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Research Article

Assessment of Antioxidant Micronutrients Levels in HIV/AIDS Patients in Sokoto, North Western Nigeria

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Abstract

Background: HIV/AIDS patients are under chronic oxidative stress and may play a critical role in the pathogenesis and chronic complications of HIV/AIDS. Micronutrients may play an important role in the management of HIV/AIDS patients.

Objective: The current study is aimed at assessing the serum antioxidant micronutrients levels in 50 controls and 200 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. CD4 cell count was enumerated using flow cytometric method, Body mass index (BMI) was calculated using weight (Kg)/height (M2), while serum concentrations of antioxidant minerals, Copper (Cu), Zinc (Zn), Manganese (Mn) and Selenium (Se) were measured using atomic absorption spectrophotometric method.

Results: In HIV-positive HAART-naive the levels of the analytes were: Cu-17.18±0.18 µmol/l, Mn-1.07±0.02 µmol/l, Se-0.28±0.01 µmol/l, Zn-13.45±0.10 µmol/l; CD4 Count-504.69 ±13.51 cells/l and BMI were 23.51±0.44 Kg/m2. In HIV-positive on HAART the levels were: Cu-17.37±0.78 µmol/l, Mn-1.25±0.05 µmol/l, Se-0.28±0.01 µmol/l, Zn-13.60±0.13 µmol/l; CD4-624.73±23.24 cells/l and BMI-25.58±0.55 Kg/m2. In Controls the levels were: Cu-19.97±0.25 µmol/l, Mn-1.68±0.07 µmol/l, Se-0.38 ± 0.01 µmol/l, Zn-16.51±0.21 µmol/l; CD4-952.02±39.15 cells/l and BMI-24.45±0.63 Kg/m2. With the exception of BMI, there were statistically significant differences between the HIV patients and Controls ($p<0.05$). The results indicated that HIV- positive patients were deficient in the vital micronutrients. A further study currently in progress will further evaluate whether supplementation with these nutrients will replenish the body micronutrient contents for improved treatment plans of HIV patients.

Keywords: HIV Patients; Micronutrients; CD4+ Count; BMI

Abbreviation List

Abbreviation	Meaning
AAS	Atomic Absorption Spectrophotometry
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine Transaminase
ANC	Antenatal Clinic
ANOVA	Analysis of Variance
ARV	Antiretroviral
ART	Antiretroviral Therapy
BDH	British Drug House
BMI	Body Mass Index
CAT	Catalase
CD4	Cluster of Differentiation Type 4
CD8	Cluster of Differentiation Type 8
CDC	Centers for Disease Control and Prevention
Cu	Copper
EDTA	Ethylene Diamine Tetra Acetic acid
FMOH	Federal Ministry of Health
FR	Free Radical
GPX	Glutathione Peroxidase
GSH	Glutathione Reduced
GSSG	Glutathione Oxidized
H^+	Hydrogen Ion
OH^-	Hydroxyl Radical
H_2O	Water
H_2O_2	Hydrogen Peroxide
HAART	Highly Active Antiretroviral Therapy
HAART- naïve	Highly Active Antiretroviral Therapy- naïve
HIV	Human Immunodeficiency Virus
Mn	Manganese
$O_2^{\cdot-}$	Superoxide Radical
%	Percentage
POD	Peroxidase
PUFA	Polyunsaturated Fatty Acids
ROS	Reactive Oxygen Species
Se	Selenium
S E M	Standard Error of Mean
SOD	Superoxide Dismutase
TBARS	Thiobarbituric Acid Reducing Substances
UDUTH	Usmanu Danfodiyo University Teaching Hospital
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization
Zn	Zinc

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 CD4+ T-cell depletion [1,5].

Inadequate micronutrients or selected 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]. Inadequate dietary intake resulting from malabsorption and diarrhoea associated with HIV infection-in addition to increased oxidative stress may contributed to antioxidant vitamins and minerals deficiencies in HIV-infected patients in Sokoto, Nigeria [4]. Micronutrients deficiencies have been found to occur at early stages of HIV infection 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. 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 [8]. Superoxide dismutase catalyses the univalent reduction and oxidation of O2.– to H2O2 and molecular O2.

