Serum Levels of Antioxidant Minerals in Relation to Severity of HIV Infection Among Patients Attending Specialist Hospital Sokoto, Nigeria

***Wasagu, I.Z. and Bunza, F.U.**

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

Received: September 26, 2017; Revised: October 31, 2017; Accepted: November 13, 2017

Abstract

Human immunodeficiency virus (HIV) positive patients are under chronic stress due to excessive production of reactive oxygen species (ROS). Micronutrients (including antioxidant minerals) deficiencies are common in HIV-related infections which may progress to acquired immunodeficiency syndrome (AIDS). The present study assessed the serum levels of antioxidant minerals in HIV-positive Highly Active Antiretroviral Therapy-naïve (HAART-naïve) patients. The study involved 128 subjects that comprised 96 HIV-positive HAART- naive patients and 32 age- and gender-matched HIV-negative controls. Levels of CD_4^+ count were enumerated using Flow Cytometry while serum levels of antioxidant minerals (Cu, Zn, and Fe) were estimated using Atomic Absorption Spectrophotometer (AAS) in controls (group1) and patients with CD_4^+ count of ≥500 cells/µl (group 2), 200-499 cells/µl (group 3) and < 200 cells/µl (group 4). Results indicated that the HIV-positive HAART-naïve patients have significantly ($p<0.05$) reduced serum levels of antioxidant minerals when compared with different stages of CD_4^+ count. The result based on gender, indicated a significantly ($p<0.05$) higher levels of CD_4^+ count and antioxidant minerals in males compared to females. A significant ($p<0.05$) positive correlation was also established between CD_4^+ and antioxidant minerals with a decline in the latter as the severity of HIV-infection increased. This study recommends that HIV-positive HAART-naïve patients be supplemented with antioxidant minerals in order to prevent the oxidative onslaught by free radicals.

Keywords: Antioxidants, minerals, HIV/AIDS patients, HAART-naive

1.0 Introduction

Antioxidant minerals including zinc, copper and iron are known co-factors needed for the synthesis, optimum catalytic activity and effective antioxidant defense of the *de novo* antioxidant enzymes [1]. Zinc (Zn) and copper (Cu) are important components of superoxide dismutase (CuZnSOD) which is an antioxidant enzyme. Therefore their deficiencies may affect the activity of the enzymes [2]. Superoxide dismutase catalyses the univalent reduction and oxidation of O_2- to H_2O_2 and O_2 [1]. Iron (Fe), an important antioxidant mineral is required for the activity of catalase, a haem-protein enzyme containing four (4) haem prosthetic groups. Catalase is concentrated mainly in the peroxisomes and mitochondria, where it catalyses the conversion of H_2O_2 to H_2O and O_2 [3].

Oxidative stress caused by increased production of free radicals and/or inadequate antioxidant protection mechanism, may be worsened in HIV-infected patients, hence compromising the immune system [4]. Antioxidant minerals protect cells and tissues against inflammatory and infectious processes while inhibiting lipid peroxidation and acting as anti-atherogenic agents [5]. Oxidative stress has also been linked to apoptosis of T-lymphocytes during HIV disease and increase the rate of HIV replication by activating the nuclear transcription factor-KB (NK-KB) cell gene [6]. Increased oxidative stress and weakened antioxidant defense system in HIV-infected patients have also been evident as reported by Allard *et al.* [7].

Antioxidant minerals play important roles in maintaining immune function and neutralizing the reactive oxygen intermediates produced by activated macrophages and neutrophils [4]. Undernourishment and micronutrients deficiencies in HIV-infected individuals exacerbate immune suppression, oxidative stress, acceleration of HIV replication and CD_4^+ count depletion [4]. CD_4^+ count helper lymphocytes are preferentially destroyed by HIV, thus the level of CD_4^+ count is reduced with the severity of HIV infection [6]. The aim of the study is to assess the serum levels of antioxidant minerals among HIV-positive HAART-naïve patients at different stages of CD_4^+ count in Sokoto, Nigeria.

***Corresponding Author: Tel: +234(0)7064565488, E-mail: ibrahimzubairu7@gmail.com**

2.0 Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used for the study were of analytical grade and are products of Randox Company Ltd., UK.

