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Serum Concentration of Selenium in Diarrheic Patients with and without HIV/AIDS in Gondar, Northwest Ethiopia

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Abstract

Background: Selenium deficiency is known to be a major public health problem, associated with increased risk of mortality with AIDS and AIDS-related complex, diarrhea included compared to those of healthy controls. In Ethiopia, there are no studies conducted on serum selenium status diarrheic patients with and without HIV infection. Therefore, the present study was aimed at determining the level of serum selenium in HIV infected and sex and aged matched HIV negative diarrheic patients..

Methods: This was a cross-sectional study of 206 (97 HIV seronegative and 109 HIV seropositive) diarrheic patients of both genders seen at University of Gondar Hospital, Gondar, Ethiopia. Serum selenium was measured by inductively coupled plasma-mass spectrometer.

Results: The mean and standard deviation of serum selenium levels in HIV seropositives was 5.90 ± 2.78 µg/dl and in HIV seronegatives was 6.99 ± 4.26 µg/dl. Deficiency of selenium was seen in 95.9% and 71.56% of diarrheic patients with and without HIV co-infection, respectively. The over all selenium deficiency was observed in 83.0% of patients included in the study irrespective of their HIV serostatus while 85.3%of the patients infected with HIV and 80.4% of patients without HIV infection had serum selenium level below 7µg/dl. The low serum selenium level was not associated with presence or absence of intestinal parasites neither with sociodemographic variables such as age, residence, marital status, occupation, monthly income. The mean serum selenium level of all male cases was statistically significantly lower than that of the females (P<0.05).

Conclusion: Our results show high prevalence of selenium deficiencies in HIV seropositive and seronegative diarrheic patients in Gondar, Ethiopia. Although this is a small group of study subjects, the findings may be used as a tool to suggest further in-depth prospective clinical trials to determine whether selenium supplements may be of public health benefit among HIV-infected populations as a stand-alone therapeutic approach and potentially as an adjuvant to antiretroviral therapy.

Keywords: Selenium; HIV/AIDS; Diarrhea; Gondar; Ethiopia

Background

The HIV pandemic has placed a great demand upon the scientific community to develop effective prevention and treatment methods. Since the beginning of the pandemic in 1981, over 25 million people are estimated to have died from the disease [1]. It is currently a leading cause of death in many parts of the world, and a disease that disproportionately affects the marginalized and socially disadvantaged. The situation is severe in sub-Saharan Africa, a region where an estimated 25.8 million adults and children are infected with HIV [2]. Many of those affected also suffer from chronic food insecurity and malnutrition; there is evidence for therapies that could potentially target both HIV disease and malnutrition, such as multivitamins, have been extensively researched for potential benefits [3]. Among such therapies, the antioxidant micronutrients theorized to have potential benefits in HIV disease, apart from correcting deficiencies, have been examined [4,5].

Diarrheal diseases are one of the most important causes of morbidity and mortality in developing countries [7]. The situation is severe in sub-Saharan Africa, a region where an estimated 25.8 million adults and children are infected with HIV [2]. 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 [7], in tropical countries is associated with weight loss and is often the presenting illness of HIV infected individuals. This diarrhea wasting syndrome in association with a positive HIV serology test is an AIDS defining illness in the World Health Organization's classification [6].

The trace mineral Selenium (Se) plays an important role in mammalian metabolism and maintenance of normal health in human populations [9]. The antioxidation function of Se in glutathione peroxidase is essential in protecting the biological system from oxidation caused by peroxides. It has been demonstrated that, when taken as a supplement, Se modulates the cellular response to oxidative stress, inducing a faster restoration of the endogenous antioxidative

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defense system against the production of reactive oxygen species [10,11]. Glutathion peroxidase controls the intracellular level of hydrogen peroxide, reducing the formation of reactive oxygen species that can induce lipid peroxidations with consequent damage to the cellular membranes [12]. Epidemiological studies suggest a low intake of Se might predispose an individual to an increased incidence of cardiovascular disorders [13]. There is increasing evidence that Se deficiency may have several serious short- and long-term medical implications, such as cardiomyopathy, cardiovascular disease, male infertility, impaired immune response, or even cancer [14,16,17]. A prospective cohort study of children born to HIV-infected women in Tanzania showed that low plasma Se levels were associated with an increased risk of mortality after adjusting for CD4+ cell counts and other measures of nutritional status [15].

