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CORRELATION OF MICRONUTRIENTS ITH IMMUNUOLOGICAL STATUS OF PEOPLE LIVING CRYPTOCOCCOSIS AND HIV/AIDS

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ABSTRACT

Micronutrients are essential trace metals which are extremely small in quantities and are often required in tissues. The measurement of these trace elements especially, Selenium (Se), Zinc (Zn) and Copper (Cu); which are known predictors of HIV disease progression were determined in plasma of People Living with HIV/AIDs (PLHA) on combination antiretroviral therapy (ART) in Abuja Nigeria, using Atomic Mass Absorption Spectrophotometry (AAS). A mean serum Zn level ($\bar{x}$Zn = 83.21±7.13µg/dL, 97.09±21.11µg/dL and 74.15±15.33µg/dL in participants at stages I, II and III immunological status. A high relationship (R^2 =0.0878) of serum Zn confirmed association of Zn deficiency with immune status and HIV, infection and disease. Similarly, mean serum Cu ($\bar{x}$Cu = 112.72±8.4µg/dL compared to 115.32±9.44 µg/dL and 94.10µg/dL in HIV infected ($p<0.05$). In stage I asymptomatic HIV disease, while serum Cu was 132±10.31µg/dL, compared to 109±8.83 µg/dL: 103.25±11.12 µg/dLin stages II and III, respectively. Thus serum Cu was significantly lower, though inversely related to severity of HIV disease with a high degree of correlation (r = 3.943) and regression (R^2 =0.7045). This confirmed Cu deficiency in participants' according

to immune status with HIV and/or cryptococcosis disease stage. Serum Selenium level of 115.14±3: 34.27µg/L compared to HIV/*Cryptococcus* positive participants whom the mean serum Se value was lower ($\bar{x}$Se = 82.05±8.42µg/L; $p<0.05$). Severe Se deficiency (Se <60 µg/dL); was found only in Stage III disease ($\bar{x}$Se = 57.43±9.17µg/dL), in HIV/*Cryptococcus* positive persons. Se deficiency was strongly dependent (R^2= 0.8892) on CD4 cell count, suggesting that other factors of immune deficiency may determine Se level in HIV and or *Cryptococcus* disease. This study highlights the need for boosting the nutritional status of infected persons for sustained viral suppression of opportunistic infections in PLHAs on cART in FCT, Abuja and perhaps north central, Nigeria.

KEYWORDS: Micronutrient, HIV/AIDS, *Cryptococcus neoformans*, Trace elements, PLWHA.

INTRODUCTION

Micronutrient metals are metals in extremely small quantities, almost at the molecular level, that reside in or are present in animal and plant cells and tissues. They are a necessary part of good nutrition, although they can be toxic if excess quantities are ingested. It has been shown that micronutrient deficiencies are common among HIV-infected persons, especially in those who are underprivileged and undernourished.[1] Under-nourished and micronutrient deficiencies in HIV-infected individuals exacerbate immunosuppression, oxidative stress, acceleration of HIV replication and CD4+ T cell depletion.[2] Deficiency of antioxidant micronutrients in HIV positive populations is probably due to increased utilization of micronutrients because of increased oxidative stress rather than inadequate dietary intake and malabsorption.[3]

Human immunodeficiency virus (HIV) infection continues to increase globally, with over 90% of new cases being reported in developing countries and regarded as the primary cause of acquired immunodeficiency syndrome.[4] in infected persons. Although major advances have been made in understanding the biology of HIV infection and significant progress in therapy has been made in the last few years, however, the basic role of host's nutrition in the pathogenesis of HIV infection still remains a major challenge in scientific knowledge. The concept that a malnourished host has a greater susceptibility to infections and a relatively worse prognosis has been generally accepted, however, it is often difficult to demonstrate that specific nutritional deficiencies contribute to poor clinical outcomes.[5] With the spread of HIV/ AIDS pandemic in developing countries where nutritional problems are common, the