2.2 Study Population

Ninety six (96) HIV-positive HAART-naïve patients attending the Antiretroviral Clinic of Specialist Hospital, Sokoto, Nigeria were used for the study. The subjects were grouped as follows: Group 1: [n=32]: HIV-positive HAART-naïve patients with CD_4^+ count ≥ 500 cells/µl, Group 2: [n=32]: HIV-positive HAART-naïve patients with CD_4^+ count 200-499 cells/µl and Group3: [n=32]: HIV-positive HAART-naïve patients with CD_4^+ cell count < 200 cells/µl.

2.3 Ethical Approval

The approval of the study was sought and obtained from the relevant Ethical Committee before the commencement of the study. The ethical clearance number of the study is SHS/SUB/218.

2.4 Sample Collection

Venous blood (5 mls) was collected from each subject out of which (4 mls) was dispensed into plain specimen bottles and allowed to clot at room temperature before centrifuging at 3000 rpm/min for 5 minutes to obtain clear unhaemolyzed serum. The serum was harvested into sterile bottles and refrigerated until the time for analysis. The remaining (1ml) was transferred into a sterile EDTA specimen bottle and used for the enumeration of CD_4^+ count within 3 hours of collection.

2.5 Analytical Methods

HIV screening was carried out using the World Health Organization (WHO) screening criteria for developing countries. The procedure involves two screening methods: HIV test using Stat Pak kit and HIV rapid screening test using Determine HIV1/2 rapid screening kit. The CD_4^+ count was carried out using Cyflow counter [8]. Serum Copper, Zinc and Iron were estimated using atomic absorption spectrophotometry (AAS) according to the method described by Steward *et al*. [9].

2.6 Statistical Analysis

The data obtained were analsed using Statistical Package for Social Science (SPSS version 2.0). The analysis of Variance (ANOVA) was used for comparisons of three (3) or more mean values and multiple comparisons were carried out using LSD. A p-value at 5% ($p \leq 0.05$) was considered statistically significant.

3.0 Results

Sociodemographic characteristics of the study subjects are presented in Table 1 consisting of 96 HIV-positive patients. The distribution of the HIV-positive participants shows that 90 (70.3%) are married, 92 (71.9%) are Hausa, 22 (17.19%) have HIV-related illnesses and 32 (25.0%) with opportunistic infections.

Table 2 shows serum levels of antioxidant minerals among HIV-positive HAART-naïve patients at different stages of CD_4^+ counts. The result indicated that Cu and Zn levels in patients which CD_4^+ counts 200–499 cells/µl were significantly low ($p<0.01$) compared to other groups. Although, Fe shows significant decrease ($P<0.001$) compared to other groups.

Table 3 presents effect of gender on CD_4^+ count and serum antioxidant minerals in HAART-naive HIV-positive patients and HIV-negative controls. Correlation coefficient (r) between CD_4^+ court and antioxidant minerals in HIV-positive HAART-naïve patient and HIV-negative controls are presented in Table 4. The result reveals that Zn significantly differ ($P<0.05$) compared to other groups.

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

Characteristics	Number 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
HIV-related illness	**22**	**17.19**
Herpes Zoster	3	2.34
Kaposis Sarcoma	2	1.56
Tuberculosis	16	12.50
Opportunistic infection	**32**	**25.0**
Recurrent Diarrhea	11	8.59
Recurrent Typhoid	8	6.25
Bronchitis	10	7.81
Candidiasis	3	2.34
Stages of CD_4^+ count	**96**	**75**
Stage I	32	25
Stage II	32	25
Stage III	32	25

Table 2: Serum Levels of Antioxidant Minerals among HIV-Positive HAART-naïve Patients at different stages of CD_4^+ counts

Parameters	Group 1 (n=32)	Group 2 (n=32)	Group 3 (n=32)	Group 4 (n=32)
Copper (µmol/L)	18±0.23	14.67 ± 0.39*	13.21 ± 0.43*	9.72 ± 0.33**
Zinc (µmol/L)	13±0.36	6.60 ± 0.35*	4.51 ± 0.33**	3.87 ± 0.34**
Iron (µmol/L)	17±0.62	14.74 ± 0.35**	11.85±0.30***	12.27±0.34***

Values are mean ± SEM, n= number of subjects, HIV= human immunodeficiency virus; CD_4= cluster differentiation type 4. The values bearing asterisk differ significantly with the respective control at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) respectively.

