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EFFECTS OF MICRONUTRIENTS SUPPLEMENTATION ON ANTIOXIDANT VITAMINS AND MINERALS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-POSITIVE SUBJECTS IN SOKOTO, NIGERIA

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ABSTRACT

Background: HIV/AIDS patients are under chronic oxidative stress and may play a critical role in chronic complications of HIV/AIDS. Micronutrients may play an important role in the improvement of the disease and in the management of HIV/AIDS patients. **Objectives:** The current study is aimed at evaluating the effects of micronutrient supplementation on antioxidant vitamins and minerals in 210 HIV-positive adult patients attending Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria. **Materials and Methods:** Participants were recruited in the Antiretroviral Therapy (ART) Clinic, Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto. Serum concentrations of antioxidant vitamins (A, C and E), were measured by standard laboratory techniques, while antioxidant mineral elements, Copper (Cu), Zinc (Zn), Manganese (Mn) and Selenium (Se) were measured by atomic absorption Spectrophotometry. **Results:** The results showed that, serum concentrations of antioxidant vitamins (A, C and E) and antioxidant mineral elements (Cu, Zn, Mn and Se) in HIV-positive HAART-naïve and HIV-positive patients on HAART were significantly ($p<0.001$) lower than the corresponding values among the age- and sex–matched controls at baseline. Mean serum concentrations of antioxidant vitamins (A, C and E) and antioxidant minerals (Cu, Zn, Mn and Se) increased significantly ($p<0.001$), in HIV-positive HAART-naïve and HIV-positive patients on HAART that received micronutrients supplement respectively compared to unsupplemented groups. **Conclusion:** The results in the

current study demonstrates that, lower serum concentrations of antioxidant vitamins and minerals observed in HIV-positive HAART-naïve patients and HIV-positive on HAART patients at baseline were reversed on micronutrients supplementation of the subjects.

KEYWORDS: Antioxidants, Micronutrients Supplementation, HIV, Nigeria.

1.0 INTRODUCTION

Infection with human immunodeficiency virus (HIV) is associated with a decline in immunity or the inability to fight infection and progresses to acquired immunodeficiency syndrome (AIDS). Several studies have shown that, deficiencies of serum antioxidant vitamins (A, C and E)) and mineral elements (Copper, Manganese, Selenium and Zinc) are common among HIV infected persons, especially those in developing countries, and injection drug users.[1-4] Thus a vicious cycle has been envisaged in which undernourished HIV- infected persons have micronutrient deficiencies, leading to further immune-suppression and oxidative stress and subsequent acceleration of HIV replication and CD_4^+ T-cell depletion.[1, 5]

Inadequate micronutrients especially Zinc, Iron, and the antioxidant vitamins A, C and E can lead to clinically significant immune deficiency and infections in children and adults infected with human immunodeficiency virus (HIV).[6] Low serum micronutrients occur in HIV-seropositive patients in developed countries despite adequate dietary intake.[7] Low dietary intake resulting from poverty and ignorance in addition to increased oxidative stress contributed to antioxidant vitamins and minerals deficiencies in HIV-infected patients in Sokoto, Nigeria.[4] These deficiencies have been found to occur at early stages and progresses with the severity of HIV infection[3-4] and may increase the risk of a poorer prognosis.

Studies have indicated the significant function of micronutrients in antioxidant defence. Vitamin A and its major precursor, β -carotenes are naturally occurring antioxidants and are known to protect cell membranes against lipid peroxidation by quenching the superoxide and peroxyl radicals.[8]

Trace elements especially zinc, copper, iron and selenium are known as co-factors needed for the synthesis, optimum catalytic activity and effective antioxidant defence of the denovo antioxidant enzymes. Thus zinc is an essential co-factor for cytoplasmic superoxide dismutase (Cu-Zn SOD) enzyme.[9] Superoxide dismutase catalyses the univalent reduction and oxidation of $O_2^{\cdot -}$ to H_2O_2 and molecular O_2.

Iron (Fe) is required for catalase (CAT) a haem-protein enzyme, containing four[4] haem prosthetic groups. Catalase is concentrated mainly in the peroxisomes and mitochondria[10] where it catalyses the conversion of hydrogen peroxide (product of superoxide dismutation) into water and oxygen. While selenium is an integral part of glutathione peroxidase (GPX) an enzyme located in the mitochondrial matrix and cytosol of animal cells.[8] Glutathione peroxidase (GPX) protect cells against oxidative damage by converting the reduced glutathione (GSH) into oxidized glutathione (GSSG) removing hydrogen peroxide (H_2O_2) to form water (H_2O).[8]

Several prospective, randomized clinical trials have suggested that, micronutrients help to strengthen the immune system, improve clinical outcomes and significantly increase $CD4^+$ cell count and reduce the severity and impact of opportunistic infections in people living with HIV/AIDS.[11-15]

The role of micronutrients supplementation in medical management of HIV/AIDS is not well defined, as some but not all studies show immunological and clinical benefits. Antioxidant micronutrients supplementation could be a relatively low cost strategy to defer the initiation of expensive, potentially toxic and lifelong antiretroviral therapy. No study has rigorously investigated the effects of micronutrient supplementation in HIV-infected population attending Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, North-Western Nigeria where HIV/AIDS is prevalent. The current study is therefore aimed at assessing whether micronutrient supplementation of HIV-positive HAART-naïve and those on treatment with HAART, using a micronutrients tablet (Centrum®) could improve the antioxidant vitamins and minerals levels in blood of these subjects compared with un-supplemented groups.

