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Levels of Serum Zinc, Copper and Copper/Zinc Ratio in Patients with Diarrhea and HIV Infection in Ethiopia

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Abstract

Introduction: Zinc and copper are essential for normal human development and functioning of the body. They have been implicated to play important roles in immuno-physiologic functions. Studies assessing the interactions between diarrheal diseases, HIV/AIDS and micronutrient status are too few in Ethiopia, as in other sub-Saharan Africa where morbidities from diarrheal diseases and HIV/AIDS are serious health problems. Hence, the present study was undertaken to investigate the level of zinc and copper as well as zinc/copper ratio among HIV positive diarrheic patients with sex and age matched HIV negative diarrheic patients.

Methods: A cross sectional study was conducted amongst 206 diarrheic patients (109 HIV seropositive and 97 HIV seronegative) patients. Concentration of serum level of zinc and copper was determined by inductively coupled plasma-mass spectrometer. Reference intervals were defined according to recommended guidelines.

Results: Mean serum zinc level were not significantly different between diarrheic patients with (68.13 ± 44.53µg/dL) and without (62.39 ± 43.64) HIV co-infection. Deficiency of zinc was seen in 69.7% and 80.4%% of diarrheic patients with and without HIV co-infection, respectively. HIV infected diarrheic patients with shigellosis (100%) and with intestinal parasites (63.3%) were deficient in serum zinc level. Unlike zinc, no diarrheic patients with or without HIV co-infection were found significantly deficient in serum copper levels.

Conclusion: Zinc deficiency is a severe public health problem in Gondar, Ethiopia, among diarrheic patients irrespective of HIV co-infection. Further studies are required to establish the role of these low concentrations in host defense against diarrheic patients with or without HIV, so that appropriate and beneficial strategies for micronutrient supplementation can be planned.

Keywords: Zinc; Copper; HIV/AIDS; Diarrhea; Gondar; Ethiopia

Introduction

Trace elements such as zinc and copper are essential for normal human development and functioning of the body [1]. They have been implicated to play important roles in immuno-physiologic functions [1,2]. For example, zinc is an integral part of more than 200 enzymes and has significant task in nucleic acid metabolism, cell replication, tissue repair, and growth. Its deprivation leads to profound alteration of thymic function with resultant loss of T-cell-mediated responses and increased susceptibility to infectious diseases [1,2]. Copper is present in cytosolic erythrocyte superoxide dismutases (Cu, Zn-SOD), which is an important scavenger of O_2^-, a free radical that causes damage to membranes and biological structures. A clear association between a history of diarrheal episodes and low plasma copper and zinc levels has been demonstrated, with a copper deficit being more prevalent than a zinc deficit [3,4].

On the other hand, micronutrient malnutrition is prevalent in many developing countries and may contribute to a weakening of immune status and, thus, a worsening of clinical condition among HIV-infected individuals [9]. Amongst the many micronutrients, the link between trace elements deficiency and morbidity and mortality from infectious diseases has been known for several years [6]. The copper and zinc are important micronutrients and they are involved in the modulation of immunity and the modification of the risk of infection [10,11].

Diarrheal diseases are one of the most important causes of morbidity and mortality in developing countries [5]. The situation is severe in sub-Saharan Africa, a region where an estimated 25.8 million adults and children are infected with HIV [6]. Diarrhea, the passage of loose or watery stools at least three times in 24 hours, is one of the clinical manifestations of HIV infection and usually tends to be chronic [7]. Chronic diarrhea, an episode that begins acutely and lasts for more than four weeks, in tropical countries is associated with weight loss and is often the presenting illness of HIV infected individuals [7]. This diarrhea wasting syndrome in association with a positive HIV serology test is an AIDS defining illness in the World Health Organization's classification [8].

In Ethiopia, as in other sub-Saharan Africa, morbidities from diarrheal diseases [2,10] and HIV/AIDS [12,13] are serious health problems. However, studies assessing the interactions between diarrheal diseases, HIV/AIDS and micronutrient status are too little. Therefore, the present study was undertaken to investigate the level of zinc and copper as well as zinc/copper ratio among diarrheic patients with and without HIV co-infection.

Methods

Study design and subjects

In this cross-sectional study, consecutive diarrheic patients

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diagnosed at the outpatient department of the University of Gondar Hospital, in Gondar, Ethiopia. Informed consent was obtained from all subjects and the study was approved by the Research Ethics Committee of the University.

