Original Article

Serum Zinc Level during and after Acute Painful Episodes in Children with Sickle Cell Anemia at the Aminu Kano Teaching Hospital, Kano, Northern Nigeria

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Abstract

Background: Acute painful crisis due to vaso-occlusive event is the leading cause of hospitalization in patients with sickle cell anemia (SCA). Zinc deficiency in children with SCA is associated with increased frequency and severity of acute painful events. We determined serum zinc level in children with SCA during acute painful crisis and compared the same with children with SCA who are in steady state and healthy non-sickle cell disease children. **Subjects and Methods:** This was a descriptive longitudinal study, involving children with SCA age 6 months to 15 years at Aminu Kano Teaching Hospital, Kano, Northern Nigeria. Subjects were recruited into three groups, which consisted of SCA in acute painful crisis, SCA in steady state, and normal subjects with hemoglobin AA (HbAA). A total of 210 subjects were recruited, 70 subjects each for SCA in acute painful crisis, SCA in steady state, and HbAA groups, respectively. Serum zinc was analyzed with atomic absorption spectrophotometery. Serum zinc levels were repeated in children with SCA and acute painful crisis 4 weeks after resolution of painful events. **Results:** The mean serum zinc level of SCA with acute painful crisis was higher than SCA in steady state, but the difference was not statistically significant (24.4 [11.0] and 23.4 [7.4]) µg/dL, respectively (t = 16.04, P = 0.54). While the HbAA control had significantly higher mean serum zinc level than SCA groups, both in acute painful and in steady state (F = 59.3, P = 0.001). Among children with SCA and acute painful crisis, repeat serum zinc level 4 weeks after resolution of acute painful events was significantly higher than during pain crisis (t = 64, P = 0.001). **Conclusion:** Zinc deficiency occurs in children with SCA and the deficiency is worsened by acute painful events Therefore, it is recommended that zinc level should be assessed and any deficiency treated. Supplementation of zinc should also be enhanced as this may reduce painful crisis in SCA.

Keywords: *Acute painful crisis, sickle cell anemia, zinc*

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Introduction

Sickle cell anemia (SCA) is the homozygous state of sickle cell disease (SCD) and it is the most common and the most severe variant of hereditary blood disorders in children.[1]

Children with SCA frequently experience acute painful crisis, the most common vaso-occlusive event. It may occur in any parts of the body, but commonly in the back, the chest, and the extremities.[2] Vaso-occlusion leads to hypoxia, ischemia, and vascular inflammation, which underlying pathophysiology of sickle cell pain.[3,4] Children with SCA have increased oxidative stress and peroxidation as well as low antioxidant potential which

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predisposes SCA to vaso-occlusive crisis.[5,6] The effects of zinc as an antioxidant is by inhibiting the lipid peroxidation which occurs in red blood cells and liver thereby stabilizing biomembranes and biostructures, thus protecting the body against oxidative stress.[6,7] These effects of zinc are believed to give zinc its ability to reduce vaso-occlusive crisis in SCA. Increased hemolysis of red blood cell in patients with SCA releases a considerable amount of zinc, which circulates in the plasma pool. This results in an increase in glomerular filtration of zinc, but its reabsorption is hampered by the renal tubular damage caused by repeated vaso-occlusive episodes.[8] The resultant hyper-zincuria due to increased hemolysis increases the daily requirement for zinc significantly in patients with SCA. This increased requirement for zinc is not met by the usual dietary intake.[9] Zinc deficiency is further worsened in children with SCA, especially those children from poor socioeconomic background as their parents may not be able to afford zinc rich diets such as meat, sea foods, milk, plantain, cocoa products, and fish.

In previous studies in the United States, Carpentieri *et al*.,[10] and Leonard *et al*.[11] reported zinc deficiency as high as 60% and 44%, respectively, in children with SCD.

Temiye *et al*.[12] in South-Western Nigeria conducted a cross-sectional descriptive study comparing the serum zinc level of SCA subjects in painful crisis and compared with SCA in steady state and healthy genotype AA children. Significantly, lower mean serum zinc was observed among children with SCA with painful event compared with the other two groups. The study compared the mean serum zinc level of each subset with 24 h dietary recall and the food was classified as low, moderate, and high zinc-containing diet. A strong correlation was observed between the type of diet of these children and the serum zinc profile. This may also explain the lowest mean serum zinc observed among the SCA in painful episodes as only 28% of these subjects had meal with high zinc as against 48% and 72% among the SCA in steady state and healthy hemoglobin AA (HbAA) subjects, respectively. However, the serum zinc status of the subjects with painful crisis was not measured after resolution of the painful episode for better comparison which forms the basis for this study.

