

# Association of Body Mass Index, Waist-Hip Ratio, Zinc, Copper, CD4<sup>+</sup> T Cells Count and Viral Load in Early HIV Infection in ART Naïve HIV Infected Adults in Taita Taveta County, Kenya

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## Abstract

During HIV/AIDS micronutrients play a key role in the host defense systems-Micronutrients such as zinc and copper have been implicated to play important roles in immuno-physiologic functions. The aim of the study was to assess the level of zinc and copper among ART naïve HIV positive adults and assess the association with their waist-hip ratio (WHR), body mass index (BMI), viral load and immunity. Descriptive cross-sectional study was conducted in the voluntary counselling and testing centre (VCT) at Comprehensive Care Clinics in Taita-Taveta County health care services, Coast, Kenya, blood samples obtained from 192 HIV sero-positive individuals, 18 years of age and gender matched healthy controls were analyzed for zinc and copper using atomic absorption spectrophotometer machine. Data were analysed by statistical (SPSS version 21) computer software. The (mean $\pm$  SD) of serum copper and zinc were (158.7 $\pm$ 51.0 $\mu$ /dl, 84.3 $\pm$ 51.1 $\mu$ /dl) in patients and (130.5 $\pm$ 17.9  $\mu$ /dl, 100.3 $\pm$ 5.5  $\mu$ /dl) in control group, respectively. Serum zinc level was significantly decreased in HIV patients (P value, 0.05) while serum copper level was significantly increased compared to control group. There was significant association between viral load and waist-hip ratio but not body mass index ( $P < 0.05$ ). Both body mass index and waist-hip ratio were not significantly associated with CD4<sup>+</sup>T cells in participants at early HIV infection.

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Serum zinc was significantly associated to waist-hip ratio in participants in early HIV infection. ( $P < 0.05$ ). This study indicates that zinc and copper levels are altered in patients in early HIV infection with more decreased Zinc level suspected with increased duration of the HIV infection. It also demonstrates that the associated characteristics in early HIV infection are different from studies in late stages. In conclusion, waist-hip ratio has been identified as a more sensitive predictor and nutrition status maker than body mass index in early HIV hence can be a decisive benchmark for monitoring HIV progression.

**Keywords:** Zinc; Copper; HIV; Immunity; CD4<sup>+</sup>, ART Naïve, Waist-hip ratio, Body mass index.

## 1. Introduction

HIV/AIDS is one of the major public health problems for the last decades. HIV continues to be a major global public health issue, having claimed more than 35 million lives since the start of the epidemic. Sub-Saharan African region is reported to be highest burden of HIV infection and HIV related deaths. <sup>[1]</sup> In 2015, 1.1 [0.94–1.3] million people died from HIV-related causes globally compared to 2M [1.3–1.7] in 2010. There were approximately 36.7 [34.0–39.8] million people living with HIV at the end of 2015 with 2.1[1.8–2.4] million people becoming newly infected with HIV in 2015 globally. Sub-Saharan Africa region is reported to have 25.5M (23.0-28.3) million people living with HIV with 12 M accessing antiretroviral therapy (ART) by end of 2015. <sup>[2]</sup> After South Africa Kenya has the largest treatment programme in Africa, with nearly 900,000 people on treatment at the end of 2015. The Fast-Track approach to HIV treatment is working but still some in remote location cannot access treatment. Global consensus and leadership have driven greater investment of financial and human capital, and mounting clinical experience and research, improved treatment regimens and diagnostics and reductions in the price of medicines have created gains in efficiency and effectiveness. <sup>[3]</sup> Patients with HIV are known to suffer from specific nutrient deficiencies, malnutrition and intestinal malabsorption <sup>[4, 5]</sup> but nutrition status earlier in the course of infection has not been widely studied. There are three key questions to answer. First, can any nutritional deficiencies be identified that might be amenable to interventions? Secondly, can specific nutritional markers be identified that predicts HIV progression? And third, are the associated characteristics in early HIV infection similar to late stages of HIV infection? Trace elements deficiencies have been observed with advanced HIV disease, and many studies reported its deficiency with higher risks of HIV disease progression and mortality, and many of these micronutrients are required for improving immune systems in HIV infected patients [6], and also it was observed that micro nutrient supplements are associated with a delay in HIV disease progression and reduce mortality in HIV positive persons not receiving highly active antiretroviral therapy. <sup>[7]</sup> Other features reported to be associated with HIV progression are body weight loss and wasting, <sup>[8]</sup> and all of these factors are considered independent predictors of HIV-related morbidity and mortality. <sup>[9, 10]</sup> Trace elements such as zinc and copper are essential for normal human development and functioning of the body. They have been implicated to play important roles in immuno-physiologic functions. Zinc is an integral part of more than 200 enzymes and has significant task in nucleic acid metabolism, cell replication, tissue repair, and growth. Its deprivation leads to profound alteration of thymic function with resultant loss of T-cell-mediated responses and increased susceptibility to infectious diseases. <sup>[11, 12]</sup> Zinc also inhibits the production of tumor necrosis factor, which is implicated in the pathophysiology of cachexia and wasting in acquired immune deficiency syndrome. Zinc deficiency is the most prevalent micronutrient