2.0 MATERIALS AND METHODS

2.1 Study Subjects and Study Site

Enrolment took place between April, 2012 and September, 2013 at the Antiretroviral Therapy (ART) Clinic, Usmanu Danfodiyo University Teaching Hospital, Sokoto Nigeria. A total of two hundred and fifty two (252) subjects were enrolled into the present study. These consisted of 106 newly diagnosed HIV-positive that are not yet on treatment with HAART (HAART-naïve) and 104 on treatment with HAART. The controls comprised 42 adult persons, sex- and age-and socioeconomic-matched HIV-negative (apparently healthy)

individuals selected as volunteers from a population of UDUTH staff and blood donors attending the blood bank of Usmanu Danfodiyo University Teaching Hospital, Sokoto.

Eligibility criteria for the patients were; asymptomatic HIV-positive adults who are HAART-naive and HIV-positive adults Who are on HAART, both with screening $CD4^+$ T lymphocytes ≥ 350 cells/µl. In all the patients and controls, informed consent was obtained from each prior to the commencement of the study. Study subjects were ineligible if they have allergy or intolerance to any study ingredient, be pregnant, have ALT greater than three times normal range, have known liver cirrhosis, have serum creatinine >133 µmol/l, smoke cigarette, abuse alcohol or be taking micronutrient or natural health product.

2.2 Study Design

The study was a prospective, interventional study where consenting eligible HIV-positive male and female patients attending ART Clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto were enrolled to receive a micronutrients supplement (Centrum®) or no supplement for 12 months, and the effect of micronutrients supplementation on antioxidant vitamins and minerals from baseline to 12 months was assessed according to the method described by Hammer and co-workers.[16] All the HIV-positive patients were evaluated clinically by the consultant Physicians and the patients allotted to different Clinical stages of HIV-infection according to the revised criteria from the Centres for Disease Control and Prevention.[17]

At enrolment a structured interviewer- administered questionnaire was administered to each patient and information on patient's demographic and socioeconomic characteristics including sex, age, marital status, occupation, family income and education were obtained. Information on the patient nutritional status, duration of HIV-infection, the type(s) and the duration of the use of HAART were also obtained. Eligible subjects were assigned to the following groups

Group A (n=42): HIV-negative (controls) not supplemented with Centrum®.
Group B (n=53): HIV-positive HAART-naïve not supplemented with Centrum®.
Group C (n=55): HIV-positive HAART-naïve supplemented with Centrum®.
Group D (n=53): HIV-positive on HAART not supplemented with Centrum®.
Group E (n=54): HIV-positive on HAART supplemented with Centrum®.

Opportunistic illness prophylaxis and treatment and ART were offered to the patients at the ART Clinic according to standard treatment guidelines for the use of antiretroviral (ARV) drugs in Nigeria.[18]

2.4 Ethical Approval

The study design and protocol were approved by the Ethics and Research Committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto. The research was carried out in accordance with the declaration of Helsinki concerning the ethical principles for medical research involving human subjects. Written informed consent was obtained from all study participants before enrolment.

2.5 Study Regimen

The study regimen used was a commercially formulated micronutrient supplement that included 23 ingredients with a trade name, Centrum® procured from Pfizer, Madison, NJ 07940, USA and distributed in Nigeria by Pfizer Specialities Limited 38, Opebi road, Adebola House Ikeja, Lagos. The subjects were randomly assigned to receive micronutrient supplement (Centrum® tablet) or not receive the micronutrient supplement (Centrum® tablet) for 12 months. The Centrum® was consumed by the study participants as one tablet daily with meals. The participants were not allowed to use another micronutrient or natural health product.

Compliance with the study regimen (Centrum®) was assessed according to the method of Kupka and colleagues[19] and Kawai and co-workers.[20] The HIV-positive HAART- naïve and HIV-positive patients on HAART that were randomly assigned to the micronutrient supplement (Centrum®) groups, were asked to bring the unused Centrum® tablets back in the next Clinic visit. Participants exchanged a used bottle with a new bottle that contained 100 Centrum® tablets. Compliance with the Centrum® supplement was calculated as the number of Centrum® tablets absent from the returned bottles divided by the total number of Centrum® tablets the subject should have taken and multiplied by 100. This was used as the indicator of the subject's compliance to the study medication.

2.6 Withdrawal from the Study

Participants were allowed to withdraw from the study at any time and for any reason, or may be withdrawn in the event of intercurrent illness, intolerance to study medication, adverse events, pregnancy, protocol violation or administrative reasons. All participants discontinued

due to an adverse event were followed up until the event resolves, or becomes stable and appropriate medical care provided.

