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Serum Zinc Concentration and C-Reactive Protein in Individuals with Human Immunodeficiency Virus Infection: the Positive Living with HIV (POLH) Study (Biological Trace Element Research)

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Serum Zinc Concentration and C-Reactive Protein in Individuals with Human Immunodeficiency Virus Infection: the Positive Living with HIV (POLH) Study

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Abstract Low zinc levels and chronic inflammation are common in individuals infected with human immunodeficiency virus (HIV). Zinc deficiency may promote systemic inflammation, but research on the role of zinc in inflammation among HIV-positive individuals taking account of antiretroviral therapy is lacking. We assessed the association between serum zinc and C-reactive protein (CRP) concentration in a cohort of HIV-positive individuals. A cross-sectional survey was conducted among 311 HIV-positive individuals (177 men and 134 women) aged 18–60 years residing in Kathmandu, Nepal. High-sensitive or regular serum CRP concentrations were measured by the latex agglutination nephelometry or turbidimetric method, and zinc concentrations were measured by the atomic absorption method. Relationships were assessed using multiple linear regression analysis. The geometric means of zinc in men and women were 73.83 and 71.93 $\mu\text{g/dL}$, respectively, and of CRP were 1.64 and 0.96 mg/L , respectively. Mean serum CRP concentration was significantly decreased with increasing serum zinc concentration across zinc tertiles (P for trend=0.010), with mean serum CRP concentration in the highest tertile of serum zinc concentration was 44.2 % lower than that in the lowest tertile. The mean serum CRP concentrations in men and women in the highest tertile of serum zinc concentrations were 30 and 35.9 % lower, respectively, than that in the lowest tertile (P for trend=0.263 and 0.162, respectively). We found a significant inverse relation between log zinc and log CRP concentrations (beta for 1 unit change in log zinc; $\beta=-1.79$, $p=0.0003$). Serum zinc concentration may be inversely associated with serum CRP concentration in HIV-positive individuals.

Introduction

Human immunodeficiency virus (HIV) infection is associated with chronic inflammation due to the chronic activation of both the adaptive and innate immune systems [1–5]. HIV infection may increase inflammation directly by viral replication and indirectly by excess levels of translocated microbial products and other chronic pathogens, by loss of immunoregulatory responses, and by hypercoagulability [1–5]. One of the bioactive molecules that may mediate the systemic effects of HIV is C-reactive protein (CRP), a pro-inflammatory biomarker. CRP has been the focus of extensive epidemiologic investigation among people infected with HIV as it is an independent predictor for survival [6]. CRP has been associated with multiple parameters of disease progression, such as CD4 cell count, HIV viral load, and a decreased time to acquired immune deficiency syndrome (AIDS) [7].

Zinc deficiency is one of the common micronutrient abnormalities seen in people infected with HIV. Low plasma zinc concentrations are frequently reported in patients with HIV [8–13] even among those who are under antiretroviral therapy (ART) [14–18]. The reported prevalence of zinc deficiency varies between 30 and 51 % among HIV-infected adult population [19–21]. Zinc deficiency alters cellular immunity, reducing the generation of T cells and depresses humoral and cell-mediated immunity, which can influence intracellular HIV-1 replication [22, 23]. Among HIV-positive individuals, zinc appears to have an important role in HIV-1 disease progression [24, 25] as its deficiency has been associated with decreases in

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CD4 counts, progression to AIDS, and mortality [8, 12, 14, 17, 26–31]. Zinc supplementation has shown to delay HIV-1 disease progression and decrease the rate of opportunistic infections in HIV-positive individuals with or without ART treatment [19, 32–35].

Studies have reported significant therapeutic benefits of zinc in reducing inflammation in HIV-negative populations. Epidemiological studies suggest that elevated concentrations of zinc are inversely associated with CRP concentrations in HIV-negative populations [36–43]. Randomized controlled trials showed decreased CRP concentrations and other inflammatory markers in zinc-supplemented healthy human populations [36, 39]. The anti-inflammatory action of zinc might be one of the important contributing factors behind such therapeutic benefits, as zinc inhibits the production of pro-inflammatory cytokines and decreases CRP [36].

