

# Serum levels of antioxidant vitamins and mineral elements of human immunodeficiency virus positive subjects in Sokoto, Nigeria

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## Abstract

**Background:** Undernourishment and micronutrient deficiencies exacerbate immunosuppression, oxidative stress, acceleration of human immunodeficiency virus (HIV) replication and CD4 T-cell depletion in HIV-infected individuals.

**Materials and Methods:** The current work reports the serum levels of antioxidant vitamins (vitamins A, C and E) and minerals (Zn, Fe, Cu) in 90 HIV positive subjects attending the Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria. The serum levels of the micronutrients were correlated with the CD4 count of the subjects.

**Results:** The results showed that the HIV positive subjects have significantly lower ( $P < 0.05$ ) levels of vitamins A, C and E. Also, serum Zn, Fe, Cu and CD4 count were also significantly ( $P < 0.05$ ) lower compared with the HIV negative subjects. Micronutrient deficiencies were more pronounced in HIV positive subjects with CD4 counts less than 200 cell/ $\mu$ l. The results based on age and sex showed no significant ( $P > 0.05$ ) difference. Vitamins A, E and C and Zn and Fe showed positive correlation with CD4 count of the HIV positive subjects.

**Conclusion:** The results suggest that the HIV subjects in the study area have lowered serum levels of antioxidant micronutrients and that the levels decrease with increase in the severity of the infection. These may increase the chances of the symptomatic and asymptomatic subjects progressing into full-blown Acquired Immunodeficiency Syndrome.

**Keywords:** Antioxidant, HIV, Nigeria

## Résumé

**Arrière-plan:** Sous-alimentation et les carences en micronutriments exacerbent immunosuppression, stress oxydant, accélération de la réplication du virus de l'immunodéficience humaine (VIH) et l'appauvrissement de la cellule T CD4 de personnes infectées par le VIH.

**Matériel et méthodes:** Travail le courant signale les niveaux de sérum de vitamines antioxygènes (vitamines A, C et E) et les minéraux (Zn, Fe, Cu) dans 90 sujets positives au VIH qui fréquentent le Usmanu Danfodiyo University Hospital de l'enseignement (UDUTH), Sokoto, Nigeria. Les niveaux de sérum des micronutriments étaient en corrélation avec le nombre de CD4 du sujets.

**Résultats:** Les résultats ont montré que les sujets de positifs au VIH ont sensiblement inférieur ( $P < 0,05$ ) niveaux de vitamines A, C et e. Aussi, sérum Zn, Fe, Cu et de CD4 nombre étaient également significativement ( $P < 0,05$ ) inférieur comparé avec les sujets négatifs du VIH. Carences en micronutriments étaient plus prononcées dans les Séropositifs sujets avec CD4 compte moins de 200 cellules/ $\mu$ l. Les résultats basés sur l'âge et le sexe a montré non significatifs ( $P > 0,05$ ) différence. Vitamines A, E et C et Zn et Fe a montré une corrélation positive avec nombre de CD4 du VIH positif sujets.

**Conclusion:** Les résultats suggèrent que les sujets du VIH dans le domaine de l'étude ont abaissé sérique de micronutriments antioxygènes et que les niveaux diminuent avec l'augmentation de la gravité de l'infection. Ces peuvent accroître les chances des sujets symptomatiques et asymptomatiques progresse en véritable immunodéficience acquise syndrome.

**Mots-clés:** Antioxygènes, le VIH, Nigeria

## Introduction

One of the greatest challenges of the 21<sup>st</sup> century is human immunodeficiency virus (HIV), the causative agent of the Acquired Immunodeficiency Syndrome (AIDS),<sup>[1]</sup> which has defied treatment a couple of decades since it was discovered and identified. The toll of HIV-AIDS is more on the sub-Saharan Africa, which is reported to have 50% (28.5 million) of all the people living with the virus in the world.<sup>[2]</sup>

