Original Research Article

Serum zinc levels of under five children with diarrheal disease

ABSTRACT

Background: Diarrhoea is a major cause of morbidity and mortality in children below five years of age especially in developing countries. One of the consequences of diarrhoea in children is zinc deficiency, which may worsen the diarrhoea itself, and thus increase morbidity and mortality.
Aim and objectives: The study aimed to determine the serum zinc status of under five children with diarrhoea attending Federal Medical Centre Owerri. Also, to compare the serum zinc level between children with diarrhoea and their age and sex-matched controls, as well as determine the relationship, if any between types of diarrhoea, and frequency of diarrhoea episodes.
Methodology: The study was a comparative cross-sectional study carried out at the Emergency Paediatric Unit, The Children's Outpatient Clinic and The Children's Ward of the Federal Medical Centre, Owerri. A total of 402 study participants were analyzed. Structured questionnaires were used to collect data including participants' socio demographic characteristics, the clinical presentation of the present diarrhoeal episode, as well as the frequency and duration of diarrhoeal episodes in the preceding year. Serum zinc was assessed using Varian AA240 Atomic Absorption Spectrophotometer. Stool microscopy and culture were done for subjects only.
Results: The prevalence of zinc deficiency in children with diarrhoea was 84.1% while that of children without diarrhoea was 56.2%. Mean serum zinc level was significantly lower in children with diarrhoea when compared to their age and sex matched controls (p=0.000). All children with persistent diarrhoea and dysentery had zinc deficiency. Children with persistent diarrhoea had significantly lower serum levels when compared to children with acute watery diarrhoea. There was a negative correlation between serum zinc level and frequency of stool, duration of the present episode of diarrhoea, frequency of diarrhoea episodes, and the duration of each episode of diarrhoea in the preceding year and the severity of dehydration.
Conclusion: The findings of this study support the present recommendation of the World Health Organization for zinc supplementation in children with diarrhoea, and recommends that children with persistent diarrhoea should have longer treatment with zinc tablets.

Keywords: [Serum zinc level; children; diarrhoeal frequency; diarrhoeal episode; persistent diarrhoea; acute watery diarrhoea, dysentery)

1. INTRODUCTION

The World Health Organization (WHO) defines diarrhoea as loose or watery stools at least three times daily or more frequently than is normal for an individual [1,2]. The word “diarrhoea” originates from two Greek words dia (which means through) and rhein (which means to flow) [3].

Despite great advances in the control of diarrhoeal disease by the use of oral rehydration salt, zinc tablets, promotion of breastfeeding, vitamin A supplementation, immunization against rotavirus, hand washing and improved water and environmental sanitation, diarrhoeal disease remains a major health problem among children under five years globally [1,2]. Each year, 1.5 million children globally die from diarrhoeal disease (more than that caused by acquired immune deficiency syndrome, malaria and measles)[1]. Globally, an estimated 2.5 billion cases of diarrhoeal disease occur among children under five years of age every year and more than half of these cases are in Africa and South Asia [1].

The incidence of diarrhoeal diseases varies with seasons (more common in the dry season) and age [1,3]. Children below five years are most vulnerable, however incidence peaks in children 6 to 12 months of life and remains high until 24 months when it begins to decline [1,2].

Nigeria is the second leading country with the highest annual child mortality due to diarrhoeal disease (150,700 deaths annually) [1].The prevalence of childhood diarrhoea in Nigeria is high, with the national prevalence rate estimated at 18.8% [4].

Diarrhoeal disease in children can lead to life threatening consequences including loss of fluid and electrolytes leading to dehydration, electrolyte imbalance, shock and even death [2,3]. Diarrhoeal disease causes loss of appetite, decreased food intake and reduced absorption of nutrients from ingested food resulting in weight loss, poor growth and micronutrient deficiencies such as zinc and vitamin A. These lead to poor nutritional state and increased risk of infections and can worsen diarrhoeal episodes [5,6].

