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Serum zinc levels in HIV infected children attending the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

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Abstract: *Background:* Zinc deficiency is common in the Paediatric age group but the extent of this disorder is unknown in HIV infected children in Nigeria prompting this study.
Objective: To determine the prevalence of zinc deficiency in HIV seropositive children, and compare this with age and sex matched controls.
Methods: A case control study of 70 HIV sero-positive and age and sex matched HIV sero-negative children was carried out in the University of Port Harcourt Teaching Hospital between 1st of June, 2009 and 31st of May, 2010. We collected demographic, clinical, haematological and biochemical parameters from cases and controls, and analysed these using SPSS 20.
Results: Sixty percent of the subjects were zinc deficient as against 41.4% of the controls, p= 0.028. Subjects that were zinc deficient were more likely to be in higher HIV disease stages, p = 0.003, in lower socio-economic classes and aged less than 60 months. We conclude that there is a high prevalence of zinc deficiency in HIV sero-positive children and they should have zinc supplementation immediately they are diagnosed to reduce their morbidity and mortality.

Key words: Zinc deficiency, HIV sero-positive, socio-economic status

Introduction

Zinc is an essential micronutrient found in every cell of every living organism and is necessary for cell growth, wound healing, mucosal epithelisation, DNA synthesis, and support of a healthy immune system.[1] Zinc is found in high concentration in flesh of beef, pork, poultry, fish and shellfish, and with lesser amounts in eggs and dairy products. Phytates in plants form complexes with zinc preventing absorption from the jejunum. Consumption of gruels made from maize, sorghum, or millet-containing phytates reduce absorption and cause deficiency of zinc. Rapid turnover of cells and consequent rebuilding during acute and chronic illnesses cause reduction in micronutrients including zinc.[2,3] Caulfield et al[2] in their analysis stated that children in developed countries have normal serum zinc levels, but most children in developing countries are deficient because they consume diets low in zinc.[2] The estimated global prevalence of zinc deficiency as at 2004 was 31%, [3] but this is lower in Nigerian children where 20% are said to be deficient in zinc. [4]

Zinc deficiency manifests clinically with increased severity and duration of diarrhoea and pneumonia, poor wound healing, dermatitis, and in severe forms, dwarfism. It is for this reason the World Health Organisation (W.H.O) recommended zinc supplementation in children with diarrhoea and pneumonia.[5] This is known to reduce the severity, frequency and duration of diarrhoea and pneumonia in children and also those infected with Human immunodeficiency virus. The prevalence of HIV infection has gradually dropped and this is especially so in Nigeria where it is now 3.4% from a high of 5.4% over a period of 6 years[6]. However, HIV has been associated with over 2.1 million deaths and 330,000 of these are children as at the end of 2007. Nte et al actually showed a 2.1% HIV associated deaths in University of Port Harcourt Teaching Hospital Nigeria.[7] HIV is associated with under-nutrition as demonstrated by studies in Africa and Nigeria, and most of these were macronutrient deficiencies.[6,8] Steenkamp et al[8] in South Africa reported zinc deficiency in some children with HIV and noticed that nutritional rehabilitation (with calorie and protein) alone did not improve serum zinc levels. For this reason, zinc supplementation programmes were initiated and sustained in East and South African children with HIV infection. The prevalence of zinc deficiency in HIV infected children is unknown in Nigeria, and it is for this reason we decided to carry out this study and make recommendations based on the results of the investigation.

Objectives

To determine the prevalence of zinc deficiency in HIV sero positive children and age and sex matched controls (HIV sero-negative children) attending the University of Port Harcourt Teaching Hospital.
To compare serum zinc levels between the subjects and the controls

Materials and Methods

This case-control study was carried out in the Paediatrics department of the University of Port Harcourt Teaching Hospital between 1st of June, 2009 and 31st of May, 2010, over a one year period. Ethical approval was obtained from the UPTH research and ethics committee prior to the commencement of the study. Included in the study, were newly diagnosed and HAART naive HIV infected children between ages 18 months and 16 years, and age and sex matched HIV negative controls. Subjects were recruited serially as they presented to the department. When a sero-positive subject who met the inclusion criteria was recruited, an age and sex matched HIV sero-negative control who met the inclusion criteria was recruited as control from the Children Outpatient clinics (CHOP), Children Emergency Ward or DTU within 72 hours. Excluded from the study were children on zinc or multivitamin supplementation. Informed consent was obtained from the parents/guardians of all eligible children. The minimum sample size (n) of 53 was calculated using the prevalence of HIV infection and zinc deficiency in children in Nigeria.[4] All children had detailed clinical history and examination performed before blood samples were collected for HIV screening and serum zinc analyses. Information obtained were demography, risk factors for HIV and zinc deficiency, and the socioeconomic status of parents/ guardians using the method recommended by Oyedeji.[9]

