

ORIGINAL ARTICLES

Serum zinc status of children with persistent diarrhoea admitted to the diarrhoea management unit of Mulago Hospital, Uganda

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ABSTRACT

Introduction: Despite great advances in the management of diarrhoeal diseases, persistent diarrhoea remains a major problem in developing countries due to its syndromic nature. Zinc depletion ranks high among the factors contributing to the detrimental effects of persistent diarrhoea on the human body. This however, has not been investigated in the Ugandan population.

Objective: To determine the serum zinc status of children with persistent diarrhoea.

Design: Cross-sectional descriptive study.

Methods: Children aged 6-36 months with persistent diarrhoea were enrolled from the diarrhoea management unit of Mulago hospital. Socio-demographic and morbidity data were collected, and laboratory investigations were carried out after recruitment. Healthy children of similar age and sex were recruited to determine reference levels of serum zinc for comparison.

Results: The mean serum zinc level in the children with persistent diarrhoea was 5.83mol/l while that of children without diarrhoea was 8.99mol/l with no age or sex difference. The serum zinc concentration of children with persistent diarrhoea was significantly lower than that of children without diarrhoea ($p < 0.001$). The prevalence of zinc deficiency in children with persistent diarrhoea was 47.9%. Of the children with persistent diarrhoea, 64 (66.7%) were stunted, wasted or both. However no significant association was observed between nutritional status and serum zinc levels. Only hypoproteinaemia was significantly associated with serum zinc levels in these children ($p = 0.03$).

Conclusion: There is a high prevalence of zinc deficiency and malnutrition among Ugandan children with persistent diarrhoea.

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INTRODUCTION

Micronutrients have for long been known to be necessary for the well being of the human body. Lately however, they have come to the forefront of nutritional research due to a better understanding of their pivotal role in body metabolism.¹

Zinc is second only to iron as an essential micronutrient. Since the 1960's when its deficiency was first described, various interactions of zinc have been elucidated. Zinc promotes growth of the human body, and this is effected through its stimulatory action on the synthesis of growth hormone mediators by the liver.¹⁻⁷

Zinc is also necessary for the integrity and normal functioning of the immune system⁸⁻¹⁰ The lymphoproliferative and anti-oxidant effects of zinc are central to this role. The latter enhance the body's natural protective mechanisms and in

combination with increased cell division, they are responsible for tissue repair and wound healing. Needless to say zinc deficiency would lead to impairment of the above functions with resultant reduction in growth rates, tissue repair and immunocompetence in children.¹⁻¹⁰

The zinc status of an individual depends on various factors namely; dietary amounts, availability for absorption, the physiological needs of the individual, as well as the extent of endogenous losses from the body. Zinc deficiency is a widespread nutritional problem affecting populations of low social economic status in both developed and developing countries¹¹⁻¹³. Consumption of staple diets high in fiber and phytate inhibits the bioavailability of zinc¹⁴⁻¹⁶. Malnutrition, a common condition in developing countries, results in deficiency of micronutrients in addition to that of protein and energy¹⁷⁻²¹. Growing children have an increased demand for all nutrients, zinc inclusive. Diarrhoea, bleeding and hemolysis have been documented to increase endogenous losses of zinc^{11,12,22}. Persistent diarrhoea due to its syndromic nature, leads to reduced absorption of all nutrients with their subsequent loss in the increased intestinal secretions and stools. This is thought to lead to a negative zinc balance^{23,24}. Persistent diarrhoea is therefore likely to have a profound effect on a population with marginal zinc status^{6,7,14,16,17}.

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In epidemiological studies, changes in growth rates of children or improvement in diarrhoeal disease following zinc supplementation are useful indices in assessing body zinc status^{1,2,4,6}. Serum zinc determination may be insensitive, however when done using atomic absorption spectrophotometry is the simplest and analytically the most reliable test for routine assessment of zinc nutrition^{25,26}. In this regard, studies in India²⁷, Bangladesh²⁸, Peru²⁹ and Pakistan³⁰ have reported reduced serum zinc levels in persistent diarrhoea. In Uganda however, no such studies have been undertaken, yet relative deficiency has been reported in certain population groups such as pre-school children and rural adolescents⁶⁻¹⁶.