Nwaoquikpe *et al*.,[13] in Owerri, Nigeria, assessed antisickling effects of some micronutrients namely, Zn, Mg and Cu and antioxidant vitamins (A, C, and E) on sickled red blood cell against extracts of Zn, Mg and Cu. The study found that zinc inhibits polymerization rate of sickled RBC, hence has antisickling effects as supported by Brewer *et al*.[9] and Chow.[7] The study[13] also documented zinc as a strong antioxidant because it improves Fe2+/Fe3+ ratio; this was also reported by Adelekan *et al*.[6]

Prasad *et al*.[14] in 1976 administered oral zinc sulfate to 10 adults' subjects who had severe vaso occlusive crisis (VOC) and found apparent symptomatic relief in 8 subjects presumably related to antisickling effect of zinc. These findings were, however, difficult to draw conclusion from, because there were no controls, and the sample size was very small.[14] Similar observation was made by Guptal *et al*.[15] in 1995 in a placebo-controlled double-blind study. A total of 145 adult sickle cell subjects were treated with either 220 mg of zinc sulfate three times daily or placebo. After 18 months, the zinc-treated subjects had an average of 2.5 crises, compared with 5.3 in the placebo group, but the severity of painful crisis was not reduced as measured by the duration of hospital stay.

Subjects and Methods

Study design

Ethical approval for the study was obtained from the Ethics Committee of Aminu Kano Teaching Hospital (AKTH), Kano [Appendix I]. Written informed consent [Appendix II] and assent where appropriate were also obtained. It was a descriptive [Appendix III] longitudinal study involving children with SCA age 6 months to 15 years who were hospitalized for acute painful crisis defined as a pain episode related to SCD in the extremities, back, abdomen, chest, or head lasting for at least 2 hours and leading to a clinic visit or hospitalization,[2] and the pain should have occurred within 48 hours as at the time of recruitment.[2]

The study period is between November 1, 2014, and June 30, 2015. Inclusion criteria for the study included the following: (1) SCA with acute painful events 6–180 months admitted into Paediatric Emergency Unit, first category as cases (those who required fluid and analgesics only for pain management), (2) second category is SCA in steady state as first control group, and (3) apparently healthy HbAA second control group age- and sex-matched.

1. Exclusion criteria included the following: (1) pyrexia (axillary temperature of >37.2°C; (2) acute infection such as respiratory tract infection and malaria; (3) localized area of swelling on the limb or any other symptoms suggesting acute or chronic osteomyelitis; (4) children on zinc or zinc-containing supplement, blood, or blood products transfusion in the preceding 3 months; (5) children known to have chronic medical conditions such as human immunodeficiency virus, tuberculosis and chronic renal

failure; (6) subjects on drugs such as captopril, proton pumps inhibitors (omeprazole, rabeprazole), thiazide diuretics, tetracycline, and sodium valproate that inhibit zinc absorption. Additionally, subjects on hydroxylurea were excluded.

The comparison groups included children with SCA who were in steady state (defined as subjects who were apparently well without any evidence of recent infection, bone pain, or other medical problems whether it is related SCA or not to for at least 4 weeks before recruitment and no transfusion of blood or blood products for at least 3 months[16]), who attended the SCD clinic at AKTH, and apparently healthy non-SCA children (HbAA), among them are siblings of SCD children and others recruited from well-baby clinic of Paediatric Outpatient Department. Children with SCA and acute painful events were followed up in sickle cell clinic after discharge, where they have their blood sample taken for repeat zinc analysis at least 4 weeks after resolution of painful crisis.

Case record form was used to obtain relevant demographic and clinical information: patient's age, age at diagnosis, sex, weight and length/height, and parents' level of education. Parental socioeconomic class was assigned using Olusanya's classification.[17] additional information included 24-h dietary recall, previous hospitalizations for painful crisis, and blood transfusions. Each participant was also examined for the presence or absence of fever, palor, jaundice, and the presence of spleen and liver enlargement.