identification and correction of micronutrient deficiencies may beincreasingly important. The use of Antiretroviral therapies (ART) is recommended globally for the management of HIV/AIDS. Periodic blood plasma viral load and CD4+T-cell count monitoring has been recommended to measure ART effectiveness since the goal of effective ART is a long term suppression of plasma viral load. While it cannot be assuming that effective ART would eliminate transmission with individual exposure, evidence from several cohorts, observational and mathematical modeling studies suggests that effective ART may be a promising way to reduce HIV transmission within a population.[1,6] Essential trace metals have a wide range of benefits among HIV-positive patients such as increased survival, improved oxidative stress, reduced hospitalization, increased weight gain, improved birth outcomes and infant immune status and reduced mother-to-child transmission.[7] The measurement of trace elements especially, selenium (se), zinc (zn), copper (cu) may be a useful marker to predict HIV infection progression. This study is therefore aimed at determining plasma level of selenium, zinc and copper with Immunuological status of People Living with HIV/AIDs, (CD4+T-cell count) levels among HIV-positive persons on ART in Abuja, Federal Capital Territory, Nigeria.

MATERIALS AND METHODS

Study area

The study area is the Federal Capital Territory (FCT) Abuja, the capital city of Nigeria, located in the center of Nigeria; lies between latitudes $8^{o}.25'$ and $9^{o}20'$ North of the Equator and Longitude $6^{o}45'$ and $7^{o}39'$ East of Greenwich Meridian. Abuja is bordered to the West by Niger State and North by Kaduna state, to the North-East by Kubwa, to the East and South and South East by Nassarawa and to the South-West by Kogi State. However, the huge influx of people into the city has led to the emergence of satellite towns such as Karu Urban Area, Suleja, Gwagwalada, Lugbe, Kuje and smaller settlements to which the planned city is sprawling towards. Thus, Abuja has a landmass of approximately 8,000 km^2 with a population of 6 million persons making it the most populous and largest urban area in Nigeria after Lagos.[8] It lies at an elevation of 840m or 2,760 ft above sea level with a tropical wet and dry climate. The FCT falls within the Guinea forest-savanna mosaic zone of the West African sub-region.

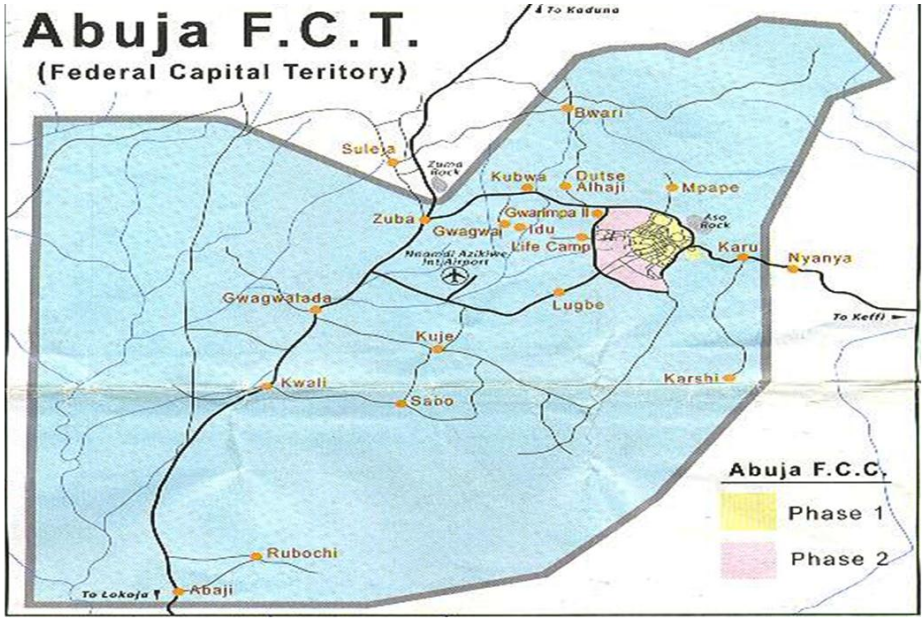


Figure 1: Map of study Area.