Malnutrition is one of the major complications of HIV infection and a significant factor in the progression of the infection into full-blown AIDS. Oxidative stress induced by the production of reactive oxygen species (ROS) may play a critical role in the stimulation of HIV replication and the development of immunodeficiency.<sup>[3]</sup> Excessive production of ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide may be related to an increased activation of polymorphonuclear leukocytes during infections or influenced by the prooxidant effect of tumor necrosis factor  $\alpha$  produced by activated macrophages during the course of HIV infection.<sup>[4]</sup> The debilitating effect of ROS can be prevented or moderated by a normal antioxidant defense largely provided by firstly the integrity of an enzymatic system that requires adequate intake of trace minerals such as selenium, copper, zinc, and manganese, and secondly adequate concentrations of vitamin E, A, and C and  $\beta$ -carotene in the cytoplasm and lipid membrane of the cells.<sup>[3]</sup>

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.<sup>[5]</sup> These deficiencies have been shown to be associated with more frequent opportunistic infections, faster disease progression, and a greater incidence of HIV-related mortality.<sup>[6,7]</sup> Possible mechanisms include increased intracellular oxidative stress, enhanced viral replication, and a reduction in the number of circulating CD4 lymphocytes associated with individual or accumulated nutrient deficiencies.<sup>[8]</sup> These mechanisms, alone or in part, may contribute to the increased morbidity, more rapid disease progression, and the higher mortality seen in HIV-infected patients with nutrient deficiencies.

There is, however, dearth of information with respect to the micronutrient status of HIV positive subjects in Sokoto, Nigeria. The current work reports the serum levels of antioxidant vitamins and minerals of HIV subjects from Sokoto, Nigeria.

## Materials and Methods

### Subjects

Ninety HIV positive subjects attending the antiretroviral clinic of Usmanu Danfodiyo University Teaching Hospital (UDUTH) were recruited for the current study. The consent of each of the subjects was sought and obtained before including them in the study. The HIV positive subjects were divided into three groups: Group 1 comprised those with CD4 count  $>500$  cells/ $\mu$ l, Group 2 had those individuals whose CD4 count was between 200 and 499 cells/ $\mu$ l and Group 3 individuals had CD4 count  $<200$  cells/ $\mu$ l. Only subjects who presented to the clinic for the first time and were not on any orthodox medication were included in the current study. A total of 25 apparently healthy, sero-negative, age-matched subjects drawn from patients on routine medical check-up, staff and students of the hospital constituted the control group.

### Sample collection

Approximately 6 ml of venous blood was collected from the subjects using standard blood collection technique and centrifuged. The serum was separated into clean labeled sample bottles and stored frozen at  $-20^{\circ}\text{C}$  for further analysis.

### Human immunodeficiency virus screening

HIV screening 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

### CD4 Count

The CD4 were counted using CYFLOW-3.

### Determination of serum vitamins

Vitamin A was estimated by a modification of the spectrophotometric method described by Neild and Pearson.<sup>[9]</sup> In this method, the conjugated double bonds of vitamin A were made to react with trifluoroacetic acid (TFA), forming a faint, short-lived, blue compound that can be read spectrophotometrically. A correction factor to account for carotene's reaction with TFA was worked out.<sup>[9]</sup> Vitamin E was determined by a modification of the micro method described by Quaife and co-workers;<sup>[10]</sup> in which tocopherols reduce ferric to ferrous ions. Carotenoids interfere with the assay and thus absorbance due to carotenes is subtracted. Serum ascorbic acid was determined by the method of Lowry *et al.*<sup>[11]</sup>

### Estimation of serum copper, zinc and iron levels

Serum levels of copper, zinc and iron were estimated using atomic absorption spectrophotometer (AA-6300).

### Statistical analysis

The data were presented as mean  $\pm$  standard error of the mean. The results were analyzed using analysis of variance (ANOVA) and multiple comparisons carried out using LSD. Significant difference was considered at  $P < 0.05$ . SPSS version 10 was used for the analyses.

### Results

The result of the CD4 count and serum levels of micronutrient of the HIV positive subjects are presented in Table 1. The results indicated significant decrease in all the micronutrients examined.

The results were delineated on the basis of age and sex of the HIV positive subjects and are presented in Tables 2 and 3, respectively. Age and sex appeared not to be factors in determining the severity of micronutrient deficiencies in the HIV subjects in the study area.