HIV Screening

Subjects that were already in care (i.e. receiving other management except antiretroviral drugs) for HIV/AIDS were not retested for HIV and were recruited after their parents gave informed consent for the study. Testing of new subjects and controls using rapid test kits (Determine® and Stat pak was done following the guidelines of the Provider Initiated Testing and Counselling (PITC).[10] Needle pricks (from the heel of a foot, or the thumb) were used to collect one drop of blood into the test kit. Initial tests used were Determine HIV 1 and 2 kits® (Abbot, Japan), and positive samples were retested using the Chembio HIV 1 and 2 Stat- pak kits® (Medford, NY, USA). If there was disparity between the two test kit results, a third kit, Gold check® was used as "tie-breaker". Information and post-test counselling were offered to all patients by the investigator or the trained assistants. All samples were tested free by the investigator and/ or trained assistants at no cost to the study groups.

Serum Zinc Analysis

The serum zinc was analysed at the Obafemi Awolowo University Central Science Laboratory, Ile Ife, using flame atomic absorption spectrophotometer Model 210, manufactured by Buck Scientific Corporation, Connecticut, USA and the laboratory reference range of normal was 80 - 120µg/dL. Each sample was done in duplicate and the mean recorded. For this study, zinc deficiency was defined as serum zinc level less than 80 µg/dL. Samples were sent in batches of 20, transported frozen, in vaccine rush containers with ice gel packs by courier to OAUTH, Ile Ife and received by the Chief scientist or his assistants at the CSL, where they were analysed.

Measurements of total WBC, AND CD4+ COUNTS

Blood in the EDTA bottle was used for White Blood Cell (WBC) count and differentials as well as the CD 4^+ cell count. Total WBC count and differentials were determined using Full Automatic Blood cell counter (ERMA INCORPORATED® Tokyo). The machine was recalibrated daily using standard solution and diluents (ERMA INCORPORATED® Control M-6) according to manufacturer's specification. The white cell count and differential counts were crosschecked with manual differential cell counter after every tenth specimen and whenever a superfluous result was obtained with autoanalyser. Lymphocyte count was calculated using total white blood cell count and lymphocyte percentage with the formula:

$$\text{Lymphocyte count} = \frac{\text{Lymphocyte percent x total WBC count } (mm^3)}{100}$$

$CD4^+$ cell count was measured in all newly diagnosed HIV sero-positive subjects, and controls. The $CD4^+$ cell count was measured in the Haematology laboratory of UPTH using Partec® Cyflow Counter 05-8401 manufactured in Germany. The $CD4^+$% were calculated, after getting the total lymphocyte count for each patient using the formula:

$$CD4^+\% = \frac{CD4^+ \text{ cell count x } 100}{\text{Lymphocyte count}}$$

$CD4^+$ count and $CD4^+$ % were used to classify HIV sero -positive subjects and controls according to immunological status.

Data analysis

The data collation and analysis were done by the investigator, with the help of statisticians. The raw data was collated into a Microsoft Excel sheet in a personal computer. The data was analyzed using SPSS version 20. HIV disease was classified using WHO clinical staging for infants and children and immune status was classified using WHO staging classification.[10] Serum zinc deficiency was defined as serum zinc level less than 80µg/dL.[4] Frequencies were measured using percentages, while arithmetic means were used for continuous variables. Test of statistical significance included

Student's t test, Chi square test, Yates correction test and Fisher's exact tests. Test of significance was assessed with 95% confidence interval and p value < 0.05 was accepted as significant.

Results

A total of 268 HIV sero-positive children were seen during the study period and of these, 102 (38%) were aged eighteen months and above. Ten (10) were excluded because they had already been started on antiretroviral therapy. Fifteen sero-positive patients who were eligible were critically ill and died in the CHEW before their parents could be counselled for inclusion into the study, while seven (7) did not give consent to participate in the study. A total number of 140 children (70 HIV sero-positive subjects and 70 age and sex matched HIV sero-negative controls) were recruited into the study. There were 40 (57.1%) males and 30 (42.9%) females in each study group, giving a male: female ratio of 1.3:1.

Clinical staging of HIV sero-positive children

Sixty two (88.6%) of the subjects presented in advanced to severe stages of diseases (stages 3 and 4). There was no child aged 60 months and above in the asymptomatic and mild HIV disease stages (Table 1).