a: Map Nigeria showing position of FCT

b: Map of FCT Abuja.[9]

Sample Size

The minimum study sample size was determined to obtain a representative study population. The number was statistically derived from *C. neoformans* prevalence in FCT[10], Abuja using the formula:

$$N = \frac{Z^2 - a\,[P\,(1-P)]}{D^2}$$

This number (413) is therefore the minimum study size required to ensure that this study used a representative sample number. This study however collected and analysed 750 samples.

Study population

This study was carried out between July, 2015 and June, 2019 at designated hospitals and HIV treatment centers of the Federal Capital Territory, Abuja. A total of 750 persons (245 Males and 505 females; aged 15 years to ≥50); whose HIV statuses were confirmed by the treatment program of the clinics and who attended these HIV clinics during the study, participated in this study.

All participants were given written informed consent which they signed before they participated in this studywith set inclusion and exclusion criteria.

Study sampling

The volunteers were drawn from clients accessing ART site in a cosmopolitan, semi-urban communities Idu, Abuja Municipal Area Council, FCT, Nigeria.

Inclusion Criteria

- All participants were confirmed HIV positive following Nigerian HIV National Testing Algorithm, HIV PCR and viral load as well as CD4 count.
- Participants having AIDS defining illness (WHO Guidelines).

Exclusion Criteria

HIV negative Subjects who were tested and confirmed following Nigerian HIV National Testing Algorithm

- Participants who did not voluntarily give consent for inclusion in the study.
- Those who didn't fulfill the selection criteria.
- Persons who are already on Multivitamins supplementation in the previous 3 months before enrolment in this study.

Laboratory Studies

Blood sample Collection

Blood samples were collected from willing volunteers and coded appropriately using Aseptic techniques. Human sweat and dirt with the samples were avoided to eliminate possible traces of our elements in the samples. All glassware and plastics ware carefully washed and rinsed in pure laboratory grade water.

Determination of *Cryptococcus neoformans*

All the collected samples were processed and analyzed using Latex-*Cryptococcus* Antigen agglutination system (IMMY Immunomycologic Inc, USA), an agglutination ELISA technique and Lateral Flow Assay **(**LFA) kit consists of immunochromatic test strips impregnated with monoclonal antibodies optimized was used to detect all four cryptococcal serotypes and diluents respectively. And all the test kits were used according to the manufacturer's instruction and result interpretation. All HIV and *C. neoformans* positive individuals were marched with healthy Control group of participants of approximate age and sex. The study was carried out on HIV and HI/*Cryptococcus neoformans* positive subjects from study site hospitals.

Determination of trace elements

Atomic Absorption spectroscopy (AAS) was used to examine for the trace elements (zinc, copper and selenium). The CD4 enumeration were measured with ParteCyflow (Sysmex, Germany) according to.[11] The threshold concentrations used were; Cu (10 mol/L), Se (0.75 mol/L) and Zn (10.7 mol/L).

Ethical Approval

Ethical approval for this study was obtained from the Federal Capital Territory Health Research Committee (FCTHRC), FCTA Secretariat, Garki Abuja (FHREC/2019/01/56/11-06-19).