Zinc is second only to iron as an essential micronutrient [7,8]. Currently, zinc deficiency is recognized as a nutritional problem worldwide, both in developing and developed countries [8,9]. Zinc is important in maintaining the integrity of the immune system, in tissue repair, antioxidant activity, growth and reproduction [5].It is abundant and easily absorbed from animal protein like red meat, dairy products and seafood and can also be found in cereals, nuts, vegetables and tubers. However, cereals, nuts, vegetables and tubers are not good sources of zinc because of the presence of phytate which chelates and prevents the absorption of zinc [5,8,9]. For many people in developing countries, cereals, vegetables and tubers represent the major sources of zinc and these sources reduce the bioavailability of zinc thereby increasing the risk of zinc deficiency in developing countries [5,8].

The association between diarrhoea morbidity and serum zinc has been documented [10,11,12]. In a systematic review of trials on the efficacy and effectiveness of zinc supplementation in children with diarrhoeal disease, the authors reported a reduction in stool frequency, volume, duration of diarrhoea, hospital stay and incidence of future episodes [13]. Okolo SN *et al* in Jos, Nigeria, reported that children with diarrhoea had significantly lower levels of serum zinc when compared with *controls [14]. Eme A et al in a retrospective study in Rivers state, Nigeria reported a reduction in diarrhoeal episodes and* reduced incidence among children who received zinc supplementation compared to children who did not [15].

This study aimed to ascertain the serum zinc status of children aged 6-59 months with diarrhoeal disease and to compare this to children of the same sex and age without diarrhoea. The study also determined the association between types of diarrhoea, frequency of diarrhoea episodes, and serum zinc levels.

2. MATERIAL AND METHODS

Study area

The study was conducted at the Paediatrics Department of the Federal Medical Centre (FMC), Owerri, Imo State, Nigeria. Imo State is in the South East Geopolitical zone of Nigeria. Imo State has a population of 3.93 million (2006 census), though in 2014, its population was projected to have increased to 5.11 million. Owerri is the capital of Imo State with 401,873 people living in the city. The residents are predominantly farmers and traders.

Federal Medical Centre is a tertiary health institution in Owerri, Imo State. The Paediatric Department is made up of the Emergency Paediatric Unit (EPU), the Special Care Baby Unit (SCBU), the Children's ward and the Children's Outpatient Clinic (CHOP). The CHOP attends to about 70 patients on each clinic day.

Study population

This included children aged 6-59 months with diarrhoea attending the Children's Outpatient Clinics, admitted into the Emergency Paediatric Unit or the Children's Ward. Age and sex-matched controls without diarrhoea were enrolled from the same units of the department.

Inclusion criteria for subjects and controls

1. Children aged 6-59 months with diarrhoea. (subjects)
2. Children whose parents/guardian gave informed and written consent.
3. Children aged 6-59 months without diarrhoea(controls)

Exclusion criteria for subjects and controls

1 Children whose parents or care giver refused to give consent.

2 Children who were taking zinc tablets.

Ethical considerations

Ethical approval for this study was sought for and obtained from the Ethics Committee of the Federal Medical Centre Owerri, while informed/written consent was obtained from the parents/guardian of the children enrolled.

Sample size

The sample size for this study was calculated using the formula for finding the significant difference between the proportions in two groups. Assuming an equal number of cases in the two groups; the sample size was calculated using:

$$n = \frac{2z^2pq}{d^2}$$

n = minimum sample size, z = normal standard deviation set at 1.96 which corresponds to the 95% confidence interval, p = prevalence of zinc deficiency in children with diarrhoea. In this study 50% was used since there is no such prevalence rate in Nigeria.

q = 1.0- p, d = degree of accuracy desired (In this study, an observed difference of 0.10 or more was considered significant at the 0.05 level). This gave a total number of subjects and controls recruited to be 422, using a non-response rate of 10%. For this study, 422 under-five children were recruited into the study but only 402 samples comprising two groups of 201 children each of those with diarrhoea and controls were analysed. Twenty children were excluded from the study because their samples spilled.

Study Design/Protocol

This was a comparative cross-sectional study. It was conducted from 17th of August 2015 to 16th of February 2016.Consecutive sampling method was used to recruit children that met the inclusion criteria from the EPU, CHOP and the children's ward. Subsequently, for every subject recruited, an age and sex-matched control who met the inclusion criteria was recruited.