Although low plasma zinc concentrations and high inflammation are frequently reported in HIV-positive individuals, no study has assessed the association between serum or plasma zinc concentration and inflammation among HIV-positive individuals taking account of ART. Only a single study has assessed the association between plasma zinc and CRP among ART-naïve HIV-positive individuals enrolled in a multi-micronutrient supplement trial in Kenya that reported the significant inverse correlation between uncorrected plasma zinc concentrations and CRP at 3 months of follow-up [44]. Studies have shown that HIV-positive individuals have persistent and low-grade inflammation, even with long-term effective ART [45], which are strongly associated with cardiovascular diseases [46–48], anemia [49], osteoporosis [50], and other non-AIDS-defining events and mortality [51, 52]. Thus, assessment of the role of zinc in inflammation among HIV-positive individuals taking account of ART is important for the potential consideration of zinc supplementation or fortification in resource-poor countries because of the importance of zinc to the immune system.

Thus, more studies are needed to assess the association between zinc and CRP in HIV-positive individuals while accounting for important confounders such as ART. As zinc is known to decrease inflammation, we hypothesized that lower concentrations of serum zinc would be associated with increased serum CRP concentrations in HIV-positive individuals taking account of ART.

Methods and Materials

Study Design and Setting

This study was conducted among HIV-positive individuals residing in the Kathmandu, Lalitpur, and Bhaktapur districts (Kathmandu Valley) of Nepal. About 50,200 people aged 15–49 years were living with HIV, with 0.3 % prevalence in the general population as of 2011 [53]. Approximately 15.7 % of the country's HIV-positive population was residing in the Kathmandu Valley at the end of 2006 [54]. Even though free ART services started in Nepal in 2004, the ART coverage among HIV-infected individuals needing ART was only 24 % as of 2011 [53].

Study Participants

As describe earlier [55–58], we recruited our study participants through the network of five non-government organizations (NGOs) working with HIV-positive individuals in the Kathmandu Valley, as recruitment of both ART-naïve and ART non-naïve HIV-positive individuals was possible using such a network. A total of five NGOs were closely working with HIV-positive individuals in the Kathmandu Valley during the study period. These NGOs were providing various need-based care and support programs including monthly support group meeting, community and home-based care services, weekly HIV clinics, in-house crisis management services, and hospital visits for consultation, CD4⁺ T cell count monitoring, and home delivery of ART to needy persons [59]. One of these NGOs also provides HIV voluntary testing and counseling services and prescribes ART. These NGOs' staff contacted approximately 360 HIV-positive individuals for our study purpose either through phone call or in person contact during their visit to NGOs 1 week prior to the data collection date. Of them, 330 individuals visited the recruitment sites. Of these 330 individuals, three people could not participate because of their work time-table, two individuals refused to participate after receiving study information in detail, and three others were not included as the evidence of their HIV-positive status was not confirmed. None of the participants received vitamin or mineral supplementation in the past 12 months.

Altogether, 322 HIV-positive individuals aged between 18 and 60 years participated in the study with their written informed consent. The Ethics Committees of the Nepal Health Research Council, Kathmandu, Nepal; National Center for Global Health and Medicine, Japan; and Waseda University, Tokyo, Japan, approved the study protocol. The Institutional Review Board of the University of Massachusetts, Amherst, USA, also approved the study procedures.

Data Collection

A structured pre-tested Nepali language questionnaire was used to collect data by in-person interviews. Trained interviewers administered the questionnaire face-to-face in a private setting with each interview lasting approximately 45–60 min. We individually informed all participants about the study procedures using a prepared information sheet. Interviewers requested participants to sign informed consent forms prior to being interviewed. They also reassured participants that their names will not be used in any documents to ensure confidentiality.