In Ethiopia, as in other sub-Saharan Africa, morbidities from diarrhoeal diseases [7] and HIV/AIDS [2,8] are serious health problems. However, studies assessing the interactions between diarrheal diseases, HIV/AIDS and micronutrient status are non existent. According to the literature, knowledge about the selenium status during diarrhea is limited and only one small study on children has shown no difference among the plasma selenium levels of the patients with persistent diarrhea, patients with acute diarrhea, and control subjects [63]. Selenium deficiency is known to be a major public health problem, associated with increased risk of mortality with AIDS and AIDS-related complex, diarrhea included compared to those of healthy controls. In Ethiopia, there are no studies conducted on serum selenium status diarrheic patients with and without HIV infection. Therefore, the present study was aimed at determining the level of serum selenium in HIV infected and sex and aged matched HIV negative diarrheic patients.

Materials and Methods

Study design and subjects

In this cross-sectional study, consecutive diarrheic patients diagnosed at the outpatient department of the University of Gondar Hospital, in Gondar, Ethiopia were included. 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 intestinal parasites

Diarrheic stool specimens were collected in sterile containers and examined microscopically for intestinal parasites following direct, concentration and modified acid fast staining procedures [20].

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 serum Se

The frozen serum samples were kept on dry ice and air freighted to Japan. Concentration of Se 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 [18]. In brief, serum sample (200 µl) was aliquoted in to teflon tube and covered with teflon ball. After adding 1 ml of concentrated HNO3 (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 HNO3 which contained 10 ng/ml internal standard elements (In, Re, and Tl). The diluted serum solution was used for analysis of serum Se 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. Selenium deficiency was defined as serum selenium level below <7µg/dl [68]

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. Comparisons of serum values of Se among HIV patients were made using a one way analysis of variance. Post hoc Tukey test was used to determine which pairs of means differ significantly. Deficiency of Se was defined at their serum levels less than 7 µg/dl [19]. P values less than 0.05 were considered statistically significant.

Results

A total of 206 patients (97HIV seronegative and 109HIV seropositive) were included in the study. Table 1 shows baseline characteristics of the patients in association with the proportion of patients with Se deficiency by HIV serostatus. Marked weight loss was observed in 55.1% and 28.9% of patients with HIV seropositive and HIV seronegative, respectively. The proportion of patients with weight loss and Se deficiency was significantly higher in those with HIV co-infection ($p< 0.05$). All the patients included in this study had diarrhea, from which more than half of HIV seropositive (55.1%) had Acute diarrhea but majority of HIV seronegative (67%) had chronic diarrhea.

Table 2 shows Mean±SD serum Se levels were slightly different between patients with (5.90±2.78 µg/dl) and without (6.99±4.26 µg/dl) HIV infection. In addition, its levels in the sera of diarrheic patients infected with intestinal parasites (6.84±5.44 µg/dl) were significantly higher compared to that in patients with weight loss (5.53±1.85) ($p< 0.05$) irrespective of HIV serostatus. Deficiency of Se was seen in 95.9% and 71.56% of diarrheic patients with and without HIV co-infection, respectively (Table 2).

Intestinal parasites were detected in 36(37.1%) and 30(27.5%) of HIV negative HIV positive diarrheic 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 Se in diarrheic patients with and without intestinal parasitoses (Table 3). The serum Se levels in the patients who were found positive for intestinal parasites was not significantly different by the presence or absence of HIV co-infection (Table 3).

