Serum zinc status in sickle cell anaemia children at the Jos University Teaching Hospital, Jos, Nigeria.

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Abstract

Background: Several clinical manifestations of sickle cell anaemia (SCA) have been associated with zinc deficiency. Determining the zinc status of children with SCA in Nigeria, a country that accounts for the highest burden of the disease worldwide, will provide a template that could assist in critically appraising the need or otherwise for zinc supplementation or fortification programmes in these children.

Methods: This was a cross-sectional comparative study conducted at the Jos University Teaching Hospital, Jos, Nigeria among 700 children (350 SCA patients and 350 age and sex matched hemoglobin AA controls). Serum zinc was analysed using the atomic absorption spectrophotometry.

Results: The median serum zinc concentration of children with SCA was 6(3-7) μmol/l and it was significantly lower than that of the controls 8(4-9) μmol/l, p = 0.04. The prevalence of zinc deficiency in this study was 67% in children with SCA compared with 34% in the control group, (p<0.0001). The proportion of zinc deficient patients was more among children from lower socio economic class (68.5%, 35.5%) than in the upper socio economic class (38.5%, 16.3%) in both cases and controls groups respectively.

Conclusion: There is a high prevalence of zinc deficiency in the study population especially among those with sickle cell anaemia. Zinc supplementation or fortification should be considered as part of intervention strategies to improve the zinc status of these children particularly those with sickle cell anaemia.

Key words: Serum zinc, Sickle cell anaemia, children, Jos, Nigeria

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Introduction

Sickle cell anaemia (SCA) is the most common genetic disorder of the black race and Nigeria accounts for the highest burden of the disease worldwide.[1, 2] It is a disorder with a high potential for oxidative damage due to a chronic redox imbalance in red cells that often results in continuous generation of reactive oxygen species (ROS).[3]The production of ROS can be amplified in response to deficiency in antioxidant vitamins and trace elements thereby contributing to the severity of sickle cell manifestations.[4, 5]

One of the essential trace elements with antioxidant properties and ability of protecting cells in the body from the potential damage caused by free radicals is zinc.[6] Zinc exerts its antioxidant action by inhibition of lipid peroxidation thereby stabilizing biomembranes and biostructures thus protecting the body against iron-catalysed free radical generation and damage.[7]Zinc is also necessary for normal growth and development through its involvement in protein synthesis, epithelial repair and synthesis of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).[8] It is also necessary for the integrity and normal functioning of the immune system.[9] Zinc deficiency has been associated with several clinical manifestations of SCA such as growth retardation, hypogonadism in males, hyperammonemia, abnormal dark adaptation, delayed wound healing and cell mediated immune disorder.[10] The primary pathogenesis of zinc deficiency in SCA patients is as a result of increased haemolysis with the consequent release of a considerable amount of zinc into the circulation thereby leading to increase in glomerular filtration of zinc. However, the reabsorption of the filtered zinc from the renal tubules is impaired as a result of renal tubular damage caused by repeated vaso-occlusive episodes that occur in SCA patients leading to excessive loss of zinc in the urine.[11] The excessive urinary zinc losses and a high protein turnover resulting from the increased hemolysis increase the daily requirement for zinc significantly in SCA patients. This increased requirement for zinc is not met by the usual dietary intake thereby predisposing SCA patients to developing zinc deficiency.[9, 12]Other mechanisms of developing zinc deficiency include poor dietary intake of zinc and hypoalbuminemia. About 80% of zinc is transferred in the plasma bound to

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albumin; therefore any condition that causes hypoalbuminemia can lead to zinc deficiency.[13]

Studies on the zinc status in SCA children are scanty in Nigeria and especially in the northern part where there is high prevalence of malnutrition.[14] This study determined the serum zinc status of sickle cell anaemia children aged 1 to 18 years and compared it with age and sex matched haemoglobin AA subjects in Jos, North Central Nigeria. The findings from this work will add to the existing knowledge on zinc nutrition among sickle cell anaemia patients and could provide a template that could assist in critically appraising the need or otherwise for zinc supplementation or fortification programmes in SCA children in Nigeria.