Information on socio-demographics, cardiovascular risk factors, smoking, alcohol intake, and ART was collected adopting the instruments from previous studies in Nepal [60–63]. ART type and adherence were collected according to the current use of medication at the time of the survey. Smoking status was measured in number of cigarettes smoked and frequency of smoking [55]. Alcohol consumption in the past 30 days was measured and categorized as yes or no alcohol intake from reported amount. Educational status was categorized as never (illiterate) or ever (literate) been to school from the reported years of formal education. Similarly, employment status was categorized as having or not having work outside of the home. Physical activity was measured in average hours spent per day in the past 12 months either on walking or any types of mild, moderate, or hard physical work. The past history of any disease was asked by a question “In the past 12 months, did you suffer from any type of diseases including minor illnesses?” with response options of yes or no. If the response was yes, the signs or symptoms of disease or disease diagnosis with details of health-seeking behavior and treatment of each disease were queried. The dietary intake was calculated from two 24-h dietary recalls in 1 week apart and on different weekdays. The daily intake of zinc was calculated using Indian food tables from the Wfood2 program version 1.0 [64].

Physical Examination

The measurements were done twice independently to estimate mean values for all anthropometric parameters. Digital scale was used to measure the body weight and stadiometer was used to measure body height. The body weight measured in kilograms and the height measured in centimeters were used to calculate the body mass index (BMI). The Omron Automatic Blood Pressure Monitor was used to measure the blood pressure after the participants had been rested for at least 10 min.

Blood Collection and Laboratory Methods

We obtained blood samples from 322 participants after an overnight fast during the survey period. Ten milliliters of

venous blood was drawn into an evacuated tube and centrifuged immediately for 15 min after which the serum samples were placed in a cooler box with dry ice and transported to a local laboratory in Kathmandu. Serum samples were stored in the same laboratory during the field work. Serum samples then were transported to a research laboratory in Tokyo, Japan. The separated serum was stored in three tubes (2.0, 1.5, and 1.5 mL) and was stored at -80°C until analysis. One tube of 2.0 mL was sent to an external laboratory (Mitsubishi Chemical Medicine Corporation, Tokyo, Japan) for testing, where high-sensitive serum CRP and zinc concentrations were measured by the latex agglutination nephelometry method and atomic absorption method, respectively. The latex agglutination turbidimetric method was used to measure the regular serum CRP concentrations of those participants whose CRP value was 5 mg/L or more. The intra-assay coefficient of variation of serum zinc was 2.9 to 6.4 %, respectively. The intra-assay coefficient of variation at CRP levels on the control serum was within 10 %. Blood samples from all study participants were drawn from mid February to mid March, so seasonal variation or difference in freezing time should not have significant effects on serum CRP and zinc levels.

The measurement of other clinical parameters such as of total cholesterol, LDL cholesterol, and HDL cholesterol were assessed by the enzymatic colorimetric method. The inter-assay coefficient of variation at total cholesterol levels of 125.48, 166.93, and 238.32 mg/dL was 1.24, 1.09, and 0.97 %, respectively. The intra-assay coefficient of variation at total cholesterol levels of 127.77, 169.90, and 241.04 mg/dL was 0.67, 0.62, and 0.77 %, respectively. CD4^{+} T cell counts were determined using a specific monoclonal antibody and fluorescence-activated cell sorter (FACS) analysis. The viral load measurement was not done due to lack of resources.

