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Evaluation of Serum Copper and Zinc Concentrations in Relation to Clinical Status among Adult People Living with HIV/AIDS in North-West Ethiopia

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAB managed the analysis of the study the literature searches. Author BKS performed the statistical analysis and wrote the first draft of the manuscript. Authors BKS, ENS, AMA and MAE designed the study and wrote the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate serum copper (Cu) and zinc (Zn) concentration in relation to clinical status among people living with HIV/AIDS for possible interventions.
Study Design: A cross-sectional study design was used.
Place and Duration of Study: The study was conducted between January and February 2013 in Felege Hiwot Referral Hospital, Bahir Dar, Ethiopia.
Methods: Serum concentrations of Cu and Zn from 150 people living with HIV/AIDS were measured using a fully automated flame atomic absorption spectrophotometer. Besides, world health organization (WHO) clinical staging, CD4+ T-cell count, CD8+ T-cell count, hemoglobin

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determination, and Body Mass Index (BMI) were performed to evaluate the clinical status of study participants.

Results: Multivariate logistic regression analysis showed that being serum Zn and Cu deficient was higher where there is no antiretroviral therapy (ART) than on ART (adjusted odds ratio (AOR)=3.56, 95% confidence interval (CI)=1.52-8.33, *P*=0.003 and 5.85, 95% CI=1.22-28.058, *P*= 0.027respectively).Similarly, the odds of being serum Zn deficient were significantly associated to lower BMI than normal (AOR=2.61, 95% CI=1.02 - 6.67, *P*= 0.046) and abnormal hemoglobin was found to be a factor to having high serum Cu/Zn ratio than normal hemoglobin (AOR=3.26, 95% CI=1.07 - 9.94, *P*= 0.038).

Conclusions: A relatively high percentage of subjects had serum Cu and Zn deficiency and high serum Cu/Zn ratio. Early evaluation of serum Cu and Zn deficiency should be carried out and mineral supplementation along with antiretroviral treatment should be provided for pre-ART, malnourished and anemic people living with HIV/AIDS.

Keywords: Zinc; copper; copper to zinc ratio; HIV/AIDS; micronutrients; antiretroviral therapy.

1. INTRODUCTION

Micronutrients are essential for normal body metabolism [1]. They are essential for host defense against infection [2] and act as activators in controlling biological functions [3]. The human immune function has been reported to depend on nutritional status [4,5]. Serum trace elements such as Cu and Zn have been reported to be useful in the diagnosis of viral diseases [6].

Cu functions as a hunter of free radicals in biological membranes and structures via its presence in cytosolic erythrocyte superoxide dismutase. Similarly, Zn plays a crucial role in nucleic acid metabolisms, cell replication, tissue repairs, and growth and thus, its deficiency leads to severe alteration of the thymic function and subsequent loss of T-cell-mediated responses and increased susceptibility to infectious diseases [7].

Human immunodeficiency virus (HIV) infection and/or ART shifts antioxidant-oxidant balance of the host towards the oxidants, with consequential poor disease outcomes [8,9]. Increased levels of oxidative stress markers were reported in HIV-infected patients [10,11] and the levels of the markers were associated with low CD4 count and increased viral load [12]. As a result, deficiencies of serum micronutrients are common among people living with HIV/AIDS due to the excessive release of pro-oxidants and cytokines leading to increased utilization of antioxidants including Cu and Zn [13]. The depletion of trace elements may also occur through increased metabolic requirements, enhanced excretion, and intestinal mal-absorption [14]. This can result in an imbalance between pro-oxidants and antioxidants which may lead to increased oxidative stress and accelerating HIV replication and mortality of the patient [15].

Evidence indicated that proper management of HIV infection needs special consideration of the nutritional status of a patient, including micronutrient nutrition. Due to the constant cellular turnover and a very large number of immune cells, the immune system needs a substantial quantity of micronutrients. Immunity and micronutrients have an interdependent relationship in which a deficiency of a micronutrient can prevent a proper response to an infection. On the other hand, an infection can negatively impact micronutrient nutritional status [16].As a result, research on Cu and Zn element deficiencies in HIV infected persons receiving ART has become a priority [17].

No studies investigating the Cu and Zn deficiency status among on-ART and pre-ART people living with HIV/AIDS have been done particularly in the study area. We assumed that understanding the major micronutrients' status in HIV/AIDS positive people would help for proper management and intervention of the disease. Therefore, the present study sets off to assess the concentration of serum Cu, Zn and, Cu to Zn ratio in relation to clinical status among adult people living with HIV/AIDS who were on-ART and Pre-ART for possible interventions.