Statistical Analysis

Data obtained were presented as $\bar{x}$ ±SD. Student's t-test and regression analysis were applied to establish the significance of the differences in value of the scores in healthy and infected group and the strength of the correlation of nutrient and disease. Correlation coefficient (r) and regression for dependence of variables were used to assess linear and interrelationships of immune status of subjects (CD4 count) and serum level of micronutrients (Zn, Cu and Se). The significance of all our observations were confirmed at 0.05 level of significance

RESULTS

Persons living with HIV/AIDS/*Cryptococus* species were examined for serum Zinc (Zn), Copper (Cu) and Selenium (Se). A total of 7(38.89%) participants were asymptomatic, stage I; HIV seropositive participants with high (≥500 Cells/µL) CD4 cell count. The stages II and III represented moderately low (200-500 Cells/µL) and very low (<200 Cells/µL), CD4 cell count respectively. Zinc level of 84.82±14.52µg/dL was recorded and compared to 127±7.66 µg/dL in matched healthy controls. The difference between HIV and/or *Cryptocccus* with the healthy control subjects was significant ($p<0.05$; Table 1.). Serum Zn level was 74.15 ±15.33 µg/dL in persons with CD4 cell count <200/dL as compared to 97.09±21.11µg/dL and 83.21±7.13µg/dL in those with CD4 cell count 200-500 Cell/dL and >500 cell/dL ($p<0.05$); at HIV disease stage II and I respectively. There was a moderate degree of correlation between serum zinc and CD4 cell count ($r=0.296$, r^2 =**0.0878**). Similarly marked Zn deficiency was observed in HIV infection at various stages of assessed HIV disease. Although the relationship was not sustained all through the disease stages, hypozincemia became more severe with late stage HIV disease "Fig. 2".

The mean levels of serum Cu in healthy controls were 112.72±8.4 µg/dL. The mean serum Copper in HIV infected participants was 115.32±9.44 µg/dL (Table 1; $p<0.05$). In stage I asymptomatic HIV disease, serum Cu was 132±10.31µg/dL compared to 109±8.83 µg/dl and 103.25±11.12 µg/dLin stages II and III, respectively. Thus serum Cu was significantly lower in advanced HIV disease when compared to healthy controls (112.72±8.4 µg/dL: 103.25±11.12 µg/dL; $p<0.01$). The decline in serum Cu level was inversely related to severity of stage of disease i.e. higher serum level in early stage of the disease with CD4 cell count >500 Cell/µL ($p<0.05$); to low levels in persons with CD4<200 cells/µL. The relationship was, however, sustained all through the disease stages.

The serum Selenium value in healthy controls was 115.14 ±3.34µg/dL as compared to HIV positive participants with cryptococcosis in whom the mean serum Se values was significantly lower($p<0.05$); with a $\bar{x}$ serum Se = 82.05±8.42µg/L. Severe Se deficiency (Se <60 µg/dL); was found only in Stage III disease ($\bar{x}$Se = 57.43±9.17µg/dL; followed by participants with stage II HIV disease plus *Cryptococcus* infection (81.13±9.03 µg/dL), and apparently healthy stage I persons with serum Se 107.59±7.07 µg/dL. In this study, 6(33.33%) HIV/*Cryptococcus* positive persons with stage III HIV disease had severe serum Se deficiency (defined by serum Se level <60µg/dL). Thus severe Se deficiency was present only in the late stage of the disease with low CD4 cell counts <200 Cells/µL, however, it followed that the lower the CD4 cell count; the lower is the serum Se level in HIV positive participants.

Table 1: Levels of Copper, Zinc and Selenium in study population in healthy and Cryptococcus positive.

		Healthy *Cryptococcus* Negative (n =18)	**Immunocompromised *Cryptococcus* Positive (n =18)**
Sex	M	5	5
	F	13	13
Mean Age in years (±SD)	M	34.96 ±5.2	34.4 ±9.03
	F	27.34 ±7.3	30.8 ±11.6
Mean micronutrients level (µg/dL)			
Serum Zinc, Zn ($\bar{x}$±SD)		127 ±7.66	84.82 ±14.52
Serum Copper, Cu ($\bar{x}$±SD)		112.72 ±8.4	115.32 ±9.44
Serum Se (µg/dL) ($\bar{x}$±SD)		115.14 ±3.34	82.05 ±8.42

Key: SD= Standard Deviation; n = number in study population group.