abnormality seen in HIV infection. Copper is present in cytosolic erythrocyte superoxide dismutases (Cu, Zn-SOD), which is an important scavenger of  $O_2^-$ , a free radical that causes damage to membranes and biological structures. <sup>[13, 14]</sup> Copper inhibits the protease from HIV by both cysteine-dependent and cysteine-independent mechanisms. Copper and zinc are involved in destruction of free radicals through cascading enzyme systems. In both, deficient and excessive intake of nutrients can have negative consequence on the immune status and susceptibility to a variety of pathogens. <sup>[15, 16]</sup> While research has been conducted in sub-Saharan Africa to the best of our knowledge, no documented evidence have been found in Taita-Taveta County, Kenya to understand the nutritional status of ART naïve HIV infected adults and give an insight of effect of zinc and copper supplementation to immunity in early HIV infection. The current study, thus aimed at further investigating the serum zinc and copper levels as well as assess the association between nutritional assessment parameters and immunity of ART Naïve HIV-seropositive patients and identifying nutritional variables that best predicts HIV Progression in early HIV infection.

## **2. Materials and Methods**

This study was a descriptive, cross-sectional hospital based study, done in Taita Taveta County Health Services comprehensive care clinics and hospitals in voluntary counselling testing (VCT) centres located in Taita Taveta County, Kenya. Study population included 192 patients with HIV seropositive and 10 HIV seronegative as control group. ARV naïve HIV infected adults in the cohort study aged between 18 and 55 years with CD4+T cell count of 400 cells/ $\mu$ L and above were recruited. They all had a BMI  $\geq 18.5$  with no evidence or history of TB, or other opportunistic infection that would necessitate treatment or initiation of ART. The adult willing to provide informed consent and residence within Taita-Taveta County, were included in the study.

### **2.1. Exclusion criteria of the study**

Patients with previous or current use of ART except for those taken at the time of delivery for women, without chronic gastrointestinal, renal or hepatic diseases and hemolyzed sample were excluded from the study.

### **2.2. Blood samples**

A 4.0ml venous blood sample was obtained from each patients using standard venipuncture technique. CD4+T cell count was determined by flow cytometer machine. Plasma and serum specimens were collected as heparinized container after centrifugation at 3000 rpm for 5 minutes. The specimen stored at freeze until analysis. Interview with the cohort group was done to obtain the clinical data; clinical data were assessed by medical doctor. Permission of this study was obtained from the local authorities in the area of the study. Ethical approval was obtained. An informed consent was obtained from each participant in the study after explaining objectives of the study. Plasma HIV RNA viral load was quantified using the Abbot M 2000rt. Zinc and copper levels were estimated by atomic absorption spectroscopy device.