2. MATERIALS AND METHODS

2.1 Study Design and Participants

Facility-based cross-sectional study was conducted consecutively on adult people living with HIV/AIDS at ART care clinic in Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia.

2.2 Blood Collection and Serum Separation

After overnight fasting, ten milliliters of blood sample was drawn from each participant using a vacutainer. From this amount 5 ml of blood was dispensed and mixed with a labeled ethylene diamine tetra acetic acid (EDTA) coated test tube for CD4/CD8 T-cell count and hemoglobin determination. The remaining 5ml of blood was collected with a labeled plain test tube and immediately after 30 minutes of collection sera were separated by centrifugation at 3000 revolutions per minute for 5 minutes then aliquot into labeled nunc tube. The aliquot was protected from light, and stored at -70°C until analysis for serum trace elements.

2.3 Immuno-hematological and Clinical Staging Analysis

CD4+ T-cell and CD8+ T-cell count were analyzed on fluorescence-activated cell scanner (FACScan) flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California) with the method as described by Tsegaye, et al. [18]. Hemoglobin was determined by using HemoCue 301 analyzer (Sweden).For this study, normal reference ranges for CD8+ T-cell count for male and female were 318-1891/µl and 273-1418/µl respectively, CD4:CD8 T-cell ratio for male and female were 0.4-2.1 and 0.6-2.7 respectively and anemic based on hemoglobin value for male and female were <14 gm/dl and <13 gm/dl respectively, all references were based on the immune-hematological reference ranges for adult Ethiopians [18]. Based on CD4 T-cell count, there are four proposed immunological HIV-related immunodeficiency classification outlines; no significant immunodeficiency (≥500 CD4 T cells /µl), mild immunodeficiency (350-499 CD4 T-cells /µl), advanced immunodeficiency (200-349 CD4 T-cells /µl) and severe immunodeficiency (<200 CD4 T-cells/ µl) [16] All immuno-hematological analytical procedure was done at Felege Hiwot Referral Hospital. The clinical stages of participants were determined based on the WHO (2006) clinical staging classification where stage I patients are asymptomatic, stage II showing mild symptoms, stage III having advanced symptoms and stage IV with severe symptoms.

2.4 Biochemical Analyses

The frozen serum samples were kept on dry ice and transported to Addis Ababa. The serum concentration of Cu and Zn was measured using a fully automated flame atomic absorption spectrophotometer version 2006 (PG-990, London, England), following the procedure described by Sumanjit et al. [19]. The measurements were done at JIJE Analytical Testing Service Laboratory (JATSL), Addis Ababa, Ethiopia. For this study, low concentration of serum Cu and Zn were defined at their serum levels less than 80µg/dl and 70µg/dl respectively (Duh and Cook, 2005). While high serum Cu to Zn ratio was defined when a Cu/Zn ratio >1 [20].

2.5 Anthropometric Measurements

Anthropometric measurements (height, weight, upper mid-arm, waist and hip circumference) were done in triplicate and the mean value was recorded. Weight and height were measured with minimal clothing and without a shoe to the nearest 01 kilogram and1mili meter respectively. Body mass index (BMI) (kg/m^2) was calculated for each participant. A cut off point less than 18.5 was used to define undernourished and a BMI of 25 or above indicates overweight or obesity [21].

2.6 Data Analysis

The information recorded from the laboratory results was entered and analyzed using statistical package for social sciences (SPSS) (Chicago, IL, USA) software, version 22.The descriptive data were analyzed with means and standard deviations, frequency counts and percentages. Cross tabulations were carried out to compare frequencies of individual characteristics and logistic regression analysis was used to test the predictability of the change in the dependent variable to each unit change of the entered independent variable. Any variable with a significant bivariate test at P-value less than or equal to 0.2 was selected as a candidate for the multivariate analysis and backward stepwise elimination model was applied [22]. Values were considered significant when P-value is less than 0.05.

2.7 Data Quality Assurance

Laboratory internal quality control was monitored during the procedure and all the measurements were carried out by the same team. All glassware and bottles for blood collection, separation of serum and further analysis were used after soaked and then rinsed them with 10% nitric acid

and de-ionized water, respectively. Contamination tests for Cu and Zn were carried out in between 10 participants. Training and procedures were given for data collectors.