Collection of stool specimens and examination for enteropathogens

Diarrheic stool specimens were collected in sterile containers and processed immediately following the standard procedures. The specimens were inoculated on plates of MacConkey agar (DIFCO) and Salmonella-Shigella agar (DIFCO). The plates were incubated at 37°C for 24 hours and then examined for non-lactose fermenting colonies which were further examined by conventional biochemical tests for the identification of *Shigella* species and *Salmonella* species [14]. The stool samples were also examined microscopically for intestinal parasites following direct, concentration and modified acid fast staining procedures [15].

Blood collection, serum separation and HIV serology

Blood samples were collected from the patients and sera were separated by centrifugation and stored at -20°C until used. The presence of HIV antibodies was determined by an enzyme linked immunosorbent assay following the manufacturer's instruction (Vironostica HIV Uni-Form II plus O, Organon Teknika, Boxtel, the Netherlands).

Determination of trace elements in serum

The frozen serum samples were kept on dry ice and air freighted to Japan. Concentration of trace elements in serum was determined using an inductively coupled plasma mass spectrometer (ICP-MS) (model 8500, Schimadzu, Tokyo, Japan), at Department of analytical chemistry, the University of Tokushima, Japan following previously published procedures [20]. In brief, serum sample (200 µl) was aliquoted in to teflon tube and covered with teflon ball. After adding 1 ml of concentrated HNO_3 (Wako Pure Chemicals, Japan), the tube was heated on an aluminum heating block (IWAKI, Asahi Techno Glass, Japan) at 120°C for 5 hours. The sample was further heated almost to dryness at 200°C after removing the teflon ball. Finally, the residue was dissolved with 2 ml of 0.1 M HNO_3 which contained 10 ng/ml internal standard elements (In, Re, and Tl). The diluted serum solution was used for analysis of the elements in ICP-MS. Commercially available single element standard solutions (1000 µg/ml) were purchased from Wako Pure Chemicals (Osaka, Japan) and used for standardization of calibration curves.

Statistical analysis

Data were analyzed using SPSS version 13 statistical package (SPSS, Inc., Chicago, IL, USA). A one-sample Kolmogorov-Smirnov test was used to assess whether the data were normally distributed. All trace element values in serum were normally distributed in patients with and without HIV co-infection and hence no transformation was done. Comparisons of serum values of the trace elements among diarrheic patients with and without HIV co-infection were made using independent T-test. Post hoc Tukey test was used to determine which pairs of means differ significantly. Deficiency of Zinc and Copper were defined at their serum levels less than 75 µg/dl and 75 µg/dl, respectively. Regression analyses was used to see the presence any association of age, sex, residence, marital status, occupation, monthly income and the status of enteropathogens as independent variables and serum level of zinc, copper and Cu/Zn ratio as dependent variable and a deficient level of Cu and Zn. P values less than 0.05 were considered statistically significant.

Results

Social demographic characteristic of study subjects

A total of 206 diarrheic patients (109 HIV seropositive and 97 sex and age matched HIV seronegative) were included in the study. Table 1 shows baseline characteristics of the diarrheic patients in association with the proportion of patients with zinc deficiency by HIV serostatus. Majority of the study subjects were in the age group 25–34 years. There was a male to female ratio of 1.61:1.00. Acute and chronic diarrhea was observed in 44.7% and 55.3% of the patients, respectively. The proportion of patients with zinc deficiency was greater among both HIV+ and HIV- patients having acute diarrhea (76.7% and 84.4%), respectively than among both HIV+ and HIV- patients having chronic diarrhea(61.2% and 78.5%), respectively .

Serum copper and zinc in relation to HIV status

The Mean ± SD serum zinc levels were not significantly different between diarrheic patients with (68.13 ± 44.53µg/dL) and without (62.39 ± 43.64) HIV co-infection (Table 2). However, its levels in the sera of diarrheic patients with *Shigella* (45.0±16.90) were significantly lower compared to diarrheic patients without *Shigella* (67.38±45.41) (Table 3). Deficiency of zinc was seen in 69.7% and 80.4%% of diarrheic patients with and without HIV co-infection, respectively. HIV infected diarrheic patients with shigellosis (100%) and with intestinal parasites (63.3%) were deficient in serum zinc level (Table 4).

Serum copper and zinc in relation to shigellosis and intestinal parasites

Shigella species and intestinal parasites were detected in 8.5% and 32.2% of the patients, respectively. While 81.8% of those with intestinal parasites were infected with single species, infection by two parasites was seen in 18.2%. Intestinal parasites detected in the stools of the patients were *Entamoeba histolytica* (27.3%), *Strongylodes stercoralis* (18.2%), *Ascaris lumbricoides* (16.7%), *Giardia lamblia* (16.7%), *Schistosoma mansoni* (12.1%), hookworm species (12.1%), and *Cryptosporidium parvum* (4.5%). There was no significant difference in the levels of serum zinc, copper and Cu/Zn ratio levels in diarrheic patients with and without shigellosis or with and without intestinal parasites (Table 3 and 4). The serum zinc levels in the patients who were found positive for *Shigella* species or intestinal parasites was not significantly different by the presence or absence of HIV co-infection (Table 1).