### **2.3. Anthropometry evaluations**

Participants were measured for anthropometry evaluations at the Nutrition Unit of the Comprehensive Care

Clinic (CCC) next to the VCT. Weight, height, waist and hip circumferences were measured in duplicate following standard procedures <sup>[17]</sup>. Participants were weighed in light clothing using a digital scale (SECA Mod 843, Hamburg, Germany) with a precision of 0.1 g. Height was determined using a portable stadiometer (SECA Mod 206, Hamburg, Germany) with a 0.1 cm precision. For the present study, obesity was considered with a BMI  $\geq 30$  kg/m<sup>2</sup>, and overweight with a BMI 25 – 29.9 kg/m<sup>2</sup> <sup>[18]</sup>. Waist and hip circumferences were measured with a 0.1 cm precision using flexible fiber glass measuring bands (SECA Mod 200, Hamburg, Germany).

## 2.4. Statistical analysis

The mean $\pm$ SD was calculated for all quantitative variables. The data collected in this study were analyzed using SPSS vs21. Binary and categorical data were presented as frequencies (percentages). Means, medians and proportions of characteristics were calculated and compared using T-tests or analysis of variance for normally distributed continuous variables and chi-square tests for categorical variables. Non-normally distributed variables were analyzed using Kruskal Wallis for categorical variables and Wilcoxon's Signed Rank for binary variables. Multivariate regression was used to access the association of serum zinc, copper levels of HIV infected adults with BMI, viral load and CD4+T cell levels. Pearson correlation was used to study association between variables. *P* value less than 0.05 was considered significant. Statistical analyses were performed using statistical package for social sciences (SPSS) versions (21).

## 3. Results

**Table 1:** Characteristics of Study Population

Table 1: Cohort Demographics and Clinical Characteristics of Participants, Stratified by Viral Load(VL)						
Characteristics	VL<150 (n=69)	VL=151-5000 (n=30)	VL>5000 (n=71)	VL=Missing (n=22)	All Patients (N=192)	p-value
Age (years), mean, ( $\pm$ SD)	36 (9)	38.8 (8)	36.3 (9.1)	33.6 (10.9)	36.3 (9.2)	0.235
Height, m	157.1(7.1)	158.6(7.7)	159.6(7.4)	161.6(8)	158.8(7.5)	0.065
Weight, kg	61.6(12)	65.7(13.6)	62.6(11.5)	60.9(8.4)	62.5(11.7)	0.395
BMI, kg/m <sup>2</sup>	25(5)	26.1(5.2)	24.6(4.3)	23.4(3.4)	24.8(4.7)	0.195
CD4 <sup>+</sup> , Cells/ $\mu$ L	551(123.1)	588(155.2)	559.3(127)	582.1(172.8)	562.9(134.4)	0.592
Log <sub>10</sub> HIV RNA, Copies/mL		1.6(0.3)	2.9(0.4)	4.3(0.5)	2.8(1)	0.00

Table1 summarizes the demographic characteristics of the 192 cohort participants stratified by viral load categories. One hundred and ninety two HIV positive ART naïve adults met the inclusion criteria after screening. Appropriate informed consent was obtained and ethical approvals from Kenyatta National Hospital, Ethics Research Committee (ERC), and IRB Tufts School of Medicine. When variables were adjusted by viral load (VL) categories there was no significant association with any of the cohort characteristics. Overall, the mean age of the cohort was  $36.3 \pm 9.2$  years, mean height was  $158.8 \pm 7.5$  cm and mean weight was  $62.5 \pm 11.7$ . The mean CD4+ T cell count was  $562.9 \pm 134.4$  cells/ml and HIV RNA was  $2.84 \pm 0.99$  log<sub>10</sub> copies/ml. Majority, 116 (60.4%) had normal BMI, while 51 (26.6%) and 25 (13.0%) were classified overweight and obese. The highest BMI was 44.53 kg/m<sup>2</sup> and the lowest was 18.6 kg/m<sup>2</sup>. The lowest CD4+ count was 402 cells/mm<sup>3</sup> and the highest was 1063 cells/mm<sup>3</sup>.

