.2
6.0 – 7.9	12.4 ± 1.2	41.1 ± 9.0	12.4 ± 1.2	306.0 ± 184.4	3.4 ± 0.9
8.0 – 9.9	12.3 ± 1.1	37.4 ± 5.9	12.0 ± 1.1	336.5 ± 168.9	3.1 ± 1.0
10.0 – 11.9	12.5 ± 2.1	40.0 ± 0.0	13.8 ± 1.8	308 ± 76.4	5.0 ± 0.0
	r = - 0.19	*r = - 0.33**	*r = - 0.03*	*r = 0.15*	*r = - 0.06*
Criss state					
2.0 – 3.9	14.1 ± 1.1	38.1 ± 5.2	13.5 ± 1.5	192.3 ± 120.3	2.9 ± 0.5
4.0 – 5.9	14.1 ± 2.9	43.8 ± 10.4	13.4 ± 1.1	193.8 ± 86.0	3.6 ± 0.4
6.0 – 7.9	12.3 ± 1.3	46.1 ± 12.2	12.0 ± 1.7	262.3 ± 138.0	3.2 ± 0.6

	PT	APTT	TT	PC	BT
8.0 – 9.9	12.1 ± 1.8	48.3 ± 11.4	12.9 ± 1.7	341.1 ± 163.3	2.9 ± 0.9
10.0 – 11.9	12.0 ± 0.0	45.0 ± 0.0	12.0 ± 0.0	635.0 ± 0.0	3.2 ± 0.0
	r = - 0.29*	*r = -0.26**	*r = - 0.20*	*r = 0.38***	*r = - 0.11*
Normal state					
8.0 – 9.9	11.0 ± 0.8	37.9 ± 7.6	9.5 ± 2.5	233.2 ± 86.5	3.4 ± 1.2
10.0 – 11.9	11.3 ± 0.8	40.3 ± 6.5	10.0 ± 0.8	206.4 ± 82.4	3.9 ± 1.0
12.0 – 13.9	10.9 ± 1.0	37.3 ± 5.1	10.0 ± 0.3	190.0 ± 60.8	3.6 ± 1.7
14.0 – 15.9	11.0 ± 0.0	13.0 ± 0.0	10.0 ± 0.0	220.0 ± 0.0	4.1 ± 0.0
	r = - 0.01	*r = - 0.21*	*r = 0*	*r = - 0.08*	*r = 0.16*

* - Significant ($p \leq 0.05$), ** - Significant($p \leq 0.01$), PT – prothrombin time, APTT – Activated Partial Thromboplastin Time, TT – Thrombin time, PC – Platelet count, BT – Bleeding time. r – correlation coefficient

Table II shows the relationship between haemoglobin concentration and coagulation variables.

Among sickle cell patients in steady state, haemoglobin concentration has a significant negative correlation with activated partial thromboplastin time r= (-0.33) and shows a negative correlation with prothrombin time. This is even lower with bleeding time and thrombin time. Haemoglobin concentration however shows a positive correlation with platelet count.

Among sickle cell anaemia patients in crisis, haemoglobin concentration shows a significant positive correlation with platelet count (r=0.38), activated partial thromboplastin time(r=0.26) and a significant negative correlation with prothrombin time(r=-0.29).

Among normal HbAA patients, haemoglobin concentration shows no significant correlation with coagulation variables.

Discussion

The findings of this study provided further evidence on the impact of coagulation abnormalities on haemoglobin concentration among children with sickle cell anaemia.
There is a significant negative correlation between haemoglobin concentration and most of the coagulation variables studied both in patients with sickle cell anaemia in crisis and steady state when compared with controls. These negative correlations portend the fact that low haemoglobin concentration may have an adverse effect on the haemostatic system. The positive correlation between haemoglobin concentration and platelet count was noted in both subjects in steady state and crisis, unlike in those with normal haemoglobin genotype.

Reduced haemoglobin concentration generates 2,3 diphosphoglcerate with reduced adenosine diphosphate. Lower aggregation threshold of adenosine diphosphate causes a significant increase in platelet number and micro-aggregate formation [12].These results are consistent with a recent report that hemolysis and decreased nitric oxide bioavailability appear to contribute to platelet activation in SCD-associated PHT [13]. Ataga et al [13] determine correlations between plasma measures of both coagulation and endothelial activation, and markers of hemolysis in SCD patients with and without PHT.

There is a negative correlation between Haemoglobin concentration and coagulation profile (PT, aPTT, TT). This is also in keeping with the study of Ataga et al who noted that hemoglobin concentration showed modest negative correlations with TAT, F1+2, D-dimers and sVCAM. In this present study we did not correlate haemoglobin concentration and TAT, F1+2, D-dimers and sVCAM due to lack of facility. Correlations observed between markers of hemolysis and plasma measures of coagulation activation suggest that the activated blood coagulation present in SCD, is due, at least in part, to hemolysis with resultant scavenging of nitric oxide by cell-free plasma hemoglobin. These results are consistent with a recent report that hemolysis and decreased nitric oxide bioavailability appear to contribute to platelet activation in and coagulation activation SCD-associated PHT [13].Several factors, including abnormal red blood cell phospholipid membrane asymmetry, with increased expression of phosphatidylserine, and ischemia-reperfusion injury appear to contribute to these phenomenom [14,15]. Children with sickle cell anaemia both in steady state and crisis have significantly lower haemoglobin concentration when compared to control. This may be due to premature haemolysis and reduced red blood cell lifespan in these subjects [16, 17].

It is however striking to note that some children with sickle cell anaemia in steady state have relatively high haemoglobin concentration. This may be due to high levels of fetal haemoglobin (HbF) as found by Ibia [18]. He noted that red blood cells which contain HbF, in addition to HbS, were less susceptible to haemolysis than the cells without HbF.

Conclusion

There are positive correlation between haemoglobin concentration (HB) and platelet count and a negative correlation between HB and PT, aPTT and TT. This shows that the lower the HB the more probable will a sickle cell pa-

tient be exposed to either thrombosis or possible bleeding; a curious paradox!

Competing Interest

The authors hereby declare that we have no competing interests.

Authors' Contribution

All the authors made substantial intellectual contributions to this study. JMC was involved in the conception, design, and data collection, interpretation of results, preparation of the manuscript, revision of the article at various stages and preparation of the final draft. JMC, SO,SU, IE and AI made substantial contributions in the design, data collection, and interpretation of the results, preparation of the manuscript, revision and preparation of the final draft. IEO undertook analysis of the data and also participated in the interpretation of the results and preparation of the final draft.

Acknowledgements

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Correspondence to:
Josephat Chinawa maduabuchi
College of Medicine
Department of Paediatrics
University of Nigeria Enugu Campus
Enugu, Nigeria