3. RESULTS

3.1 Clinical Characteristics of the Study Participants

According to the ART status, 75 (50%) of the study participants had started ART more than a year ago. Among currently available combinations of antiretroviral (ARV) drugs, the most frequently prescribed drugs were azidothymidine-lamivudine-nevirapine (AZT-3TC-NVP) combination which accounts for 27/75(36%) of study participants (Table 1). Based on WHO 2006 clinical staging classification, 76 (50.6%) of the study participants were WHO clinical stage III followed by 40 (26.7%) in WHO clinical stage II. Mean of CD4T-cells count/µl of the study participants were 358.13 ±199.92 with a minimum count of 7 and a maximum count of 891 CD4T-cells/µl. More than a quarter (26.7%) of the study participants had a CD4+T-cell count within 200-349 cells/µg, followed by 38 (25.3%) within 350-500 cells/µg. Furthermore, according to CD4:CD8+T-cell count ratio, most 116 (77.3%) of the study participants were below a normal CD4:CD8+T-cell count ratio (Table 1). Regarding to the BMI analysis, 28 (18.7%) of the study participants had a BMI of lower than 18.5 and showed signs of undernourishment (Table 1). The mean hemoglobin value of study participants was 13.77±1.707 gm/dl with a minimum value of 7 gm/dl and a maximum value of 17 gm/dl. In addition, the proportion of anemic participants based on hemoglobin value was 37 (24.7%) (Table 1).

3.2 Distributions of Serum Cu, Zn and Cu to Zn Ratio Concentration Based on Clinical Status

3.2.1 Low serum Cu concentration distribution based on clinical status

According to the ART status, the proportion of low serum Cu concentration (<80µg/dl) was higher 10/75 (13.3%) in those pre-ART study

Table 1. Clinical characteristics of study participants in Felege Hiwot Referral Hospital, Bahir Dar from January to February, 2013

Characteristics		Frequency	Percent
Total		150	100.0
ART status	Pre-ART	75	50.0
	On-ART	75	50.0
ARV drugs	D4T-3TC-NVP	10	13.3
	D4T-3TC-EFV	6	8.0
	AZT-3TC-NVP	27	36.0
	AZT-3TC-EFV	10	13.3
	TDF-3TC-NVP	10	13.3
	TDF-3TC-EFV	10	13.3
	ABC-3TC-NVP	1	1.4
	ABC-3TC-EFV	1	1.4
WHO clinical stage	I	30	20.0
	II	40	26.7
	III	76	50.6
	IV	4	2.7
CD4+T-cell count/µl	>500	36	24.0
	350-500	38	25.3
	200-349	40	26.7
	<200	36	24.0
CD4:CD8 T-cell count ratio	Normal	34	22.7
	Abnormal	116	77.3
BMI	Nourished	115	76.6
	Malnourished	28	18.7
	Obese	7	4.7
Hb	Normal	113	75.3
	Anemic	37	24.7

participants than those on-ART study participants which accounted for 2/75 (2.7%). The mean serum Cu concentration of on-ART study participants was higher 123.63 ±22.97 µg/dl than in those pre-ART study participants with the mean of 120.67±35.1µg/dl. The percentage of low serum Cu concentration was found in 1/4 (25%) of WHO stage IV, while in WHO stage I were accounted for 2/30 (6.7%).Higher low serum Cu concentration was observed in 5/36 (13.9%) of the study participants who had a CD4+ T-cell count >500 cells/µl than 2/36 (5.6%) in those a CD4+ T-cell count < 200 cells/µg. Regarding the hemoglobin value, low serum Cu concentration was observed in 2/37 (5.4%) of anemic study participants and 10/37 (8.8%) in those who had normal hemoglobin value (Table 2).

3.2.2 Low serum Zn concentration distribution based on clinical status

The mean serum Zn concentration of study participants who are on-ART was higher 98.43 ±32.47µg/dl than pre-ART with the mean of 78.97±28.20 µg/dl. The proportion of low serum Zn concentration was higher 33/75 (44%) in pre-ART study participants than in 13/75 (17.3%) on-ART (Fig. 1).

On the other hand, the mean serum Zn concentration of study participants with WHO clinical stage I was higher 77.77±29.64µg/dl than WHO clinical stage IV with the mean of 75.00±32.91µg/dl. The percentage of low serum Zn concentration in relation to WHO clinical stage revealed that majority 56.7% and 50% was found in WHO stage I and WHO stage IV respectively, while WHO stage II and WHO stage III was found to be 22.5% and 23.7% respectively.

