ORIGINAL ARTICLE

SERUM ANTIOXIDANT VITAMINS AND MINERALS AMONG HIV-POSITIVE HAART-NAIVE PATIENTS AT DIFFERENT STAGES OF CD4+ CELL COUNTS IN SOKOTO, NIGERIA.

Wasagu IZ Bunza FU

Department of Chemical Pathology, Faculty Medical Laboratory Sciences,
Usmanu Danfodiyo University, Sokoto, Nigeria.

Correspondence and reprint request to: **Ibrahim Zubairu Wasagu,**
Department of Chemical Pathology, Faculty Medical Laboratory Sciences,
Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.
eMail: ibrahimzubairu7@gmail.com **Phone No.:** +2347064565488.

ABSTRACT

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Background: Human Immunodeficiency Virus (HIV) belongs to retrovirus family. Infection with HIV induces chronic oxidative stress resulting from excessive production of free radicals. The excessive amounts of free radicals have the potential to cause oxidative damage to cells and tissues in humans. **Objectives:** To assess the serum levels of antioxidant vitamins and minerals in HIV-positive HAART-naive patients at different stages of CD_4^+ cell counts and controls in Sokoto, Nigeria. **Method:** This cross-sectional study was conducted on 96 HIV-positive HAART-naive patients and 32 age- and sex- matched HIV-negative controls. Plasma levels of CD_4^+ cell counts were assessed using Flow Cytometry while serum antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) were assessed using Spectrophotometry and Atomic Absorption Spectrophotometry respectively. The HIV-positive HAART-naive patients were divided into three groups based on CD_4^+ count. **Results:** Majority of the HIV-infected patients in the study population were married (70.3%) and predominantly Hausas (71.9%). Compare to the HIV negative control, the three groups of HIV-positive HAART-naïve patients have statistically different serum levels of antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn). Also there was statistically significant difference in the serum levels of antioxidants vitamins (A, C and E) and minerals (Cu, Zn and Fe) between males and females in both the HIV-positive HAART-naïve patients and the controls. **Conclusion:** The serum levels of antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) were significantly different between HIV-positive HAART-naive patients and HIV negative control.

Keywords: Antioxidant, Vitamins, Minerals, Human Immunodeficiency Virus

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a member of the retrovirus family that causes Acquired Immunodeficiency Syndrome (AIDS).[1] Human Immunodeficiency Virus (HIV) remains a global health problem of unprecedented dimensions. Globally, 36.7 million people were infected with HIV, 1.8 million people became newly infected and 1 million people died from AIDS-related illness in 2016. In 2016, 3.4 million Nigerians were infected with HIV and HIV prevalence rate in Sokoto was 6.4% respectively. [2]

Infection with HIV induces chronic oxidative stress resulting from excessive production of reactive oxygen species (ROS) or "free radicals".[3] The excessive amounts of free radicals have the potential to cause oxidative damage to cells and tissues in humans.[4] Oxidative stress linked to apoptosis of T-Lymphocytes during HIV disease.[5] Increased oxidative stress and weakened antioxidant defense system in HIV-positive patients were also observed.[6]

Studies have indicated the significant function of micronutrients in antioxidants defence.[7] 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.[8,9]

Observational studies in Baltimore, Washington DC among HIV-positive persons that are yet to receive highly active antiretroviral therapy (HAART) showed that low or deficient serum concentrations of several micronutrients including thiamine, selenium, zinc and vitamins A, C and E to be individually associated with either low CD_4^+ cell counts, advanced HIV-related diseases, faster disease progression or HIV-related mortality.[10,11,12] Among the cross-sectional studies, concentrations of -tocopherol, vitamin A and folate but not of vitamin A, zinc or selenium were significantly higher in HIV-positive receiving HAART.[13]