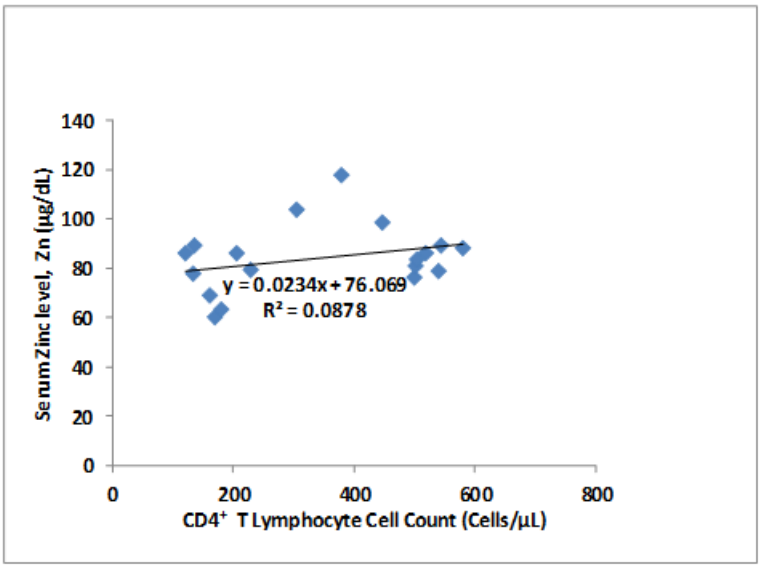
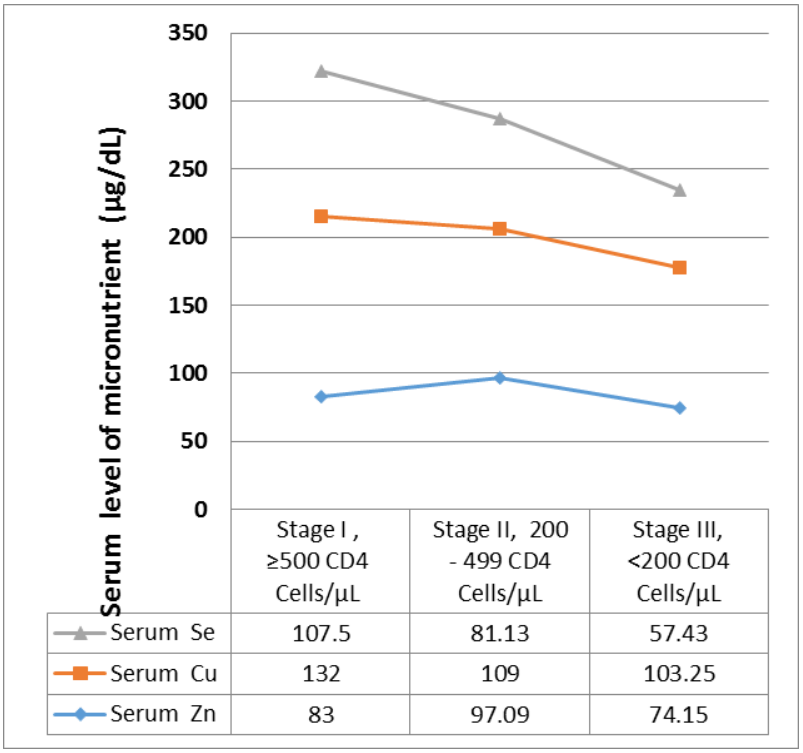


Fig. 2: Scatter-plot showing correlation between serum Zinc level and CD4 cell counts of HIV infected subjects in the study population.