Regression analyses of age, residence, marital status, occupation, monthly income and the status of intestinal parasitoses as independent variables and serum level of Se as dependent variable did not show any significant association between the parameters and a deficient level of

		patients with HIV infection		patients without HIV infection	
		No. of patients	No. (%) with Se deficiency	No. of patients	No. (%) with Se deficiency
Age					
	15-24	11	9(81.8)	41	31 (75.6)
	25-34	54	47(87)	30	25(83.3)
	35-44	33	28(84.8)	13	11(84.6)
	>45	11	9(81.8)	13	11(84.6)
Sex					
	Male	65	55(84.6)	62	45(72.6)
	Female	44	38(86.4)	35	33(94.3)
Address					
	Rural	78	67(85.9)	64	49(76.6)
	Urban	31	26(83.9)	33	29(87.9)
Marital Status					
	Married	53	44(83)	47	38(80.9)
	Single	33	28(84.8)	44	35(79.5)
	Divorced	23	21(91.3)	6	5(83.3)
Occupation					
	Government Employee	25	25(100)	22	19(86.4)
	Farmer	46	38(82.6)	17	13(76.5)
	Student	17	15(88.2)	10	10(100)
	Housewife	7	5(71.4)	32	24(75)
	Other	14	10(71.4)	16	12(75)
Income*					
	Low	59	50(74.6)	67	48(81.4)
	Medium	34	18(94.7)	19	30(88.2)
	High	16	10(90.9)	11	15(93.8)
Diarrhea					
	Acute	60	49(81.7)	32	27(84.4)
	Chronic	49	44(89.8)	65	51(78.5)
Weight loss					
	Yes	60	54(90)	28	25(89.3)
	No	49	39(79.6	69	53(76.8)
Intestinal Parasitosis					
	Yes	30	25(83.3)	36	28(77.8)
	No	79	68(86.1)	61	50(82)

* Low: less than 1000 Ethiopian Birr/month. Medium: 1000 – 5000 Ethiopian Birr, High: more than 5000 Ethiopian Birr/month. 1 Ethiopian Birr = 0.12 US Dollar.

Table 1: Baseline characteristics of diarrheic patients in relation to selenium deficiency

Selenium (µg/dl)	HIV seronegative (n=109)	HIV seropositive (n=97)	Patients with marked weight loss (n=88)
Mean±SD	6.99±4.26	5.90±2.78	5.53±1.85
Median(Range)	6.58 (0.05-40.57)	5.46 (1.73-21.77)	5.42 (1.39-11.19)
<7, no. (%)	78(71.56)	93(95.9)	79(89.8)*

* P < 0.05 compared to HIV seronegatives.

Table 2: Serum levels of selenium in patients with and without HIV co-infection, apparently healthy controls and asymptomatic HIV infected blood donors.

serum Se. The mean serum Se level of all male cases was statistically significantly lower than that of the females (P<0.04).

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 [21]. One of the major manifestations of the HIV disease in the region is the diarrhea-wasting syndrome [22,23]. Observational studies have mostly shown an association between decreasing serum Se and progression through HIV disease stages to poorer outcomes [23-25].

Variables		HIV seronegative	HIV seropositive	Total	*p*-value
Positive for intestinal parasites	Number of patients	36	30	66	0.4
	Mean ±SD	7.3±6.35	6.29±4.13	6.84±5.44	
	Range	0.05-40.57	1.99-21.77	0.05-40.57	
Negative for intestinal parasites	Number of patients	61	79	140	0.3
	Mean ±SD	6.81±2.34	5.76±2.08	6.21±2.25	
	Range	1.39-12.81	1.73-11.24	1.39-12.81	

Table 3: Serum levels of selenium (µg/dl) in diarrheic patients with and without HIV co-infection by status of Intestinal parasitoses

In this study, the over all Se deficiency was observed in 83.0% of patients included in the study irrespective of their HIV serostatus and 85.3%of the patients infected with HIV, 80.4% of patients without HIV infection had serum Se level below 7µg/dl. This observation is higher than our previous report, 21.9 % [26]. Inline with our finding, a small study done in the U.S reported that decreased Se levels in AIDS patients compared to controls, along with malabsorption was found, as defined by the D-xylose test, in 60% of cases; however, it also found that inadequate intake was seen in 71% of cases [27]. Other studies demonstrated that Se deficiency in AIDS increase risk of death [28,29].

