

Research Article

Assessment of Some Micronutrients and Vitamins of HIV Positive Patients in South-South Nigeria

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Abstract

Background: Human Immunodeficiency Virus (HIV) infection continues to be a serious health issue in most parts of the world, with a global prevalence of 0.7% among adults, the majority being in developing countries with limited healthcare resources.

Objective: The objectives of this study was to determine the Iron, calcium, zinc, copper, Vitamins D and B12 of HIV positive subjects compared with the control, with respect to gender, age and with respect to duration of treatment.

Materials: A total of fifty (50) HIV-positive subjects and fifty (50) HIV-negative subjects (control) were used in this study. Zinc, iron and calcium were evaluated using atomic absorption spectrophotometer, Vitamins D and B12 was done using ELISA, while calcium was estimated using O-cresolphthalein complexone method. The results were presented in tables and chart as mean $\pm$ standard deviation. Statistical analysis was done using one way Analysis of Variance (ANOVA) and Student's t-test using Statistical Package for Social Sciences (SPSS) version 21.0. A p-value of <0.05 was considered significant.

Results: Among the study population, 23(46.0%) were males and 27(54.0%) were females. With respect to age, 29(58.0%) were aged 20 -40 years, while 21(42.0%) were aged 41 years and above. Based on treatment period, 28(56.0%) were less than one year, while 22(44.0%) were more than one year. Among the control subjects, 32(64.0%) were males and 18(36.0%) were females. With respect to age, 24(48.0%) belonged to age group 20 -40 years, while 26(52.0%) belonged to age group 41 years and above. The results obtained showed that Calcium, zinc, copper, Iron, Vitamins B12 and D were significantly lower in HIV positive subjects compared with control ($p<0.05$). Calcium, Iron, Vitamins B12 and D were significantly lower ($p<0.05$) in age group 41 years and above in comparison with age group 20 -40 years. Calcium, Iron, Vitamins B12 and D

were significantly ($p<0.05$) higher in HIV positive subjects undergoing treatment for over a year compared with those undergoing treatment for less than one year. There was a significant positive correlation between Calcium and Iron ($r=0.840$, $p=0.000$), Calcium

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and Vitamin B12 ($r=0.744$, $p=0.000$), Calcium and Vitamin D ($r=0.825$, $p=0.000$), Iron and Vitamin B12 ($r=0.743$, $p=0.000$), Iron and Vitamin D ($r=0.765$, $p=0.000$) and Vitamin B12 and Vitamin D ($r=0.562$, $p=0.000$).

Conclusion: The study concludes that the decrease in vitamins and micronutrients that accompanies HIV infection suggests a potentially important role of nutritional supplementation and good nutrition in the proper management of HIV/AIDS.

Keywords: HIV; Iron; Calcium; Zinc; Copper; Vitamin D; Vitamin B₁₂

Introduction

Human Immunodeficiency Virus (HIV) is a lenti virus of the retrovirus family which is the aetiologic agent of Acquired Immunodeficiency Syndrome (AIDS). In 1981, the first case of AIDS was discovered among homosexual men in the United States [1]. The men had an unusual type of pneumonia called pneumocystic carinii pneumonia and rare skin tumours called Kaposi's sarcoma. Shortly thereafter, the disease was recognized in Western Europe and Africa [1]. Today, it has become a global pandemic. HIV infects about 0.6% of the world's population. UNAIDS and the WHO has estimated that AIDS has killed more than 25million people since it was first recognized in 1981, making it one of the most destructive pandemics in recorded history(UNIADS/WHO Estimates, 2024). Since the beginning of the epidemic, 88.4 million [71.3-112.8 million] people have been infected with the HIV virus and about 42.3 million [35.7-51.1 million] people have died of HIV. Globally, 39.9 million [36.1-44.6 million] people were living with HIV at the end of 2023. An estimated 0.6% [0.6-0.7%] of adults aged 15-49 years worldwide are living with HIV, although the burden of the epidemic continues to vary considerably between countries and regions. The WHO African Region remains most severely affected, with one in every 30 adults (3.4%) living with HIV and accounting for more than two-thirds of the people living with HIV worldwide (UNIADS/WHO Estimates, 2024). HIV infects primarily vital cells in human immune system such as helper T cell (CD4+ T cells), macrophages and dendritic cells. HIV infection leads to low level of CD4+ T-cells. When CD4+ T-cells numbers decline below a critical level, cell-mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections. HIV is transmitted by three main routes which are:sexual, blood product and vertical(mother-to-child) transmission [2,3].