While selenium is an integral part of glutathione peroxidase (GPX) an enzyme located in the mitochondrial matrix and cytosol of animal cells. Glutathione peroxidase (GPX) protect cells against oxidative damage by converting the reduced glutathione (GSH) into oxidized glutathione (GSSG) removing hydrogen peroxide (H2O2) to form water (H2O) [9]. 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 [10- 14].

The current study is aimed at assessing the serum antioxidant micronutrients levels in 200 HIV-positive adult patients attending Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria. This will serve as a baseline data for additional study on the effects of micronutrients supplementation on antioxidant status in HIV-positive adults on highly active antiretroviral therapy and treatment naïve in Sokoto, North Western Nigeria.

Materials and Methods

Study Subjects and Study Site

Enrolment of subjects took place from July to September 2013 at the Antiretroviral Therapy Clinic, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto Nigeria. The subjects were recruited as part of the ongoing study on the effects of micronutrients supplementation on antioxidant status in HIV-positive adults on highly active antiretroviral therapy and treatment naïve in Sokoto, North Western Nigeria. A total of two hundred (200) subjects were enrolled into the study. These consisted of 100 newly diagnosed HIV-positive HAART-naïve and 100 HIV-positive on HAART. The controls comprised of 50 gender, age and socioeconomically-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 treatment with HAART. 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 are 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.

Study Design

The study was a cross sectional study where consenting eligible HIV-positive male and female patients attending Antiretroviral Therapy Clinic of Usmanu University Teaching Hospital, Sokoto were enrolled into the study. 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 [15].

At enrolment a structured interviewer-administered questionnaire was administered to each patient and information on patient's demographic and HIV- related characteristics including gender, age, marital status and stage of HIV infection were obtained.

Eligible subjects were assigned to the following groups:

Group A (n=50): HIV-negative controls

Group B (n=100): HIV-positive HAART-naïve

Group C (n=100): HIV-positive on HAART

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.

Blood Samples Collection and Processing

A total of 5 millilitres (ml) of blood were collected from each subject. Out of this, 3ml was 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°C until assayed in batches; for serum levels of antioxidant minerals, while 2ml of the blood was collected into an EDTA blood specimen bottles from BDH Laboratory supplies, United Kingdom and used to enumerate the CD4+ within 4 hours of sample collection.

Estimation of CD4+and Antioxidant Mineral Elements

Serum antioxidant mineral element, Cu, Zn, Mn and Se were measured using atomic absorption Spectrophotometry (AAS) as described by Kaneto [16], 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. CD4 cell count was enumerated by flow cytometry, using Cyflow Counter manufactured by Partec, Munster, Germany, while BMI was calculated using weight (Kg)/height (M2).

Statistical Analysis

The data obtained was analysed using Microsoft Office Excel 2007 and Graphpad InStat® statistical soft ware Version 3.10, 32 Bit for windows (2009). 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 0.0.05 ($P \leq 0.05$) was considered as significant.

Results

The demographic and HIV-related characteristics of the study population were presented in Table I. The result indicates that, majority of the HIV-infected patients are married and about 56.7% of the study population are in the stage I of HIV infection. Table 2 showed the CD4+ cell count and anthropometric parameters in HIV-positive patients and controls. The mean CD4+ cell count in HIV-positive HAART-naïve (504.69 ±13.51cells/µl) and HIV-positive patients on HAART (624.73±23.24 cells/µl) were significantly lower ($P<0.001$) than the corresponding values in controls (952.02±39.15 cells/µl). The mean serum levels of the antioxidant mineral elements in controls and HIV-positive patients were presented in Table 3. The mean serum concentrations of Cu (17.18±0.18 µmol/l), Zn (13.45±0.10 µmol/l), Mn (1.07±0.02 µmol/l) and Se (0.28±0.01 µmol/l) in HIV-positive HAART-naïve and mean serum Cu (17.37±0.78 µmol/l), Zn (13.45±0.14 µmol/l), Mn (1.25±0.05 µmol/l) and Se (0.28±0.01 µmol/l) in HIV-positive patients on HAART were significantly lower ($P<0.001$) than the corresponding values in controls.