Statistical Analyses

Out of 322 participants, we excluded 11 participants from the analysis as they did not have information on serum zinc and CRP concentration or socio-demographic or anthropometric information, resulting in a final study population of 311 participants (177 men and 134 women). The linear regression analysis and chi-square tests were used to assess the difference in demographic, lifestyle, anthropometric, and clinical parameters across tertiles of serum zinc concentrations, for continuous variables and categorical variables, respectively. The relationship between zinc concentrations and serum CRP was assessed using multiple linear regression analysis. All analyses were done for men and women separately, as well as combined. To better approximate normal distributions, serum zinc and CRP concentrations were log-transformed prior to analysis. The means and their 95 % confidence intervals (CIs) of log-transformed serum CRP concentrations were also

calculated for each tertile of serum zinc concentrations, then back-transformed.

Major socio-demographic characteristics and other mediators having previously established or plausible associations with the dependent variable were included as covariates in the analyses. Age (years, continuous), sex (men or women), alcohol intake (never or ever), smoking (never or ever), physical activity (≤ 3.5 or > 3.5 , h/day), body mass index (kg/m^2 , continuous), history of any disease in the past 12 months including minor illnesses (yes or no), cholesterol (mg/dL , continuous), CD4^+ T cell count (cells/uL ; continuous), ART (yes or no), and zinc intake (mg , continuous) were adjusted for in the multivariate models. The ordinal numbers 0–2 assigned to tertile categories of serum zinc concentrations were used to calculate trend associations. The two-sided p values less than 0.05 were considered statistically significant. SAS statistical software version 9.1 (SAS Institute, Inc., Cary, NC) was used to analyze the data.

Results

The geometric means of serum zinc in men and women were 73.83 and 71.93 $\mu\text{g}/\text{dL}$, respectively, and of serum CRP concentrations in men and women were 1.63 and 0.96 mg/L , respectively. The mean age of participants was 35.7 years for men and 32.5 years for women. A total of 93 of our participants (30.1 %) had $\text{CRP} \geq 3 \text{ mg}/\text{L}$. The socio-demographic and lifestyle characteristics and other clinical parameters across tertiles of serum zinc concentrations are presented in Table 1. The variables such as younger age, no ART medication, lower body mass index, and cholesterol were associated with the lowest serum zinc concentrations.

The relationship between serum zinc and CRP concentrations is shown in Table 2. The multivariate adjusted geometric mean of serum CRP concentration was significantly decreased with an increasing serum zinc concentrations across zinc tertiles (P for trend = 0.010), with mean serum CRP concentrations in the highest tertile of serum zinc concentrations being 44.2 % lower than that in the lowest tertile (Table 2). The mean serum CRP concentrations in men and women in the highest tertile of serum zinc concentrations were 30 and 35.9 % lower, respectively, than that in the lowest tertile (P for trend = 0.263 and 0.162, respectively).

The multiple linear regression analysis also showed that log serum zinc concentration was inversely associated with log serum CRP concentration after adjustment for demographic, anthropometric, lifestyle, and HIV-related clinical factors (beta for 1 unit change in log zinc; $\beta = -1.79$, $p = 0.0003$). This inverse association remained significant in both men ($\beta = -1.25$, $p = 0.033$) and women ($\beta = -2.80$, $p = 0.003$) in sex-specific analysis. The inverse relationship between serum zinc concentrations and CRP remained significant among

participants with a history of ART ($\beta = -2.12$, $p = 0.001$). This relationship did not change in further analysis in either with or without any history of disease in past 12 months including minor illnesses (data not shown in the tables).

Discussion

To our knowledge, this is the first study exploring the association between serum zinc and CRP concentrations among HIV-positive individuals taking account of the use of ART. We observed significant association of lower concentrations of serum zinc with increased serum CRP concentration in our study participants.

Our findings are in line with those studies previously highlighting an inverse association between zinc concentrations and inflammatory markers in different populations. Among HIV-negative populations, numerous studies have shown the benefits of zinc with respect to many chronic disorders that have been related to chronic inflammatory markers [36–43]. For example, several randomized, double-blind, placebo-controlled trials suggested that zinc supplementation increased plasma zinc concentrations and decreased the plasma CRP concentrations and other inflammatory and oxidative markers in healthy human subjects [36, 38–40]. Among ART-naïve HIV-positive population, a single trial in Kenyan HIV-positive adults with food supplement or the food plus a micronutrient capsule containing 15 mg zinc/day reported the significant inverse correlation between uncorrected plasma zinc concentrations and CRP at 3 months of follow-up [44].