KEY :
Group 1 = Control subjects
Group 2 = HIV-positive HAART-naive patients with CD_4^+ count > 500 cells/µl
Group 3 = HIV-positive HAART-naive patients with CD_4^+count 200-499 cells/µl
Group 4 = HIV-positive HAART-naive patients with CD_4^+ count < 200 cells/µl

Table 3: Effect of Gender on CD_4^+ Count and Serum Antioxidant Minerals in HAART-naïve HIV-Positive Patients and HIV-Negative Controls

Parameters	HIV-Negative Controls		HIV-Positive HAART-naive Patients	
	Male (n=32)	Female (n=32)	Male (n=32)	Female (n=32)
CD_4^+ Count (cells/µL)	906.00±52.74	798.13 ± 37.05**	358.46±12.62	326.02±12.74**
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= cluster differentiation type 4; M=male; F=female; HIV= human immunodeficiency virus. There was statistically significant difference at ($p < 0.05$) (*), $p < 0.01$ (**)

Table 4: Correlation Coefficient (r) between CD_4^+ Count and Antioxidant Minerals in HIV-Positive HAART-naïve Patients and HIV-Negative Controls

Parameters	Group 1 (r)	Group 2 (r)	Group 3 (r)
CD_4^+/Cu	0.042	0.126	0.116
CD_4^+/Zn	0.25	0.415*	0.044
CD_4^+/Fe	0.121	0.124	0.109

The correlation coefficient (r) values bearing (*) differs significantly at $p < 0.05$.

4.0 Discussion

Human immunodeficiency virus (HIV) is capable of modulating the machineries of immune system leading to derangement in metabolic activities of macro and micronutrients in the body. Micronutrients deficiencies are prevalent in many HIV-Infected individuals and numerous studies have reported that these deficiencies impair immune responses, weaken epithelial integrity and accelerated HIV disease progression [10, 11]. Copper, iron and zinc are essential antioxidant minerals that are required in minute amount for proper health of the body. They play important roles in the activities of several antioxidant enzymes that protect body cells against highly toxic reactive oxygen species and also enhance the immunologic activities of phagocytes and lymphocytes [12].

The reduced antioxidant minerals (Cu, Zn and Fe) observed in group 2 and 3 where due to severity of HIV infection which is in agreement with previous studies. Bilblis *et al*. [11] reported lower levels of Cu, Zn and Fe in HIV-positive HAART-naïve patents and associated the levels with the severity of HIV-infection. In a study conducted by Akiibinu *et al*. [10], significantly lower levels of Co, Fe, Zn, Mn, Cu and Se were observed in symptomatic HIV/AIDS patients. Baum *et al*. [13] reported a significantly lower level of Zn in HIV/hepatitis C virus co-infected patients when compared with non-infected controls. The profound decrease in the number and functions of circulating CD_4^+ count reported by Ammann *et al*. [14] was associated with the consequences of trace metal deficiency in HIV-infected patients. The result of this study is also consistent with the report of Arinola *et al*. [15] in which Cu and Zn were significantly lower in HIV-positive patients.

The deficiencies of antioxidant minerals could however be reversed by supplementation with micronutrients [16]. The deficiencies of the antioxidant minerals 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. It could, however, be due to mal-absorption and diarrhoea, associated with HIV infection Bilbis *et al*. [11].

The CD_4^+ count in HIV-positive HAART-naive patients was significantly decreased in females than males. The levels of antioxidant minerals (Cu, Zn and Fe) in HIV-positive HAART-naïve patients when compared to controls were significantly decreased in females than males. This could be attributed to the fact that HIV-positive female patients in the study area were adamant and reluctant to access health care services on time due to physiological fear of discrimination and stigmatization.

A significant positive correlation between CD^+_4 counts and serum antioxidant minerals was observed in HIV-positive HAART-naïve patients in this study. This is in agreement with the report of Bilbis *et al*. [11].

Conclusion and Recommendation

In conclusion, it has been observed in this study that, serum levels of the antioxidant minerals (Cu, Zn and Fe) decreased significantly with increased severity of HIV infection. We therefore, recommend the incorporation of the minerals as supplement in the management of HIV infected patients in order to prevent oxidative onslaught by free radicals.