This study was designed to test the hypothesis that Ugandan children with persistent diarrhoea had low serum zinc levels.

METHODS

This was a cross-sectional descriptive study carried out from October 1999 to January 2000, in the paediatric diarrhoea management unit, of Mulago hospital. Patients aged 6 to 36 months with a diagnosis of persistent diarrhea (lasting 14 days or more) were selected. Healthy children with no diarrhoea, no clinical illness and normal nutritional status were also recruited into the study to establish reference standard for serum zinc levels in the population. These children were drawn mainly the population in Kampala city and Mpigi district. Children with sickle cell disease or those who presented with malaria or helminthiasis were excluded from the study. Parental/caretaker informed consent was obtained before enrollment.

Using the formula by Kish and Leslie³¹ a sample size of 96 was chosen to detect at a 20% prevalence of persistent diarrhoea²⁹ with precision of 8% and 95% confidence.

One of us (EB) collected data at the time of admission/enrollment into the study. Information regarding family socio-economic and demographic characteristics was obtained by interviewing the parent/caregiver who had accompanied the child to hospital.

Measurements

Clinical examination including anthropometry was done on all the children. The children were weighed to the nearest 0.1 kg using a standardized Salter spring scale while height was measured using a stadiometer to the nearest millimeter. Using the

mid-point between the acromion and olecranon process, the mid upper arm circumference was measured using a non-stretch fiberglass tape.

Laboratory procedure

For the zinc analysis, 3ml of blood were collected into plastic tubes (Nalgene Sybron Corporation, Rockester, New York) and centrifuged within one hour to avoid haemolysis. The serum obtained was frozen at -20°C until the zinc analysis. Calibration of the atomic absorption spectrophotometer (Perkin - Elmer 2380) was done using zinc stock standards diluted with 5% glycerol. Zinc concentration was then measured using an air-acetylene flame at a wavelength of 213.9 nm and a slit width of 0.7nm.

Three milliliters of the fresh blood were collected into a sequestrene bottle for hematological studies. A full haemogram (erythrocyte, leukocyte and platelet counts, and the various cell parameters) was done using an automated counter (Coulter Onyx 4237217 - A). It was re-calibrated daily using standard solutions (4C® Plus Coulter Cell Control). A sickling test was done using sodium metabisulphite added volume for volume to blood on a glass slide and sealed under a cover slip. A blood smear for malarial parasites was done using Leishman's stain and subsequently examined under the high power lens of a light microscope.

Three milliliters of blood was collected into a plain bottle for protein assays. Both total protein and serum albumin were quantified by an automated spectrophotometer (550 Express Plus Spectrophotometer) that was calibrated daily using a control (Auditriol Control, Biotec Laboratories, United Kingdom). Total serum protein was estimated using the Biurette method while serum albumin was estimated using the Dye Color bind (BCG - Bromocresol Green) method.

Direct stool microscopy for ova and cysts was done on fresh specimens.

Data management and analysis

Data analysis was done using EPI-Info, Minitab and BMDP Statistical software. Proportions and frequency tables were used to summarize the distribution of categorical variables in the study population. For continuous variables, analysis of variance was used. Cross tabulations, chi-square tests and odds ratio were used to test association between serum zinc concentration and individual factors. Regression analysis was done to determine the effect of nutritional status and other factors on the zinc levels.

The lower limits for the various measurements were; serum zinc- 4.73 mmol/l, Serum protein- 60 g/l,

serum albumin- 35 g/l, weight and height for age less than - 2 Z scores. Significant p value was considered to be less than 0.05.