It showed that antioxidants vitamins and minerals deficiencies are common among HIV-infected patients, especially in those who are less privileged.[14] Micronutrients play important roles in maintaining immune function and neutralizing the reactive oxygen intermediates produced by activated macrophages and neutrophils. CD_4^+ cell counts are helper lymphocytes which are preferentially destroyed by HIV, thus the level of CD_4^+ cell count is reduced with the severity of HIV infection.[4] Micronutrient deficiencies in HIV-infected individuals exacerbate immune suppression, oxidative stress, acceleration of HIV replication and CD_4^+ cell count depletion. The main objective of this study was to assess the serum levels of antioxidants vitamins and minerals among HIV-positive HAART-naïve patients and controls at different stages of CD_4^+ cell counts in Sokoto, Nigeria.

MATERIALS AND METHOD

Study Population

The target populations were adults aged 18 to 50 years who were presenting to the Antiretroviral Therapy Clinic (ART) of Specialist Hospitals, Sokoto. A total of 128 subjects which consisted of 96 HAART-naive people living with HIV infection and 32 age- and sex-matched controls were recruited for this study.

Inclusion Criteria

Human Immunodeficiency Virus (HIV) HAART-Naïve patients that were 18 to 50 years old both males and females who have no clinical conditions likely to affect serum concentrations of antioxidant vitamins and minerals.

Exclusion Criteria

The HIV HAART-Naïve patients who have clinical conditions likely to affect serum concentrations of antioxidant vitamins and minerals such as liver disease, gastroenteritis, renal disease and other related conditions were excluded from the study. Pregnant women and cigarette smokers were also excluded from this study.

Sample Size Determination

The sample size for the study was determined using the formula below.[15]

$$n = \frac{(Z^2 Pq)}{d^2}$$

Sampling Technique.

A convenient sampling technique was utilized where HIV- positive patients attending Antiretroviral Therapy Clinic (ART), and those apparently healthy persons (control) were recruited consecutively for the study until the desired sample size is attained.

Ethical Consideration

Approval for the study was obtained from the Ethics and Research Committee of SMOH, Sokoto in accordance with the Helsinki declaration. Consequently, the following ethical issues were observed in the conduct of the study.

Study Design

It is a cross sectional study design. This study was conducted on ninety six (96) HAART-naïve people living with HIV infection attending the Antiretroviral Therapy Clinic of the Specialist Hospital, Sokoto. The controls comprised of thirty two (32) age- and sex- matched apparently healthy individuals drawn from staff of specialist Hospital, Sokoto and University Community. The subjects were grouped as follows:

Group I: [n=32]: HIV-negative (controls)
Group II: [n=32]: HIV-positive HAART-naïve with CD^+_4 cell count $\geq$ 500 cells/µl
Group III: [n=32]: HIV-positive HAART-naïve with CD^+_4 cell count, 200-499 cells/µl
Group IV: [n=32]: HIV-positive HAART-naïve with CD^+_4 cell count < 200 cells/µl

Blood Samples Collection and Processing

From each subject 5 ml of venous blood sample was collected using a sterile disposable syringe and

needle. The blood (4 ml) was dispensed into a plain specimen bottle and allowed to clot at room temperature after which it was centrifuged at 3000 rpm/min for 5 minutes to obtain à clear serum. The sera was harvested into sterile serum bottles and stored at - 20°C until the time for analysis.

The remaining 1 ml of the blood was transferred into a sterile EDTA specimen bottle and used for the enumeration of CD_4^+ cell count within 3 hours of the blood collection.

ANALYTICAL METHODS

HIV Screening for Patients and Controls

This was carried out using the World Health Organization (WHO) screening criteria for developing countries, which entails the screening with two different rapid screening methods:

i. **HIV test using Stat Pak kit**

ii. **HIV rapid screening test using Determine HIV1/2 rapid screening kit.**

CD_4^+ Cell Counts for Patients and Controls

The CD_4^+ cell counts were counted using Cyflow counter[16].