Despite the reported effect of HIV infection on serum Se status, [26-29] we did not observe a significant difference in the mean serum Se concentrations between patients with and without HIV infection. Se deficiency observed in patients with and without HIV infection may suggest that gastrointestinal absorption of Se was so altered or inadequate intake of oral Se. In addition, HIV/AIDS malabsorption can also deplete levels of many nutrients; including Se. Se deficiency is associated with decreased immune cell counts, increased disease progression, and high risk of death in the HIV/AIDS population [30,31]. HIV/AIDS gradually destroys the immune system, and oxidative stress may contribute to further damage of immune cells. Antioxidant nutrients such as Se help protect cells from oxidative stress, thus potentially slowing progression of the disease [32]. Selenium also may be needed for the replication of the HIV virus, which could further deplete levels of Se [33] and increased pathogenesis and viral mutations, was reported in the Se-deficient mice due to a decrease in glutathione peroxidase activity, leading to increased host oxidative stress [65]. In addition, identification of a glutathione peroxidase homologue in HIV-1 leaves little room for doubt that a direct interaction between HIV and Se can occur, particularly since the same gene has now been identified in several other viruses [66].

Our results show that in adults there is a significant difference between men and women ($p < 0.04$) with a higher concentration of Se in men. This suggests a sex linked hormonal influence over serum level of Se. It has previously been shown that Se is essential for spermatogenesis [34]. This trace element is present in the protein of the capsule surrounding the sperm mitochondria and may have a structural function [35]. Our data also show a lightly positive correlation between a lower concentration of Se in serum and age in men.

The low serum level of Se in the sera of the diarrheic patients can be due to reduced dietary intake resulting from anorexia or decreased absorption and low selenium in the soil is also a contributing factor but Ethiopia is among the medium group countries in the

international comparisons [67]. Even though the Se status of Ethiopia in general seems satisfactory, Se deficiencies of local nature may exist and measures to correct it may be needed. Furthermore, Serum Se concentrations seem to decrease with age [36,37] and are lower in people with chronic diseases [37]. In our previous study a significantly low level of serum Se was also observed in TB patients [38]. Se is known to be an essential component of antioxidative selenoenzymes such as glutathione peroxidase which are known to protect host cells from oxidative damage in inflammatory conditions [39]. Although the mechanisms involved have yet to be elucidated, the important role of Se in normal immune responses is well established [39]. Se also has immunopotentiating effects as its deficiency appears to result in immunosuppression, whereas supplementation with low doses of Se appears to result in augmentation and/or restoration of immunologic functions [40].

Our observation that about 80.4% of HIV seronegative controls and 85.3% of HIV seropositive patients had low serum Se levels indicates that Se is a public health problem in adult population of the region. In line with this, a review of limited number of studies involving HIV patients and pregnant women in Africa indicated the severity of Se deficiency [26,38]. Further, the occurrence of Se deficiency during diarrhea and HIV infection may have clinical micronutrient for normal immune function [25]. An examination of 125 HIV-positive men and women linked Se deficiency with a higher rate of death from HIV [41]. In a small study of 24 children with HIV who were observed for five years, those with low Se levels died at a younger age, which may indicate faster disease progression [42]. Results of research studies have led experts to suggest that Se status may be a significant predictor of survival for those infected with HIV [43]. Furthermore, Researchers continue to investigate the relationship between Se and HIV/AIDS, including the effect of Se levels on disease progression and mortality. There is insufficient evidence to routinely recommend Se supplements for individuals with HIV/AIDS, but physicians may prescribe such supplements as part of an overall treatment plan. It is also important for HIV-positive individuals to consume recommended amounts of Se in their diet.