RESULTS

A total of 96 children with persistent diarrhoea and an equal group of healthy children were recruited for the study. There was a male to female

ratio of 1.6: 1. Reported ages ranged from 6 - 36 months with a median age of 13 months. The majority of children (85.4%) in both groups were in the age range 6-24 months. There was no statistically significant difference in age between the two groups ($t = 0.356$; $p = 0.71$). The mean age at weaning was 4.6 months in both groups and there was no statistically significant difference between them ($p = 0.983$). Table 1 shows the socio-demographic characteristics of the children.

Table 1: Socio-demographic characteristics: children

Characteristics	Children with Persistent Diarrhoea	Healthy children with no diarrhoea	t - value	p value for difference between groups
Age ¹ (months)	13.79 ± 6.7	14.14 ± 6.9	0.35	0.72
Sex M: F (numbers)	60: 36	60: 36	-	0.95
M: F (ratio)	1.6: 1	1.6: 1	-	
Age at weaning ¹ (months)	4.62 ± 3.7	4.63 ± 1.5	0.21	0.98

¹ Mean ± standard deviation

None of the socio-demographic characteristics showed a statistically significant difference between the two groups.

Biochemical characteristics

The mean serum zinc concentration of children with persistent diarrhoea was 5.83 (± 3.83, 95% CI 2.00-9.66) µmol/l (statistical range 0.00-13.49 µmol/l), which was significantly lower than that of the healthy children (8.99 (± 2.13, 95% CI 6.86-11.12) µmol/l (statistical range 4.73 - 13.26 µmol/l). Serum zinc levels were considered to be low if they were less than - 2 SD of the mean or < 4.73 µmol/L. Subsequent evaluation of serum zinc levels revealed that 46 (47.9%) of children with persistent diarrhoea had low serum zinc levels (< 4.73 µmol/L) as compared to 3 (3.1%) of healthy children. There were no sex differences in the distribution of serum zinc between both the healthy children with no diarrhoea ($p = 0.79$) and in those with persistent diarrhoea ($p = 0.065$).

Serum protein and albumin were significantly lower in children with persistent diarrhoea compared to those without diarrhoea ($p = 0.00$, $p = 0.00$ respectively).

Among the children with persistent diarrhoea, 46 (47.9%) had low total protein (< 60 g/l) while 67 (69.7%) of them had low serum albumin (< 35 g/l). Mean hemoglobin levels were significantly

lower in children with persistent diarrhoea as compared to healthy children ($p = 0.00$).

All the bio-chemical indices reviewed were statistically significantly lower in the children with persistent diarrhoea compared to the healthy ones.

On logistic regression none of the child or parental socio-demographic characteristics was statistically significantly associated with serum zinc levels.

In the three months preceding this study, 49 (51%) of the children with persistent diarrhoea had at least one episode of persistent diarrhoea while 68 (70.8%) had at least one episode of acute diarrhoea. Measles infection had occurred in 19 (19.8%) of the children with persistent diarrhoea. There was a high occurrence (94.7%) of non-enteric illness in the month prior to enrollment into the study. Ninety-one of the children had suffered from fever (40.7%), respiratory tract (19.8%), skin (1.1%) or urinary tract (9.8%) infections. Some of these children (28.6%) had a mixture of the above infections.

Using Waterlow's³² classification of malnutrition only 32 (33.3%) of children with persistent diarrhoea were of normal height and weight for age. The rest had varying degrees of protein- energy malnutrition. None of the above morbidity factors showed a statistically significant association with serum zinc levels.

Table 2: Biochemical indices

Characteristics	Children with Persistent Diarrhoea	Healthy children with no diarrhoea	t - value	p value for difference between groups
Serum zinc	5.829 ± 3.83	8.993 ± 2.133	6.43	0.00 *
Serum protein ¹	5.742 ± 1.094	6.401 ± 0.684	5.02	0.00 *
Serum albumin ¹	2.887 ± 0.774	3.375 ± 0.365	5.60	0.00 *
Hemoglobin ¹	10.455 ± 2.383	12.371 ± 1.132	7.139	0.00 *

¹ Mean + standard deviation

* Significant p value

Of the biochemical factors reviewed, both total serum protein and albumin were found to be significantly lower in children with persistent diarrhoea (p = 0.00). Only total protein was however found to be significantly associated with serum zinc levels (p = 0.032). See table 3.