2.7 Screening, Baseline, and Follow-up Assessments

All the HIV-positive patients were consecutively selected from the population of HIV positive patients attending ART Clinic, Usmanu Danfodiyo University Teaching Hospital Sokoto. Potential participants were identified by preliminary screening at routine Clinic visits. At the ART Clinic, HIV- positive patients who satisfied the study inclusion criteria were consecutively selected until the desired sample size was attained. All the patients were evaluated by the consultant Physicians at the Clinic. Following informed consent, a structured interviewer-administered questionnaire was used to elicit data on subject's socioeconomic and demographic characteristics, including age, sex, marital status, tribe, HIV-related characteristics, opportunistic infection and stage of HIV infection were obtained.

The screening visit was followed in two to four weeks by baseline visit. At baseline visit, eligible participants were enrolled to the study groups and baseline blood samples were collected for laboratory analysis as indicated in the study design. Follow-ups were conducted on the HIV-positive patients enrolled into this study at 3-monthly Clinic visits in which consultant Physicians carried out a complete clinical examination. During each Clinic visit, the HIV-positive patients were asked about their health status, including questions on the incidence of signs and symptoms of HIV disease (e.g. presence of diarrhoea, oral thrush, wasting and opportunistic infections).

2.8 Blood Samples Collection and Processing

Blood samples (about 5 millilitres) were collected into a sterile plain vacutainer blood specimen bottles from BDH Laboratory supplies, United Kingdom and allowed to clot at room temperature and later centrifuged at 3000rpm/min for 5 minutes to obtain clear unhaemolyzed serum. The sera were harvested into sterile serum-separation tubes and rapidly stored at -20^{o}C until assayed in batches; for serum levels of antioxidant vitamins and minerals.

2.9 Estimation of Biochemical Parameters

Antioxidant vitamins A, C and E and antioxidant mineral elements including copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) were measured initially at baseline and every 3 months for a total of 12 months. The serum vitamin A concentration was measured by the

method of Bessey and co-workers[21], serum vitamin C (ascorbic acid) concentration was estimated using the method of Natelson[22] and serum vitamin E concentration was estimated using the method of Hashim and Schuttringer.[23]

Serum concentrations of antioxidant mineral elements (Cu, Zn, Mn and Se) were estimated by atomic absorption spectrophotometric method (AAS) as described by Kaneto[24], using atomic absorption spectrophotometer, Buck model 205 manufactured by Buck Scientific Inc. 58 Fort Point St. East Norwalk, CL 06855. The principle of the flame AAS is based on the dissociation of the element from its chemical bonds. In this process a solution containing a suitable compound of the metal is converted into an aerosol which is then injected into a flame which then converts the sample into an atomic vapour and molecular vapour. This is then placed in an unexcited or ground state (neutral atom). Thus, the neutral atom is at a lower energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum. The amount of radiant energy absorbed at a characteristic wavelength in the flame is proportional to the concentration of the element present in the sample.

For manganese, 1:4 dilutions of serum specimens were prepared with water and aspirated to AAS. While a dilution of manganese working standards (1ppm and 3 ppm Mn) and blank were prepared using 5% glycerine. For Copper (Cu): The serum was diluted 1:1 with water, aspirated and read in AAS. Standards and blanks were prepared with 10% glycerine (recommended standards are 5 ppm and 15 ppm Cu; however, the lowest standard alone could be used). For Zinc (Zn): The serum was diluted 1:4 with water and aspirated to AAS. Standards and blanks were prepared by diluting with 5% glycerine (series of standards 1, 3 and 6 were recommended, however, 1 and 3 ppm were enough which have comparable concentration with sample). Selenium (Se): This element was read from samples prepared for Zn or Cu analysis. Standards and blanks were prepared accordingly.

2.10 Statistical Analysis

The data obtained were analysed using Microsoft Office Excel 2007 and Graphpad InStat® statistical soft ware Version 3.10, 32 Bit for windows (2009). The results are expressed as mean ± SEM. Group comparisons were made using one-way analysis of variance (ANOVA), paired comparisons were carried out using the Student's t-test, analysis and p-value of equal to or less than 0.05 ($P \leq 0.05$).

3.0 RESULTS

The demographic and HIV-related characteristics of the study population were presented in Table 1. Majority of the HIV-infected patients in the study population are married (71.1%), predominantly Hausa and about 56.7% of the study populations are in the CDC stage I of HIV infection. The effect of sex on serum concentrations of antioxidant vitamins (A, C and E) and antioxidant minerals (Cu, Zn, Mn and Se) in HIV-positive patients and controls was shown in Tables 2 and 3. Mean serum concentrations of vitamins A, C and E were significantly (P<0.001) higher in female patients (0.39±0.01 µmol/l, 45.10±1.84 µmol/l and 12.06±0.35 µmol/l respectively) compared with male patients (0.38±0.01 µmol/l, 39.92±1.87 µmol/l and 11.10±0.30 µmol/l respectively). It should be noted that in the course of follow-ups during the research some of the patients declined to continue with the research while others absconded. These cases were excluded from the analysis and final computation.