Sample collection and laboratory methods

The skin overlying the vein was thoroughly cleaned with providone iodine and methylated spirit in circular manner and allowed to air dry. Blood was obtained by inserting size 21-gauge stainless steel needle with attached propyl syringe into any prominent superficial vein in the dorsum of the hand or antecubital fossa. Three millilitres of blood was drawn, dry cotton wool was applied at the site, and gentle pressure was applied till bleeding stopped. The blood was inoculated into zinc-free plain bottle (which has been soaked overnight in 10% nitric acid to avoid zinc contaminants[18] before blood sample collection).

Blood sample in the plain bottles was centrifuged at 3000 rpm for 10 min within 1 h of sample collection in the Paediatric Department side laboratory. The serum was removed with zinc-free pipette prepared using the same method with zinc-free bottle and the serum was transferred inside another zinc free plain bottle. Serum sample was kept in a refrigerator at a temperature of −22°C pending analysis.[18]

Determination of serum zinc level

Participants' serum zinc levels were estimated using flame atomic absorption spectrophotometer (AAS), Buck Scientific Model 210VGP® AAS. Instructions written in the manual were carefully followed. Zinc analysis was carried following Smith *et al*.[19] Quality control was done with zinc powder and nitric acid before and after 10 samples.

Statistical analysis

Data were analyzed using SPSS version 16. Descriptive statistics (mean and standard deviation) were used to summarize baseline demographic data that were continuous and normally distributed. Categorical data were described as frequencies and percentages. For variables that were continuous and normally distributed, Student's *t*-test was used to compare the means of two variables. Paired *t*-test was used to compare the means of two variables of the same group, that is, serum zinc during pain crisis and after resolution of painful episodes. Analysis of variance was used to compare means of more than two variables. A *P*-value of less than or equal to 0.05 was considered significant.

Participants Enrolment

A total of 297 families were approached to participate in the study, of which 71% (210) eligible particicpants consented and were enrolled as shown in Figure 1.

Results

A total of 210 participants were enrolled, of which 140 were children with SCD (70 with pain events and 70 in steady state) and 70 non-SCD (HbAA) controls were enrolled in the study. Among participants with SCA and acute painful events, 5 were excluded from the study (3 due to recurrent acute painful events and 2 were lost to follow up) and 65 were included in the final analysis. The mean age of study participants in the three groups was 90.7, 89.7, and 91.3 months, respectively. No statistically significant differences were observed in age and sex in the sickle cell groups and the non-SCD controls.

Subjects with SCA (inclusive SCA in acute painful crisis and those in steady state) had significantly lower mean weight compared with HbAA controls and the difference was statistically significant ($F = 8.27$, $P = 0.001$). No statistically significant differences were observed in the parental socioeconomic status considering the three groups together [Table l]. But the socioeconomic status was significant when compared with the SCA group.

The mean serum zinc level of subjects with SCA in acute painful crisis was higher than those of children with SCA in steady state, but the difference was not

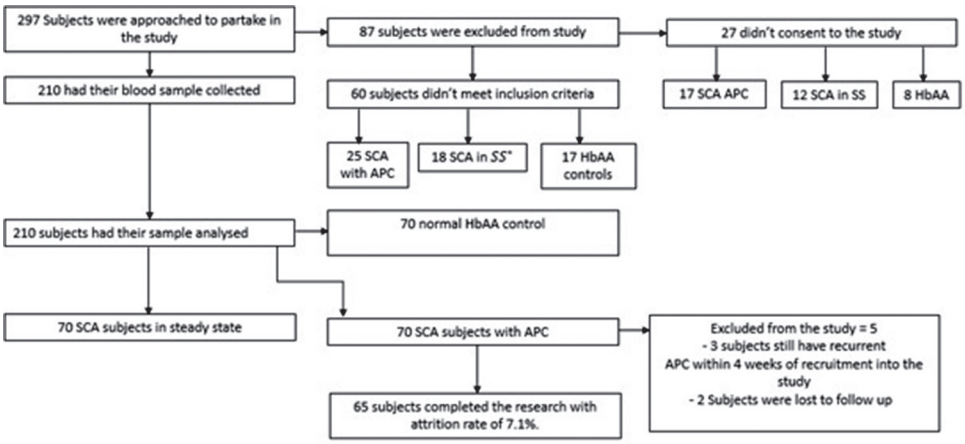

Figure 1: Breakdown of participantsæ enrollment

Table 1: Sociodemographic characteristics of the study population (*n*=210)