Clinical consequences in HIV infected patients encompass all organs and systems and include a host of infections and malignancies that rarely cause illnesses inimmunocompetent individuals. Clinical features vary according to age, sex, race, geographic location, treatment status and life style [4]. In these patients, virus replication and worsening of immunological status continues throughout the course of the disease. Reduced cobalamin level have been reported in 10 -35% of patients infected with HIV and, although many of these patients have gastrointestinal symptoms or late stage HIV disease, reduced levels are also described in early asymptomatic disease [5]. The serum transcobalamin II cobalamin-binding capacity is significantly raised in asymptomatic patients with HIV infection regardless of vitamin B12 level;this suggests a stage of negative cobalamin balance. Cobalamin levels tend to fall along with CD4 cell counts in those patients progressing to AIDS and thus falling cobalamin levels are predictors of disease progression [6,7]. Vitamin A plays an important role in maintaining the structural and functional integrity of mucosal epithelial cells and this is essential in preventing microbial invasion [8]. The functions of vitamin A include increasing the levels of acute phase reactants in response to infection, regulating monocyte function, improving the cytotoxicity of natural killer cells and increasing total lymphocyte, especially the CD4 counts [9,10].

Zinc deficiency affects both non-specific and specific immunity [11]. Deficiency of zinc also affects other mediators of non-specific immunity, such as polymorphonuclear leucocyte and natural killer cell function [12,13]. The cytokines interleukin-1, interleukin-2(IL-1, IL-2) and interferon- γ have been reported to be suppressed during zinc deficiency. Zinc deficiency also results in decreased T and B lymphocyte concentrations and depressed T and B-lymphocyte functions and decreased CD4+:CD8+ ratios are also seen [13-15].

Impairment of cell mediated immunity has been described in iron-deficient humans [16]. Iron deficiency has been demonstrated to reduce the bactericidal effects of neutrophils [17]. Other immunological abnormalities that have been observed with iron deficiency include impaired natural killer cell activity, reduced production of macrophage inhibitor activity, depression of T-lymphocyte numbers, defective T-lymphocyte induced proliferative response, impaired interleukin-2 production by lymphocytes and impairment of delayed cutaneous hypersensitivity [16,18]. There are many potential causes of poor nutritional status (growth performance and micronutrient status) in HIV-infected patients and these include reduced dietary intake, increased nutrient losses and increased nutrient requirements [19]. Micronutrient deficiencies have been shown to be more severe

in HIV-infected patients, compared to uninfected patients and several micronutrient deficiencies often coexist [20]. Due to the importance of these vitamins and trace elements it is therefore necessary to assess their levels in HIV patients in Benin City. This study is motivated by the need to ascertain the prevalence of calcium, zinc, copper, cobalamin, vitamin D and iron deficiency among HIV-infected patients. This will lead to a better management of patients infected with HIV, without proper research, the prevalence of calcium, zinc, copper, cobalamin, vitamin D and iron deficiency may be underestimated, thereby giving little attention to the affected people.

Material and Methods

Study Design

This study was carried out in Benin City, Edo State. Benin City is the capital of Edo state in southern Nigeria. The study design was a prospective cross-sectional study. Specimens(blood) were collected from HIV-positive patients attending HIV clinic of Central Hospital Benin city and also from HIV-negative individuals who were recruited from Benin City. This study was carried out within six months, September, 2023 to February 2024. A complete record of medical history was obtained for each subject, including name, age, gender and period of HIV infection with the use of the questionnaire. The samples obtained were taken to the laboratory for analysis. The results generated were further used to make comparisons with their control counterparts. The serum was used to assay serum cobalamin(vitamin B12), vitamin D, calcium, Iron, zinc and copper.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Study Population

The study population was made up of HIV-positive adult population who were on anti-retroviral drugs and HIV-negative persons aged between 18 and 60 years and of both sexes. They were divided into two groups: Test group -subjects for this study were recruited consecutively from HIV clinic of the Central hospital Benin. This group was made up of confirmed HIV-positive patients. A total of 50 HIV patients were recruited for the study. The second group the control subjects were selected consecutively from general out-patient department and general public. This group was made up of HIV-negative individuals. A total of 50 control subjects were used for this study.