The baseline characteristics of HIV-positive patients and controls were shown in Table 4. The mean serum concentrations of antioxidant vitamins (A, C and E) and minerals (Cu, Zn, Mn and Se) were significantly (p<0.001) lower in HIV-positive patients compared with the corresponding control values. Table 5 showed the effects of micronutrients supplementation on serum concentrations of antioxidant vitamins (Vitamin A, C and E) in HIV-positive patients and controls at baseline, 3, 6, 9, and 12 months post supplementation. The mean serum levels of antioxidant vitamins A, C and E at baseline were significantly (P< 0.001) lower in HIV–positive HAART-naïve patients (group B and C) (0.38±0.01µmol/l and 0.42±0.01 µmol/l respectively) and HIV–positive patients on HAART (group D and E) (0.37±0.01µmol/l and 0.36±0.01µmol/l respectively) compared with the control (1.66±0.02 µmol/l) at baseline. Micronutrient supplementation showed significantly (p<0.001) increased serum vitamins A, C and E levels in HIV-positive HAART-naïve patients (group C) and HIV-positive on HAART patients (group E) at 3, 6, 9 and 12 months compared with the unsupplemented groups (group B and D).

The effects of micronutrients supplementation on serum concentrations of antioxidant minerals (Cu, Zn, Mn and Se) in HIV-positive patients and controls at baseline, 3, 6, 9, and 12 months post supplementation was presented in Table 6. The mean serum levels of antioxidant minerals Cu, Zn, Mn and Se measured at baseline were significantly (P< 0.001) lower in HIV – positive HAART-naïve patients (group B and C) and HIV–positive patients on HAART (group D and E) compared with the corresponding control values (group A)

(Table 6). Micronutrient supplementation showed significantly ($p<0.001$) increased serum concentrations of Cu at 6, 9 and 12 months; while serum Zn, Mn and Se concentrations were significantly ($p<0.001$) increased at 3, 6, 9 and 12 months in HIV – positive HAART-naïve patients (group C) compared with the corresponding values in the unsupplemented (group B) (Table 6 and Figures 4 and 5) The mean serum concentrations of Cu, Zn and Se were significantly ($p<0.001$) increased at 6, 9 and 12 months while serum Mn concentrations significantly increased at 9 and 12 months in HIV-positive on HAART patients supplemented with micronutrients (group E) compared with the unsupplemented group (group D

Table 1: Demographic and HIV-related Characteristics of the Study Population

Characteristic	Number of Subjects	Percentage (%)
Marital Status	252	100
Married	180	71.4
Single	52	20.6
Widowed	15	6
Divorced	5	2
Tribe	252	100
Hausa	181	71.8
Fulani	7	2.8
Igbo	19	7.5
Yoruba	5	2
Others	40	15.9
HIV-related Illness	40	15.9
Herpes Zoster	3	1.19
Kaposi Sarcoma	2	0.79
Tuberculosis	35	13.9
Opportunistic Infection	89	35.3
Recurrent Diarrhoea	13	6.2
Recurrent Typhoid	12	5.7
Bronchitis	14	6.7
Candidiasis	4	1.9
Otitis Media	2	0.9
Others	44	21
Stage of HIV Infection	210	83.3
Stage I	143	56.7
Stage II	56	22.2
Stage III	8	3.2
Stage IV	3	1.2

Majority of the HIV-infected patients in the study population are married (71.1%) followed by single (20.6%), the subjects are predominantly Hausa and most of them in CDC stage I (56.7%) of HIV infection.

Table 2: Impact of Sex on Serum Concentrations of Antioxidant Vitamins (Vitamin A, C and E) in HIV-Positive Patients and Controls.

	HIV-Negative Controls		HIV-Positive Patients		
Parameter	**Male (n=20)**	**Female (n=22)**	**Male (n=20)**	**Female(n=22)**	**P value**
Vitamin A (µmol/l)	1.66 ± 0.04^{c1d1}	1.66 ± 0.04^{c2d2}	0.38 ± 0.01^{a1b1}	0.39 ± 0.01^{a2b2}	P<0.001
Vitamin C (µmol/l)	69.53 ± 5.21^{c1d1}	75.29 ± 5.00^{c1d2}	39.92 ± 1.87^{a1b1}	45.10 ± 1.84^{a2b2}	P<0.001
Vitamin E (µmol/l)	21.46 ± 0.91^{c1d1}	20.43 ± 0.96^{c2d2}	11.10 ± 0.30^{a1b1}	12.06 ± 0.35^{a2b2}	P<0.001

Values are mean ± SEM; n=number of Subjects; Significant differences: **a1**(p<0.001)=male controls versus male patients; **a2**(p<0.001)=male controls versus female patients; **b1**(p<0.001)=female control versus male patients; **b2**(p<0.001)=female control versus female patients; **c1**(p<0.001)=male patients versus male controls; **c2**(p<0.001)=male patients versus female controls; **d1**(p<0.001)=female patients versus male controls; **d2**(p<0.001)=female patients versus female controls by Bonferroni multiple comparison Test.