Methods

Study location

The study was conducted at the Paediatric Haematology/Oncology clinic and General Paediatric Outpatient clinic of the Jos University Teaching Hospital, (JUTH) Jos, North Central Nigeria. The hospital provides primary, secondary and tertiary health care to the people of Plateau State and seven other neighbouring States. The Paediatric Haematology/ Oncology clinic runs weekly with an average clinic attendance of forty- five patients per week

Jos, the capital of Plateau State of Nigeria, is located in the middle belt of the country. It stands at a height of about 1,250 metres above the sea level. Based on the 2006 population census [15] Plateau State and Jos have an estimated population of about 3.2 million and 900,000 respectively. The staple foods grown in Jos include acha, maize, millet, Irish potato, yam, cassava, fruits and vegetables.[16.]

Study design

This was a cross-sectional comparative study of sickle cell anaemia and haemoglobin AA children conducted from January to March, 2012.

Ethical consideration

Written informed consent was obtained from the parents. The aims and objectives of the study were explained to the parents or caregivers, and the procedure to be done was explained to the patients if up to 6 years of age. Approval for the study was obtained from the Ethics Committee of the Jos University Teaching Hospital. Participation in the study was voluntary and at no cost to the patients.

Estimation of sample size

Using the plasma zinc concentration of 31μg/dl from a previous Nigeria study [17], an absolute standard error of 0.05, a standard normal deviate of 1.96 and an attrition rate of 5%, a minimum sample size of 350 was calculated for each group using the appropriate formula.[18]

Inclusion and exclusion criteria

Subjects aged one to 18 years with haemoglobin SS and haemoglobin AA were recruited into the study as cases and controls respectively after a written consent had been obtained from the parents/caregivers and assent from the older children. Subjects on zinc supplements or those with any chronic illnesses which could affect zinc level e.g. Down syndrome, liver cirrhosis, protein energy malnutrition, malabsorption syndrome, diabetes mellitus, acquired immunodeficiency syndrome, cystic fibrosis etc. were excluded from the study.

Study population

Study population consisted of SCA and hemoglobin AA children aged between one and 18 years attending the Haematology/Oncology Clinic (cases) and the General Paediatric Out-patient clinic (controls) of the Jos University Teaching Hospital (JUTH) respectively.

Patient selection and data collection

The haemoglobin genotypes of consecutively consenting subjects and, age and sex paired controls were determined using cellulose electrophoresis.

For each selected SCA patient, a healthy subject with haemoglobin genotype AA matched for age and gender was selected as control. The controls were consecutively selected from the General Paediatric Out-patient clinic of JUTH. They were well children on follow up who had been treated for non-chronic illnesses, at least 4 weeks prior to recruitment. A standard consent form was read to the parents or care givers in their language. Further clarifications were given on request.

Personal data, medical and social information were obtained using questionnaire directly administered by the investigators. The occupational and educational status of the parents were obtained from which their socioeconomic status were classified using the Olusanya et al [19] index scoring method.

Specimen collection

Five millilitres of blood was collected from the antecubital vein or any other easily accessible superficial vein on the forearm from each patient after thorough cleaning of the overlying skin with 70% alcohol. A new plastic syringe with a stainless steel needle was used for each venepuncture. The blood specimens were collected into a plain bottle, and the serum separated after spinning at 3000 revolution per minute (rpm) within 30 minutes of collection. The serum samples were analysed for zinc using Atomic Absorption Spectrophotometer (PU9100X, Philips, Holland).[20] Serum zinc value less than 7.65μmol/l was categorised as low (zinc deficient) while serum zinc value greater than or equal to

7.65µmol/l was categorised as normal (not zinc deficient).[21]