Variable	SCA-APC (*n*=70)	SCA-SS* (*n*=70)	HbAA subjects (*n*=70)	Test statistic	*P*
Age (months)					
Mean (SD)	90.7 (45.6)	89.7 (44.6)	91.3 (46.0)	0.22[1]	0.98
Range	7-180	6-180	6-180		
Gender					
Male, *n* (%)	38 (54.3)	38 (54.3)	38 (54.3)	0.00[2]	1.00
	32 (45.7)	32 (45.7)	32 (45.7)		
Weight (kg)					
Mean (SD)	20.2 (6.6)	19.8 (6.7)	24.3 (8.3)	8.27	0.001
Range	5 to 38	7 to 40	8 to 43		
Height (cm)					
Mean (SD)	113.5 (20.7)	111.9 (22.1)	116.4 (23.3)	0.75	0.47
Range	69 to 152	63 to 153	69 to 158		
Female, *n* (%)					
SES, *n* (%)					
Upper class	25 (35.8)	10 (14.3)	15 (21.4)	14.41[2]	0.072
Middle class	23 (32.9)	23 (32.9)	24 (34.3)		
Lower class	22 (31.4)	37 (52.9)	31 (44.3)		

n=Frequency; SCA=Sickle cell anemia, SS*=Steady state; APC=Acute painful crisis; HbAA=Hemoglobin AA; SES=Socioeconomic status, [1]F – analysis of variance, test; [2]Chi-square test; *SCA in steady state

Table 2: Comparison between the mean serum zinc of children with SCA in acute painful crisis and those in steady state

Age (months)	Freq (*n*)	Mean serum Zn APC (SD), µg/dL	Mean serum Zn SCA-SS (SD), µg/dL	Test statistic	*P*
6-30	8	22.9 (14.5)	24.9 (6.4)		
31-56	8	24.1 (7.5)	25.7 (7.9)		
57-82	17	26.7 (11.2)	23.9 (7.5)		
83-108	13	25.2 (12.7)	24.9 (7.6)		
109-134	10	25.9 (10.0)	23.5 (7.7)		
135-160	9	22.0 (11.1)	17.7 (4.3)		
161-180	5	18.7 (7.8)	21.4 (9.1)		
Total		24.4 (11.0)	23.4 (7.4)	16.04[1]	0.54

MSZ=Mean serum zinc; *n*=frequency; SCA=Sickle cell anemia; SS* = SCA in steady state; APC=Acute painful crisis, Zn=Zinc, [1]Independent *T*-test

statistically significant (F = 16.04, P = 0.54) [Table 2]. The control (HbAA) subjects had significantly higher mean serum zinc level than SCA subjects (both in steady state and with painful crisis) and the difference was statistically significant (F = 59.3, P = 0.001) [Table 3]. Among subjects with SCA with acute painful events,

Table 3: Comparison between the mean serum zinc of HbAA subjects with that of SCA subjects with painful crisis and SCA in steady state

Age (months)	Freq (*n*=70)	MSZ APC (SD) µg/dL	MSZ SCA SS* (SD) µg/dL	MSZ HbAA (SD) µg/dL	Test statistic	*P*
6-30	8	22.9 (14.5)	24.9 (6.4)	42.1 (6.8)		
31-56	8	24.1 (7.5)	25.7 (7.9)	50.4 (12.6)		
57-82	17	26.7 (11.2)	23.9 (7.5)	49.1 (15.6)		
83-108	13	25.2 (12.7)	24.9 (7.6)	49.4 (15.4)		
109-134	10	25.9 (10.0)	23.5 (7.7)	44.4 (11.8)		
135-160	9	22.0 (11.1)	17.7 (4.3)	48.0 (11.9)		
161-180	5	18.7 (7.8)	21.4 (9.1)	51.6 (28.3)		
Total		24.4 (11.0)	23.4 (7.4)	48.9 (14.4)	59.3[1]	0.001

n=frequency; MSZ=Mean serum zinc; SCA=Sickle cell anemia; SS*=SCA in steady state; APC=Acute painful crisis; HbAA=Hamoglobin AA, [1]*F* – analysis of variance

Table 4: Mean serum zinc level during painful crisis and 4 weeks after resolution of painful episodes among SCA subjects (*r*=65)

Variables	SCA-APC* *n*=65	SCA-SS *n*=65	Test statistic	*P*
Serum Zn, µg/dL Mean (SD)	24.6 (11.0)	29.8 (11.2)	64.0[1]	0.001

SCA-APC*=SCA with acute pain crisis; SCA-SS=SCA ≥4 weeks after resolution of APC following discharge; Zn=Zinc, [1]Paired *T*-test

repeat serum zinc level 4 weeks after resolution of acute painful crisis was higher than during acute painful events and the difference was statistically significant (t = 64, P = 0.001) [Table 4].