Sample Size

The sample size for this study was determined using the Fisher's formula equation(Araoye, 2004) for cross sectional study:

$$n = Z^2 pq / d^2$$

Where n is the sample size,

Z is the standard normal deviate, set at 1.96 which corresponds to the 95% confidence level.

P is the prevalence, deduced as 3.4%(0.034), being prevalence of HIV in Edo State(Federal Ministry of Health, 2019)

q is given as 1 -p

d is the degree of accuracy desired, set at 0.05 level. Mathematically, sample size

$$n = (1.96)^2 \times 0.034 \times (1-0.034) / (0.05)^2 = 3.8416 \times 0.034 \times 0.966 / 0.0025$$

$$n = 50.41 \approx 50$$

A total of one hundred samples comprising of 50 HIV-positive subjects(test subjects) and 50 HIV-negative subjects(control) was recruited for this study.

Study Criteria

Inclusion Criteria: HIV-positive subjects confirmed by repeated positive ELISA test, HIV confirmed positive subjects aged between 18 and 60 years with confirmed HIV infection as test subjects and HIV-negative subjects aged between 18 and 60 years were recruited as control subjects for the study.

Exclusion Criteria: Individuals who smoke, drink alcohol, hypertensive, had earlier been diagnosed of any acute or chronic inflammatory diseases, cardiovascular, diabetes, sickle cell or pulmonary diseases and those who did not give consent were excluded from the study.

Sample Collection

After proper cleaning of the venipuncture site with cotton wool soaked with methylated spirit, 5 millilitres(mls) of blood was collected from both the test and control groups. The blood was dispensed into plain bottles for serum biochemical assay. The collected samples were transported to the laboratory within 30 minutes after collection. The blood sample in the plain bottles was allowed to clot and the sera were separated from the clot after spinning with the speed of 2500 to 3000 rpm for 15 minutes at room temperature. The sera were stored in aliquots at -4°C till analyzed.

Sample Analysis

The samples and controls were analyzed with atomic absorption spectrophotometer after digestion.

Determination of Copper, Zinc and Iron

Principle:The sample digest is first aspirated in to the flame whose high temperature converts the analyte ions into atoms in vapours state. Absorption occurs when a ground state atom absorbs energy in form of a light at a specific wavelength and is elevated to an excited state. The relationship between the amount of light absorb and the concentration of the analyte present in known standard can be used to determine unknown concentration by measuring the amount of light absorbed

Method:Atomic Absorption Spectrophotometer(Alan Walsh, 1955). The analysis of the serum copper, zinc and iron was carried out using the following method of Nitricperchloric Acid Digestion (Qal/Am/23). This is a method for rapid determination of analytes such as Cu, Zn, Fe, Hg, Pb, Ca, Mg as well as SO₄ in samples using Atomic Absorption Spectrophotometer after digestion. In this technique, the Animal or plant samples are completely digested.

Determination of Vitamins D and B₁₂

Method:Enzyme Linked Immunosorbent Assay (ELISA) [20].

Principle:The ELISA is based on the competitive binding enzyme immunoassay technique. Themicrotiter plate provided in the kit has been pre-coated with an antibody specific to Methylmalonic Acid(MMA). During the reaction, MMA in the sample or standard competes with a fixed amount of biotin-labelled MMA for sites on a pre-coated monoclonal antibody specific to MMA. Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a 3,3',5',5'-Tetramethylbenzidine (TMB) substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of MMA in the samples is then determined using the formula:OD of sample/OD of standard X concentration of standard.

Calcium Estimation

Method:Colourimetric method using O-cresolphthalein complexone

Principle:Calcium forms a violet complex with O-cresolphthalein complexone in an alkaline medium.