Table 1: WHO clinical staging of subjects by age group

WHO Clinical staging	1 n (%)	2 n (%)	3 n (%)	4 n (%)	Total n (%)
Age group (months)					
18-59	3(4.3)	5 (7.1)	15 (21.4)	24 (34.3)	47 (67.1)
60-119	0 (0)	0 (0)	14 (20)	6 (8.6)	20 (28.6)
120-192	0 (0)	0 (0)	1 (1.5)	2 (2.8)	3 (4.3)
Total	3 (4.3)	5 (7.1)	30 (42.9)	32 (45.7)	70 (100)

Age related immunological status of HIV sero-positive children.

Only 6(8.6%) subjects had normal immunity and 46 (65.7%) of subjects had advanced to severe immune suppression.

Table 2: Immunological status of subjects by age groups

Immunological status	Normal n (%)	Mild n (%)	Advanced n (%)	Severe n (%)	Total n (%)
Age group (months)					
18-59	2(2.9)	8(11.4)	12(17.1)	25 (35.7)	47 (67.1)
60-119	3 (4.3)	8(11.4)	8 (11.4)	1(1.4)	20 (28.6)
120-192	1 (1.4)	2 (2.9)	0 (0.0)	0 (0.0)	3 (4.3)
Total	6 (8.6)	18 (25.7)	20(28.6)	26 (37.1)	70 (100)

Serum zinc status and study groups

Forty-two (60%) of the subjects and 29 (41.4%) of the controls were zinc deficient and this difference was significant, $\chi^2 = 4.830$, p = 0.028.

Serum zinc levels and age groups of subjects and controls

For each age group, controls had higher serum zinc levels than the subjects, though this was only significant for the 18-59 months age group. Table 3 shows age-related prevalence of zinc deficiency in subjects and controls. Of the 47 subjects aged 18-59 months, 28(59.6%) were zinc deficient as against 17 (36.1%) in the control group.

Table 3: Age-related prevalence of zinc deficiency in subjects and controls

Age groups (Months)	Subjects N	Deficient n (%)	Controls N	Deficient n (%)	χ^2	p
18 – 59	47	28 (59.6)	47	17 (36.1)	5.16	0.02
60 – 119	20	14 (70.0)	20	11(55.0)	0.96	0.33
120 – 192	3	0 (0.0)	3	1 (33.3)		0.99*
Total	70	42 (60)	70	29 (41.4)		

Fishers exact test

Serum zinc Levels and HIV disease

The mean serum zinc levels decreased as the WHO HIV clinical staging worsened. Subjects in stages 1 and 2 had normal mean serum zinc levels while those with advanced disease, had mean serum zinc levels lower than normal (Table 4). There was significant difference in mean serum zinc levels between subjects in stages one and two (95.319 ± 24.01 ug/dL) and those in stages three and four (62.04 ± 28.80 ug/dL), with the latter having a lower mean, t = 3.1, p = 0.003.

Table 4: Mean serum zinc levels according to WHO clinical staging

WHO Stage	n (%)	Mean zinc levels ug/dL ±SD
1	3 (4.3)	113.9 ±34.1
2	5 (7.1)	84.2 ±2.9
3	30 (42.9)	73.8 ±23.5
4	32 (45.7)	51.0 ±30.1
Total	70 (100)	65.8 ±33.3

In subjects, the mean serum zinc level in those with normal immune status was within normal levels, but it was low in those who had evidence of immune suppression. The mean serum zinc levels were higher in the controls than in the subjects across various immune categories except in those with normal immune status. The differences were significant in the mild and severe immune suppression categories. Table 5

Table 5: Mean Serum zinc level and immune status of subjects and controls

Immune Status	Subjects n (%)	Subjects Mean zinc µg/dL (±SD)	Controls n (%)	Controls Mean zinc µg/dL (±SD)	t	p
Normal	6 (8.6)	110.4(±27.6)	22 (31.4)	92.5(±27.4)	1.4	0.168
Mild	18 (25.7)	69.3 (±23.7)	27 (38.6)	84.8(±25.5)	-2.1	0.046*
Advanced	20 (28.6)	70.8 (±22.5)	15 (21.4)	79.0(±14.6)	-1.2	0.235
Severe	26 (37.1)	49.3(±28.1)	6 (8.6)	86.6(±18.7)	-3.9	0.002*
Total	70 (100)	65.8(±33.3)	70 (100)	86.1(±23.8)		

* p ,< 0.05

In the control group, majority 49 (70%) had normal or mild immune suppression whereas majority of the subjects 46 (66%) had advanced or severe immune suppression. One of the controls with advanced immune suppression had a very low serum zinc level of 56 µg/dL which reduced the mean serum zinc in the group to below normal (Table 6).