Regression analyses of age, sex, residence, marital status, occupation, monthly income and the status of enteropathogens as independent variables and serum level of zinc, copper and Cu/Zn ratio as dependent variable did not show any significant association between the parameters and a deficient level of these trace elements.

Discussion

HIV infection has become the dominant health problem in many parts of sub-Saharan Africa, with the worst affected areas in central, south and eastern parts of the subcontinent including Ethiopia [6]. One of the major manifestations of the HIV disease in the region is the diarrhea-wasting syndrome [15,16]. Patients with wasting syndrome were reported to have low levels of plasma micronutrients, including serum level of zinc and copper compared to that in non-wasting patients [16,17].

In the present study, 69.7% of the diarrheic patients co-infected with HIV, 80.4% of diarrheic patients without HIV co-infection had serum zinc levels below 75 µg/dL. This may be because zinc

Variable	Total	Zinc deficiency (ZD) in HIV diarrheic patients			
		HIV + 109(52.9%)	HIV + with ZD 76(69.7%)	HIV -97(47.1%)	HIV - with ZD 78(80.4%)
Age					
15-24	52 (25.2%)	11 (10.1%)	7 (63.3%)	41 (42.3%)	32 (78%)
25-34	84 (40.8%)	54 (49.5%)	37 (68.5%)	30 (30.9%)	27 (90%)
35-44	46 (22.3%)	33 (30.3%)	24 (72.7%)	13 (13.4%)	10 (76.9%)
>45	24 (11.7%)	11 (10.1%)	8 (72.7%)	13 (13.4%)	9 (69.2%)
Sex					
Male	127 (61.7%)	65 (59.6%)	48 (73.8%)	62 (63.9%)	49 (79%)
Female	79 (38.3%)	44 (40.4%)	28 (63.6%)	35 (36.1%)	29 (82.9%)
Marital status					
Married	100 (48.5%)	53 (48.6%)	42 (79.2%)	47 (48.5%)	36 (76.6%)
Single	77 (37.4%)	33 (30.3%)	19 (57.6%)	44 (45.4%)	37 (84.1%)
Divorced	29 (14.1%)	23 (21.1%)	15 (65.2%)	6 (6.2%)	5 (83.3%)
Occupation					
Government Employee	47 (22.8%)	25 (22.9%)	18 (72%)	22 (22.7%)	15 (68.2%)
Farmer	63 (30.6%)	46 (42.2%)	28 (60.9%)	17 (17.5%)	16 (94.1%)
Student	27 (13.1%)	17 (15.6%)	13 (76.5%)	10 (10.3%)	9 (90.0%)
Housewife	39 (18.9%)	7 (6.4%)	6 (85.7%)	32 (33.0%)	26 (81.3%)
Other Income	30 (14.6%)	14 (12.8%)	11(78.6%)	16 (16.5%)	12 (75%)
Low	126 (61.2%)	59 (54.1%)	40 (67.8%)	67 (69.1%)	57 (85.1%)
Medium	53 (25.7%)	34 (31.2%)	27 (79.4%)	19 (19.6%)	14 (73.7%)
High	27 (13.1%)	16 (14.7%)	9 (56.3%)	11 (11.3%)	7 (63.6%)
Diarrhea					
Acute	92 (44.7%)	60 (55%)	46 (76.7%)	32 (33%)	27 (84.4%)
Chronic	114 (55.3%)	49 (45%)	30 (61.2%)	65 (67%)	51 (78.5%)
Shigella					
Positive	18 (8.7%)	4 (3.7%)	4 (100%)	14 (14.4%)	13 (92.9%)
Negative	188 (91.3%)	105 (96.3%)	72 (68.6%)	83 (85.6%)	65 (78.3%)
Intestinal parasite					
Positive	66 (32%)	30 (27.5%)	19 (63.3%)	36 (37.1%)	30 (83.3%)
Negative	140 (68%)	79 (72.5%)	57 (72.2%)	61 (62.9%)	48 (78.7%)

Table 1: Characteristics of diarrheic patients in relation to zinc deficiency.

deficiencies predispose patients to diarrhea irrespective of their HIV status. This observation is in agreement with a few previous reports in some developed countries and African patients [16,18,19]. Plasma zinc levels consistent with deficiency has been reported to occur in 63% of Zambian patients with persistent diarrhea [16], in 66.7% of the Ethiopian pregnant women irrespective of their HIV serostatus [20]. Abnormally low serum zinc level were reported in 29% on HIV infected American hospitalized patients [19], in 45% of HIV infected south African adults [21] and in 47.9% of Ugandan children with persistent diarrhoea [22]. The higher prevalence of zinc deficiency found in the present study could be due to an inadequate zinc intake and poor bio-absorption [37]. It is worthy to note that several dietary factors are known to affect zinc absorption as a result of physico-chemical interactions in the intestine [48].