Zinc can function as an anti-inflammatory agent. Zinc deficiency may induce apoptosis and endothelial cell dysfunction in humans due to an increase concentration of inflammatory cytokines and oxidative stress [38–40, 65–67]. Cell culture studies suggested that zinc may decrease nuclear transcription factor kB activation and pro-inflammatory cytokine generation whereas it may increase peroxisome proliferator-activated receptor- α (PPAR- α) and anti-inflammatory proteins A20 in human aortic endothelial cells and monocytic leukemia cells compared with zinc-deficient cells [36]. Zinc was proposed to inhibit NF- kB activation via A20 [38], a zinc finger-transactivating factor that plays an important role in reducing IL-1 β - and TNF- α -induced NF- kB activation [68]. The activation of PPAR- α and PPAR- γ and the downregulation of inflammatory cytokines and endothelial cell adhesion molecules in endothelial cells were reported to be zinc dependent [69]. Thus, zinc may decrease inflammatory cytokines due to the downregulation of NF- kB activation through A20 and PPAR signaling pathways [36].

Interestingly, our result suggests that higher serum zinc concentrations are more strongly associated with reduced inflammation in participants with a history of ART. However, the interaction between serum zinc and ART use was not

Table 1 Baseline characteristics of HIV-positive individuals of the Positive Living with HIV (POLH) Study ($n=311$)

	Serum zinc concentrations			<i>p</i> value*
	T1 (lowest)	T2	T3 (Highest)	
Serum zinc levels (median, range; ug/dL)	61 (29–68.99)	74 (69–79.99)	88 (80–157)	
Subjects (<i>n</i>)	105	101	105	
Age (mean±s.d., year)	33.19±7.14	34.81±7.26	35.11±6.92	0.11
Sex (male, %)	58 (55.24)	47 (46.53)	72 (68.57)	0.005
Smoking (never, %)	58 (55.24)	59 (58.42)	49 (46.67)	0.21
Alcohol consumption, last 30 days (none, %)	92 (87.62)	93 (92.08)	86 (81.90)	0.09
Anti-retroviral therapy (yes, %)	66 (62.86)	79 (78.22)	81 (77.14)	0.02
History of any disease in the past 12 months (yes, %)	68 (64.76)	60 (59.71)	67 (63.81)	0.69
Body mass index (mean±s.d., kg/m ²)	21.10±3.20	21.68±2.62	22.40±2.90	0.005
Physical activity (>3.5, h/day)	48 (45.71)	50 (49.50)	55 (52.38)	0.62
Cholesterol (mean±s.d., mg/dL)	137.06±30.98	151±42.85	166.62±45.98	<0.001
CD4 ⁺ T cell count (median, range; cells/uL)	326 (15–1007)	342 (39–1551)	367 (60–1412)	0.35
Zinc intake (median, range; mg)	7.29 (1.33–18.83)	7.72 (1.30–16.20)	8.03 (2.91–27.12)	0.009

*P for trend values were based on the Mantel-Haenszel chi-square test for categorical variables and linear regression analysis for continuous variables, with ordinal numbers 0–2 assigned to tertile categories of serum zinc concentrations

statistically significant. Further studies are needed in identifying patients that would benefit most from zinc supplementation.

Contrary to our expectations, only one third of our study participants had CRP >3 mg/L. Despite having CRP <3 mg/L among majorities of participants, the significant association of lower concentrations of serum zinc with increased serum CRP concentrations even after taking account of ART in our study suggests that zinc might have a potential role in reducing the

levels of inflammation in HIV-positive individuals. Studies have shown that HIV-positive individuals have persistent and low-grade inflammation, even with long-term effective ART, which are strongly associated with cardiovascular diseases [46–48], anemia [49], osteoporosis [50], and other non-AIDS-defining events and mortality [51, 52].