Table 6: Immune Status of Zinc Deficient Subjects and Controls

Immune Categories	Subject N	Subject Deficient n(%)	Controls N	Controls Deficient n(%)	χ^2	P
Normal	6	0(0)	22	8(36.4)	1.53*	0.216
Mild	18	8(44.4)	27	12(44.4)	0.00	1.00
Advanced	20	14(70)	15	7(46.7)	1.94	0.163
Severe	26	20(76.9)	6	2(33.3)	2.52*	0.112
Total	70	42(60)	70	29(41.4)		

Yates correction test

Serum zinc and Socioeconomic Status of study group
In subjects, only those in class 1 had normal mean serum zinc level, and though there were fluctuations, the lower the classes, the lower the mean serum zinc levels. The fluctuation was also noted in the control group, but mean serum zinc level was normal in classes 1, 2, and 3. It was also noticed that the mean serum zinc was lower in subjects than controls within the same socio economic class, but the differences were not significant as shown in Table 7.

Table 7: Mean serum zinc levels and socioeconomic status of subjects and control

Socioeconomic Status	Subjects n (%)	Subjects Mean zinc µg/dL (±SD)	Controls n (%)	Controls Mean zinc µg/dL (±SD)	t	p
1	4 (5.7)	88.6 (±20.2)	3 (4.3)	124.7 (±32.3)	-2.1	0.091
2	14 (20)	71.4 (±34.3)	18 (25.7)	86.7 (±26.1)	-1.4	0.163
3	17 (24.3)	79.8 (±24.1)	35 (50)	89.6 (±19.3)	-1.6	0.119
4	28 (40.0)	58.5 (±26.5)	11 (15.7)	66.7 (±13.4)	-1.0	0.339
5	7 (10)	37.1 (±31.0)	3 (4.3)	75.5 (±25.8)	-1.8	0.098
Total	70 (100)	65.8 (±33.3)	70 (100)	86.1(±23.8)		

Discussion

Zinc deficiency was seen in 50.7% of the study population and this was higher than the 20% previously reported by Maziya-Dixon et al [4] and the estimated global prevalence of 31%.[2] The higher prevalence in this study can be accounted for by the fact that the study population were ill children as against the study by Maziya-Dixon et al [4]where apparently healthy household children (children living in a household i.e. with a family as against street children were investigated. Serum zinc levels are reduced during acute and chronic infection as there is reduced intake, and increased utilization for healing.[1] Another reason for the higher prevalence was the relatively small sample used in the study compared to the larger population by Maziya-Dixon et al.[4] In the study, only serum zinc level was used to determine zinc deficiency state, whereas the estimated global prevalence pooled results from various populations (affluent and impoverished) with diverse cultures and feeding practices, used degree of stunting in areas where serum zinc levels were not available and zinc contents in various diets to arrive at their result. The HIV status of the children investigated by Hotz et al [3] and Maziya-Dixon et al[4] was not ascertained and this methodological difference could have also accounted for the higher prevalence in the study.

In the HIV sero-positive children, the prevalence of zinc deficiency was 60%. This was higher than the prevalence of 54.3% reported by Ndeezi et al[11] in Uganda and the 20% by Eley et al[12] in South Africa. The study investigated only HAART naïve HIV sero-positive children while Ndeezi et al combined children on HAART and those that were HAART naïve. Studies have shown that HAART improves serum zinc levels probably by reducing viral load, improving general well being, immune status, and invariably, overall nutrition in HIV patients, thus reducing the number of HIV sero-positive children that will be zinc deficient.[13,14] HAART also reduces opportunistic infections, and thus reduces the frequency of repeated infections and excessive utilization of zinc. The higher prevalence of zinc deficiency in HIV sero-positive children in this study as against that by Eley et al, can be explained by the fact that the children investigated by Eley et al were clinically stable, and received nutritional rehabilitation, thus improving their macro- and micro- nutrient status. In the present study, no attempt was made at nutritional rehabilitation before they were recruited.

The prevalence of zinc deficiency in the control group was 41.4% and this was lower than the 47.5% from Bilbis et al[15] in Sokoto, North West Nigeria, and 72% by Muller et al [16] in rural Burkina Faso, but higher than 11% by Takyi et al[17] in Ghana. While the present study investigated children between 18 and 192 months, Bilbis et al,[15] studied only children less than 60 months. Children less than 60 months have lower zinc levels than older children because of the higher demand for growth and reduced intake from diet.[2] The higher prevalence in the study by Muller et al can be attributed to the fact that a sub-sample of a population rather than the whole population was used. The method of recruitment of this sub sample was not stated and it may have caused some bias in favour of zinc deficiency. Another reason for the higher prevalence in the study by Muller et al, was that

the children investigated were said to have consumed mainly cereals with little protein in their diet and this can account for the reduction of zinc in that population as zinc is known to be low in cereals.[2,3] Takyi et al[17] had a much lower prevalence possibly because they used hair zinc levels for their study which reflects chronic zinc nutrition rather than acute as serum zinc levels determines.