This study was carried out by the researcher with two other doctors and three laboratory scientists. The two doctors were trained by the researcher on the proper filling of the questionnaire, anthropometric measurements and collection of samples for serum zinc one week before commencement of study. Two laboratory scientists, in the haematology and microbiology department of Federal Medical Centre Owerri, and one laboratory scientist in the chemical pathology department of the Nnamdi Azikiwe University, Awka assisted by the researcher separated and analysed the samples.

Structured questionnaires were administered by the research team to caregivers of each child recruited into this study. Information obtained included: bio data of the child, level of education of the parents, occupation of the parents (for both subjects and controls), frequency of stools/day, duration of the present diarrhoeal episode, frequency of diarrhoeal episodes and duration of the episodes in the preceding year, presence of blood in stool, and interventions used (for subjects only). Socioeconomic class was determined using the method proposed by Oyedeji [16].

Examination and Anthropometric Measurements

The researcher examined the subjects and controls, checking for signs of dehydration as well as their anthropometric measurements (weight, length or height).

Weight and Length/ Height

Children were weighed to the nearest 0.1 kilogramme (kg). Those below 24months were weighed nude in a Salter® bassinet scale, while those >24months were weighed with a Surgifield® medical spring balance scale, wearing only inner clothing and without shoes. The children stood on the weighing scale with their head held erect and hands by their side. The reading was taken twice in kilogram and the average recorded. The scale was standardised using an object with a known weight.

The length of the children <24months was measured with an infantometer, with heads in anatomic position and crown touching the fixed board. The movable board at the lower end of the infantometer was brought to the heel, with the knees in full extension. While the height of children of those >24 months was measured with a Surgifield® Standiometer to the nearest centimetre. They stood straight without their shoes, head held erect, and arms hanging by their side. The readings were taken twice in centimetre and the average recorded.

Sample Collection and Laboratory Analysis

Serum Zinc

After obtaining consent and proper disinfecting, 3mls of venous blood was collected from the antecubital vein of each study participant. The sample was put into a pre-labelled sterile anticoagulant free bottle that had been made free of trace elements by immersing in 10% nitric oxide overnight then rinsed in deionised water. The samples were put in vaccine rush containers with ice packs to prevent haemolysis of red blood cells, and taken to the haematology laboratory of the Federal Medical Centre Owerri; where they were centrifuged for 10minutes, to separate the serum. The serum was later put in another trace element decontaminated bottle and stored at -20^0C until they were ready for transportation. The serum samples were transported in batches of 50 at appropriate temperature to the Chemical Pathology department of the Nnamdi Azikiwe University, Awka where they were stored at -20^0C until they were analysed. The samples were analysed by a laboratory scientist assisted by the researcher within 4 weeks of collection. Serum samples were diluted 5-fold with deionised water and then 200µl of sample added to 800µl of reagent, and mixed lightly. The mixture was then kept for 30 minutes at room temperature after which it was analysed using Varian AA240 Atomic Absorption Spectrophotometer. Serum zinc level of less than 65µg/dl was regarded as zinc deficiency.

Stool Microscopy

Fresh faecal samples were collected after consent was obtained from the parents/guardian of the children and put in a pre-labelled sterile screw cap bottle. Samples were stored at 4^0C until they were analysed. Faecal samples for microscopy were mixed with a drop of normal saline, a cover slip placed on the preparation and then examined under the microscope unstained. Thereafter, 3g of stool sample (measured with a pre-calibrated teaspoon) was put into a container and 50ml of floatation fluid (distilled water, potassium iodide and powdered iodine crystals) added. The mixture was strained into another test tube until the test tube was full. A cover slip was placed on the test tube and the sample allowed to stand for 20 minutes, after which the cover slip was lifted off with the drop of fluid adhering to it and placed on a slide. The slides were examined under the microscope.

Stool Culture

Faecal samples were emulsified in sterile peptone water and a loopful was inoculated on xylose lysine deoxycholate (XLD), MacConkey (MCA) and Salmonella-Shigella Agar plates. These were incubated overnight at 37^0C and read the next day.