Statistical Analysis

Data collected from this study were subjected to statistical computations by a computerizing the Statistical Package for the Social Science(SPSS) software, version 21. The Mean and standard deviation of the results obtained were calculated. Student's t-test and ANOVA(LSD) was used for the analysis using SPSS package version 21. Values with p<0.05 will be considered statistically significant in this study. Pearson correlation was used. Pearson correlation was used for correlation.

Results

Demographic Characteristics of the Subjects

In this study, 50 HIV positive subjects and 50 healthy individuals as control subjects were recruited for this study. Among the study population, 23(46.0%) were males and 27(54.0%) were females. With respect to age, 29(58.0%) were aged 20 -40 years, while 21(42.0%) were aged 41 years and above. Based on treatment period, 28(56.0%) were less than one year, while 22(44.0%) were more than one year. Among the control subjects, 32(64.0%) were males and 18(36.0%) were females. With respect to age, 24(48.0%) belonged to age group 20 -40 years, while 26(52.0%) belonged to age group 41 years and above(Table 1).

The results showed that the Calcium(mg/dL) level in HIV positive subjects and healthy control was 5.64±1.31 and 8.83±0.45,

Zinc(mcg/ml) was 0.60 ± 0.21 and 0.85 ± 0.11 , Copper(mg/L) was 0.83 ± 0.18 and 1.23 ± 0.14 , Iron(mcg/dl) was 60.50 ± 10.91 and 92.65 ± 18.91 , Vitamin B12(pg/ml) was 204.30 ± 69.28 and 228.10 ± 63.08 , while Vitamin D(ng/ml) was 13.70 ± 3.94 and 25.95 ± 4.63 respectively. Calcium, zinc, copper, Iron, Vitamin B12 and Vitamin D were significantly lower in HIV positive subjects compared with healthy control($p < 0.05$)(Table 2). The results also showed that the Calcium(mg/dL) in male and female HIV positive subjects was 5.63 ± 1.49 and 5.60 ± 1.07 , Zinc(mcg/ml) was 0.65 ± 0.22 and 0.49 ± 0.14 , Copper(mg/L) was 0.88 ± 0.21 and 0.80 ± 0.19 , Iron(mcg/dl) was 63.00 ± 10.02 and 60.25 ± 7.38 , Vitamin B12(pg/ml) was 219.25 ± 83.63 and 206.00 ± 66.89 , while Vitamin D(ng/ml) was 14.62 ± 4.65 and 12.63 ± 2.19 respectively. Calcium, zinc, copper, Iron, Vitamins B12 and D were non-significantly higher in male HIV positive subjects in comparison with female subjects($p > 0.05$)(Table 3).

The results obtained showed that the Calcium(mg/dL) level in HIV positive subjects aged 20 -40 years and age 41 years and above was 6.79 ± 0.57 and 4.41 ± 0.69 , Zinc(mcg/ml) was 0.62 ± 0.16 and 0.55 ± 0.23 , Copper(mg/L) was 0.86 ± 0.16 and 0.74 ± 0.13 , Iron(mcg/dl) was 68.22 ± 7.17 and 52.67 ± 9.42 , Vitamin B12(pg/ml) was 258.11 ± 62.71 and 155.67 ± 31.25 , while Vitamin D(ng/ml) was 16.22 ± 2.38 and 10.89 ± 2.93 respectively. Calcium, Iron, Vitamins B12 and D were significantly lower($p < 0.05$) in age group 41 years and above in comparison with age group 20 -40 years, while Zinc and Copper were non-significant($p > 0.05$)(Table 4). The results obtained showed that the Calcium(mg/dl) level in HIV positive subjects undergoing treatment for less than one year and more than one year was 4.93 ± 0.50 and 6.87 ± 0.48 , Zinc(mcg/ml) was 0.63 ± 0.26 and 0.56 ± 0.16 , Copper(mg/L) was 0.89 ± 0.23 and 0.82 ± 0.11 , Iron(mcg/dl) was 57.78 ± 7.20 and 67.78 ± 7.17 , Vitamin B12(pg/ml) was 168.33 ± 44.56 and 254.56 ± 63.87 , while Vitamin D(ng/ml) was 11.67 ± 2.29 and 17.11 ± 2.09 respectively. Calcium, Iron, Vitamin B12 and Vitamin D were significantly higher in HIV positive subjects undergoing treatment for over a year compared with those undergoing treatment for less than one year(Table 5).