Measurement of Serum Antioxidants Vitamins (A, C & E) and Minerals (Cu, Fe and Zn) for Patients and Controls

The serum antioxidant vitamins (A, C and E) were estimated based on the method described by Bessey *et al*.[17], Natelson[18] and Hashim and Schuttringer[19] respectively.

The serum antioxidant minerals (Cu, Fe and Zn) were estimated using atomic absorption spectrophotometer (AAS) by the method of Steward *et al*.[20].

Statistical Analysis

Each parameter of CD_4^+ cell count and serum antioxidants vitamins and minerals were analyzed and expressed as mean plus/minus standard error of the mean (mean SEM). The results were analyzed using analysis of variance (ANOVA) for comparisons of three (3) more mean values and multiple comparisons was carried were carried out least significant difference (LSD). Paired two-tailed student's t-test was used for matched samples. A p-value less than or equal to 0.05 ($p \leq 0.05$) was regarded as statistically significant. SPSS version 20 and Microsoft office excel were used for the analysis. Data on CD_4^+ cell count and serum levels of antioxidants vitamins and minerals were also presented in form of tables.

RESULTS

The results of the demographic and HIV related characteristics of the study population were presented in Table 1. The results indicated that the majority of the HIV-infected patients in the study population were married (70.3%) and were predominantly Hausa.

The results of the serum levels of antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) of the HIV-positive HAART-naïve patients were presented in Table 2. Compare to the HIV negative control, the three groups of HIV-positive HAART-naïve patients have statistically significant lower serum levels of antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***)

The results of CD_4^+ cell counts and antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) as delineated on the basis of gender of the HIV-positive HAART-naïve patients and HIV-negative (Controls) were presented in Table 3. There were statistically significant difference at ($p < 0.05$) (*), $p < 0.01$ (**) in the mean CD_4^+ cell count, antioxidants vitamins (A, C and E) and minerals (Cu, Zn and Fe) between males and females in both the HIV-positive HAART-naïve patients and the controls.

There is a positive correlation between CD_4^+ cell counts and serum antioxidants vitamins and minerals at different stages of CD_4^+ cell counts in HIV-positive HAART-naive patients which was statistically significant in serum level of zinc and the HIV-positive HAART-naïve patients group III and serum level of Vitamin E and the HIV-positive HAART-naïve patients group IV. This is shown in Table 4.

Two hundred and thirty six patients had SAB for Caesarean section out of which 52 (22.03%) patients had PDPH. The mean age of patients was 30.98 ± 6.34 years. The highest incidence of PDPH was within the age group 31-40 with 29 (24.58%) patients. Age group 15-19 years did not record any incidence, though only 4 patients belonged to this group as shown in Table 1

One hundred and thirty four (56.78%) of the 236 patients were done as emergencies and 36 (26.86%) of them had PDPH; 16 (15.67%) of the 102 elective cases had headaches as shown in Table 2.

Twenty nine (34.52%) patients who had 2 attempts at lumbar puncture before success had PDPH. The PDPH rate among those who had 4 attempts was 2(25 %) as shown in Table 3.

All the subarachnoid blocks were performed with the Quincke needle type. Size 24G was the most commonly used; out of the 116 patients who had lumbar puncture with this needle, 27 (23.28%) developed headaches. A similar percentage (43; 23.26%) of headache was seen in those in whom 23G was used. Sizes 26G surprisingly had the highest incidence of PDPH (8, 26.93%) as shown in Table 4.

The headaches were mild and moderate in 13 (61.9%) and 8 (38.1%) patients respectively as shown in fig 1. None of the patients complained of severe PDPH. All the patients who had PDPH did well on oral fluids and analgesics; none of them required epidural blood patch.

Table 1: Demographic and HIV Related Characteristics of the Study Population

Characteristics	**No. of Subjects**	**Percentages (100%)**
Marital status	**128**	**100**
Married	90	70.3
Single	22	17.2
Widowed	10	7.8
Divorced	6	4.7
Tribe	**128**	**100**
Hausa	92	71.9
Fulani	12	9.4
Igbo	18	14.1
Yoruba	6	4.6
Stages of CD_4^+ Cell Count	**96**	**75**
Stage I	32	25
Stage II	32	25
Stage III	32	25

The majority of the HIV-infected patients in the study population were married (70.3%) followed by single (17.2%), the subjects were predominantly Hausa.