Intestinal parasitism occurs widely throughout Ethiopia [45]. In line with this; we found a high prevalence of intestinal parasites in diarrheic patients included in the present study. 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 [46]. In our study, no significant difference in serum Se concentrations was observed between the two groups which are inline with previous research done in children with acute and persistent diarrhoea as compared with the control group [63]. Abnormalities in the mucosa of intestinal tract were observed in children infected with *Ascaris lumbricoides* by jejunal biopsy, which disappeared rather rapidly after deworming [47]. In addition to intestinal parasitosis, the presence of reactive oxygen species may contribute to the etiology of diarrheal disease by promoting hypersecretion in the small intestine [41]. It is possible that selenium deficiency contributes to this pathway by failing to detoxify reactive oxygen species. Inadequate selenium status may also increase risk of childhood diarrhea by weakening immunity and thus increasing susceptibility to etiologic agents of childhood diarrhea. For example, impaired antibody responses tend to increase susceptibility to bacterial infections such as Escherichia coli [48]. Animal studies indicate that selenium is important for resistance to dysentery in swine [64]. Rotavirus infection, a common cause of childhood diarrhea, may be facilitated by impaired antibody and cytolytic T-lymphocyte defense mechanisms due to selenium deficiency [48].

Impaired bioavailability of Se in adults and children with diarrhoea has been reported in Zambia [49]. It is worth mentioning that clinical trials of oral, high dose Se supplementation for HIV-infected persons suggest that Se reduces rates of hospital admission and reduced health-related costs during a 2-year follow-up period [50]. Furthermore, a study of Se supplementation in Tanzanian HIV infected HIV infected pregnant showed a reduced risk of low birth weight and secondary outcomes for this trial showed a reduction in diarrheal morbidity [51]. A 2004 randomized controlled trial performed with 400 participants in Kenya found that supplementation resulted in higher CD4+ (+23 cells/mL, P = 0.03) and CD8+ (+74 cells/mL, P = 0.005) cell counts compared with placebo (potentially beneficial outcomes), but there was no change in plasma viral load [52]. A small trial conducted in 2008 in Nigeria found non significant increases in T-cell counts for subjects on a Se and aspirin regimen versus those receiving Se alone, but there were problems with the randomization scheme. [53]. These indicate that adequate supply of Se, either through supplementation or adequate diet, has a major role in preventing morbidity and mortality as well as in offering some modest benefits in terms of birth outcomes and diarrheal morbidity.

An association between the chronic diseases and alterations in the serum concentration of Se has been demonstrated previously [54-63]. Observational studies indicate that death from cancer, including lung, colorectal, and prostate cancers, is lower among people with higher blood levels or intake of Se [54-58]. Research suggests that Se affects cancer risk in two ways. As an anti-oxidant, Se can help protect the body from damaging effects of free radicals. Se may also prevent or slow tumor growth. Certain breakdown products of Se are believed to prevent tumor growth by enhancing immune cell activity and suppressing development of blood vessels to the tumor [59]. Some population surveys have also suggested an association between lower antioxidant intake and a greater incidence of heart disease and rheumatoid arthritis [60-62].

In conclusion, Se deficiency is a severe public health problem in Gondar, Ethiopia, among diarrheic patients irrespective of HIV infection. Although this is a small group of study subjects, the findings may be used as a tool to suggest further in-depth prospective clinical trials to determine whether selenium supplements may be of public health benefit among HIV-infected populations as a stand-alone therapeutic approach and potentially as an adjuvant to antiretroviral therapy. We also recommended that the findings may be used as a tool to assist health professionals to develop and implement health and nutrition education curricula and design public health interventions aimed at preventing or correcting micronutrient deficiencies.

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