Table 3: Impact of Sex on Serum Concentrations of Antioxidant Minerals (Cu, Zn, Mn, Se) in HIV-Positive Patients and Controls.

	HIV-negative Controls		HIV-positive Patients		
Parameter	**Male (n=20)**	**Female (n=22)**	**Male (n=94)**	**Female(n=116)**	**P value**
Copper (µmol/l)	20.16 ± 0.42^{c1d1}	20.08 ± 0.30^{c2d2}	16.72 ± 0.25^{a1b1}	17.28 ± 0.17^{a2b2}	P<0.001
Zinc (µmol/l)	16.66 ± 0.31^{c1d1}	16.20 ± 0.29^{c2d2}	13.38 ± 0.12^{a1b1}	13.51 ± 0.11^{a2b2}	P<0.001
Manganese (µmol/l)	171.02 ± 9.07^{c1d1}	162.34 ± 11.20^{c2d2}	117.41 ± 4.89^{a1b1}	112.95 ± 3.17^{a2b2}	P<0.001
Selenium (µmol/l)	0.39 ± 0.02^{c1d1}	0.38 ± 0.02^{c2d2}	0.28 ± 0.01^{a1b1}	0.28 ± 0.00^{a2b2}	P<0.001

Values are mean ± SEM; n=number of Subjects; Significant differences: **a1** (p<0.001) =male controls versus male patients; **a2** (p<0.001) =male controls versus female patients; **b1**(p<0.001) =female controls versus male patients; **b2** (p<0.001) =female patients versus female controls; **c1**(p<0.001)=male patients versus male controls; **c2**(p<0.001)=male patients versus female controls; **d1**(p<0.001)=female patients versus male controls; **d2**(p<0.001)=female patients versus female controls by Bonferroni multiple comparison Test.

Table: 4 Baseline Characteristics of HIV-Positive Patients and Controls

Characteristics	Group A (n=42)	Group B (n=52)	Group C (n=54)	Group D (n=51)	Group E (n=53)
Male	22	23	25	21	25
Female	20	29	29	30	28
Age(Years)	31.14±1.44	33.06±1.04	33.02±1.12	34.24±1.10	34.79±1.11
Body Weight(Kg)	63.68±1.76	61.51±1.59	65.18±1.63	68.84±2.08	69.88±2.24^{b2}
Height(m)	1.61±0.01	1.65±0.01	1.65±0.01	1.66±0.01	1.63±0.01
BMI (Kgm^{-2})	24.45±0.63	22.70±0.55	23.98±0.60	25.06±0.73	26.24±0.83^{b2}
Vitamin A (µmol/l)	1.66±0.02	0.38±0.01^{a1}	0.42±0.01 ^{a2}	0.37±0.01 ^{a3}	0.36±0.01 ^{a4c3}
Vitamin C (µmol/l)	72.54±3.59	45.97±3.21 ^{a1}	52.54±2.48 ^{a2}	39.19±2.04 ^{a3c2}	33.17±1.95^{a4bc3}
Vitamin E (µmol/l)	20.92±0.66	11.22±0.47 ^{a1}	12.74±0.57 ^{a2}	12.09±0.41 ^{a3}	10.45±0.36 ^{ac3}
Copper (µmol/l)	20.12±0.25	17.23±0.26^{a1}	17.39±0.24 ^{a2}	16.31±0.38 ^{a3}	17.15±0.26 ^{a4}
Zinc (µmol/l)	16.42±0.21	13.30±0.13 ^{a1}	13.45±0.14 ^{a2}	13.44±0.21 ^{a3}	13.61±0.15 ^{a4}
Manganese (nmol/l)	166.47±7.23	101.48±1.98^{a1}	111.02±3.98 ^{a2}	135.00±8.32^{a3b1c2}	112.86±5.40^{a4}
Selenium (µmol/l)	0.38±0.01	0.24±0.01 ^{a1}	0.28±0.01 ^{a2}	0.31±0.02^{a3b1}	0.28±0.02 ^{a4}

Values are mean ± SEM measured at baseline; n= number of subjects; BMI= body mass index; CD4= cluster of differentiation type 4; Significant differences: **a1** (P<0.001) = Control versus Group B; **a2** (P<0.001) = Control versus Group C; **a3** (P<0.001) = Control versus Group D; **a4** (P<0.001) = Control versus Group E; **b1** (P<0.001) = Group B versus Group D; **b2** (P<0.001) = Group B versus Group E; **c1** (P<0.001) = Group C versus Group B; **c2** (P<0.001) Group C versus Group D; **d** (P<0.001) = Group D versus Group E.

Table 5: Effects of Micronutrients Supplementation on Serum Concentrations of Antioxidant Vitamins (Vitamin A, C and E) in HIV-Positive Patients and Controls at Baseline, 3, 6, 9, and 12 Months Post Supplementation.