The results obtained also showed that there was a significant positive correlation between Calcium and Iron($r = 0.840$, $p = 0.000$), Calcium and Vitamin B12($r = 0.744$, $p = 0.000$) and Calcium and Vitamin D($r = 0.825$, $p = 0.000$). There was significant positive correlation between Iron and Vitamin B12($r = 0.743$, $p = 0.000$), Iron and Vitamin D($r = 0.765$, $p = 0.000$) and Vitamin B12 and Vitamin D($r = 0.562$, $p = 0.000$) respectively(Table 6).

Variables	Subjects(%) (n = 50)	Control(%) (n = 50)
Gender		
Male	23(46.0%)	32(64.0%)
Female	27(54.0%)	18(36.0)
Age(years)		
20 -40	29(58.0%)	24(48.0%)
41 and above	21(42.0%)	26(52.0%)
Treatment Period		
< 1 year	28(56.0%)	-
> 1 year	22(44.0%)	-

Table 1: Demographic characteristics of the study subjects.

Parameters	Subjects(n = 50)	Control(n = 50)	t-value	p-value
Calcium(mg/dl)	5.64 ± 1.31	8.83 ± 0.45	9.600	0.000
Zinc(mcg/ml)	0.60 ± 0.21	0.85 ± 0.11	5.712	0.000
Copper(mg/L)	0.83 ± 0.18	1.23 ± 0.14	8.516	0.000
Iron(mcg/dl)	60.50 ± 10.91	92.65 ± 18.91	6.632	0.000
Vitamin B12(pg/ml)	204.30 ± 69.28	228.10 ± 63.08	1.116	0.278
Vitamin D(ng/ml)	13.70 ± 3.94	25.95 ± 4.63	7.875	0.000
KEY: N=Sample size; $p > 0.05$ = Not significant; $p < 0.05$ = Significant				

Table 2: Calcium, zinc, copper, iron, vitamin D and B12 levels of HIV positive subjects and control.

Parameters	Male Subjects (n = 23)	Female Subjects (n = 27)	t-value	p-value
Calcium(mg/dl)	5.63 ± 1.49	5.60 ± 1.07	0.031	0.976

Zinc(mcg/ml)	0.65±0.22	0.49±0.14	2.234	0.061
Copper(mg/L)	0.88±0.21	0.80±0.19	0.753	0.476
Iron(mcg/dl)	63.00±10.02	60.25±7.38	0.516	0.621
Vitamin B12(pg/ml)	219.25±83.63	206.00±66.89	0.311	0.765
Vitamin D(ng/ml)	14.62±4.65	12.63±2.19	0.947	0.375
KEY: N=Sample size; p>0.05= Not significant; p<0.05= Significant				

Table 3: Calcium, Zinc, Copper, Iron, Vitamins D and B12 levels of HIV positive Subjects with respect to gender.

Parameters	20 -40 years (n = 29)	41 years and above (n = 21)	t-value	p-value
Calcium(mg/dl)	6.79±0.57	4.41±0.69	9.233	0.000
Zinc(mcg/ml)	0.62±0.16	0.55±0.23	0.788	0.454
Copper(mg/L)	0.86±0.16	0.74±0.13	2.120	0.067
Iron(mcg/dl)	68.22±7.17	52.67±9.42	3.406	0.009
Vitamin B12(pg/ml)	258.11±62.71	155.67±31.25	4.209	0.003
Vitamin D(ng/ml)	16.22±2.38	10.89±2.93	4.619	0.002
KEY: N=Sample size; p>0.05= Not significant; p<0.05= Significant				

Table 4: Calcium, Zinc, Copper, Iron, Vitamins D and B12 levels of HIV positive Subjects with respect to age.