Discussion

The mean serum zinc level observed in this study in children with SCA was lower than the World Health Organization (WHO) normal reference value.[20] Recurrent hemolysis, defective zinc homeostasis, and increase urinary loss due to abnormal renal tubular reabsorption are known predisposing factors to low serum zinc in children with SCA. In addition, acute painful crisis has been shown to cause further decrease in serum zinc level.[12] These may have been responsible for the lower serum zinc level in children with acute painful episode. This finding was like that reported by Temiye *et al*.,[12] in Lagos, who reported significantly low mean serum zinc among children with SCA during painful episodes. The lowest mean serum zinc was observed in the oldest age category [161–180 months] compared with other age group in children with SCA during pain crisis in this study. This finding may be explained by the facts that the older SCA have more painful crisis. These recurrent pain events might have been a predisposition to the much lower serum zinc among the older age group as reported by Olabode *et al*.[21] The mean serum zinc level among subjects with SCA in acute painful crisis was surprisingly found to be higher than those in steady state, although the difference was not significant (P = 0.54). Studies have shown that serum zinc level assumes a diurnal peak once in the early hours of the day[22-24] and its level was said to be lower following repeated feeding as suggested by Hotz *et al*.[21] Although the confounding effect of diurnal variability was controlled for in this study, as all samples were taken at the same time of the day between 8 am and 12 noon. Another possible explanation could be that in our study we found more children with acute painful crisis from a relatively upper socioeconomic class compared with those in steady state. This could imply better nutrition for children with acute painful crisis. Kehinde *et al*.,[24] from Lagos, South western Nigeria, reported similar lower serum zinc among subjects with SCA in steady state relative to those with acute painful episodes. However, this contrasts with the study by Temiye *et al*.[12] who found significantly higher mean serum zinc in children with SCA in steady state compared with those in acute painful event. Similarly, 24 h dietary recall revealed that most subjects in steady state were on high zinc diet compared with children with acute painful crisis whom were on moderate zinc diet. This could have accounted for the higher mean serum zinc levels found in subjects in steady state in the study.[12]

A significantly higher serum zinc level was observed among HbAA controls compared with the levels in SCA patients in steady state, and sickle cell anemic children with painful crisis. Reasons may be that HbAA controls do not have additional demand for zinc and they are not prone to increase loss as found in children with SCA. This finding of higher mean serum zinc among the normal HbAA subjects corroborates what was observed by Temiye *et al*.[12] and Idonije *et al*.[25] Additionally, highest zinc level was found among the oldest age group [161–180 months]. HbAA children in this study compared with the younger age, although the difference was not statistically significant. Phytate has been shown

to form a complex with zinc and limits its intestinal absorption,[26,27] hence wide use of phytate-rich grains as weaning diet coupled with low concentration of zinc in breast milk[27] may have resulted in lower serum zinc levels among the toddlers. Similarly, Hotz *et al*.[23] also showed a lower serum zinc level among toddlers, with a steady increase across the older age groups with the peak found in the adolescent age group. The finding of higher mean serum zinc levels among the HbAA subjects in this study was like what was observed in Benin study,[25] southern Nigeria. The difference may be due to the geo-regional differences in the availability and consumption of zinc-rich diet. Food items rich in zinc,[28] for example, groundnut, red meat, and porridge (millet and sow milk) are routinely consumed in the Northern parts of the country. Similarly, plantain and melon soup (important sources of zinc) are widely consumed in South-South region of the Nigeria. In contrast, in the South-Western region, there exists cultural believes that prevents children from being fed with proteins such as fish and meat which[29] may have been accounted for the lower serum zinc levels observed in the Lagos study. Nevertheless, the mean serum zinc levels in the three-study group were lower than the WHO reference value.[20] Excessive cooking, which is a norm in our society, has been shown to deplete the zinc content of food[8] and may have been responsible for the lower mean serum zinc noted in this study.