CD4 count of the HIV positive subjects were correlated with the serum micronutrient levels and the regression coefficients ( $r$ ) are presented in Table 4. The results indicated positive correlation for all the parameters with vitamin E, Zn and

Cu showing significant ( $P < 0.05$ ) positive correlations.

### Discussion

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.<sup>[6]</sup> These deficiencies have been shown to be associated with more frequent opportunistic infections, faster disease progression, and a greater incidence of HIV-related mortality.<sup>[7,12]</sup>

Although serum and plasma levels of micronutrients are assumed to be imperfect indicators of body fluids, it has been shown that their deficiencies are common among HIV-infected persons.<sup>[13]</sup> Micronutrients play important roles in maintaining immune function and neutralizing the reactive oxygen intermediates produced by activated macrophages and neutrophils.

Lower level of vitamin E in HIV patients compared with controls is a result of its increased utilization in quenching free radicals. Poor dietary intakes, poor absorption and diarrhea in HIV subjects may contribute to the reduced level of vitamin E and other micronutrients in the HIV subjects of the subject area. It is also possible that the recycling mechanism of biologically active vitamin E through ascorbate is impaired in HIV patients. Coutsoudis *et al.*<sup>[14]</sup>

**Table 1: Serum levels of antioxidant vitamins and some microelements of HIV positive subjects based on CD4 count**

Parameter#	HIV with CD4 count >500 cells/ $\mu$ l (n = 7)	HIV with CD4 count 200–499 cells/l (n = 46)	HIV with CD4 count <200 cells/ $\mu$ l (n = 47)	Controls (n = 25)
Age (years)	29.33 $\pm$ 3.49	30.89 $\pm$ 1.37	29.02 $\pm$ 1.55	29.32 $\pm$ 2.05
CD <sub>4</sub> count (cell/ $\mu$ l)	558.17 $\pm$ 15.43*	317.83 $\pm$ 11.76***	112.85 $\pm$ 6.68***	856 $\pm$ 46.21
Vitamin A ( $\mu$ g/dl)	5.78 $\pm$ 1.20***	8.52 $\pm$ 1.05***	6.15 $\pm$ 0.77***	46.67 $\pm$ 1.33
Vitamin C (mg/dl)	0.63 $\pm$ 0.12	0.73 $\pm$ 0.07	0.84 $\pm$ 0.18	0.890 $\pm$ 0.05
Vitamin E (mg/dl)	0.41 $\pm$ 0.03*	0.34 $\pm$ 0.02*	0.32 $\pm$ 0.02*	0.885 $\pm$ 0.06
Zinc ( $\mu$ g/dl)	23.97 $\pm$ 6.42**	0.34 $\pm$ 2.75***	9.86 $\pm$ 1.76***	84.34 $\pm$ 1.66
Iron ( $\mu$ g/dl)	50.23 $\pm$ 7.61*	57.97 $\pm$ 3.43*	51.54 $\pm$ 2.97*	84.207 $\pm$ 1.86
Copper ( $\mu$ g/dl)	38.07 $\pm$ 4.06**	32.67 $\pm$ 2.19**	37.82 $\pm$ 1.85**	80.655 $\pm$ 1.53

Values are mean  $\pm$  SEM; n = number of subjects, #All the parameters differ significantly ( $P < 0.05$ ), except the age, using ANOVA, The values bearing asterisk differ significantly with the respective control at  $P < 0.05$ (\*);  $P < 0.01$ (\*\*); and  $P < 0.001$ (\*\*\*)