Serum zinc appears to be a marker of susceptibility to infections in patients with AIDS [19]. Periodic serum levels may assist health-care providers in risk stratifying of patients. Despite some studies reported that more advanced disease stage and lower $CD4^+$ lymphocyte counts have been associated with lower zinc concentrations [23-26], we did not observe a significant difference in the mean serum zinc concentrations between diarrheic patients with and without HIV co-infection. This observation may suggest that the ongoing diarrheal illness has a greater impact on zinc status than the effect of HIV infection by itself. However, further studies are needed to fully understand the inconsistency on whether low plasma zinc concentrations are a consequence, confounder or contributing cause of more severe HIV-related disease. The low serum level of zinc in the sera of the diarrheic patients can also be due to reduced dietary intake resulting from anorexia or decreased absorption. Furthermore, reduction in plasma zinc that occurs during infection is attributable to redistribution within the body, [27] or induced by pro inflammatory cytokines have each been linked to specific host-defense mechanisms [28].

In this study, we found a high prevalence of intestinal parasites in diarrheic patients, which is in line with the wide occurrence of intestinal parasitism in Ethiopia. Earlier data suggest that intestinal parasite infections can affect the nutritional status of infected people by modifying the key stages of food intake, digestion and absorption [34]. Abnormalities in the mucosa of intestinal tract were observed in children infected with *A. lumbricoides* by jejunal biopsy, which disappeared rather rapidly after deworming [35]. Trophozoites of *G. lamblia* were shown to damage the brush borders of enterocytes and impair the activity of mucosal enzymes, particularly the disaccharidases, causing carbohydrate and fat malabsorption [36]. It is also hypothesized that the increased intestinal absorption of zinc associated with anti-*Giardia* treatment may be explained by the restoration of intestinal mucosa that had been impaired by giardiasis [37]. However, our cross-sectional data analysis showed no significant differences between the parasite-free and the infected groups in this study. It is worth mentioning that in a randomized, double-blind, placebo-controlled trial, vitamin A and zinc supplementation to children was associated with distinct parasite-specific health outcomes. Vitamin A plus zinc reduces *G. lamblia* incidence and improves immune status among Mexican children [38]. Furthermore, there are reports that low maternal zinc status during pregnancy or delivery has been shown to be associated

	Serostatus		
Analyte (cutoff)	HIV+	HIV-	P-value
Copper (75 µg/dL)			
Mean±SD	126.8 ± 34.9	113.5 ± 38.3	0.6
Median(range)	126.1 (39.5-211.3)	109.0 (3.3-277.9)	
<75, no (%)	7 (6.4)	8 (8.2)	
Zinc (75 µg/dL)			
Mean±SD	68.1 ± 44.5	62.4 ± 43.6	0.1
Median(range)	52.9 (18.8- 252.6)	52.73 (19.6-315.5)	
<75, no (%)	76 (69.7)	78(80.4)	
Cu/Zn			
Mean±SD	2.4 ± 1.2	2.37 ± 1.37	0.3
Median (Range)	2.21 (0.5-6.5)	2.16 (0.1-6.4)	

Table 2: Serum copper and zinc in relation to HIV status.

	***Shigella* species**		
Analyte (cutoff)	*Shigella* species +Ve	*Shigella* species –Ve	p-value
Copper (75 µg/dL)			
Mean±SD	120.5 ± 34.8	120.6 ± 37.3	0.9
Median (Range)	121.58 (34.6-195.5)	118.52 (3.3-277.9)	
<0.75, no. (%)	1 (5.6)	14 (7.4)	
Zinc(75 µg/dL)			
Mean±SD	45.0 ±16.9	67.4 ± 45.4	0.04
Median (Range)	40.6 (19.6-79.3)	53.66 (18.8-315.5)	
<0.75, no. (%)	17 (94.4)	137 (72.9)	
Cu/Zn			
Mean±SD	3.0 ± 1.4	2.3 ± 1.3	0.03
Median (Range)	2.9 (0.8-6.1)	2.13 (0.1-6.5)	

Table 3: Serum copper and zinc in relation to shigellosis.