Data analysis

The data collected was analysed using the Statistical Package for Social Sciences (SPSS) version 19.0. The mean and standard deviation of quantitative variables were determined. Correlation between serum zinc level and frequency of stools per day, duration of the present episode of diarrhoea, episodes of diarrhoea in the preceding year, duration of the episodes in the preceding year and the severity of dehydration were determined using Pearson correlation coefficient. The student's T-test was used to compare the mean of two variables and p values <0.05 were regarded as significant. The results of children were made known to the parents or guardian of the participants, and the children with diarrhoea who had zinc deficiency were given prescriptions for zinc supplementation.

3. RESULTS AND DISCUSSION

Sociodemographic Parameters

Table 1 shows the age group, gender and socioeconomic class distribution of the study population. The mean age of children with diarrhoea and the controls were 17.6 ± 11.8 months and 18.3 ± 11.1 months respectively. Children between the ages of 6 – 12 months had the highest prevalence of diarrhoea accounting for 92 (45.8%) of cases. There were more males in both subjects 109 (54.2%) and controls 112 (55.7%). The greater proportion of the study population were in the upper and middle socioeconomic class, however more children with diarrhoea were from the lower socioeconomic class compared to the controls (9.5%). The observed differences were not statistically significant (p values = 0.631, 0,764 and 0.292 respectively).

Table I: Sociodemographic characteristics of the study population

Socioeconomic Variables	Children withdiarrhoea (%)	Controls (%)	X^2	P value
Age group (months)				
6 – 12	92 (45.8)	80 (39.8)		
13 – 24	73 (36.3)	78 (38.8)		
25 – 36	20 (10.0)	27 (13.4)	2.579	0.631
37 – 48	9 (4.5)	11 (5.5)		
49 – 59	7 (3.5)	5 (2.5)		
Gender				
Male	109 (54.2)	112 (55.7)	0.090	0.764
Female	92 (45.8)	89 (44.3)		
Socioeconomic class				
Upper	86 (42.8)	94 (46.8)		

Middle	86 (42.8)	88 (43.8)	2.462	0.292
Lower	29 (14.4)	19 (9.5)		
Total	201(100)	201(100)		

X^2=Chi-square, %=Percentage

Clinical presentation of the children with diarrhoea

The clinical presentation of the children with diarrhoea is shown in Table II. One hundred and twenty three children (61.2%) presented with passage of loose stool 3–4 times each day, one hundred and seventy (84.6%) had symptoms lasting for less than a week, 12 (6.0%) had blood in their stools and one hundred and forty one (70.1%) had fever.In the preceding year, 114 children (56.7%) had diarrhoea;of these, 78 (38.8%) had an average of 1–2 episodes per year, while only one child (0.5%) had more than six episodes per year. The highest duration of diarrhoeal episodes reported was 2–3 days in 57 (28.4%) of the children.

Table II: Clinical presentation of the children with diarrhoea

Clinical Presentation	**Number of Children n = 201(%)**
Frequency of stool per day	
3-4 times	123(61.2)
5-6 times	65(32.3)
≥7 times	13(6.5)
Duration of present episode	
<1 week	170(84.6)
1-2 weeks	22(10.9)
≥2 weeks	9(4.5)
Presence of blood in stool	
Yes	12 (6.0)
No	189 (94.0)
Presence of fever	
Yes	141 (70.1)
No	60 (29.9)
Episodes of diarrhoea in the preceding year	
None	87(43.3)
1-2	78(38.8)
3-4	33(16.4)
5-6	2(1.0)
≥6	1(0.5)
Average duration of diarrhoea episodes in the preceding year	
None	87(43.3)
<2 days	12(6.0)

2-3 days	57(28.4)
4-5 days	33(16.4)
>6 days	12(6.0)

%=Percentage

Types of diarrhoea in the study subjects

Amongst the 201 children with diarrhoea, 182 (90.5%) had acute watery diarrhoea, 12(6%) had dysentery and 7(3.5%) had persistent diarrhoea. Out of the 12 children with dysentery, 10 had acute watery diarrhoea while 2 had persistent diarrhoea. Table III shows the frequency of the types of diarrhoea.

Table III: Types of diarrhoea in the study subjects

Types of diarrhoea	**Number of children (%)**
Acute watery diarrhoea	182 (90.5)
Dysentery	12 (6.0)
Persistent diarrhoea	7 (3.5)
Total	201(100)

%=Percentage

Prevalence of zinc deficiency in the study population

Serum zinc level below 65µg/dl was seen in 169 children with diarrhoea and 113 children without diarrhoea, giving a prevalence rate for zinc deficiency of 84.1% and 56.2% in subjects and controls respectively. Zinc deficiency was significantly more in children with diarrhoea than in the controls (X^2 = 37.254; p < 0.001) as shown in Table IV.