Whole blood was also collected into a heparinised capillary tube. This was placed in the microhaematocrit machine and centrifuged at a speed of 1200 rpm for 5 minutes. The packed cell volume (PCV) was determined by measuring the red cell column on the haematocrit reader. The PCV values were categorized as normal, mild and moderate anaemia based on the World Health Organisation cut-offs for anaemia.[22]

Statistical analysis

The initial data cleaning was done using Epi-Info 3.5.1 and final data cleaning, recoding and analysis was done using SAS version 9.1. A p-value <0.05 was considered to be statistically significant. The median values of continuous variables such as the age, serum zinc concentrations of the cases and controls were compared using Mann Whitney- U test while categorical variables such as the gender, socioeconomic status of the parents of the subjects were compared using the Chi-square test.

Results

Characteristics of the study population

Seven hundred children aged 1 to 18 years were recruited into the study of which 350 were sickle cell anaemia patients (cases) and 350 were age and sex-matched controls. There were 358 (51.1%) males and 342 (48.9%) females with a male to female ratio of 1.1: 1.0. The median ages of the cases and controls were 8 (4-10) years for both groups, (p= 0 .69). About two third of the children in both the subjects and controls were between ages of 1 and 10 years (Table 1).

Table 1 also shows that the male to female ratios in the subjects and controls were comparable (p=0.88). Over 40% of the children were from the lower socioeconomic class. The subjects and controls were however similar in terms of socioeconomic class (p=0.34)

Median serum zinc concentration of the study population

The median serum zinc concentration of children with sickle cell anaemia was 6 (3-7) µmol/1 and was lower than that of the controls 8 (4-9)µmol/l, the difference was significant (p = 0.04). The sickle cell anaemia children had lower mean serum zinc levels than the controls in all the age groups (Table 2).

Prevalence of zinc deficiency in the study population

Two hundred and thirty five (67%) children in the sickle cell anaemia group had low serum zinc (less than 7.65 µmol/l) compared with one hundred and twenty children (34%) children in the control group, this was statistically significant (p<0.0001) (Table 2).

Comparison of median serum zinc concentration and degree of anaemia

The cases have lower median serum zinc concentrations than the controls at different levels of anaemia and the difference was statistically significant (p<0.05). In both cases and controls, moderate anaemia was associated with the lowest median serum zinc concentrations. Table 2.

Table 1: Characteristics of the study population

Variable	Cases N=350(%)	Controls N=350(%)	P - value
Age group (years)			
1-5	113 (32.3)	100 (28.6)	
6-10	117 (33.4)	134 (38.3)	
11-15	87 (24.9)	82 (23.4)	
>15	33 (9.4)	34 (9.7)	0.55[a]
Median age (IQR) years	8 (4-10)	8 (4-10)	0.69[b]
Gender			
Male	178 (50.9)	180 (51.4)	
Female	172 (49.1)	170 (48.6)	0.88[a]
Socio-economic class of parents			
Lower	162 (46.3)	169 (48.3)	
Middle	101 (28.9)	110 (31.4)	
Upper	87 (24.8)	71 (20.3)	0.34[a]

[a]Chi square test, [b]Mann Whitney- U test, IQR = Interquartile range

Table 2: Serum zinc concentration in the study population.

Parameters	Cases	Controls	P value
Level of Zinc concentration	Frequency (%)	Frequency (%)	
<7.65 µmol/l (low)	235(67)	120(34)	
>7.65 µmol/l (normal)	115(33)	230(66)	<0.0001[a]
Zinc concentration by age group	Median serum zinc (IQR) (µmol/l)	Median serum zinc (IQR) (µmol/l)	
1-5 years	6 (2-7)	8 (5-10)	0.04[b]
6-10 years	6 (3-8)	8 (4-9)	0.04[b]
11-15 years	6 (2-7)	8 (5-9)	0.03[b]
>15 years	7 (2-8)	9 (5-10)	0.03[b]
ALL	6 (3-7)	8 (4-9)	0.04[b]
Zinc concentration by degree of anaemia	Cases	Controls	
None	6 (3-8)	9 (4-10)	0.03[b]
Mild	6 (2-7)	8 (4-10)	0.04[b]
Moderate	5 (2-6)	7 (3-9)	0.03[b]