**Table 2:** Comparison of copper and zinc levels between HIV patients with control groups (mean  $\pm$  S.D).

Parameter(mean $\pm$ S.D)	HIV patients (n=192)	Parameters Control (n=10)	(P. Value)
Serum Zinc $\mu$ g/dL	$84.3 \pm 51.1$	$100.3 \pm 5.5$	0.00
Serum Copper $\mu$ g/dL	$158.7 \pm 51.0$	$130.5 \pm 17.9$	0.00

\* Significant ( $P < 0.05$ )

The results of copper and zinc levels in serum of HIV patients and control groups are shown in Table.[2] The results indicate that, in HIV infected patients, copper is significantly reduced and zinc is significantly increased ( $P < 0.05$ )

**Table 3:** Association between Waist-Hip Ratio (WHR) and Age with Serum Zinc concentration

Parameter	B	Std error	P-Value
Age	1.183	0.605	0.053
Waist Hip Ratio (WHR)	-175.840	80.829	0.032

Table [3] shows that waist-hip ratio was significantly ( $P < 0.05$ ) associated with serum zinc. Additionally, there was no significant association between age of participants and serum zinc concentration.

**Table 4:** Association between Waist-hip Ratio (WHR) and Body Mass Index (BMI) with CD4+ T Cells

Parameter	B	Std error	P-Value
Waist Hip Ratio (WHR)	-311.750	166.254	0.062
Body Mass Index (BMI) Group	-17.052	20.007	0.395

Table [4] shows waist-hip ratio and body mass index were not significantly associated with CD4+T cell counts ( $P < 0.05$ ).

**Table 5:** Association between Waist-hip Ratio (WHR) and Body Mass Index (BMI) with viral load

Parameter	Odd Ratio	95% Confidence Interval	P-Value
Age, years	0.963	0.922,1.006	0.092
Waist Hip Ratio (WHR)	18.48	1.536,222.5	0.038
Body Mass Index (BMI) Group	1.914	0.869,4.219	0.107

Table [5] shows that waist-hip ratio is a significant predictor of viral load, (OR 18.48; 95% CI: 1.536, 222.5,  $P < 0.05$ ) while body mass index was not significantly associated. Waist-hip ratio makes the strongest unique contribution to explaining the viral load, when the variance explained by other variables in the model is considered.

#### 4. Discussions

HIV infection is a condition caused by the human immunodeficiency virus (HIV). HIV/AIDS remains one of the clinical conditions of public health important with high morbidity and mortality worldwide, especially in developing countries. Reference [1] The condition gradually destroys the immune system, which makes it harder for the body to fight infections. During HIV/AIDS micronutrients play a key role in the host defense systems-Micronutrients such as zinc and copper have been implicated to play important roles in immunologic functions. They are necessary component of many enzymes. References [6, 19] Zinc deficiency is the most prevalent micronutrient abnormality seen in HIV infection. In both, deficient and excessive intake of nutrients can have negative consequence on the immune status and susceptibility to a variety of pathogens. The results of the present study showed that, there is significant decrease in the mean serum zinc levels in patients

