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Plasma Antioxidant Micronutrients and Oxidative Stress in People Living with HIV

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Abstract: Increased lipid peroxidation induced by reactive oxygen species has been implicated in several aspects of HIV disease pathogenesis including loss of immune function, chronic weight loss, inflammatory response and decreased immune cells proliferation. The aim of this study was to evaluate plasma antioxidant micronutrients and lipid peroxidation indices in HIV positive clients (50 untreated-Not on Antiretroviral Therapy (NART), 50 on treatment with Antiretroviral Therapy-ART) and 28 sero-negative control subjects (≥27 age-matched). Plasma antioxidant vitamins and lipid profile were measured. Beta-carotene levels were Control (3.44±0.46 RE), NART (5.17±0.67 RE) and ART (7.53±1.83 RE) with ART, being significantly ($p<0.05$) higher in ART subjects than controls. The corresponding ascorbic acid levels (mg/dl) were 1.40±0.10, 2.30±0.20 and 1.58±0.10. Levels of Superoxide Dismutase (SOD) were significantly increased in NART subjects compared with ART and controls. In contrast, the uric acid level was much lower ($p<0.05$) in NART subjects than ART and control groups. Malondialdehyde (MDA) was significantly higher in subjects on ART (2403.73±310.99 μ/ml) than NART (1628.11±111.56 μ/ml) and control (1459.58±119.11 μ/ml). The lipid profile showed substantially elevated levels of total cholesterol, triglyceride and Low Density Lipoprotein (LDL)-cholesterol in NART subjects compared with ART and controls. High Density Lipoprotein (HDL)-cholesterol showed no significance among the groups. Results showed increase in oxidative stress and a weakened antioxidant defense system in HIV positive persons, especially those not on antiretroviral therapy.

Key words: Antioxidant, lipid profile, oxidative stress, HIV, PLWH

INTRODUCTION

Several human studies in Human Immunodeficiency Virus (HIV) individuals have reported that micronutrient deficiencies and lipid peroxidation may exacerbate stress induced by the disease. The infection and its evolution often results in increased requirement for nutritional micronutrients, especially antioxidants (Gil, 2005). Lipid peroxidation-the oxidative degradation of lipids in cell membranes (Mayes, 2000) is due to inflammation associated with infection that leads to activation of inflammatory cells. This activation involves neutrophils, endothelial cells and other phagocytic cells which might promote oxidative stress (Murray, 2000). Levy (2005) reported that lipid peroxidation in HIV infected persons was a result of abnormalities in lipid metabolism potentially induced by the disease itself and medication used for the treatment thereby resulting in oxidative stress. Gil *et al*. (2005) considered oxidative stress a pathologic phenomenon that results from imbalance between the systems producing active oxygen species and those defending the organism. These defense systems function synergistically to prevent or destroy the active oxygen species. Under normal circumstances oxidative stress is deleterious to normal cell function (Pasupathi *et al*., 2009).

Oxidative stress in HIV infection has nutritional importance because lipids are absorbed in the small intestines into the blood stream and carried by lipoproteins to their target sites (Grundy *et al*., 2004). Lipids serve as an efficient source of energy both directly and potentially when stored in adipose tissues (Pasupathi *et al*., 2009). They also serve as thermal insulator in the subcutaneous tissues and around certain organs. Non-polar lipids are reported to act as insulators that allow waves along myelinated nerves (Grundy *et al*., 2004; