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MICRONUTRIENTS AND MARKERS OF OXIDATIVE STRESS IN SYMPTOMATIC HIV-POSITIVE/AIDS NIGERIANS: A CALL FOR ADJUVANT MICRONUTRIENT THERAPY

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ABSTRACT

Background: *The status of micronutrients and oxidative metabolites have not been fully explored in Nigerian symptomatic HIV-positive/AIDS patients, despite the existing evidences linking micronutrient deficiencies with immune dysfunctions. The present study assessed the plasma levels of selected micronutrients and markers of oxidative stress in symptomatic HIV-seropositive/AIDS patients.* ***Methods:*** *Seventy newly diagnosed HIV-positive patients (37 males and 33 females) volunteered to participate in this study. Sixty-five age matched HIV-seronegative, apparently healthy individuals (35 males and 30 females) served as controls. The plasma levels of cobalt (Co),copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), selenium (Se), vitamin C, vitamin E, total antioxidant potential (TAP), total plasma peroxide (TPP), oxidative stress index (OSI) and malondialdehyde (MDA) were determined in them using atomic absorption spectrophotometer and spectrophotometric methods respectively.* ***Results:*** *The plasma levels of TAP, Cu, Zn, Fe, Co, Se, vitamin C and vitamin E were significantly lower ($p< 0.05$) in symptomatic HIV-positive/AIDS patients when compared with the controls. The mean values of MDA, TPP and OSI increased significantly ($p<0.01$) in symptomatic HIV-positive/AIDS patients when compared with the controls. Interestingly, there was no significant change in the mean level of Mn ($p>0.05$) when compared with the controls. In this study, symptomatic HIV-positive/AIDS patients demonstrated oxidative stress.* ***Conclusion:*** *Since most antioxidants regulating the oxidative stress are micronutrient dependent, this study strengthens the evidence and will convince the remnant skeptics that micronutrient supplementation is important in the management of AIDS.*

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KEY WORDS

symptomatic HIV-infection; Nigerians; micronutrients; oxidative stress

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[I] INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is the resultant effect of progressive loss of cellular immunity due to metabolic dysfunctions associated with human immunodeficiency virus (HIV) infection [1, 2]. Previous workers associated the progressive loss of cellular immunity in HIV-infection and AIDS with the defect in immunologic activities of monocytes, neutrophils, natural killer cells and significantly decreased level of circulating CD4 +T-cells [2]. Several lines of evidences show that certain micronutrients are important in man and animal due to their dual function for immune modulation and antioxidants or as ligands in the antioxidant enzymes [1, 2, 3]. Previous workers have reported the effects of deficiencies of selenium, zinc, iron and copper on the activities of neutrophils, monocytes, lymphocytes and macrophages [3]. The complex immune dysfunction in HIV-positive individuals predisposes them to both pathogenic and opportunistic infections [4-8]. In the reports of Kochanowski et al [9] and Sherman [10], iron deficiency was implicated as the cause of low cellular immune response and decreased secretion of interferon-γ, tumor necrotic factor–α and interleukin–2. Arinola et al [11] also reported deficiencies in Cu, Mg, Fe, Se and Cr in HIV-positive patients and associated these with the complications of HIV-infection. Samba et al [1] reported significantly lower levels of Zn and Se in HIV-positive patients and associated the adverse clinical outcome during HIV infection with lower levels of vitamins and micro-mineral deficiencies. Se is a co-factor of several key enzymes, the plasma levels of which determine the activities of glutathione peroxidase, thioredoxin reductase and deiodinase (an enzyme involved in thyroid hormone activation) [12].

HIV is an intracellular pathogen that causes continuous cellular activation which results to free radical and cytokine generation [13, 14]. A number of cytokines up-regulate the HIV expression and cause anorexia in both acute and chronic HIV-infection [14]. The free radicals generation must be controlled at physiological level by the antioxidant system to avoid oxidative stress. Vitamins A, C and E are free radical scavengers while certain trace metals (Fe, Zn, Se, Cu, Mn) are essential in the activities of several antioxidant metalloproteins

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that protect the cells against highly toxic reactive oxygen species [3]. Deficiency of selenium reported in HIV-infection has been linked with decrease of glutathione peroxidase activity and the susceptibility of tissues to injury by reactive oxygen species [16]. HIV-infection represents a serious risk factor for the development of metabolic and cellular immune disturbances. Most previous workers only determined the status of micronutrients in uncomplicated HIV-infection, it is therefore necessary to identify the effects of complicated HIV-infection/AIDS on the plasma levels of Cu, Mn, Fe, Co, Zn, Se, vitamin C, vitamin E, total antioxidant potential (TAP), total plasma peroxide (TPP), oxidative stress index (OSI) and malondialdehyde (MDA) in the present study.

[II] MATERIALS AND METHODS

Seventy newly diagnosed HIV-positive patients (37 males and 33 females) with clinical features of AIDS (i.e. chronic diarrhea, weight loss, skin rashes, and persistent fever) were recruited to participate in this study. Another sixty-five age matched HIV-seronegative, apparently healthy individuals (35 males and 30 females) served as controls. The participants (HIV-positive patients and controls) did not test positive to hepatitis B or C viruses and Mycobacterium tuberculosis infections at the time this study was conducted. Five milliliters of blood was withdrawn from each patient and control into a lithium heparin bottle. 0.5 mL of the plasma was immediately transferred into the bottle containing phosphoric acid for the estimation of ascorbic acid. The remaining plasma for the estimation of Cu, Mn, Fe, Co, Zn, Se, vitamin E, TAP, TPP was stored at -200C until analyzed. The experimental protocol was approved by the Research Ethical Committee of the Ministry of Health, Oyo state, Nigeria.

2.1. Determination of ascorbic acid (vitamin C)

Vitamin C concentration was determined by using the method of Briggs [17]. In brief, ascorbic acid in the plasma is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2, 4 – dinitrophenylydrazine to form a reddish – hydrazone, which is measured at 520nm.

2.2. Determination of vitamin E

Vitamin E (α-tocopherol) was assayed by the method of Desai [18]. Briefly, vitamin E was extracted from plasma by addition of 1.6ml ethanol and 2.0ml petroleum ether to 5.0ml plasma and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2ml of 2% α-α-dipyridyl, 0.2ml of 0.5% ferric chloride was added and kept in the dark for 5 min. An intense red coloured layer obtained on addition of 4 ml butanol was read at 520 nm.

2.3. Determination of MDA

Level of lipid peroxidation was determined by measuring the formation MDA using the method of Varshney and Kale [19]. The principle is based on the fact that malondiahydehyde (MDA) produced from the peroxidiation of membrane fatty acid reacts with the chromogenic reagent; 2-thiobarbituric acid (TBA) under acidic conditions to yield a pink–coloured complex measured spectrophotometrically at 532nm. 1, 1, 3, 3-tetramethoxylpropane was used as standard.

2.4. Determination of TAP

TAP was determined using the ferric reducing / antioxidant power (FRAP) assay [20, 21]. 1.5 ml of working pre-wormed (370C) FRAP reagent (300mM acetate buffer - pH 3.6, 10mM 2,4,6- tripyridyl-s-triazine in 40mM HCl and 20mM $FeCl_3$ at ratio 10:1:1) was vortex mixed with 50µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv. / L.

2.5. Determination of total plasma peroxide (TPP)

Ferrous-butylated hydroxytoluene-xylenol orange complex reacts with plasma hydrogen peroxide to form a colour complex measured spectrophotometrically at 560mm. H_2O_2 was used as standard. 1.8ml of reagent 6 (F0X2) was mixed with 200µ1 of plasma. This was incubated at room temperature for 30 minutes. 100µMol H_2O_2 was used as standard. The mixture was centrifuged and the supernatant separated for reading at 560nm [21].

2.6. Determination of oxidative stress index (OSI)

OSI, an indicator of the degree of oxidative stress is the percent ratio of the TPP to the TAP [15].

2.7. Determination of Co, Cu, Mn, Fe, Zn, Se

Trace metals (Fe, Zn, Mn, Co, Cu and Se) were determined using atomic absorption spectrophotometer (AAS) as described by Kaneko et al [22]. The atomization of the element aspirated into the AAS results in the absorption of light of the same wavelength as that emitted by the element when in the excited state.

2.8. Statistical analysis

The statistical analysis of data generated in this study was carried out using SPSS version 10 and the values expressed as mean + 1 SD. Student (t) test was used for comparison. The changes were considered significant, when p-values were less than 0.05.

[III] RESULTS

The results obtained in this study show deregulated levels of certain trace metals in symptomatic HIV-positive patients. The levels of Co, Cu, Zn, Fe and Se **[Table– 1]** were significantly lower ($p< 0.05$) while no significant change was observed in the mean level of Mn ($p>0.05$) in the symptomatic HIV-positive/AIDS patients, when compared with controls. Significantly ($p< 0.05$) lower levels of TAP, vitamin C and vitamin E; with significantly higher ($p<0.05$) levels of TPP, MDA and OSI **[Table–2]** observed in symptomatic HIV-positive/AIDS patients when compared with the controls is an indication of oxidative stress in them.

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Table: 1. levels (Mean + SD) of trace metals in symptomatic HIV-positive/AIDS patients and controls

	N	Co (µg/dL)	Fe (µg/dL)	Zn (µg/dL)	Mn (µg/dL)	Cu (µg/dL)	Se (µg/dL)
Controls	65	63.5+6.8	75.8+8.2	122.1+18.3	65.4+13.1	71.5+16.0	57.8+11.7
HIV-patients	70	38.0+6.5	67.0+11.6	97.4+22.0	62.8+9.5	59.4+12.5	42.3+8.2
p values		<0.01*	<0.05*	<0.05*	>0.05	<0.05*	<0.01*

* = significantly different from the controls. N = number of subjects used in the study

Table: 2. levels (Mean + SD) of antioxidant vitamins and markers of oxidative stress in symptomatic HIV-positive/AIDS patients and controls

	N	TAP (µMol Trolox equiv./ L)	TPP (µMol H_2O_2/ L) (%)	OSI (nM/ml)	MDA (mg/L)	Vitamin C (mg/L)	Vitamin E (mg/L)
Controls	**65**	1652+380	11.8+4.3	0.71+0.46	8.2+3.2	22.6+8.7	12.7+5.3
HIV-patients	**70**	930+370	33.9+12.0	3.70+1.50	17.5+5.8	12.5+4.1	8.3+4.0
p values		<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.05*

* = significantly different from the controls. N = number of subjects used in the study.

[IV] DISCUSSION

Corneal HIV-infection is capable of modulating the machineries of immune system and causing deranged metabolic activities of macro- and micronutrients molecules. Most of the intra- and extracellular metabolic processes require micronutrients for their optimal activities. The present study demonstrated significantly lower levels of plasma Fe, Co, Zn, Cu and Se in the symptomatic HIV-positive/AIDS patients, when compared with the controls. Meanwhile, there was no significant change in the plasma level of Mn in the symptomatic HIV-positive/AIDS patients, when compared with the controls. Fe, Zn, Se, Cu, Mn are essential in the activities of several antioxidant enzymes that protect the cells against highly toxic reactive oxygen species, and also enhance the immunologic activities of phagocytes and lymphocytes [3]. Since HIV requires large amount of selenium for its replication in the cells [23], the deficiencies of Se observed in these symptomatic HIV-positive/AIDS patients could be due to increased utilization by the HIV. The chronic gastroenteritis and mal-absorption [1] commonly encountered in HIV-positive patients may contribute to micronutrients metabolic disturbances observed in our HIV-positive/AIDS patients. This observation appears to corroborate the report of Bilbis et al [24] who reported lower levels of Fe, Cu and Zn in HIV-positive patients, and associated the levels with the severity of HIV-infection. A considerable number of previous investigators have reported significantly lower levels of certain micronutrients in HIV-positive patients [25, 26]. In a study conducted by Baum et al [25], a significantly lower level of Zn was observed in HIV/hepatitis C virus co-infected patients when compared with non-infected controls. In another study conducted in Co te d 'Ivoire by Djinhi et al [26], a significantly lower level of Se was reported in HIV–infected patients. The profound decrease in the number and functions of circulating CD_4+T-cells reported by Ammann et al [2] and Groux et al [4] 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 [27] that Cu and Zn were significantly lower in HIV-positive patients.

To the knowledge of the authors, this study is the first to report cobalt deficiency in AIDS patients. The fact that cobalt is an essential trace metal linking the four pyrrol rings of cobalamin for effective synthesis of red blood cells [28] makes cobalt deficiency the novel of this study. The megaloblastic anaemia reported earlier by Koduri [29] which responded well to danazol therapy in a patient with AIDS could be ascribed to the cobalt deficiency observed in this study. Therefore, the deficiency of cobalt in our AIDS patients may contribute to anaemia commonly encountered in AIDS patients. Also, cobalt deficiency has been implicated in several neurological disorders such as myeloneuropathy [30], myelopathy [31], myeloneuroencephalopathy and myeloencephalopathy [32]. Neurological and behavioural disorders have been reported in advanced cases of AIDS. This study being the first to report cobalt deficiency in AIDS patients, may suggest that cobalt deficiency has a critical role to play in the development of neurological disorders in AIDS patients.

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We observed significantly lower levels of vitamins C and E in our symptomatic HIV-positive /AIDS patients when compared with the controls. These are antioxidant vitamins that scavenge for free radicals in the system. Vitamin E is the cell membrane antioxidant protecting the cell membrane from free radical attack and cell lysis [3]. Depression of vitamins C and E in these patients may be due to increased demand in the detoxification / neutralization of free radicals. The lower levels of these antioxidant vitamins may also be due to mal-absorption and diarrhea that are common complications of AIDS, and secondary to gastro-enteritis and altered gut barrier function. This result agrees with previous workers who reported significantly lower levels of vitamins C and E in HIV-1 sero-positive patients [33]. In the study conducted by Mehta et al [34], significantly lower levels of lipid soluble vitamins were reported in HIV-positive patients. Bilbis et al [24] also reported lower levels of vitamins A, C and E in HIV-positive patients. Significantly lower levels of vitamins E have been reported earlier in asymptomatic HIV-positive/AIDS patients by Djinhi et al [26]. Also, significantly lower levels of vitamins A, C and E were reported in HIV-positive children by Srinivas et al [35]. The significantly lower levels of vitamins A, C and E in the HIV-positive children were associated with increased utilization of antioxidant micronutrients in the neutralization of free radicals in HIV-positive patients [35]. The combination of malnutrition, mal-absorption and exhaustion during detoxification of free radicals could account for the significantly lower levels of vitamins C and E in our symptomatic HIV-positive/AIDS patients.

The symptomatic HIV-positive/AIDS patients showed significantly higher plasma levels of markers of oxidative stress (TPP, OSI and MDA) when compared with the controls. These significantly higher levels of markers of oxidative stress could be due to high free radical load and insufficient antioxidant micronutrients in the HIV-positive patients. High free radical load may cause lipid peroxidation, fragmentation or aggregation of protein and deamination of guanine and adenine in DNA chain to cause gene mutation and cell membrane damage [36]. The data of the present study corroborates the reports of previous workers that markers of oxidative stress are higher in HIV- infected patients. Kameoka et al [37] reported that the reactive oxygen species stimulate oxygen responsive transcription factors that induce HIV replication in the infected T-lymphocyte. Baum et al [28] reported significantly higher level of oxidative stress in HIV-positive patients when compared with non-infected controls. This result also agrees with Suresh et al [33] which reported higher level of MDA in both symptomatic and asymptomatic HIV-1 infected patients in both conditions. Mandas et al [38] reported deranged level of oxidative stress in AIDS patients on anti-retroviral therapy and associated the higher level of oxidative stress to the pro-oxidant effect of the anti-retroviral drugs.

A significantly lower level of TAP was observed in the symptomatic HIV-positive/AIDS patients recruited for this study, when compared with the controls. This study agrees with Suresh et al [33] who reported significantly lower level of total antioxidant capacity in both symptomatic and asymptomatic HIV infections. Stromajar-Racz et al [39] also reported significantly lower level of antioxidant enzymes in HIV-1 infected patients. The lower level of TAP in the present study could be due to excessive free radical generation, mal-absorption due gastro-enteritis, diarrhea and nutritional antioxidant deficiency commonly encountered in HIV-positive patients. Since TAP is an index of all classes of antioxidants, the significantly lower level of TAP observed in this study could be due to significantly lower levels of vitamin C, vitamin E, Cu, Zn, Se and Fe.

In conclusion, micronutrient deficiency possibly contributing disproportionately to the oxidative stress is a feature of AIDS. Therefore, adjuvant micronutrients therapy should be seriously considered in the management of AIDS patients to avert or ameliorate the complications of AIDS and slow the progression of the disease.

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No financial assistance was received for this work.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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