Table 2: Serum Levels of Antioxidant Vitamins and Minerals among HIV-Positive HAART-naïve Patients at Different Stages of CD_4^+ Cell Counts.

Parameters	**Group II (n=32)**	**Group III (n=32)**	**Group IV (n=32)**
Vit A (µmol/L)	0.96 ± 0.023*	0.52± 0.022**	0.26±0.012***
Vit C (µmol/L)	20.65 ± 1.12**	10.20 ± 0.50***	6.37± 0.24***
Vit E (µmol/L)	8.66 ± 0.43*	5.06 ± 0.27***	3.61± 0.21***
Cu (µmol/L)	14.67 ± 0.39*	9.72 ± 0.33**	13.21 ± 0.43*
Zn (µmol/L)	6.60 ± 0.35*	3.87 ± 0.34**	4.51 ± 0.33**
Fe (µmol/L)	14.74 ± 0.35**	12.27±0.34***	11.85±0.30**

Key:
Group II = HIV-positive HAART-naive patients with CD_4^+ cell count > 500 cells/µl
Group III = HIV-positive HAART-naive patients with CD_4^+ cell count 200-499 cells/µl
Group IV = HIV-positive HAART-naive patients with CD_4^+ cell count < 200 cells/µl

Values are mean ± SEM, n = number of subjects, HIV = human immunodeficiency virus; HAART = highly active antiretroviral therapy; CD_4^+ cell count = cluster differentiation type 4; Vit A = vitamin A; Vit C = vitamin C; Vit E = vitamin E; Cu = Copper; Fe = Iron; Zn = Zinc. All the parameters differ significantly ($p < 0.05$), using ANOVA. The values bearing asterisk differ significantly with the control (HIV negative) at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) respectively.

Table 3: Effect of Gender on CD_4^+ Cell Counts and Serum Antioxidant Vitamins and Minerals in HIV-positive HAART-naïve Patients and HIV-negative Controls

Parameters	HIV^- Controls		HIV^+ HAART-naive Patients	
	Male (n=16)	Female (n=16)	Male (n=48)	Female (n=48)
CD_4^+ Cell Count(cells/µL)	906.00±52.74	798.13 ± 37.05**	358.46±12.62	326.02±12.74**
Vit A (µmol/L)	1.80 ± 0.053	1.66 ± 0.049*	0.59 ± 0.027	0.56 ± 0.026*
Vit C (µmol/L)	43.83 ± 1.04	41.06 ± 0.70*	13.00 ± 0.96	11.78 ± 0.73*
Vit E (µmol/L)	15.62 ± 0.53	15.19 ± 0.49*	6.09 ± 0.45	5.47 ± 0.40*
Cu (µmol/L)	20.54 ± 0.88	17.73 ± 0.53**	13.53 ± 11.36	11.54 ± 0.58*
Zn (µmol/L)	16.92 ± 0.37	14.89 ± 0.42*	5.98 ± 0.41	4.01 ± 0.42*
Fe (µmol/L)	27.82 ± 0.46	25.54 ± 0.33*	14.04 ± 1.32	11.86 ± 0.31*

Values are mean ± SEM; n = number of subjects; CD_4^+ cell count = cluster differentiation type 4; M = male; F = female; HIV = human immunodeficiency virus; HAART = highly active antiretroviral therapy; HIV^+ = HIV positive; HIV^- = HIV negative; there were statistically significant difference at ($p < 0.05$) (*), $p < 0.01$ (**) in the mean CD_4^+ cell count, antioxidants vitamins (A, C and E) and minerals (Cu, Zn and Fe) between males and females, using paired two-tailed student t-test.