Parameter	Group A (Control) (n=42)	Group B (HIV^+) (n=52)	Group C (HIV^+ +Centrum®) (n=54)	Group D (HIV^+ +HAART) (n=51)	Group E (HIV^+ +HAART + Centrum®) (n=53)
Vitamin A (µmol/l)					
Baseline	1.66±0.02	0.38±0.01^{a1}	0.42±0.01^{a2}	0.37±0.01^{a3}	0.36±0.01^{a4c3}
3 Months	-	0.37±0.01^{c1}	0.46±0.01	0.36±0.01^{c2}	0.39±0.01^{c3}
6 Months	-	0.34±0.01^{c1}	0.52±0.02	0.34±0.01^{c2}	0.45±0.01^{bc3d}
9 Months	-	0.31±0.01^{c1}	0.60±0.02	0.31±0.01^{c2}	0.53±0.01^{bd}
12 Months	-	0.15±0.01^{c1}	0.64±0.02	0.16±0.01^{c2}	0.56±0.01^{bd}
Vitamin C (µmol/l)					
Baseline	72.54±3.59	45.97±3.21^{a1}	52.54±2.48^{a2}	39.19±2.04^{a3c2}	33.17±1.95^{a4b3}
3 Months	-	42.77±3.22^{c1}	57.54±2.46	35.73±2.04^{c2}	37.75±1.94^{c3}
6 Months	-	42.06±3.27^{c1}	66.17±2.48	33.62±2.04^{c2}	44.98±2.03^{c3d}
9 Months	-	40.22±3.24^{c1}	76.44±2.49	31.26±2.04^{c2}	54.36±2.26b^{cd}
12 Months	-	38.77±3.48^{c1}	83.70±2.73	29.30±2.07^{c2}	62.24±2.44b^{cd}
Vitamin E (µmol/l)					
Baseline	20.92±0.66	11.22±0.47^{a1}	12.74±0.57^{a2}	12.09±0.41^{a3}	10.45±0.36^{ac3}
3 Months	-	10.54±0.47^{c1}	15.34±0.57	11.20±0.41^{c2}	12.66±0.36^{bc3}
6 Months	-	10.11±0.48^{c1}	18.20±0.57	10.77±0.41^{c2}	15.36±0.38^{bc3d}
9 Months	-	9.83±0.50^{c1}	21.18±0.56	10.32±0.41^{c2}	17.96±0.41^{bc3d}
12 Months	-	9.43±0.52^{c1}	23.47±0.66	9.08±0.34^{c2}	19.79±0.65^{bc3d}

Values are mean ± SEM; n=number of Subjects; Significant differences: **a**^{1} (P<0.001) = Control versus Group B; **a**^{2} (P<0.001) = Control versus Group C; **a**^{3} (P<0.001) = Control versus Group D; **a**^{4} (P<0.001) = Control versus Group E; **b** (P<0.001) = Group B versus Group E; **c**^{1}(P<0.001) = Group C versus Group B; **c**^{2} (P<0.001) = Group C versus Group D; **c**^{3} (P<0.001) = Group C versus Group E; **d** (P<0.001) = Group D versus Group E by Bonferroni multiple comparison Test.

Table 6: Effects of Micronutrients Supplementation on Serum Concentrations of Antioxidant Minerals (Cu, Zn, Mn and Se) in HIV-Positive Patients and Controls at Baseline, 3, 6, 9, and 12 Months Post Supplementation.

Parameter	Group A (Control) (n=42)	Group B (HIV^+) (n=52)	Group C (HIV^+ + Centrum®) (n=54)	Group D (HIV^+ + HAART) (n=51)	Group E (HIV^+ +HAART + Centrum®) (n=53)
Copper (µmol/l)					
Baseline	20.12±0.25	17.23±0.26^{a1}	17.39±0.24 ^{a2}	16.31±0.38 ^{a3}	17.15±0.26 ^{a4}
3 Months	-	16.86±0.26	17.88±0.24	15.85±0.40^{c2}	17.44±0.27^{a4d}
6 Months	-	16.34±0.27^{c}	18.54±0.23	15.42±0.40^{c2}	18.03±0.27^{b2d}
9 Months	-	15.76±0.26^{c}	19.46±0.23	15.06±0.32^{c2}	18.50±0.29^{b2d}
12 Months	-	15.28±0.27^{c}	19.72±0.24	14.93±0.32^{c2}	18.68±0.29^{b2d}
Zinc (µmol/l)					
Baseline	16.42±0.21	13.30±0.13 ^{a1}	13.45±0.14 ^{a2}	13.44±0.21 ^{a3}	13.61±0.15 ^{a4}
3 Months	-	12.99±0.13^{c1}	13.72±0.14	13.19±0.21	13.85±0.15 ^{b2}
6 Months	-	12.67±0.14^{c1}	14.21±0.14	12.91±0.20^{c2}	14.21±0.15 ^{b2d}
9 Months	-	11.99±0.14^{c1}	14.99±0.16	12.30±0.21^{c2}	14.64±0.14 ^{b2d}
12 Months	-	11.73±0.14^{c1}	15.11±0.16	12.10±0.20^{c2}	14.79±0.15 ^{b2d}
Manganese (nmol/l)					
Baseline	166.47±7.23	101.48±1.98^{a1}	111.02±3.98 ^{a2}	135.00±8.32^{a3b1c2}	112.86±5.40^{a4}
3 Months	-	92.81±2.10 ^{c1}	117.74±4.03	130.00±8.18^{b1}	117.04±5.36^{b2}
6 Months	-	90.55±2.00 ^{c1}	130.10±3.98	126.54±8.32^{b1}	122.27±5.61^{b2}
9 Months	-	78.12±2.04 ^{c1}	149.75±4.77	114.27±8.10^{b1c2}	139.02±6.44^{b2d}
12 Months	-	76.61±3.25 ^{c1}	159.08±4.06	108.12±7.22^{b1c2}	144.76±6.38^{b2d}
Selenium (µmol/l)					
Baseline	0.38±0.01	0.24±0.01 ^{a1}	0.28±0.01 ^{a2}	0.31±0.02^{a3b1}	0.28±0.02 ^{a4}
3 Months	-	0.22±0.01^{c1}	0.31±0.01	0.29±0.02 ^{b1}	0.30±0.02^{b2}
6 Months	-	0.19±0.01^{c1}	0.34±0.01	0.26±0.02 ^{b1c2}	0.32±0.02^{b2d}
9 Months	-	0.16±0.01^{c1}	0.40±0.01	0.23±0.02 ^{b1c2}	0.36±0.02^{b2d}
12 Months	-	0.15±0.01^{c1}	0.40±0.01	0.22±0.02 ^{b1c2}	0.37±0.02 ^{b2d}
12 Months	-	0.15±0.01^{c1}	0.40±0.01	0.22±0.02 ^{b1c2}	0.37±0.02 ^{b2d}