	Stage I , ≥500 CD4 Cells/µL	Stage II, 200 - 499 CD4 Cells/µL	Stage III, <200 CD4 Cells/µL
Serum Se	107.5	81.13	57.43
Serum Cu	132	109	103.25
Serum Zn	83	97.09	74.15

Fig. 3: Serum level of micronutrients in relation to CD4 cell count and HIV/AIDS disease stages in study population.

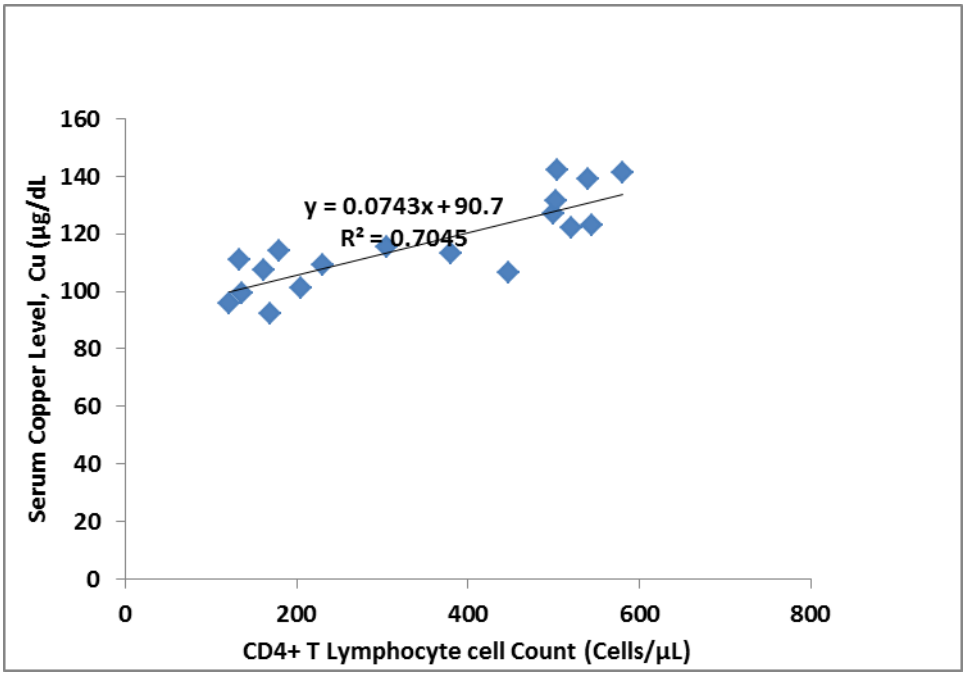


Fig. 4: Scatter-plot showing correlation between serum Copper level and CD4 cell Counts of HIV infected in study population.