Table 3: Logistic regression of various factors and serum zinc levels in persistent diarrhoea

Factor	p value
<i>Parental</i>	
Age	0.52
Formal Education	0.77
Maternal occupation	0.49
<i>Children</i>	
<i>a) Demographic</i>	
Sex	0.19
Age	0.59
Age of weaning	0.73
Breastfeeding	0.64
<i>b) Morbidity</i>	
Vaccination status	0.28
Previous Measles infection	0.64
Recent non-enteric illness	0.78
Previous persistent diarrhoea	0.84
Previous acute diarrhoea	0.67
Duration of diarrhoea	0.30
Weight for height	0.07
Weight for age	0.18
Height for age	0.51
Mid upper arm circumference	0.33
Temperature	0.36
<i>c) Biochemical</i>	
Albumin	0.68
Protein	0.032 *
Haemoglobin	0.61
Constant	0.037

* Significant p value; goodness of fit chi square = 9.027

The model used was; $\log \left(\frac{p}{1-p} \right) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots$ Where α = a constant; β = Regression coefficient of dependent variable; X = Independent variable under investigation and p = the probability.

DISCUSSION

This cross-sectional descriptive study was designed to establish the serum zinc status of children with persistent diarrhoea admitted to Mulago hospital. This was necessary given the current evidence that the Ugandan population is relatively zinc deficient^{6,16} and persistent diarrhoea is an emerging new problem known to exacerbate both micro- and macro nutrient deficiency^{23,27-30}. For this purpose, 96 children with persistent diarrhoea aged 6-36 months were recruited into the study. It was also necessary to recruit 96 healthy children for the purpose of establishing the current normal serum zinc status in the children around Kampala.

Although laboratory criteria for the assessment of body zinc status are not well established, serum zinc concentration was used to screen for zinc deficiency because it is the best single choice^{25, 26} available in comparison to the other options^{1, 2,4,6,33,34}.

The range of serum zinc of healthy children in the current study was 4.73 - 13.26 $\mu\text{mol/l}$ with a mean of 8.98 and no age or sex difference ($p = 0.063$, $p = 0.79$ respectively). These serum zinc levels are lower than what has been established by other researchers. An earlier study in Kampala found a serum zinc range of 8.4 - 20.9 $\mu\text{mol/l}$ (mean 10.1 \pm 3.2) among children aged 4 -14 years with no age or sex difference²². Gatheru *et al.*, in Nariobi found a range of 10-17 $\mu\text{mol/l}$ (mean 12.9) in children aged 1 to 3 years¹⁷. The findings of the current study however, agree with other Uganda studies that reveal varying degrees of zinc deficiency among relatively healthy individuals. Kikafunda and Tumwine carried out a zinc supplementation trial in pre-school children using the principal that the best indicator of a deficient state may be the clinical response to supplementation⁶. Indeed, a significant increment in mid upper arm circumference and a greater weight gain were recorded in a section of the study population. In Bachou's study of rural adolescents in the West Nile region of Uganda, up to 90% of the participants had low hair zinc levels¹⁶. This reflects a relatively low serum zinc status in the populations from which these children were sampled. These studies therefore bring to light a potential public health concern by revealing a trend of low zinc status in a cross-section of communities in Uganda. The high endemicity of malaria in Uganda and the predominance of vegetarian diets with a high phytate content may

explain the poor zinc status^{9, 16}.

The current study detected a profound negative effect of persistent diarrhoea on the serum zinc status. The serum zinc concentration of children with persistent diarrhoea ranged from 0.00-13.49 $\mu\text{mol/l}$ (95% CI 2.00-9.66) with a mean of 5.83. Despite a degree of overlap in levels between the children with persistent diarrhoea and the healthy children, the difference between the mean serum zinc levels in these two groups of children was highly significant ($p = 0.00$). The comparatively lower serum zinc concentrations in children with persistent diarrhoea are in agreement with what other studies have shown^{23,27-30,35}.