( $84.3 \pm 51.1$   $\mu\text{g/dl.}$ ) when compared with mean serum zinc level of control group ( $100.3 \pm 5.5$   $\mu\text{g/dl.}$ ) with ( $P$ -value  $< 0.05$ ). This finding agrees with a study done by (Khalili and his colleagues 2008) who reported that there was a significant decrease between zinc levels in patients and zinc level of control group. Reference [20, 21] Micronutrients play a critical role in the proper functioning of the immune system. Thus in HIV where there is profound immune-suppression, there occur deficiency of many micronutrients. Micronutrient deficiencies, which are commonly observed with advanced HIV infection, have been associated with higher risks of HIV progression and mortality. Our results also indicate towards this truth. In HIV cases there is zinc deficiency, an essential element for the functioning of CD4+ cell counts compared to the control group. Similarly marked zinc deficiency has been observed in HIV infection at various stages of illness. Reference [22] Therefore Zn deficiency may be a cofactor for progression of disease. In this study patients with HIV revealed significant increase in the mean serum copper level ( $158.7 \pm 51.0$   $\mu\text{g/dl.}$ ) when compared with the control subjects ( $130.5 \pm 17.9$   $\mu\text{g/dl.}$ ). These findings are consistent with previous study done by Malviya and his colleagues 2008, who reported that there was a significant increase in the mean of serum copper concentration when compared with control group. References [20, 22] Serum Copper (Cu) levels have been reported to be significantly higher in infection and inflammatory states. The rise in serum copper level was inversely related to severity of stage of disease i.e. higher serum level in early stage of the disease. This rise has been attributed to an increase hepatic synthesis and release of ceruloplasmin. Serum copper is high in both symptomatic as well as in asymptomatic cases but prevalence is more in early stage of disease though many studies have corroborated to the finding obtained in this study, only few correlated it to the stage of the disease and or CD4+ T cell counts. To some extent our study has shown that the rise of serum copper correlated inversely with diminution in serum zinc level which is consistent with study by Malviya and his colleagues 2008. This serum Cu: Zn ratio may be a useful predictor of survival and disease progression in HIV patients. Moreover, there were no significant differences in serum Cu levels among the three groups following 1993 criteria of the Center for Disease Control and Prevention (Atlanta, USA) although serum copper enhancement occurred at the beginning of the infection process (group A). This increase then remains constant in advanced stages of HIV infection (groups B and C).[22, 23] It was noted that all patients in this study were in asymptomatic category as per the eligibility criteria were most variables have not changed compared to the later stages of HIV infection. In this study there was no significant relationship between CD4+ T Cell with body mass index and waist-hip ratio. Despite there being no significant association from the results between the BMI (general adiposity) and WHR (central adiposity) the trend is developing between the WHR and CD4+ T cell count which may be more likely indication of metabolic state or metabolic syndrome in early HIV infection. This may be a likely indication that in early stages of HIV infection most of the parameter have not changed and most of the population under study contains characters of a healthy population. All patients had a BMI  $\geq 18.5$  which is considered as a marker of normal category with CD4 count 400 cells/ $\mu\text{L}$  and above. This is in contrast to most studies which have focused on the late stages of HIV infection. The association between micronutrient deficiencies and obesity is particularly important in populations where micronutrient deficiencies are widely spread. Micronutrient deficiencies may increase the risk of fat deposition and thus, of obesity and related diseases. Zinc is an integral part of many enzymes and has significant task in nucleic acid metabolism, cell replication, tissue repair, and growth. Its deprivation leads to profound alteration of thymic function with resultant loss of T-cell-mediated responses and increased susceptibility to infectious diseases. Copper is present in cytosolic erythrocyte superoxide dismutase (Cu, Zn-