Low serum Zn concentration was observed in 15/37(40.5%) of anemic study participants while 31/113(27.4%) was observed in those study participants who had normal hemoglobin value. The mean serum Zn concentration of study participants with anemic hemoglobin value was lower (77.73±24.99) than those study participants with normal hemoglobin value (92.29±33.09) (Fig. 2).

The mean serum Zn concentration of study participants who had a CD4+ T-cell count >500 cells/µl was higher (96.08±32.8 µg/dl) than in those study participants who had a CD4+ T-cell count < 200 cells/µl(with the mean of 85.06±29 µg/dl).Low serum Zn concentration was observed in 11/36 (30.6%) of those study participants who had a CD4+ T-cell count < 200 cells/µl while 9/36 (25%) was observed in a CD4+ T-cell count >500 cells/µl.

Based on the BMI of study participants, higher 12/28 (42.9%) and 3/7 (42.9%) of low serum Zn concentration were observed in both malnourished and obese study participants respectively than in nourished study participants which accounted 31/115 (27%).The mean serum Zn concentration of study participants with a BMI of malnourished were lower 78.46±22.55 than in those study participants with a BMI of well-nourished 91.69 ± 33.04 (Fig. 3).

3.2.3 High serum Cu/Zn ratio distribution based on clinical status

According to the ART status, the proportion of high serum Cu/Zn ratio (>1) was higher in 58/75 (77.3%) of pre-ART study participants than in those on-ART study participants 56/75 (74.7%).The mean serum Cu/Zn ratio concentration of pre-ART study participants was higher (1.77±0.98µg/dl) than in those on-ART study participants with the mean of 1.37±.47µg/dl (Table 2). High serum Cu/Zn ratio was observed in26/30 (86.70%) of study participants of the WHO clinical stage I while participants in the WHO clinical stage IV had 3/4 (75%). Higher serum Cu/Zn ratios were observed in 28/36 (77.8%) of the study participants who had a CD4+ T-cell count <200 cells/µg than in 25/36 (69.4%) of the study participants who had a CD4+ T-cell count of >500 cells /µg. Higher 24/28 (85.7%) of high serum Cu/Zn ratios were detected in undernourished study participants than 84/115 (73%) of nourished participants (Table 2). Furthermore, Higher serum Cu/Zn ratios were observed in 33/37 (89.20%) of anemic study participants than 81/113 (71.70%) in those with normal hemoglobin value (Table 2).

3.3 Logistic Regression Analysis of Variables Associated with Serum Cu, Zn and Cu to Zn Ratio Concentration

3.3.1 Low serum Cu concentration based on regression analysis of clinical variables

Pre-ART study participants were almost six times more likely at risk of low serum Cu concentration than those on-ART study participants (AOR=5.85, 95% CI=1.22-28.058, *P*= 0.027). Regression analyses of WHO clinical stage and CD4 T-cell count /µl as independent variables

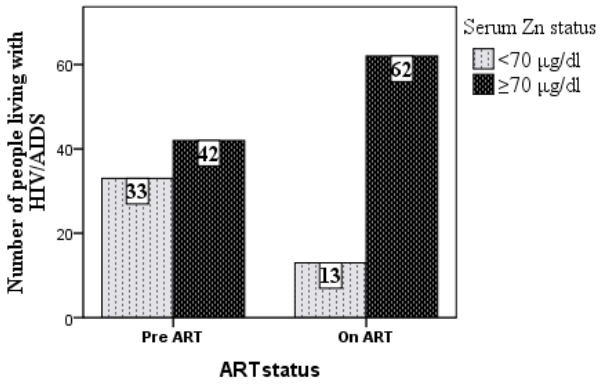

Fig. 1. The distribution of low serum Zn concentration with reference to ART status of study participants (N = 150)

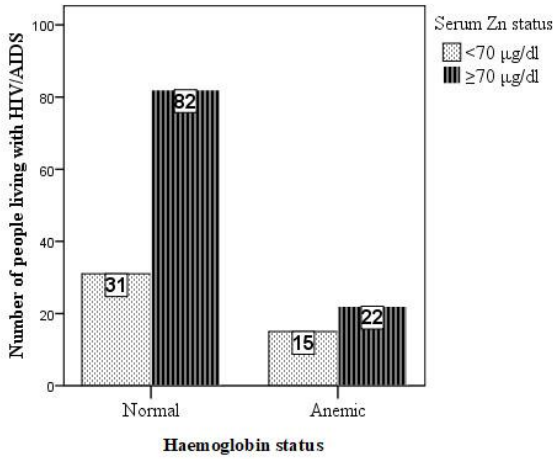

Fig. 2. The distribution of low serum Zn concentration with reference to hemoglobin status of study participants (n = 150)