Table IV: Prevalence of zinc deficiency in the study population

Zinc status	**Diarrhoea**	**Controls**	**X^2**	**P value**
	Number (%)	**Number (%)**		
Deficient	169(84.1)	113(56.2)		
Normal	32(15.9)	88(43.8)	37.254	<0.001*
Total	201(100)	201(100)		

(*****) =Statistically significant, X^2=Chi-square, %=Percentage

Prevalence of zinc deficiency according to the types of diarrhoea

All the children with persistent diarrhoea and dysentery had zinc deficiency. Zinc deficiency was observed in 82.4% of children with acute watery diarrhoea. However, no statistically significant association was found between types of diarrhoea and zinc deficiency (p=0.137) as shown in Table V.

Table V: Prevalence of zinc deficiency according to the types of diarrhoea

Diagnosis	Zn Deficient n = 169(%)	Normal Zn n = 32(%)	X^2	P value
Acute watery diarrhoea	150 (82.4)	32(17.6)		
Dysentery	12 (100)	0 (0.0)	3.973	0.137
Persistent diarrhoea	7 (100.0)	0 (0.0)		

X^2=Chi-square, %=Percentage

Relationship between serum zinc level and the types of diarrhoea

Serum zinc levels were lower in children with persistent diarrhoea and dysentery compared to children with acute watery diarrhoea, but the difference was statistically significant only when children with persistent diarrhoea were compared with children who had acute watery diarrhoea. ($p < 0.001$) (Table VI).

Table VI: Relationship between serum zinc level and the types of diarrhoea

Types of diarrhoea	Serum zinc level X± SD (µg/dl)	*P* value
Acute watery diarrhoea	48.7 ± 14.9	
Dysentery	37.4 ± 8.5	<0.001*
Persistent diarrhoea	31.1 ± 5.7	

(*) = Statistically significant

Relationship between serum zinc level and the frequency of diarrhoea episodes in the preceding year

The serum zinc level declined as the frequency of diarrhoea episodes in the preceding year increased. However, the observed difference was not statistically significant (p = 0.282) (Table VII).

Table VII: Relationship between serum zinc level and the frequency of diarrhoea episodes in the preceding year

Diarrhoea episodes in the	Serum zinc level	Test statistic	*P* value

Preceding year	X ± SD (µg/dl)		
None	50.0 ± 12.9		
1 - 2	50.0 ± 17.7		
3 - 4	44.6 ± 12.5	1.126	0.282
5 – 6	43.6 ± 6.8		
>6	38.1 ± 5.2		

X=Mean, SD= Standard deviation

Spearman correlation between serum zinc level and some variables.

As shown in Table VIII, a negative correlation existed between serum zinc level and duration of diarrhoea, as well as,stool frequency ($r = -0.262$, $p < 0.001$ and $r = -0.169$, $p = 0.017$ respectively). Similarly serum zinc level also showed a negative correlation with episodes of diarrhoea in the preceding year, the duration of each episode and severity of dehydration ($r = -0.190$; $p = 0.007$, $r = -0.258$; $p < 0.001$ and $r = -0.171$; $p= 0.015$ respectively).

Table VIII: Correlation between serum zinc level and some variables.

Variables	r	P value
Frequency of stools per day	-0.169	0.017
Duration of the present episode of diarrhoea	-0.262	< 0.001
Episodes of diarrhoea in the preceding year	-0.190	0.007
Duration of diarrhoeaepisodes	-0.258	<0.001
Severity of dehydration	-0.171	0.015

4. DISCUSSION

The prevalence of zinc deficiency in children with diarrhoea in this study was 84.1%. Like many other studies, the high prevalence of zinc deficiency in children with diarrhoea observed in the study could be explained by the increased fecal zinc excretion during diarrhoea since zinc is metabolized and absorbed through the intestinal tract[17,18].This may also be explained by reduced intake during diarrhoeal episodes. A lower prevalence was reported in Iran, India and Uganda and this difference may be due to the fact that the researchers observed children with only one type of diarrhoeal disease (either acute or persistent diarrhoea) unlike in this study where children with acute, persistent and bloody diarrhoea were all recruited[19,20,21]. Furthermore, the study in India recruited older children (0 to 12 years) into their study and zinc deficiency is known to be more likely in children under five years of age due to decreased absorptive capacity for zinc and

increased likelihood of diarrhoea[21].This finding indicates need for strict adherence of zinc supplementation in children with diarrhoea.