Various studies have found malnutrition in 40-83% of children with persistent diarrhoea^{36,37,38} while the results of the current study show that 66.7% of the children with persistent diarrhoea were either stunted, wasted or both. The observed lack of significant correlation between nutritional states and serum zinc levels has also been documented in a Ghanaian study of preschool children³⁹ and in hospitalized patients with the Acquired Immune Deficiency syndrome (AIDS) in the United States⁴⁰. This may probably be due to a cytokine directed internal redistribution of zinc that could also be responsible for the lack of a direct correlation between serum albumin and zinc⁴¹.

In contrast, hypoproteinaemia was significantly associated with serum zinc levels. Sarker *et al.*, similarly found that plasma protein mass concentration had a direct proportional relationship to serum zinc levels in children with diarrhoea⁴². The low levels seen in persistent diarrhoea may in part be due to the hypoproteinaemia resulting from malnutrition or from protein loss secondary to the impaired absorption and increased secretions from the diseased gut.

Neither the duration of persistent diarrhoea nor history of a prior episode (acute or persistent) was significantly associated with serum zinc levels. These findings agree with studies in Peru²⁹ and Pakistan³⁰ and may probably imply that most zinc losses occur during the on-going episode of diarrhoea, and they may also suggest that during states of zinc depletion, homeostatic mechanisms attempt to maintain a steady state of circulating levels of zinc by re-distribution in the body^{41,43}.

In this study, history of previous measles infection was not significantly associated with serum zinc concentration among the children with persistent diarrhoea. This is contrary to what Sarker *et al.*, found in Bangladeshi children with post-measles diarrhoea⁴². The differing results observed in the current study may probably be due to the longer time period (3 months) within which measles infection was considered to have occurred.

The findings of this study reveal significant zinc deficiency among children with persistent diarrhoea. However, given that most of the children were severely malnourished, it could not be established whether the deficiency was solely due to the diarrhoea or to the poor nutritional status. Of the factors associated with persistent diarrhoea in the study group, only low serum protein was significantly related to serum zinc levels. What is clear though is that the integrated management approach to children with persistent diarrhoea should specifically address the issue of zinc supplementation given the high prevalence (47.9%)

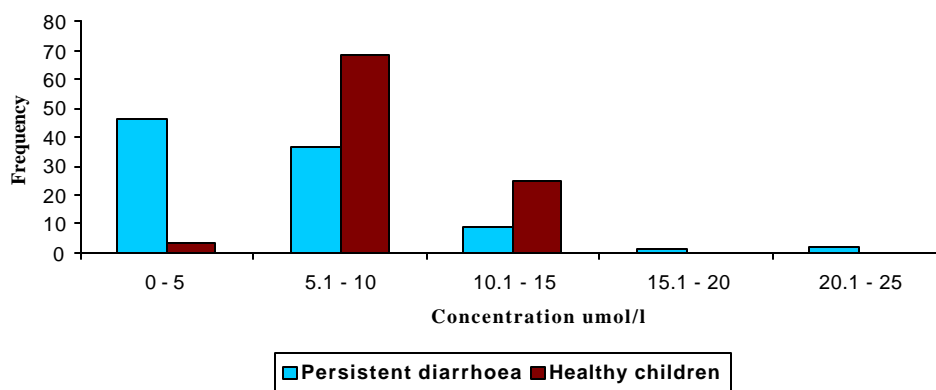
of zinc deficiency in children with persistent diarrhoea admitted to Mulago hospital.

Hypoproteinaemia is significantly associated with serum zinc levels in children with persistent diarrhoea and its presence should alert to the severity of hypozincaemia.

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Figure 1: Comparison of Serum Zinc Levels between Children with Persistent Diarrhoea and those without Diarrhoea.



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