Values are mean ± SEM; n=number of subjects; Significant differences:
a^{1} (P<0.001) = Control versus Group B;
a^{2} (P<0.001) = Control versus Group C; **a**^{3} (P<0.001) = Control versus Group D; **a**^{4} (P<0.001) = Control versus Group E;
b^{1} (P<0.001) = Group B versus Group D; **b**^{2} (P<0.001) = Group B versus Group E; **c**^{1} (P<0.001) = Group C versus Group B;
c^{2} (P<0.001) = Group C versus Group D; **d** (P<0.001) = Group D versus Group E by Bonferroni multiple comparison Test.

4.0 DISCUSSION

In the current study, the finding of 33.77±0.55 years as mean age of HIV-positive patients and 56.7% (143/210) of the patients being in Centres for disease control (CDC) and prevention, stage I of HIV infection are noteworthy, indicating that HIV-infection is predominantly found in the middle aged group (Tables 2 and 3). This may be attributable to the middle age group involvement in economically productive ventures, coupled with physical well being with a quest for sexual adventure which makes it easy for the spread of HIV-infection in the study area. This finding is in agreement with the earlier reports in Nigeria, where most HIV-infected men and women were between the ages of 20 and 39 years.[25]

Females formed the majority of the HIV-infected patients and constituted 55.24% (116) of the total group (210), while males constitute 44.76% (94) of the total group (Table 2). This finding is consistent with the increasing rate of women living with HIV/AIDS which rose globally from 43 % in 1999 to 50% in 2010.[26] Similar findings were also recorded in Sub-Saharan Africa where women constituted about 59% of the adults' population living with HIV/AIDS in 2010.[2] This also compares favourably with the reported cases in Northern Nigeria where about 53.2% of women were reported infected with HIV.[27]

Other associated risk factors that can augment the rate of women becoming infected with HIV may include certain traditional practices like female genital mutilation (FGM) and unfaithful multiple partners in sexual relationships.[28] Moreover poverty and ignorance in addition to other socioeconomic conditions may be the driving forces increasing the risk of women getting infected through increased commercialization of sex.[25] Micronutrient deficiencies are prevalent in many HIV-infected populations, and numerous studies have reported that these deficiencies impair immune responses, weaken epithelial integrity, and are associated with accelerated HIV disease progression.[29]

In this study, the effect of supplementation of micronutrients during a twelve months[12] period on serum concentrations of antioxidant vitamins (A, C and C) and mineral elements, copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) status was significant for all the studied nutrients.

When the subjects were classified according to sex, the mean serum concentrations of antioxidant vitamins (A, C and E) and minerals (Cu, Zn, Mn and Se) in HIV-positive patients

and control groups were statistically significant ($p<0.001$), with lower serum concentrations of antioxidant vitamins (A, C and E) and minerals (Cu, Zn, Mn and Se) in HIV-positive patients compared with similar values in controls (Tables 2 and 3).

The result in the current study showed that at baseline, there were significant decrease in the serum levels of antioxidant vitamins A, C and E in HIV-positive HAART-naïve and HIV-positive on HAART patients compared with control (Table 4). This is in agreement with the previous studies (3-4, 12, 30-31). It is possible that the low levels of antioxidant vitamins levels could be due to either increased utilization of the antioxidant vitamins in quenching excessive free radicals and thereby prevents peroxidation of polyunsaturated fatty acids (3-4) or inadequate dietary intake, malabsorption and diarrhoea that are commonly seen in HIV subjects may also contribute to the decreased levels of vitamins C and E in HIV-positive subjects (3).