**Table 2: Serum levels of antioxidant vitamins and some microelements of HIV positive subjects based on age**

Age class (years)	n	Vitamin A ( $\mu$ g/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Zn ( $\mu$ g/dl)	Fe ( $\mu$ g/dl)	Cu ( $\mu$ g/dl)
11–20	17	12.8 $\pm$ 2.62	0.65 $\pm$ 0.08	0.39 $\pm$ 0.01	14.32 $\pm$ 6.02	59.56 $\pm$ 4.61	34.57 $\pm$ 4.1
21–30	34	13.9 $\pm$ 3.65	0.74 $\pm$ 0.05	0.37 $\pm$ 0.01	18.21 $\pm$ 5.67	64.92 $\pm$ 5.77	35.92 $\pm$ 2.54
31–40	26	13.8 $\pm$ 3.02	0.60 $\pm$ 0.09	0.34 $\pm$ 0.01	19.42 $\pm$ 3.01	66.11 $\pm$ 3.11	34.17 $\pm$ 5.60
41–60	13	13.5 $\pm$ 2.12	0.70 $\pm$ 0.14	0.36 $\pm$ 0.02	20.51 $\pm$ 6.97	58.15 $\pm$ 7.94	39.49 $\pm$ 6.55

n = number of subjects

**Table 3: Serum levels of antioxidant vitamins and some microelements of HIV positive subjects based on sex**

Parameter	Male (n = 40)	Female (n = 50)	Total (n = 90)
Age (years)	31.57 ± 10.46	28.55 ± 9.22	29.91 ± 9.86
CD4 count	246.93 ± 151.31	225.60 ± 138.63	235.08 ± 144.04
Vitamin A	13.34 ± 6.16	13.13 ± 6.26	13.23 ± 6.18
Vitamin C	0.70 ± 1.29	0.66 ± 0.51	0.68 ± 0.94
Vitamin E	0.34 ± 0.11	0.33 ± 0.11	0.34 ± 0.11
Zinc	18.68 ± 18.18	13.71 ± 14.88	16.17 ± 16.68
Copper	36.14 ± 14.31	34.89 ± 13.36	35.04 ± 13.74
Iron	58.40 ± 21.76	51.28 ± 21.21	54.45 ± 21.69

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n = number of subjects

**Table 4: Correlation coefficients (r) of CD4 count and serum antioxidant micronutrients levels of HIV subjects**

Parameter	Correlation coefficient (r)
Age (years)	0.05
Vitamin A	0.18*
Vitamin C	0.03
Vitamin E	0.24*
Zinc	0.32*
Copper	0.23*
Iron	0.004

r Values bearing \* differ significantly ( $P < 0.05$ )

reported low vitamin E, along with vitamin A and  $\beta$ -carotene, as part of biochemical manifestation in HIV/AIDS patients. In the current study, progressive reduction in vitamin E level in HIV subjects was observed compared with the control. Studies have shown that double intake of vitamin E decreased the risk of progression to AIDS.<sup>[15]</sup>

Zinc level may be normal or high in asymptomatic HIV-infected patients and a steady decline is expected as immunodeficiency sets in. In the current study, there is progressive decline in serum Zn levels in HIV subjects compared with controls. Casselli and Biochi<sup>[16]</sup> demonstrated an increase in CD4 cell count and reduction in progression of the disease when zinc sulfate supplementation was given to AIDS patients with low zinc level.

HIV infection has been reported to be associated with increased proinflammatory cytokines,<sup>[17]</sup> which was reported to lower the plasma levels of Fe and Zn but raises the level of Cu.<sup>[18]</sup> In the current study, however, we reported a reduced level of Cu, which could be due to reduction in the synthesis of ceruloplasmin by liver, possibly due to malnutrition which may be aggravated by HIV.

Similar results have been reported elsewhere. It has been reported that HIV positive children have lowered antioxidant vitamins A, C and E compared to age-matched HIV negative controls.<sup>[19]</sup> Johane

*et al.*<sup>[3]</sup> also reported a decreased serum Vitamin C, alpha-tocopherol,  $\beta$ -carotene and selenium in HIV-positive patients. They, however, reported normal levels of vitamin A and zinc for the HIV positive subjects contrary to the report of the current study. The deficiencies could, however, be reversed by supplementation with micronutrients,<sup>[20]</sup> in addition to aggressive disease control and management. The deficiencies of the antioxidant vitamins and microelements of HIV subjects in the study area may be due to decreased intake of the nutrients as a result of poverty and ignorance that are widespread in African communities and due to increased utilization of antioxidant micronutrients because of increased oxidative stress associated with HIV infection.

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