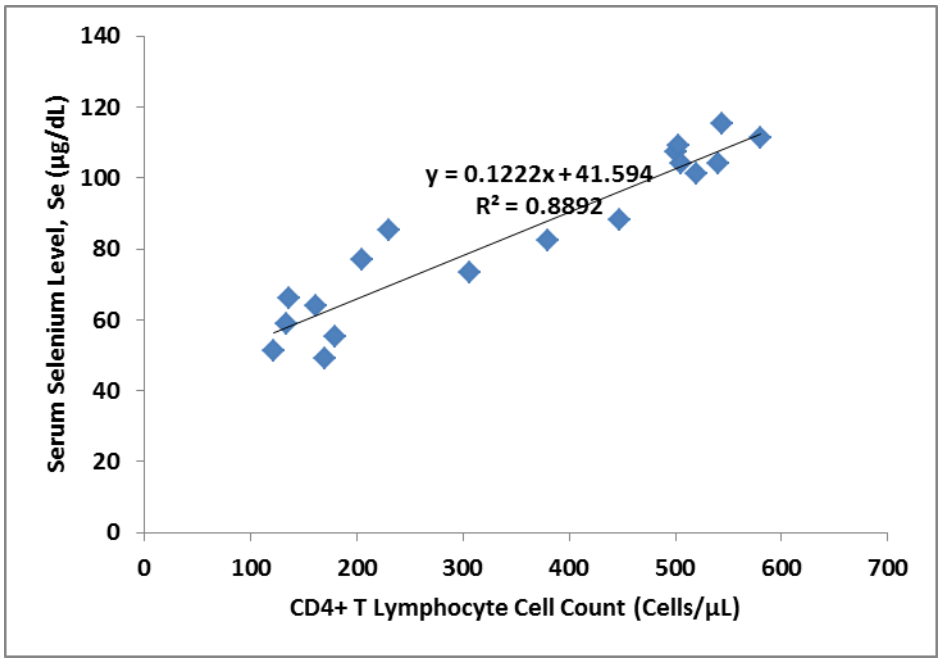


Fig 5: Scatter-plot showing correlation between serum Selenium and CD4 cell counts in HIV positive participants.

DISCUSSION

Micronutrients are comprised of trace elements vital for the maintenance of human immunological function although deficiencies of some of these minerals have been associated with impaired functions. For instance, zinc deficiency has been reported to decrease

lymphocyte concentrations, copper deficiency reduced cytokine response while selenium deficiency negatively impacted on proper functioning of the neutrophils and T-lymphocytes.[11,12,13] In this study, marked Zn deficiency was observed in HIV infected individuals at various stages of illness. A significant low serum zinc level (hypozincemia) were recorded in People Living With HIV and AIDS (PLWHA) with or without cryptococcosis when compared with healthy controls ($p<0.05$) (84.82±14.52µg/dl: 127±7.66 µg/dl) respectively. The mean serum zinc concentration in persons at stages I, II and III HIV immunological status with a CD4 T-cell count >500cells/µl, 200-500cell/µl and < 200cells/µl were 83.21: 97.09: 74.15.33µg/dL respectively. This result agrees with earlier reports of global HIV and CD4 levels.[14,15,16,17,18,19] In addition, we observed that serum Zn level were directly proportional to the CD4 cell count. That is, those with lower CD4 cell count; had lower serum Zn level. Similarly, marked serum Zn deficiency were reported in HIV infection at various stages of illness with low CD4+ T-cell count, advanced disease progression or death.[10,21,22,23,24] Zinc deficiency is also linked with impaired immune function and an increased receptiveness to infection.[25,26] In the present study however, Zinc deficiency, became more severe with HIV/cryptococcsis disease progression. This finding may be due to the high demand in zinc, an essential HIV nucleocabsid and intergrase proteins that are necessary for assembly of infectious virus, which contain zinc fingers required for normal structure and functioning.[23,27,28,29] Some studies on the other hand have reported that Zn deficiency is not a common contributory factor for HIV/AIDS or clinical expression and that HIV infection doesn't induce Zn deficiency.[26] However, this finding is in agreement with high serum Zn among HIV subjects earlier reported in Addis Ababa, Ethiopia.[30] The disparity in Zn intake from natural sources may account for the differences in Serum Zn levels. This finding agrees with a previous study by.[31] Furthermore, there was a moderate degree of correlation between serum zinc and CD4 cell count ($r=0.296$, $r^2=0.0878$).

Similarly, mean serum Cu of asymptomatic HIV+ persons in stage I was 132±10.31µg/dL as compared to 109±8.83 µg/dL: 103.25±11.12 µg/dL recorded in stages II and III, respectively. Serum Cu confirmed Cu deficiency in participants' according to HIV disease and immunological status. This agrees with reports of other scientists.[31,32,33] Thus, serum Cu was however, significantly lower, though inversely related to severity of HIV disease with a high degree of correlation ($r = 3.943$) and regression ($R^2=0.7045$). However, the values were not significantly correlated with age, sex or community population ($p>0.05$). Many studies have associated micronutrients level to HIV disease as observed in this study, including its

correlation to disease stage and immunological status.[34] Significantly, higher serum Cu levels have been reported in HIV positives with opportunistic infection[35,36] and inflammatory states which was attributed to an increased hepatic synthesis and release of Ceruloplasmin.[32, 36,37,38] However[39,40,41], did not observe any difference in serum (Cu) level in HIV infected compared to healthy individuals.