Analytic (cutoff value)	**Intestinal parasites**		p-value
	Parasite positive	Parasite negative	
Copper (75 µg/dL)			
Mean ± SD	121.5 ± 44.5	120.1 ± 33.1	0.4
Median (Range)	114.9 (3.3-277.9)	119.35 (34.6-198.7)	
<0.75, no. (%)	6 (9.1)	9 (6.4)	
Zinc (75 µg/dL)			
Mean ± SD	61.9 ± 38.1	67.1 ± 46.7	0.9
Median (Range)	51.0 (18.8-183.4)	53.6 (19.6-315.5)	
<0.75, no. (%)	49 (74.2)	105 (75.0)	
Cu/Zn			
Mean±SD	2.5 ± 1.5	2.3 ± 1.2	0.7
Median (Range)	2.4 (0.1-6.5)	2.15 (0.3-6.1)	

Table 4: Serum zinc and copper in relation to intestinal parasites.

with a 3.5–7 fold increased risk for premature rupture of membranes [39,40]. A prospective, randomized, controlled trial on the effect of zinc supplementation on mortality of infants born small for gestational age indicated reduction in deaths of infants with a rate ratio of 0.32 (95% confidence interval: 0.12– 0.89) [40]. These indicate that adequate supply of zinc, either through supplementation or adequate diet, has a major role in preventing morbidity and mortality [41].

Shigellosis remains one of the most severe enteric infections affecting children and adults in developing countries, including Ethiopia [5]. It results in the frequent passage of bloody mucoid stools, abdominal cramps, and tenesmus caused by ulceration of the intestinal epithelium. Such extensive damage of the intestinal epithelial tissues leads to malabsorption of nutrients including zinc. Even though we did not see significant difference in serum zinc levels in patients with and without shigellosis, a study in Bangladesh found that Zinc supplementation significantly shortens the duration of acute shigellosis promotes better weight gain during recovery and reduces diarrhoeal morbidity during the subsequent 6 months [42].

In this study, unlike zinc, no diarrheic patients with or without HIV co-infection were found significantly deficient in serum copper levels. Our present findings are consistent with those in our two previous studies on the relationship between the levels of trace elements and tuberculosis and HIV infections [46], and in pregnant and non-pregnant women [20] in Gondar, northwest Ethiopia. The elevated serum levels of copper may reflect a nonspecific increase in serum concentration of copper binding protein, ceruloplasmin [28]. Plasma concentrations of ceruloplasmin and copper increased as an acute-phase response in a variety of infections and inflammatory conditions [26]. High serum levels of copper were also reported in HIV-infected patients suggesting its possible role as a useful marker of HIV activity and progression to AIDS [29]. The level of serum copper also increases in other infectious diseases. A study on serum level of copper in neonates suffering from pneumonia in demonstrated that pneumonia patients had high serum levels of copper in the infection phase [47].

It is interesting to note that an association between acute phase response to infection and alteration in dynamics of many trace elements, particularly zinc and copper, has been recognized for many decades [43]. The falls in serum zinc, and rise in serum copper, are brought about by changes in the concentration of specific tissue proteins such as C-reactive protein (CRP) that are controlled by cytokines [44]. Although, the changes are generally believed to be beneficial aspects of the early acute-phase response, the assessment of status for these elements is particularly difficult, since plasma concentration may bear little relationship to tissue status. Therefore, simultaneous assessment of acute-phase proteins, for example, serum CRP, together with zinc and copper and monitoring changes in concentrations has been suggested as an important step in the interpretation of serum level of trace elements [45]. A limitation of this study was the failure to include parameters for the acute-phase response or inflammation (such as CRP) and markers for progression of HIV infection ($CD4^+$ T cells count) and cross-sectional design and the lack of serum data from a similar population without diarrhea or HIV. Inclusion of such parameters may help to delineate whether the observed values of serum levels of zinc, copper and Cu/Zn ratio in diarrheic patients with or without HIV co-infection were either due to the acute phase response or due to micronutrient status. In addition, inclusion of data on dietary intake of the patients and clinical evaluation for specific signs of the deficiency may provide useful information to better explain the situation of those trace elements deficiency in the study population.

Conclusion

Zinc deficiency is a severe public health problem in Gondar, Ethiopia, among diarrheic patients irrespective of HIV co-infection. The low concentrations of zinc could result from preceding deficiencies that enhanced susceptibility to infection, and/or from their high demands in overt infections causing diarrhoea. Finally, this study recommends further studies to establish the role of these low concentrations in host defense against diarrheic patients with or without HIV, so that appropriate and beneficial strategies for micronutrient supplementation can be planned.

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