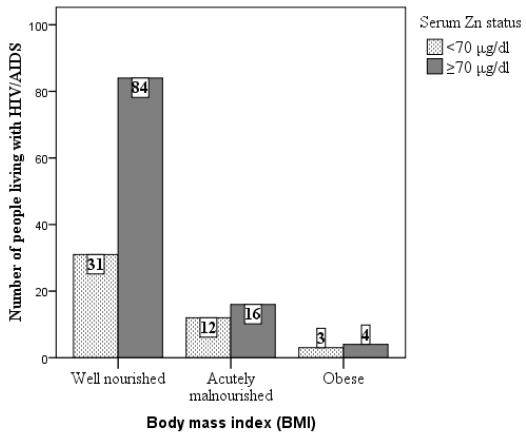

Fig. 3. The distribution of low serum Zn concentration with reference to BMI of study participants (n = 150)

Table 2. Distributions of serum Cu and Cu to Zn ratio concentration based on clinical characteristics (N=150)

Characteristics		Serum Cu status N (%)		Cu/Zn ratio status N (%)	
		≥80µg/dl	<80µg/dl	Normal(≤1)	High(>1)
Total		138 (92.0)	12 (8.0)	36 (24.0)	114 (76.0)
ART status	pre-ART	65 (86.7)	10 (13.3)	17 (22.7)	58 (77.3)
	On-ART	73 (97.3)	2 (2.7)	19 (25.3)	56 (74.7)
WHO clinical stage	I	28 (93.3)	2 (6.7)	4 (13.3)	26 (86.7)
	II	34 (85.0)	6 (15.0)	13 (32.5)	27 (67.5)
	III	73 (96.1)	3 (3.9)	18 (23.7)	58 (76.3)
	IV	3 (75.0)	1 (25.0)	1 (25.0)	3 (75.0)
/CD4 T-cell count /µl	>500	31 (86.1)	5 (13.9)	11 (30.6)	25 (69.4)
	350-500	35 (92.1)	3 (7.9)	7 (18.4)	31 (81.6)
	200-349	38 (95.0)	2 (5.0)	10 (25.0)	30 (75.0)
	<200	34 (94.4)	2 (5.6)	8 (22.2)	28 (77.8)
CD4:CD8 T-cell count ratio	Abnormal	106 (91.4)	10 (8.6)	28 (24.1)	88 (75.9)
	Normal	32 (94.1)	2 (5.9)	8 (23.5)	26 (76.5)
BMI	Nourished	104 (90.4)	11 (9.6)	31 (27.0)	84 (73.0)
	Malnourished	27 (96.4)	1 (3.6)	4 (14.3)	24 (85.7)
	Obese	7 (100.0)	0 (0.0)	1 (14.3)	6 (85.7)
Hb	Normal	103 (91.2)	10 (8.8)	32 (28.3)	81 (71.7)
	Anemic	35 (94.6)	2 (5.4)	4 (10.8)	33 (89.2)

Table 3. Logistic regression analysis of selected clinical variables associated with the distribution of low serum Cu concentration (N = 150)

Characteristics	Serum CU status N (%)		OR (95% CI)		*P*-value
	≥80µg/dl	<80µg/dl	COR	AOR	
ART status					
Pre-ART	65 (86.7	10 (13.3)	**5.61 [1.18 - 26.58]Ʊ**	**5.85 [1.22 - 28.06]**	**0.027Φ**
On-ART	73 (97.3)	2 (2.7)	1.00*	1.00*	
WHO clinical stage					
I	28 (93.3)	2 (6.7)	1.00*	1.00*	
II	34 (85.0)	6 (15.0)	2.47 [0.46 - 13.21]	2.97 [0.53 - 16.60]	0.215
III	73 (96.1)	3 (3.9)	0.57 [0.09 - 3.63]	1.50 [0.20 - 11.32]	0.694
IV	3 (75.0)	1 (25.0)	4.67 [0.32 - 68.03]	3.79 [0.24 - 59.07]	0.341
CD4 T-cell count/µl					
>500	31 (86.1)	5 (13.9)	1.00*	1.00*	
350-500	35 (92.1)	3 (7.9)	0.53 [0.12 - 2.41]	0.37 [0.06 - 2.23]	0.277
200-349	38 (95.0)	2 (5.0)	0.32 [0.06 - 1.81]	0.19 [0.02 - 1.39]	0.102
<200	34 (94.4)	2 (5.6)	0.36 [0.06 - 2.02]	0.25 [0.04 - 1.69]	0.156