Micronutrients supplementation leads to significantly increased serum concentrations of vitamins A, C and E in HIV-positive HAART-naïve patients and HIV-positive on HAART patients. However, the effectiveness of the micronutrients supplementation on serum antioxidant vitamins A, C and E levels began at three[3] months and persisted through twelve[12] months (Table 5). This is consistent with the earlier findings indicating that micronutrients supplementation in HIV-positive patients resulted in the restoration of the serum vitamins A, C and E concentrations.[2,30] Studies of Jaruga and co-workers[30] and Austin and co-researchers[2] independently reported that, micronutrients supplementation in HIV-positive patients resulted in the restoration of the serum vitamins A, C and E concentrations and activities of antioxidant enzymes, superoxide dismutase and catalase to The levels observed for the control subjects, but $CD4^+$ cell counts remain unchanged. Allard and co-workers[12] also demonstrated in a randomized controlled trial that, daily supplementation of 800 IU vitamin E and 1000 mg vitamin C in HIV infected subjects in Canada significantly decreased oxidative stress and produces a trend towards a reduction in viral load.

Our results as well as those of others have shown that, the beneficial effect of micronutrients supplementation on the serum vitamins A, C and E levels was more pronounced in HIV-positive HAART-naïve patients than in HIV-positive on HAART patients. Gil and co-workers[32] reported an increased oxidative stress in addition to persistent redox imbalance associated with HIV infection during apparently successful HAART. The study by Mandas

and co-researchers[33] also observed that HIV-1 infected patients that showed maximum HAART adherence have a significantly higher oxidative status than those with poorer HAART adherence. Ngondi group, working in Cameroon, reported lower serum concentrations of vitamins A, C and E in HIV-positive patients on HAART and attributed this to the increased oxidative stress often associated with HIV infection and the use of antiretroviral combination therapy that compounds the oxidative stress by increasing lipid peroxidation.[34] Hulgan and co-workers[35] also demonstrated that, oxidant stress was increased during the treatment of human immunodeficiency virus infections.

In this study, significantly decreased serum levels of antioxidant minerals (Cu, Zn, Mn and Se) were demonstrated in both HIV– positive HAART-naïve and HIV-positive on HAART patients compared with HIV negative controls at baseline (Table 6). Others have also reported reduced serum concentrations of antioxidant minerals (Cu, Zn, Mn and Fe) in HIV– positive HAART-naïve and HIV-positive on HAART patients in Sokoto[4] and decrease serum copper and selenium.[36] The possible reasons for the lower serum concentrations of antioxidant minerals at baseline could be due to increased utilization of the antioxidant micronutrients due to increased oxidative stress associated with HIV infection.[32] Antioxidant mineral deficiencies were also attributed to low dietary intake of the nutrients resulting from poverty and ignorance that is widespread in African communities[4], malabsorption and diarrhoea associated with HIV-infection.[1]

In the current study, supplementation with 23 micronutrients significantly increased the serum concentrations of copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) in HIV– positive HAART-naïve and HIV-positive patients on HAART compared with the unsupplemented groups (Table 6). This is in agreement with Jaruga and co-workers[30], who reported that, micronutrients supplementation in HIV-positive subjects partially, restored the activities of antioxidant enzymes, superoxide dismutase and catalase to the levels observed for controls.

Antioxidant vitamins (A, C and E) are free radical scavengers and are continuously being utilised in response to the increased rate of free radical generation due to HIV infection. Antioxidant minerals (Cu, Zn, Mn and Se) are co-factors to the *denovo* antioxidant enzymes and the increase utilization of the enzymes in response to the excessive amount of reactive oxygen species (ROS) production that accompany HIV infection may result into lower serum levels of the minerals in HIV-positive male and female patients compared with the male and

female controls. Among the controls, no difference was found between male and female for serum antioxidant minerals (Cu, Zn, Mn and Se) concentrations. This is consistent with the earlier findings indicating that plasma zinc, selenium and vitamin E levels were similar between males and females[37] and also mean serum copper, zinc and iron were also similar in both sexes.[4]

CONCLUSION

The results in the current study demonstrates that, lower serum concentrations of antioxidant vitamins and minerals observed in HIV-positive HAART-naïve patients and HIV-positive on HAART patients at baseline were reversed on micronutrients supplementation of the subjects. Micronutrients supplementation had improved the general well being and the quality of life of HIV-positive HAART-naïve and HIV-positive patients on HAART in the study area. Additional research should focus on a follow-up study involving a long term randomized control clinical trial of micronutrients supplementation derived from dietary sources or nutriceuticals and compare with the standard of care is needed to fully ascertain the time taken for natural micronutrients to delay the decline in $CD4^+$ count or commencement of ART in HIV/AIDS patients.

Conflicts of interest

The authors disclose no conflict of interest, including any financial, personal or other relationships with people or organizations that could inappropriately influence the finding of this study.

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