Some limitations of our study deserve comment. First, the possibility of reverse causality must be considered, namely that inflammation may influence the level of serum zinc

Table 2 Multivariate-adjusted means of serum CRP concentrations according to the tertile of serum zinc concentrations in HIV-positive individuals of the Positive Living with HIV (POLH) Study ($n=309$)

	Serum zinc concentrations			<i>P</i> for trend*
	T1 (Lowest)	T2	T3 (highest)	
All participants	105	101	103	
Univariate model	1.67 (1.36–1.98)	1.17 (0.85–1.49)	1.12 (0.81–1.44)	0.074
Multivariate model ^a	1.74 (1.43–2.05)	1.23 (0.92–1.54)	0.97 (0.65–1.29)	0.010
Men	58	60	57	
Univariate model	2.13 (1.70–2.57)	2.01 (1.52–2.49)	1.16 (0.77–1.55)	0.033
Multivariate model ^a	1.90 (1.46–2.34)	1.59 (1.18–2.00)	1.33 (0.89–1.76)	0.263
Women	47	40	47	
Univariate model	1.24 (0.81–1.66)	0.73 (0.33–1.13)	1.05 (0.54–1.56)	0.502
Multivariate model ^a	1.39 (0.96–1.83)	0.67 (0.21–1.13)	0.89 (0.67–1.12)	0.162

Values are geometric means of C-reactive protein (mg/L); 95 % confidence interval in parentheses (all such values)

*P for trend values were based on the multiple linear regression analysis, with ordinal numbers 0–2 assigned to tertile categories of serum zinc concentrations

^a All multivariate models adjusted for age (years, continuous), sex (men or women), alcohol intake (never or ever), smoking (never or ever), physical activity (≤ 3.5 or > 3.5 , h/day), body mass index (kg/m², continuous), history of any disease in the past 12 months (yes or no), cholesterol (mg/dL, continuous), CD4⁺ T cell count (≤ 200 or > 200 ; cells/uL), anti-retroviral therapy (yes or no), and dietary zinc intake (mg, continuous)

concentration, a possibility which the cross-sectional design of our study prevents us from ruling out. However, our hypothesis was based on previous study findings including clinical trials showing that zinc deficiency may increase inflammation [36, 38–40]. The information on past history of any minor illnesses over the past 12 months was adjusted in the multivariate models. We also did further analysis excluding participants with any history of disease including minor illnesses in the past 12 months. The protective effect of a serum zinc concentration against inflammation remained the same in either analysis.

Second, although we adjusted for factors known to influence serum CRP and zinc concentrations, the possibility of residual confounding cannot be excluded. Third, we assessed physical activity level by self-report rather than via direct methods. It is therefore possible that some residual confounding by physical activity persists, though in prior studies, associations between physical activity and zinc concentrations are quite modest [70, 71]. Future studies should consider using the use of accelerometers to minimize confounding by physical activity. Finally, although the physiologic relation between serum zinc concentrations and inflammation may not be different regardless of the differences in geographic location, recruitment strategy, and other social variables, some caution should be taken in generalizing our study findings to the entire population of HIV-positive individuals in the country, as our participants were not selected using random sampling method. Specifically, our results may be applicable to HIV-positive individuals in networks of NGOs or support groups, as exist in various countries in Asia as well as in other regions.

In conclusion, the present study suggests that higher concentration of serum zinc may be associated with low-grade inflammation among HIV-positive individuals even after taking account of ART. This finding is important in that this may lead to potential new intervention strategies to reduce inflammation, thereby improving health and quality of life of HIV-positive individuals. Further prospective studies are warranted to confirm the role of serum zinc concentrations against inflammation in HIV-positive populations.

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