The mean serum zinc level in subjects was significantly lower than that found in controls. This was comparable to other studies in Nigeria,[15] and Kenya.[16] Though the other studies investigated adults, the difference between subjects and controls may be attributed to the fact that HIV, being a chronic infection, predisposes infected individuals to under-nutrition and other opportunistic infections, putting excessive stress on the immune system and lowering the zinc levels in serum. The low serum zinc levels in some of the controls may be attributable to their ill-health poor appetite, reduced dietary zinc intake and the high zinc turnover rate during illness, and since there are no readily available stores in the body, that which is available for assay will be low.

Mean serum zinc level in subjects was noticed to be lower, though not significantly, in the younger age groups than in the older age groups. Bilbis et al, [12] in Northern Nigeria also noted similar findings in their study. Within the same age group, mean serum zinc levels were lower in subjects than in the controls. This may be a reflection of the HIV disease burden on the subjects. Lower serum zinc levels in younger children may be a consequence of higher turnover of zinc as it is needed for growth, metabolism and to fight infection, thereby reducing that which is available in serum for analysis.[2] Again, younger children who are being weaned off milk onto other diets are usually given cereals that are poor sources of zinc and these contain high levels of phytate that reduce the absorption of zinc from the diet.[2]

Advanced HIV disease stage was associated with low mean serum zinc levels. This significant difference was also seen in Uganda, South Africa,[11] and Kenya,[16] even though adult population was used in the Kenyan study.[16] Advanced HIV disease stage is more likely to be associated with recurrent and chronic infections thus reducing intake and increasing zinc turnover. There were also significantly lower serum zinc levels in subjects who had evidence of immune suppression than those who did not have evidence of immune suppression. This was also reported by Eley et al in South Africa.[11] It is worth noting here, that immune categorization is better made with CD4% and not absolute count in children,[10] as the total white blood cell count is relatively low in children and following HAART administration, CD4+ counts recover slowly and may eventually reach optimal levels.

$CD4^+$ count and percentage were significantly lower in subjects than controls. The low $CD4^+$ count and percentage in HIV is a well known phenomenon, as these cells are very susceptible to the HIV and they get depleted fast in the course of the disease.[1, 2,] This finding of reduced $CD4^+$ count with advanced disease was similar to reports from other parts of Nigeria and other developing countries.[6,8,13] Zinc is required for the enzymes (protease and integrase) activities in HIV and as the virus proliferates and disease progresses, the zinc levels in serum decrease. The proliferation also causes release of oxygen free radicals and superoxide dismutase is used to mop up these free radicals to prevent their accumulation to toxic levels.

Another significant finding in the present study was that the lower the socioeconomic status of the guardians, the lower the serum zinc levels of study groups though there were fluctuations in some classes, and this was seen in other studies.[4, 17] Subjects in the same socioeconomic class with controls had lower mean serum zinc levels. The association has not been looked at in other studies, and though there were more subjects in lower socioeconomic classes, the finding shows that there is reduced serum zinc levels in children of low socioeconomic class.[17] Children of lower socioeconomic status have low access to food rich in zinc, and consume diets rich in phytate, thereby decreasing their serum zinc levels. These children are also prone to recurrent infections, and as their immune system attempts to fight these infections, there is reduction in serum zinc levels.[2,3,19] Those parents in lower socioeconomic classes whose children presented in advanced disease stages may have poor health seeking behaviour and thus not present until the illness was advanced. Poverty may also be a contributing factor to their late presentation.[18]

Conclusion and Recommendation

Our study showed a high prevalence of zinc deficiency in HIV infected children (60%), and also in controls (40%). Most of the zinc deficient children were in low socioeconomic class, ages less than 60 months and in advanced HIV disease stage. The mean serum zinc levels were also low in HIV sero-positive. It is therefore our recommendation that all HIV infected children should be supplemented with zinc immediately after being diagnosed to reduce the co-morbidities associated with the infection and improves their immunity.

Limitation of the study

The trend or change of serum zinc levels could not be ascertained as this was a case- control study. A longitudinal study might have assessed this change over time. Dietary intakes of nutrients were not assessed, as this is one of the main ways of assessing zinc levels. The cost of the study was high.

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