Parameters	Control (n = 50)	Treatment Duration		t-value	p-value
		< 1 year (n = 28)	> 1 year (n = 22)		
Calcium(mg/dl)	8.83±0.45	4.93±0.50	6.87±0.48	13.865	0.000
Zinc(mcg/ml)	0.85±0.11	0.63±0.26	0.56±0.16	0.981	0.355
Copper(mg/L)	1.23±0.14	0.89±0.23	0.82±0.11	0.747	0.477
Iron(mcg/dl)	92.65±18.91	57.78±7.20	67.78±7.17	3.818	0.005
Vitamin B12(pg/ml)	228.10±63.08	168.33±44.56	254.56±63.87	4.702	0.002
Vitamin D(ng/ml)	25.95±4.63	11.67±2.29	17.11±2.09	6.519	0.000
KEY: N=Sample size; p>0.05= Not significant; p<0.05= Significant					

Table 5: Calcium, Zinc, Copper, Iron, Vitamins D and B12 levels of HIV positive Subjects with respect to treatment duration.

		Calcium	Zinc	Copper	Iron	Vit. B12	Vit. D
Calcium	Pearson Correlation		-0.219	0.246	0.840**	0.744**	0.825**
	Sig.(2-tailed)		0.353	0.296	0.000	0.000	0.000
	N		50	50	50	50	50
Zinc	Pearson Correlation	-0.219		0.241	-0.302	-0.373	-0.266
	Sig.(2-tailed)	0.353		0.306	0.195	0.106	0.256
	N	50		50	50	50	50
Copper	Pearson Correlation	0.246	0.241		0.339	0.062	0.143
	Sig.(2-tailed)	0.296	0.306		0.144	0.796	0.547
	N	50	50		50	50	50
Iron	Pearson Correlation	0.840**	-0.302	0.339		0.743**	0.765**
	Sig.(2-tailed)	0.000	0.195	0.144		0.000	0.000
	N	50	50	50		50	50
Vit B12	Pearson Correlation	0.744**	-0.373	0.062	0.743**		0.562**
	Sig.(2-tailed)	0.000	0.106	0.796	0.000		0.010
	N	50	50	50	50		50
Vit D	Pearson Correlation	0.825**	-0.266	0.143	0.765**	0.562**	

	Sig.(2-tailed)	0.000	0.256	0.547	0.000	0.010	
	N	50	50	50	50	50	
**. Correlation is significant at the 0.01 level(2-tailed).							

Table 6: Pearson Correlation of Calcium, Zinc, Copper, Iron, Vitamin D and B12 levels of HIV positive subjects.

Discussion

Human Immunodeficiency Virus(HIV) infection continues to be a serious health issue in most parts of the world, with a global prevalence of 0.7% among adults, the majority being in developing countries with limited healthcare resources. Nowadays, micronutrients have become a part of the immunotherapeutic approach against cancer, inflammatory diseases, autoimmune diseases, etc [19,20]. In recent years, the importance of nutrition in human health has received growing attention. Therapeutic and preventive supplementation with vitamins and micronutrients has been used successfully for a long time for many clinical conditions [21,22]. The aim of this study is to assess the total Iron, Vitamin D, Vitamin B12 and some micro nutrients(copper, zinc and calcium) levels in HIV positive subjects in Central Hospital, Benin City, Edo State.

In this study vitamin B12 was significantly lower in HIV positive subjects compared normal healthy control($p < 0.05$). Multiple factors are responsible for the low levels of serum vitamin B12 in HIV positive patients, like chronic diarrhea leading to malabsorption, gastric mucosal damage by HIV and opportunistic infections and intrinsic factor due to injury of the gastric mucosa [23]. During the early stages of HIV infection, due to increased lymphocyte turnover, fast dividing immune cells cause increased consumption of micronutrients contributing towards one of the etiologies of vitamin B12 deficiency in HIV patients [20-25]. Deficiency of vitamin B12 contributes to disorders in methylation affecting the immune function and NK Cell activity [26]. It increases the number of cytotoxic T cells and also participates in antibody synthesis. Low serum vitamin B12 levels affect the composition of intestinal microbiota in the gastrointestinal tract altering the gut barrier defense mechanism. A recent study has also revealed that vitamin B12 is a cofactor of transcriptional regulators and anti-repressors [27]. The finding of this study is in agreement with previous studies which reported significant decrease in vitamin B12 of HIV positive subjects in comparison with normal healthy control [28-30].