-reference category for the independent variable, **Ʊ -statistically significant at COR, **Φ-** statistically significant at AOR (p ≤0.05) where OR= Odds ratio, COR= crude odds ratio, AOR= adjusted odds ratio*

and low serum Cu concentration as a dependent variable did not show any significant association at 95% CI (Table 3).

3.3.2 Low serum Zn concentration based on regression analysis of clinical variables

Three factors were the strongest predictor of low serum Zn concentration. The odds ratio of being low serum Zn concentration by pre-ART study participants were more likely at risk of low serum Zn concentration than those on-ART study participants (AOR=3.56, 95% CI=1.52-8.33, *P*= 0.003).The odds ratio of being low serum Zn concentration was found to be more likely at risk of low serum Zn concentration than those nourished study participants (AOR=2.61, 95% CI=1.02 - 6.67, *P*= 0.046).On the other hand, participants of WHO clinical stage II were less likely to serum Zn deficient as compared to WHO stage I participants (AOR=0.22, 95% CI=0.07 - 0.64, *P*= 0.005).Regression analyses of CD4 T-cell count/μland Hb as independent variables and low serum Zn concentration as a dependent variable did not show any significant association at 95% CI (Table 4).

3.3.3 High serum Cu/Zn ratio based on regression analysis of clinical variables

One factor was the strongest predictor of high serum Cu/Zn ratio. Anemic study participants were more than three times likely at risk of high serum Cu/Zn ratio than those study participants with normal Hb value (AOR=3.26, 95% CI=1.07 - 9.94, *P*= 0.038). Regression analyses of sex, age, monthly income, WHO clinical stages, CD4 T-cell count /μl and BMI as independent variables and high serum Cu/Zn ratios as a dependent variable did not show any significant association at 95% CI (Table 5).

4. DISCUSSION

The overall mean value of serum Cu concentration of this study's participants was 122.15± 29.601μg/dl. The result of the present study is higher than a similar study in India with a mean of serum Cu concentration of 119.95±7.94μg/dl [23]. The overall low serum Zn (< 70 μg/dL) concentration of the current study was 30.70% which is higher than a similar study conducted in Argentina which was 23% [24]. However, it was lower than previous similar findings in Iran (65%) [25], in Boston (USA) reported as 42% [26], South Africa with 45% [27], in Addis Ababa (Ethiopia) with 53.6% [28], Gondar (Ethiopia) with 69.7% [29]. In this study, the overall high serum Cu/Zn ratio of study participants was 76%.The result of this study was much higher than a similar study in Argentina, 21% of HIV infected patients showed a Cu/Zn ratio higher than 1.0 [24]. Selecting different cut off points for determining low serum Zn concentration could be a reason for this variation. Other reasons could be variation in sample size, the commencement of ART and intake of dietary items and frequency.

In the current study, mean serum Cu concentration of study participants was higher (123.63 ±22.97μg/dl) in on-ART participants than 120.67 ±35.1 μg/dl in pre-ART participants. This is in agreement with a study in Nigeria 19.750 ± 6.82 and 17.70 ± 3.91 of on-ART and pre-ART study participants respectively [30]. On the other hand, the proportion of serum Zn deficiency in this study which was higher (44%) in pre-ART study participants than 17.3% in on-ART is slightly comparable with a similar study in Uganda where serum Zn deficiency was higher in 59.6% of pre-ART than in 29.5% of on-ART [31]. However, almost an equal distribution of low serum Zn concentration was reported in a study in Germany where 25% and 22% of on-ART and pre-ART individuals [32] and a 50/50% deficiency in both groups in India [33].

In the present study, the percentage of high serum Cu/Zn ratio was observed in 26/30 (86.70%) of WHO clinical stage I study participants while in 3/4 (75%) of WHO clinical stage IV. This finding is consistent with previous reports in India by [23] a lower serum Zn level reported in patients at late stage than the early stage of the disease but a rise in serum Cu level was found in the late stage of the disease. Other similar studies showed patients in later stages of HIV-1 infection may experience an increase in plasma Cu levels [34,35].