[a]Chi square test, [b]Mann Whitney- U test, IQR = Interquartile range

Serum zinc levels and the socio-economic class of the study population

Comparing the proportion of the study population that were zinc deficient across the social classes, more children from lower socioeconomic class 111 (68.5%) vs 60 (35.5%) were zinc deficient compared with those from upper socioeconomic class 25 (38.5%) vs 15 (16.3%) in both the cases and controls groups respectively, $p<0.0001$.

Discussion

The result of this study reveals a high prevalence rate of zinc deficiency among children with sickle cell anaemia.

The serum zinc concentration in the SCA subject is lower than the internationally acceptable normal (7.65–22.95 μmol/L) [21] and lower than that of the healthy controls. This is in agreement with previous studies that found low serum zinc level in SCA patients compared with healthy controls, [7, 23-26] but different from some studies, [12, 27] that did not find any statistically significant difference in serum zinc levels between SCA patients and controls.

The reason for the low mean serum zinc level in SCA children in this study is unknown. However, many factors may be responsible. First, red cell is an important storage site for zinc, therefore, chronic haemolysis which occurs in SCA patients usually results in the loss of large amount of zinc from the red cells and this could account for the low serum zinc in the SCA patients.[12]This study found that SCA subjects with moderate anaemia have lower serum zinc levels than SCA subjects with mild or no anaemia. Second, impaired zinc homeostasis as a result of excessive excretion of zinc in the urine of the sickle cell anaemia subjects may also explain the low serum zinc. Defective reabsorption of zinc due to recurrent vaso-occlusive events in the renal tubules occurs in sickle cell anaemia patients leading to excessive urinary loss of zinc.[9] However, urinary zinc was not analysed in this study.

The prevalence rates of zinc deficiency of 67% and 34% in SCA children and the control group respectively in this study are higher than the 20% prevalence set by the International Zinc Nutrition Consultative Group, as an indicator of zinc deficiency risk of significant public health importance.[22] This high prevalence rates in this study calls for attention to be focused on strategies aimed at improving the zinc status of children generally in Nigeria. The prevalence rate in this study is higher than what has been reported in some studies [28-30] but lower than the 74.3% reported in Brazil.[31] The high prevalence of zinc deficiency in this study may, in addition to chronic haemolysis and impaired zinc homeostasis, be due to inadequate dietary intake of bioavailable zinc; [21] though the dietary intake of the study participants was not assessed. The staple foods in our setting, like other low income countries comprise primarily of cereals, tubers and legumes which contain significant amount of phytate, a compound that inhibits the absorption of zinc and few sources of animal based diets which are rich in zinc and free of phytates.[21, 32] This inadequate dietary intake of zinc could be related to poor nutrition knowledge, poverty and food insecurity. Our study shows that the proportion of zinc deficient patients was more in children from lower socio economic class than in the children from upper socio economic class thus corroborating the influence of poverty and food insecurity on the zinc status of the children, as people from low socioeconomic class tend to be poorer and more food in secured.

Limitations of the study

The dietary intake and nutritional status of the study participants were not assessed. However, the cases and the controls were comparable in terms of their socioeconomic classes suggesting that there may be no difference in their dietary intakes across the social classes. Furthermore, the study was conducted in a single institution, a multicenter study could enhance the external validity of the findings.

Conclusion

The serum zinc concentration is significantly lower in patients with SCA compared with the controls. There is a high prevalence of zinc deficiency among the study population especially those with sickle cell anaemia. Zinc supplementation or fortification should be considered as part of intervention strategies to improve the zinc status of these children particularly those with sickle cell anaemia with the aim of possibly reducing the morbidity and mortality associated with the disease.

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Conflict of interest: None

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