SOD), which is an important scavenger of  $O_2^-$ , a free radical that causes damage to membranes and biological structures as reported by Baum and his colleagues 2000. Reference [24] Zinc also inhibits the production of tumor necrosis factor, which is implicated in the pathophysiology of cachexia and wasting in acquired immune deficiency syndrome. The findings from this study indicated that waist-hip ratio can be an independent predictor of serum zinc concentration. These findings are consistent with previous study done by Garcia and his colleagues 2012, who reported that there was a significant association between serum zinc concentration, vitamins A and C with obesity, adiposity and leptin concentration. The relationship between micronutrients especially zinc, and obesity might be affected by leptin, an adipokine associated with satiety. Reference [25] This association could be explained by the effect of zinc-alpha2- glycoprotein (ZAG) on leptin concentrations. ZAG is an adipokine involved in the metabolism of lipids in the adipocyte that is down-regulated in obesity, probably due to the inflammation process associated with obesity or HIV infection. In obese individuals, low ZAG gene expression is associated with low serum adiponectin and high plasma leptin levels, and may play an important role in the development of obesity [26, 27]. The influence zinc has on the adipocyte through the expression of leptin, by promoting free fatty acid release and glucose uptake, may also be controlled through the expression of a number of zinc-transporters in the adipocyte that may be altered in obesity [28, 29]. The findings have also indicated that WHR and BMI are not independent predictors of CD4<sup>+</sup> T cell count in early HIV infection which is different from the late stages of infection towards AIDS. In our study we have noticed that the more severe the infection as indicated by viral load the less will be the CD4<sup>+</sup> T cells count. The obvious implication of these observation is, that clinical diagnosis is usually made late in our setup. It is neither mandatory nor economically viable to propose the use of CD4<sup>+</sup> T cell count for these purposes of diagnosing stage of HIV infection/AIDS in our limited resource set up in sub-Saharan Africa. CD4<sup>+</sup> lymphocyte counts are a standard laboratory marker of disease progression in HIV infection, but expense precludes their use in many resource-poor settings in Africa. We demonstrated that WHR for HIV patients was a stronger predictor of viral load and serum zinc in this study population hence can be used together with CD4<sup>+</sup> count for better care and management of HIV patients.

The finding from this study has identified waist-hip ratio as an independent predictor of viral load in early HIV infection. These findings are consistent with previous study done by *Kwiatkowska* and his colleagues 2013, who reported that HIV infected patients have significantly lower BMI and higher WHR values. At the same time the HIV infection is significantly more often accompanied by features of central adiposity, expressed as abnormal waist circumference value. Higher WHR in the HIV patients is due to low hip circumference. HIV infected women usually have normal body weight and are significantly more likely than not infected women to show the features of central obesity as a result of increased waist circumference and low hip circumference. Men infected with HIV, compared with not infected ones, are characterized by lower, normal body weight, and their significantly higher WHR is determined by low value of hip circumference. Reference [30] This study results have also shown that body mass index is not an independent predictor of viral progression in early stage of HIV infection where most of the variables have not changed and the patients are generally clinically asymptomatic. These findings are in contrast to many studies of late stages of HIV infection where BMI is used to assess HIV progression. The CD4<sup>+</sup> cell count is less sensitive in early HIV infection where most parameters in the body are within normal range but a lot of viral replication and metabolic reactions are happening hence the association



between WHR (central adiposity) and Viral load. As the viral load increases more damages of the immune system function is done leading to its decline as T cells function is altered. This increase in viral load suggest further pronounced decrease of serum zinc due to disturbed protein synthesis, decreased internal absorption and massive destructions associated with this HIV infection as indicated in many studies of late stages of HIV infection. Viral infection is characterized by increased oxidation stress and other metabolic activities hence affecting the central adiposity. The high viral load diminishes the capacity of the antioxidant system to control oxidative stress. Oxidative stress refers to a state in which there is an overabundance of molecules called free radicals. Free radicals can damage cells and are involved in the processes of inflammation and scarring leading to further damage of immune system. Glutathione and other antioxidants such as zinc are involved in controlling oxidation stress. Glutathione (GSH) is a small, ubiquitous antioxidant that plays key regulatory roles in metabolic and cell-cycle-related functions (Dong-Ho and his colleagues 2012). This cysteine-containing tripeptide (gamma glutamylcysteinylglycine) provides the principal intracellular defense against oxidative stress caused by reactive oxygen species such as free radicals and peroxides and participates in detoxification of many physiological molecules. Deficiency of glutathione can lead to immune suppression, decline of immune system function, and an increase in HIV replication. HIV-infected people tend to have subnormal GSH levels in plasma. In vitro studies show that lowering intracellular GSH levels decreases cell survival, alters T cell functions and increases HIV replication and sensitivity to tumor necrosis-inducing cell death [31].