References

[1] Stocker, R. and Keaney, J.F. (2004). Role of Oxidative Modification in Atherosclerosis. Physiological Review, Vol. 84, pp. 1381-1478.

[2] Ishigani, N., Isoda, K. and Adachi, T. (2011). Deficiency of CuZn superoxide dismutase promotes inflammation and alters medial structure following vascular injury. Journal of Atherosclerosis Thrombosis, Vol. 18, pp. 1009-1017.

[3] Evans, P. and Halliwell, B. (2001). Micronutrients; Oxidant/Antioxidant Status. Biological Journal of Nutrition, Vol. 85, No. 2, pp. 567-574.

[4] Seifried, H.E., Anderson, D.E., Sorkin, B.C. and Costello, R.B. (2004). Free radicals: the prons and cons of antioxidants. American Society of Nutrition Sciences Journal of Nutrition, Vol. 134, pp. 3143-3163.

[5] Maggini, S, Wintergerst, E.S., Berevidge, S. and Horning, D.H. (2007). Selected vitamins and trace elements support immune function by strengthing epithelial barriers, cellular and humoral immune responses. British Journal of Nutrition, Vol. 98, pp. 29-35.

[6] Conner, E.A., Terwilliger, E.F., Sodroski, J.G. and Haseltine, W.A. (1998). Identification of a protein encoded by the vpu gene of HIV-1. Nature, Vol. 334, pp. 532-534.

[7] Allard, J.P., Aghdassi, E., Chau, J., Salit, I. and Walmslay, S. (1998). Oxidative Stress and Plasma Micronutrients in Humans with HIV infection. American Journal of Clinical Nutrition, Vol. 67, pp. 143-147.

[8] Cassens, U., Golde, W., Kuling, G., Schlenke, P., Lehman, L.G., Traore, Y., Servais, J. and Henin, Y. (2004). Simplified volumeric Flow Cytometry Allows Feasible and Accurate Determination of CD_4 T-Lymphocytes in Immunodeficient Patients Worldwide. Antiviral Therapy, Vol. 9, pp. 395-405.

[9] Steward, E.A., Max, H.G., John, A.P. and Christopher, Q. (1989). Inorganic Constituents and Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, London, pp. 234-235.

[10] Akiibinu, M.O., Adeshiyan, A. and Olalekan, A.O. (2012). Micronutrients and Markers of Oxidative Stress in Symptomatic HIV-positive Nigerians. Institute of Integrative Omics and Applied Biotechnology Journal, Vol. 3, No. 2, pp. 7-11.

[11] Bilbis, L.S., Idowu, D.B., Saidu, Y., Lawal, M. and Njoku, C.H. (2010). Serum Levels of Antioxidant Vitamins and Mineral Elements of Human Immunodeficiency Virus Positive Subjects in Sokoto, Nigeria. Annal of African Medicine, Vol. 9, No. 4, pp. 235-239.

[12] Prasad, A.S. (1998). Zinc and Immunity. Molecular Cell Biochemistry, Vol. 188, pp. 63-69.

[13] Baum, M., Sales, S., Jayaweera, D.L., Bradwin, G., Rafie, C., Page, J. and Campa, A (2011). Co-infection with Hepatitis C Virus, Oxidative Stress and Antioxidant Status in HIV-positive Drug Users in Miami. Human Immunodeficiency Virus and Medicine, Vol. 12, No. 2, pp. 78-86

[14] Amman, A.J., Abraham, D., Conant, M., Chulldwin, D. and Cowan, M. (1983). Acquired Immune Dysfunction in Homosexual Men. Clinical Immunology and Immunopathology, Vol. 22, pp. 315-325.

[15] Arinola, O.G. and Akiibinu, M.O. (2005). Effect of HIV Urinary Schistosomiasis or Malaria on the Levels of Nutritionally Essential Trace Elements in Nigerians. European Journal of Scientific Research, Vol. 6, No. 1, pp. 65-73.

[16] Drain, P.K., Kupkka, R., Mugusi, F. and Fawzi, W.W. (2007): Medical Laboratory Manual for Tropical Countries 2nd edition ELSB Cambridge, pp. 465-567.