In this study, the percentage of low serum Zn concentration was 56.7% and 50% in WHO stage I and WHO stage IV respectively. The current finding was higher compared to a previous similar study from New Jersey (USA) where at early stage and late stage of disease low serum Zn concentration was found to be 14.7% and 36% respectively [36]. Similarly, a study from South Africa reported lower serum Zn concertration of 20% at early clinical stages of HIV infection and 36% and 45% at clinical stages

Table 4. Logistic regression analysis of selected socio-demographic and clinical variables associated with the distribution of low serum Zn concentration (N = 150)

Characteristics	Serum Zn status N (%)		OR[95% CI]		P-value
	≥70 µg/dl	<70 µg/dl	COR	AOR	
ART status					
Pre-ART	42 (56.0)	33 (44.0)	**3.75 [1.77 - 7.95]Ʊ**	**3.56 [1.52 - 8.33]**	**0.003Φ**
On-ART	62 (82.7)	13 (17.3)	1.00*	1.00*	
WHO clinical stage					
I	13 (43.3)	17 (56.7)	1.00*	**1.00***	
II	31 (77.5)	9 (22.5)	**0.22 [0.08 - 0.62]Ʊ**	**0.22 [0.07 - 0.64]**	**0.005Φ**
III	58 (76.3)	18 (23.7)	0.24 [0.09 - 0.58]	0.41 [0.15 - 1.08]	0.072
IV	2 (50.0)	2 (50.0)	0.76 [0.09 - 6.17]	0.76 [0.09 - 6.66]	0.81
CD4 T-cell count/µl					
>500	27 (75.0)	9 (25.0)	1.00*	1.00*	
350-500	22 (57.9)	16 (42.1)	2.18 [0.81 - 5.88]	3.18 [0.88 - 11.41]	0.076
200-349	30 (75.0)	10 (25.0)	1.00 [0.35 - 2.83]	1.64 [0.42 - 6.36]	0.477
<200	25 (69.4)	11 (30.6)	1.32 [0.47 - 3.72]	1.17 [0.32 - 4.24]	0.813
BMI					
Nourished	84 (73.0)	31 (27.0)	1.00*	1.00*	
undernourished	16 (57.1)	12 (42.9)	2.03 [0.86 - 4.77]	**2.61 [1.02 - 6.67]**	**0.046Φ**
Obese	4 (57.1)	3 (42.9)	2.03 [0.43 - 9.60]	1.98 [0.37 - 10.64]	0.425
Hb					
Normal	82 (72.6)	31 (27.4)	1.00*	1.00*	
Anemic	22 (59.5)	15 (40.5)	1.80 [0.83 - 3.92]	2.11 [0.82 - 5.40]	0.119

**-reference category for independent variable, Ʊ -statistically significant at COR, Φ- statistically significant at AOR (p ≤0.05)*

Table 5. Logistic regression analysis of selected socio-demographic and clinical variables associated with the distribution of high serum Cu to Zn ratio (N = 150)

Characteristics	CU to ZN ratio N (%)		OR(95% CI)		*P*-value
	≤1(Normal)	>1(High)	COR	AOR	
WHO clinical stage					
I	4 (13.3)	26 (86.7)	1.00*	1.00*	
II	13 (32.5)	27 (67.5)	0.32 [0.09 - 1.11]	0.35 [0.09 - 1.29]	0.114
III	18 (23.7)	58 (76.3)	0.49 [0.15 - 1.61]	0.52 [0.15 - 1.79]	0.301
IV	1 (25.0)	3 (75.0)	0.46 [0.04 - 5.60]	0.65 [0.05 - 8.86]	0.747
CD4 T-cell count /µl					
>500	11 (30.6)	25 (69.4)	1.00*	1.00*	
350-500	7 (18.4)	31 (81.6)	1.95 [0.66 - 5.76]	2.14 [0.59 - 7.67]	0.244
200-349	10 (25.0)	30 (75.0)	1.32 [0.48 - 3.61]	1.40 [0.39 - 5.00]	0.603
<200	8 (22.2)	28 (77.8)	1.54 [0.53 - 4.44]	0.97 [0.28 - 3.42]	0.969
BMI					
Well nourished	31 (27.0)	84 (73.0)	1.00*	1.00*	
Acutely malnourished	4 (14.3)	24 (85.7)	2.21 [0.71 - 6.89]	2.42 [0.73 - 7.98]	0.147
Obese	1 (14.3)	6 (85.7)	2.21 [0.25 - 19.14]	2.04 [0.22 - 19.15]	0.532
Hb					
Normal	32 (28.3)	81 (71.7)	1.00*	1.00*	
Anemic	4 (10.8)	33 (89.2)	**3.26[1.07 - 9.94]Ʊ**	**3.26 [1.07 - 9.94]**	**0.038Φ**