Table 4: Correlation Coefficient (r) Between CD_4^+ Cell Counts and Antioxidant Vitamins and Minerals in HIV-positive HAART-naïve Patients

Parameters	Group II (r)	Group III (r)	Group IV (r)
Vit A	0.075	0.065	0.057
Vit C	0.018	0.339	0.177
Vit E	0.117	0.104	0.498**
Cu	0.042	0.126	0.116
Zn	0.265	0.415*	0.044
Fe	0.121	0.124	0.109

Key:
Group II = HIV-positive HAART-naive patients with CD_4^+ cell count > 500 cells/µl
Group III = HIV-positive HAART-naive patients with CD_4^+ cell count 200-499 cells/µl
Group IV = HIV-positive HAART-naive patients with CD_4^+ cell count < 200 cells/µl

CD^+_4= cluster differentiation type 4; HIV= human immunodeficiency virus; HAART = highly active antiretroviral therapy; Vit A = vitamin A; Vit C = vitamin C; Vit E = vitamin E; Cu = Copper; Fe = Iron; Zn = Zinc. The correlation coefficient (r) values bearing (*) and (**) differ significantly at $p < 0.05$ and $p < 0.01$ respectively, using Pearson's linear correlation analysis.

DISCUSSION

This study showed that antioxidant vitamins and minerals (micronutrients) deficiencies are common among HIV-infected individuals.[14] In this study, the significantly ($p < 0.05$) lower serum levels of antioxidant vitamins (A, C and E) in HIV-positive HAART-naïve patients was in agreement with the previous studies.[21,22,23,24] The lower levels of these antioxidant vitamins may be due to mal-absorption and diarrhea that are common complications of HIV/AIDS. The combination of malnutrition, mal-absorption due to gastro-enteritis, diarrhea and excessive free radicals generation could account for the significantly lower levels of antioxidant vitamins (A, C and E) in HIV-positive HAART-naïve patients. Also there were measurable difference between the values obtained in the HIV-positive HAART-naïve patients (Group II, III and IV) when compared with the different stages of CD_4^+ cell count.

Copper, iron and zinc are essential antioxidants minerals that are required in minute amount for proper health of the body. They play important roles in the activities of several antioxidants enzymes that protect the cells against highly toxic reactive oxygen species and also enhance the immunologic activities of phagocytes and lymphocytes.[25] In this study, the serum concentrations of antioxidant minerals (Cu, Fe and Zn) measured in HIV-positive HAART- naïve patients were significantly ($p < 0.05$) lower compare to those in HIV negative control. This is in agreement with the previous studies.[21,22]

The mean CD_4^+ cell counts in HIV-positive HAART-naive patients was significantly decreased in females than males.

The mean serum levels of antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) in HIV-positive HAART-naïve patients when compared with controls were significantly decreased in females than males. This was in agreement with previous study[21]. There were statistically significant differences ($p < 0.05$) in the mean CD_4^+ cell count and serum levels of antioxidant vitamins (A, C and E) between males and females.

The finding of a significantly positive correlation between CD_4^+ cell counts and serum antioxidant vitamins and minerals meant that, a decreased in CD_4^+ cell counts in HIV-positive HAART-naïve patients is accompanied with a decreased in antioxidant vitamins and minerals. These findings are consistent with previous authors who reported positive correlation for the parameter with vitamin E, Zn and Cu showed significantly ($p < 0.05$) positive correlation in HIV-positive HAART-naïve patients.[21]

CONCLUSIONS

The serum concentrations of antioxidant vitamins (A, C and E) and minerals (Cu, Fe and Zn) were significantly decreased in HIV-positive HAART-naive patients. The serum concentrations of antioxidant vitamins and minerals were significantly decreased in females than males of the HIV-positive HAART-naive patients and controls. There is a positive correlation between CD_4^+ cell counts and serum antioxidants vitamins and minerals at different stages of CD_4^+ cell counts in HIV-positive HAART-naive patients.

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