Similarly, the study observed statistically significant lower mean serum zinc level in children with diarrhoea compared to the controls (47.4±14.9µg/dl vs. 60.4±16.6µg/dl). Increased loss of zinc in diarrhoea, reduced intake and increased demand during intestinal infections may explain this finding. Several other studies have also reported lower mean serum zinc level in children with diarrhoea when compared to their age and sex matched controls[14,19,20].

The prevalence of zinc deficiency among the controls in this study was 56.2%. This prevalence is comparable to that documented by many Nigerian and African authors[22,23,24,25]. This could be attributed to the fact that children in developing countries eat meals made up of mainly grains and tubers which are either low in zinc or high in phytate. However, comparatively, the observed prevalence is higher than the global prevalence of 31% documented by Caulfield *et al* and 28.1% documented in Iran[8,26]. This variance could be attributed to the difference in the methodology used for zinc estimation and the age of children sampled in the studies. While Caulfield *et al* estimated zinc deficiency by assessing the bioavailability of zinc in countries' food supply, and calculating the proportion of individuals in each country with intake below the daily zinc requirement without actually assaying for serum zinc level as in this study; Fesharakinia*et al* measured serum zinc level in older children aged 9 to 11 years, and zinc deficiency is known to be less common in the older age group[8,26].

Children with dysentery and persistent diarrhoea had lower levels of serum zinc when compared to children with acute watery diarrhoea this difference was statistically significant for persistent diarrhoea. Persistent diarrhoea and dysentery are more likely to be of bacterial origin, cause systemic disease, and lead to increased demand for zinc, but in persistent diarrhoea, there is disruption and loss of intestinal epithelia which leads to defective fluid absorption by the intestine and loss of fluid containing essential micronutrients including zinc[7,13,27].This prolonged loss of zinc in persistent diarrhoea may be accountable for the finding in this study. Mahyar*et al* and Chaudhary *et al* in Iran and India respectively also reported significant reduction in serum zinc level in children with dysentery and persistent diarrhoea[20,28]. Longer zinc supplementation in children with dysentery and persistent diarrhoea may have to be considered.

In this study, serum zinc level declined as the frequency of diarrhoea episodes in the preceding year increased. A similar trend has been documented by researchers in Uganda[19]. This may imply that zinc losses are cummulative (rather than occurring only during the current episode of diarrhoea), and that some level of replenishment could occur in between diarrhoeal episodes to help maintain serum zinc level. In contrast, Ferazz*et al* in Brazil documented that the frequency of diarrhoea episodes in the preceding year did not affect serum zinc level[29].

There was a negative correlation between serum zinc level and stool frequency, duration of the present episode of diarrhoea, episodes of diarrhoea in the preceding year and the duration of each episode in that year. This means that serum zinc level declined as stool frequency or duration of diarrhoea episodes increased. Increased frequency of stool would result in increased loss of zinc in stool, more so when the duration of diarrhoeal episode is increased, prolonged loss of zinc will occur, thus creating a vicious cycle.

Serum zinc level also showed a negative correlation with severity of dehydration. Children with a greater number of bowel motions per day or longer duration of diarrhoea are more likely to have low serum zinc level due to increased loss in stool; as well as increasing risk of dehydration. A similar finding was reported by researchers in Iran [20].

Conclusion

The prevalence of zinc deficiency in under-five children with diarrhoea in this study was high at 84.1%; and of public health significance, thus strengthening the WHO zinc supplementation in children with diarrhoea. Children with persistent diarrhoea had significantly lower serum zinc levels when compared to those with acute watery diarrhoea indicating the need for longer supplementation of zinc in these children.

Data Availability

The anthropometric data used to support the findings of this study are available from the corresponding author upon request.

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