In this study vitamin D was significantly lower in HIV positive subjects compared normal healthy control($p < 0.05$). Well-known risk factors impairing bone maintenance and attributing to low levels of vitamin D in HIV infection include minimized exposure to sunlight and nutritive intake, insufficient absorption, fatty liver and renal damage encouraged anomalous vitamin D activation, altered bioavailability of non-hydroxylated vitamin D in adipose tissue and the antiretroviral treatment intervening vitamin D metabolism [29,30]. Since the virus itself may induce hypovitaminosis D through many mechanisms(increased levels of TNF- α , inhibition of renal hydroxylation and induction of 25-hydroxyvitamin D consumption by the macrophages and lymphocytes), the abnormal biochemical features found in our HIV-infected subjects compared with the controls, in particular the lower vitamin D levels, may support the hypothesis that HIV per se might play a role in the pathogenesis of bone loss [31].

Vitamin D was significantly lower($p < 0.05$) in age group 41 years and above in comparison with age group 20 -40 years($p > 0.05$). This finding is consistent with prior findings in HIV carriers [22-25]. Allavena, et al., and Okparaku, et al., observe substantial correlation between age and vitamin D, one of the justifications may comprise the samples from younger HIV cohorts with few patients over 60 years of age, identical to the current research, which consisted majority of patients ranging within 23-47 years of age [32,33]. The current work revealed non-significant difference vitamin D among HIV positive subjects with respect to gender which is consistent with other studies conducted on HIV infected individuals [32-36].

Hypovitaminosis D is a risk factor for developing comorbidities including infectious diseases as well as immune disorders in patients infected with HIV [33]. Studies have shown that though ART is associated with a decreased prevalence of opportunistic gastrointestinal diseases and incidence of malnutrition, gastrointestinal infections and severe gastroenteritis, which alter micronutrient absorption, may persist after ART initiation [24]. Several HIV medications, particularly nucleoside reverse transcriptase inhibitors, can inhibit the replication of mitochondrial DNA and cause vomiting and diarrhea that can reduce the absorption or increase the losses of several micronutrients [35,36].

Calcium was significantly lower in HIV positive subjects compared with healthy control($p < 0.05$). Studies have shown that some minerals/trace elements may be key factors in maintaining health despite human immunodeficiency virus infection and in

reducing mortality [34]. Each of the mineral/trace elements examined in this study may contribute to the general well-being of HIV-infected persons. Calcium has been shown to reduce diarrhoea in HIV-positive/AIDS patients. Therefore, the results obtained from this study tend to suggest that patients with a high dietary intake might be able to replace lost calcium and in turn reduce the burden of diarrhoea. This finding is in agreement with previous studies which reported significant decrease in calcium among HIV positive subjects compared with healthy control [37-39].

Iron was significantly lower in HIV positive subjects compared with healthy control ($p < 0.05$). Iron is one of the micronutrients that is commonly deficient in HIV infection [11]. Iron is essential for the formation and functioning of red blood cells and vitamin C is known to promote the absorption of iron [15]. In male patients, the iron intake was higher than in female subjects. The reason for this discrepancy is not clear, but it may be related to the fact that iron is lacking in women's food due to a lack of knowledge, consuming foods/diets deficient in iron or because of extra demands during the menstrual cycle (women need extra iron until they pass the menopause stage) [38-40]. This finding is in agreement with previous studies which reported significant decrease in iron among HIV positive subjects compared with healthy control [24-27].

The present study demonstrated that HIV-infected subjects had significantly lower zinc concentration when compared with the control subjects. This observation is in agreement with previous reports [29-33]. This finding may be attributed to high demand in zinc because HIV nucleocapsid and integrase proteins that are essential for assembly of infectious virions contain zinc fingers that require zinc for normal structure and functioning [5]. Zinc deficiency is associated with impaired immune function and an increased susceptibility to infection. Zinc deficiency may result due to malnutrition [30]. One of the factors responsible for malnutrition in an HIV-infected person is reduced appetite, which could be due to difficulty in ingesting food as a result of infections such as oral thrush or oesophagitis caused by *Candida*, a common opportunistic infection in HIV-infected people and fever, side effects of medicines or depression. Poor absorption of nutrients may be due to accompanying diarrhea which may be because of bacterial infections such as *Salmonella* or *Mycobacterium avium* intercellular; viral such as CMV or parasitic infections such as *Giardia*, *Cryptosporidium parvum* and *Enterocytozoon bieneusi*; due to nausea/vomiting as a side effect of medications used to treat HIV or opportunistic infections [7].