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. CD4+ Cell Count and Anthropometric Parameters in HIV-Positive Patients and Controls.

Characteristics	Group A (n=50)	Group B (n=100)	Group C (n=100)
Male	22	45	46
Female	28	55	54
Age(Years)	34.14±1.44	33.06±1.04	33.02±1.12
Body Weight(Kg)	68.68±1.76	65.51±1.59	65.18±1.63
Height(m)	1.61±0.01	1.65±0.01	1.65±0.01
BMI (Kgm^{-2})	24.45±0.63	23.51±0.44	25.58±0.55
CD4 (cells/µl)	952.02±39.15	504.69 ±13.51 [a1]	624.73±23.24[a2]

Values are mean ± SEM measured at baseline; n= number of subjects; BMI= body mass index; CD4=cluster of differentiation; Significant differences: **a1** (P<0.001) = Control versus Group B; **a2** (P<0.001) = Control versus Group C.

GROUP A = HIV – Negative Control

GROUP B = HIV – Positive HAART-naïve

GROUP C = HIV – Positive on HAART

Table 3. Serum Levels of Antioxidant Minerals in HIV-positive Patients and Controls.

Characteristics	Group A (n=50)	Group B (n=100)	Group C (n=100)
Copper (µmol/l)	19.97±0.25	17.18±0.18[a1]	17.37±0.78[a2]
Zinc (µmol/l)	16.51±0.21	13.45±0.10 [a1]	13.45±0.14 [a2]
Manganese (µmol/l)	1.68±0.07	1.07±0.02[a1]	1.25±0.05[a2]
Selenium (µmol/l)	0.38 ± 0.01	0.28± 0.01 [a1]	0.28±0.12 [a2]

Values are mean ± SEM measured at baseline; n= number of subjects. Significant differences: **a1** (P<0.001) = Control versus Group B; **a2** (P<0.001) = Control versus Group C

GROUP A = HIV – Negative Control

GROUP B = HIV – Positive HAART-naïve

GROUP C = HIV – Positive on HAART

Discussion

Previous study reported by our research group [4] indicated a deficiency of antioxidant vitamins and minerals in Sokoto. As a follow- up to that study, baseline serum concentrations of antioxidant mineral elements (Cu, Zn, Mn and Se) and CD4+ cell count were measured and the results presented here were part of the ongoing study on the effects of micronutrients supplementation on antioxidant status in HIV-positive adults on highly active antiretroviral therapy and treatment naïve in Sokoto, North Western Nigeria. In this case, we also measured selenium instead of iron because of the pro-oxidant effect of iron if used in supplementation studies.

The finding of 33.77±0.55 years as mean age of HIV-positive patients and 66.5% (133/200) 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 (Table 1). 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 [17].

Females formed the majority of the HIV-infected patients and constituted 54.5% (109) of the total group (200), while males constitute 45.5% (91) of the total group (Table 1). 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 [18]. Similar findings were also recorded in Sub-Saharan Africa where women constituted about 59% of the adults' population living with HIV/AIDS in 2010 [18]. This also compares favourably with the reported cases in Northern Nigeria where about 53.2% of women were reported infected with HIV [19].

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). 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 [14].

The result of this study showed a decreased CD4+ cell count in HIV-positive HAART-naïve and HIV-positive on HAART patients compared with controls. This is in agreement with the reports of previous studies [4, 13, 20] that reported similar decrease in CD4+cell count at baseline.

Significantly decrease 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 (Table 3). 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]. 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 [20].

Conclusion

These results indicated that HIV-positive patients were deficient in the vital micronutrients. A further study currently in progress will further evaluate whether supplementation with these nutrients will replenish the body micronutrient contents for improved treatment plans of HIV 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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