## **5. Conclusion**

In conclusion, this study indicates that zinc and copper levels are altered in HIV infected patients with more decreased zinc level suspected with increased duration of HIV infection and damage to the immune system. This observation can help to guide the supplementation of zinc in seropositive patients. This study demonstrated significant decreased serum zinc in HIV seropositive cases when compared with the controls, conversely copper were significant increased. It may be concluded that the assessment of the above parameters can be of great help to know the prognosis of the disease which may be supportive in trimming the morbidity as well as delaying the mortality of HIV seropositive patients. These data suggest that copper which is an acute phase reactant, may be a useful marker of HIV activity and progression to AIDS as in other chronic infective diseases.

In this study, zinc has been associated with obesity, central adiposity and leptin concentration in HIV infected patients hence may play an important role in fat deposition. In conclusion, zinc may be playing an important role in fat deposition and the pathogenesis of obesity. The findings have also indicated that waist-hip ratio and body mass index are not independent predictors of CD4+ T cell count in early HIV infection which is different from most studies of the late stages of HIV infection conducted in sub-Saharan Africa. In early HIV infection viral load measurement association to waist-hip ratio may suggest it is the actual course of infection while CD4 T cell count is a response to the damage hence seen more pronounced in later stages of infections. This may also be a likely indication that in early HIV infection there is a high intensity of metabolic activity or metabolic syndrome that cause central adiposity compared to late stages of infection that causes general adiposity affecting the body mass index. In conclusion, associated factors in early HIV infection are different from studies in late HIV infection. Finally, the findings have demonstrated waist-hip ratio as an independent predictor of both serum zinc level and viral load.

## **6. Constraints/Limitations**

A major limitation of this study is that cross-sectional studies cannot establish causality. More studies are needed to understand the causes and consequences of micronutrient status to patients in early HIV infection. Longitudinal studies are needed to evaluate cause and effect, and to design interventions to improve the antioxidant capacity by stimulating the enzymatic antioxidant system or supplementing effective antioxidants in HIV infected patients. The relationship of copper and zinc with waist-hip ratio and body mass index could be different in populations with a high prevalence of these micronutrients deficiencies. Future research should focus on studying causality and the effect of supplementation with multiple micronutrients on central and general adiposity in randomized clinical trials in populations with a high prevalence of micronutrient deficiencies since this study was limited to two micronutrients. Although the research has reached its aims, there were some unavoidable limitations. There were challenges especially where sample collection was not properly done or had few cells, this led to inconclusive results due to various reasons such as insufficient blood specimens, compromised sample integrity, internal control failed and clot error thereby repeat sample collection had to be done to some participants. Another challenge faced was the level of education each patient had in terms of understanding, some of our patients were below average. Therefore, their misguided concern about patient's safety discouraged our patients and their relatives to be enlisted in the study.

## **7. Recommendations**

Waist-hip ratio measurement is economically viable and fast to take since measuring the viral load for most patient is expensive though for better monitoring and management viral load test should be done during enrollment in the comprehensive care clinic or any HIV patient clinic. It can be concluded that, waist-hip ratio measurement is more sensitive than body mass index as a nutrition status marker hence decisive bench mark. Waist-hip ratio seems to be an appropriate diagnostic criteria for monitoring HIV progression in early stages of HIV infection. Waist-hip ratio can be used for clinical assessment with CD4+ T cell count since viral load test are expensive. It is recommended to clinicians in comprehensive care clinic (CCC) such as it this study site for improved monitoring of patients in early HIV infection or newly recruited in limited resource setting in Kenya. In deed for countries like Kenya and others in sub-Saharan Africa there is a need to revise these definition criteria in clinics immediately to add sensitivity and specificity reliance on CD4+T cell count be promoted by including anthropometric such as waist hip ratio. Inclusion of at least baseline viral load or waist-hip ratio measurements should be considered in assessment of patients in early HIV infection in limited resource setting such as in sub-Saharan Africa.

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