It was observed that the repeat serum zinc level after resolution of acute painful event was higher than the mean serum zinc during acute painful crisis in the cases. The difference was statistically significant compared with the value during painful events. This may be due to associated increased demand and utilization of zinc during acute painful episode.[12,25] Furthermore, as suggested by Omoti in Benin,[30] painful crisis may cause elevation of white cell count and cytokine production. Cytokines have been shown to stimulate hepatic metallothionein, a low-molecular-weight binding protein that facilitates the intracellular shift of circulating zinc into the hepatic cell. Similarly, painful episodes also interfere with nutrition which may lead to lower serum zinc levels.[12] Hence, the increase in serum zinc levels after resolution of the crisis may depict recovery from painful crisis.

Conclusion

Sickle cell anemic subjects with acute painful crisis had lower mean serum zinc levels, and the value increased after resolution of the painful events. Subjects who are HbAA control group had highest mean serum zinc compared with children with SCA both during acute painful events and in steady state. The mean serum zinc levels observed in this study was lower in both sickle cell group and HbAA subjects compared WITH WHO reference value of 65 μg/dL for African children.

Limitation of the study

1. The type and quality of zinc content in the diet were not classified
2. The influence of some factors, which could affect serum zinc levels, such as malaria, degree of anemia, HbF levels, and use of hydroxyurea, was not assessed.

Recommendation

From this study, it is recommended that

1. Serum zinc level should be ascertained in sickle cell anemic patients
2. Health education and public enlightenment should be launched to prevent zinc deficiency in children with SCA
3. Further research should be undertaken for zinc supplementation trial in subjects with SCA to see whether there will be changes in the number of painful episode or reduction in duration or intensity of painful events.

Line of future research

1. To evaluate the effects of zinc therapy on the severity and resolution of acute painful crisis
2. Role of zinc supplementation on the frequency of acute painful episode in children with SCA.

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Conflicts of interest

There are no conflicts of interest.

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AKTH/MAC/SUB/12A/P-3/VI/1207 **30th December, 2013**

Dr. Ahmed A. Kudirat
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AKTH, Kano

Ufs:

The Head of Department
Paediatrics
AKTH, Kano

ETHICAL APPROVAL

Further to your application in respect of your research proposal titled "Serum Zinc Level during Acute Painful Crisis among Children with Sickle Cell Anaemia at Aminu Kano Teaching Hospital, Kano", the Committee reviewed the proposal and noted same as a Prospective Study.

In view of above, Ethical approval is given to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Ethical Committee.

Regards

Barrister Bara'atu Aliyu-Datti
Secretary, Research Ethical Committee
For: Chairman

Appendix I: Ethical approval

ASSENT FORM

I _____ have been fully informed about the study titled SERUM ZINC LEVEL DURING ACUTE PAINFUL CRISIS AMONG CHILDREN WITH SICKLE CELL ANEAMIA AT AKTH KANO, and I have agreed to participate in the research with the right to opt out anytime. I am aware the study will involve taking blood samples and it is part of my diagnostic investigation and the risks are the same for any patient going for any test that involves taking blood samples.

Information obtained will be confidential and the outcomes of the research would help improve the care of patients with sickle cell anaemia. None participation in this study would not jeopardise treatment.

Name of patient: _____

Signature/thumbprint: _____

Name of witness: _____

Date: _____

Appendix III: Assent forma 13

Consent Form

This is to satisfy that I willingly agreed for my child/dependant to be enrolled into the study titled SERUM ZINC LEVEL DURING ACUTE PAINFUL CRISIS AMONG CHILDREN WITH SICKLE CELL ANEAMIA AT AKTH KANO

The details of the study and its implications have been explained to me by the researcher Dr Kudirat Ahmed that I will be ask some questions about my child/dependant. That my child/dependant will be examined by a doctor, the study will involve taken 3mls of blood from my child/dependant. That I will not be asked to pay for the test and it is not compulsory for me to participate in the study. The information and details about my child/dependant will remain confidential. Data obtained from me about my child's condition will not be disclosed or used to identify me or my child/dependant for any other reason besides in treating and caring for my child/dependant.

Name of patient: _____ Name of Guardian: _____

Signature/thumbprint: _____ Signature of the witness: _____

Name of witness: _____

Date: _____

Appendix II: Consent form