The serum zinc level of the HIV-infected subjects on ART for more than one year and did not show any significant difference compared with HIV positive subjects on ART for less than one year. This finding is in consonance with previous report [31-34]. The ART treatment and its duration seem to have no effect on zinc level. Deficiencies of these micronutrients may result in fatigue, depression and widespread abnormalities in connective tissue, such as inflamed gingivae, petechiae, perifollicular hemorrhages and impaired wound healing, coiled hairs, hyperkeratosis and bleeding into body cavities [35-37]. Furthermore, the association between serum zinc level with respect to age and gender was not statistically significant ($p > 0.05$). This shows that gender and age may not be predictor/risk factor of zinc deficiency among HIV positive subjects. This observation is in agreement with previous reports [10,15,18].

Serum copper in this study was significantly lower in HIV-positive subjects compared with HIV sero-negative subjects. This finding is agreement with another study in Ethiopia [2]. However, this observation is in contrast with previous reports that found significantly higher serum copper in HIV-seropositive subjects than in controls but considerably lower than in pre-HAART levels [1,13,16,17]. Lawal, et al., and Nwegbu, et al., both reported a significant increase in the mean plasma copper concentration of HIV-infected subjects when compared with the control group. The mean serum copper level of HIV-infected subjects on ART for less than one year was non-significantly lower than HIV-positive patients on drugs for over one year. Copper is required for immune complex formation, blood and coagulation factors formation. It is a major micronutrient required by the body in HIV infection [14,17]. Copper has been established as an acute phase reactant and its serum level has been shown to change significantly in a range of acute and chronic infective, inflammatory and neoplastic processes owing to increased reproductive of ceruloplasmin [4]. Serum copper has been shown to return to normality after overcoming the initial acute phase of the associated disease [18].

In general, there was significantly lower vitamins and micronutrients among HIV-positive subjects compared with healthy control. This is probably due to their high consumption of carbohydrate sources like maize meal, which contains only low levels of these nutrients [28]. The results in this study are consistent with findings by Onyango, et al., that there was a lower intake of energy as well as most of the other nutrients among HIV seropositive patients. Energy intake is related to the stage of the

infection, rapid weight loss, anorexia, opportunistic infections, malabsorption and altered metabolism [8]. Thus, it is clear that HIV-infected patients are at increased nutritional risk. Energy requirements increase significantly as the HIV disease progresses [42-54]. This may thus be viewed as a bad trend for patients with low energy intake as reported in this study. The higher energy intake assists to a certain degree in reducing wasting and improves the well-being of the patients [43].

Conclusion

In conclusion, calcium, zinc, copper, Iron, Vitamin B12 and Vitamin D were significantly lower in HIV positive subjects compared with healthy control ($p < 0.05$). Calcium, zinc, copper, Iron, Vitamins B12 and D were non-significantly higher in male HIV positive subjects in comparison with female subjects ($p > 0.05$). Calcium, Iron, Vitamins B12 and D were significantly lower ($p < 0.05$) in age group 41 years and above in comparison with age group 20 -40 years, while Zinc and Copper were non-significant ($p > 0.05$). Calcium, Iron, Vitamins B12 and D were significantly higher in HIV positive subjects undergoing treatment for over a year compared with those undergoing treatment for less than one year. Calcium, Iron, Vitamins B12 and D were significantly lower ($p < 0.05$) in age group 41 years and above in comparison with age group 20 -40 years, while Zinc and Copper were non-significant ($p > 0.05$). The decrease in vitamins and micronutrients that accompanies HIV infection suggests a potentially important role of nutritional supplementation and good nutrition in the proper management of HIV/AIDS. It is therefore recommended that HIV-infected Patients should be investigated and treated for micronutrients deficiency, to reduce the morbidity and mortality associated with HIV infection. Further studies should be carried out on other micronutrients and vitamins not covered in this study among HIV positive subjects.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Consent to Participate

Informed consent was obtained from each participant prior to specimen collection.

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Data Availability

Data is available for the journal. Informed consents were not necessary for this paper.

Author's Contribution

The authors contributed equally.

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