-reference category for the independent variable, **Ʊ -statistically significant at COR, **Φ**- statistically significant at AOR (p ≤0.05)*

III and IV respectively [27]. The mean serum Zn concentration of study participants with WHO clinical stage I was higher (77.77±29.641µg/dl) than in those of the WHO clinical stage IV (75.00±32.914µg/dl).The Indian study mentioned previously [23] also showed a lower serum Zn level reported in patients at a late stage than the early stage of the disease.

The higher serum Cu/Zn ratio of this study was higher in 89.2% of anemic study participants than 71.7% in those with a normal hemoglobin value.This difference might be due to the antagonism effect of iron on the absorption of Zn in the gastrointestinal tract. This study is in accordance with a similar study in India that showed a decrease in serum Zn level and an increase in serum Cu level in the anemic individuals [37]. Another similar study in Egypt revealed that, Cu level was higher in the anemic than the control group [38].

In the present study, low serum Zn concentration was higher in (42.9%) of undernourished study participants than 27% in nourished study participants. This study was in agreement with a study in Iran found that higher (41.7%) in undernourished study participants than 33.6% in nourished study participants [25].

In logistic regression analysis, the finding of this study revealed that pre-ART study participants were more likely at risk of low serum Zn concentration than those on-ART study participants (AOR=3.56, 95% CI=1.52-8.33 *P*= 0.003).The differences might be due to the role of combination therapy with ARVs that reduces viral replication, pro-oxidants and cytokines. In addition to this the difference could be variation in health education. This study is in accordance with a study in Uganda which showed that pre-ART study participants were more likely at risk of low serum Zn concentration than those on-ART study participants (AOR=3.7, 95% CI=1.8-7.7) [31].

In the current study, CD4+ T cell count had no association with Zn status. This finding is similar to what was reported in a similar study in India [31].In this study, participants of the WHO clinical stage II were less likely to serum Zn deficient as compared to the WHO clinical stage I participants (AOR=0.22, 95% CI=0.07 - 0.64, *P*= 0.005).The differences might be due to the role of ART commencement at clinical stage II. This finding disagreed with study in South African, where the prevalence of Zn deficiency increased with HIV disease staging but on-ART was excluded from the study population [27].

In this study, BMI status was found to be one of the clinical determinants for low serum Zn concentration where undernourished study participants were significantly and more likely at risk of low serum Zn concentration than nourished study participants (AOR=2.61, 95% CI=1.02-6.67, *P*= 0.046).This association was also reported in previous studies in South Africa (AOR=1.19, 95% CI=1.09 - 1.30, *P*< 0.05) [27].

Furthermore, anemic study participants were more likely at risk of high serum Cu/Zn ratio than those study participants with normal hemoglobin value (AOR=3.259, 95% CI=1.068-9.944, *P*= 0.038).The association between anemia and low serum Zn concentration was also reported in a similar study in India [37].

5. CONCLUSIONS

The percentage of overall serum Cu and Zn deficiency and high serum Cu/Zn ratio was 76 %, 30.7%and 8% respectively. In the multiple logistic regression analysis, ART status was identified as one of the clinical factors that had an association with low serum Cu and Zn concentrations. On the other hand, body mass index and hemoglobin values had positive association with low serum Zn concentrations and high Cu/Zn ratio respectively. Thus, for the proper onset of ART treatment, early evaluation of biochemical analyses for serum Cu and Zn deficiency should be carried out and appropriate mineral supplementation along with anti-retroviral treatment may be required especially for those who are in pre-ART, undernourished and anemic groups living with HIV/AIDS. In addition, regular health education and counseling about maintaining adequate healthy diet and nutrition care should be applied to people living with HIV/AIDS especially for those on pre-ART living with HIV/AIDS.

CONSENT

Written informed consent was obtained from the study participants after the purpose of the study was explained. Participants were informed that all the data obtained from them would be kept confidential using codes instead of any personal identifiers.

ETHICAL APPROVAL

Before data collection, ethical approval was obtained from the ethical board of Bahir Dar University. Permission was obtained from Amhara Regional Health Bureau and from Felege Hiwot Referral Hospital ethical board.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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