In this study, Serum Selenium level in healthy controls was 115.14 ±3.34.27µg/L as compared to HIV/*Cryptococcus* positive participants whom mean serum Se values were lower (x̄Se = 82.05±8.42µg/L and 57.43±9.17µg/L respectively ($p<0.05$). Severe Se deficiency (<60 µg/dL) was found only in Stage III disease (x̄Se = 57.43±9.17µg/L) of HIV/*Cryptococcus* positive individuals. Thus, severe Se deficiency was recorded only in the late stage of the disease and was strongly dependent (R^2= 0.8892) on CD4 cell count. This implies that the lower the CD4 cell count; the lower is the serum Se in HIV positive participants.In agreement with our study, many scientists have reported a low level of serum Se in HIV infected individuals.[42,43] Thus, suggesting that other factors of immune deficiency may determine Se level in HIV and or *Cryptococcus* disease persons.[44,45,46] This is consistent with reported observations by.[47,48] where Selenium deficiency was responsible for HIV progression and mortality. Additionally, some researchers have also observed that Se deficiency was severe only in AIDS and not in asymptomatic HIV persons.[49,50]. On the contrary, previous researchers have documented that Se deficiency was independent of malabsorption, CD4 cell, mortality and OIs in HIV positive individuals.[51,52,53,18] Serum Se from this study was not associated with sex or age. Similar studies have also shown that AIDS is the most important risk factor for cryptococcosis, due to the suppression of immune responses by the HIV virus.[54,55,56] This agrees with reports that trace elements deficiency leads to malnutrition effects on the immune system[57] and anaemia[41], while[30] observed **a**lterations in the serum levels of trace elements in tuberculosis and HIV co-infection.Trace elements often serve as cofactors in enzymatic reactions.[58,59,60,16, 18]

Many investigators have utilized plasma Cu/Zn ratio for clinical assessment of Zn deficiency in severe disease.[47] In a study of >100 positive homosexual men Cu: Zn ratio of >1 was associated with increased mortality[61,62], hence plasma Cu: Zn ratio may be a useful predictor of survival in HIV disease progression.The correlation of *C. neoformans*, an Opportunistic Infection (OI) and its widely expressed markers of virulence and pathogenesis, just as micronutrient deficiency (represented in important trace elements of Zn, Cu and Se); is

consistent with the hallmark of HIV infection and subsequently AIDS pathogenesis.This in turn is the progressive depletion of $CD4^+$ T-cell populations in close association with progressive impairment of cellular immunity and increasing susceptibility to opportunistic infections.[33,34,44,63]

CONCLUSIONS

This study demonstrated high level of zinc deficiency. More so, the zinc status of the HIV-infected subjects on ART treatment was comparable with HIV-infected subjects with *Cryptococcus*. This showed that the ART treatment does not complement zinc status; rather it may improves $CD4^+$ T-cell count of PLHA on cART. Zinc has potential to boost immune system in more than one way. But ART treatment centers on boosting the immune system as shown by increased $CD4^+$ T-cell count, while the subjects may still suffer zinc deficiency amid improved $CD4^+$ T-cell count. Hence, zinc supplementation is still being used in the management of PLWHA along with cART. Selenium depletion is also known and important in HIV disease, with a high risk for OIs. As many more HIV infected persons are initiated on cART, the need to sustain long-term viral suppression is increasingly becoming very important. Since micronutrient status play important role in HIV/Cryptococcus disease, boosting the nutritional status of infected persons should be added to the management of PLWHA for sustained viral OIs suppression and boost cART.

ACKNOWLEDGEMENT

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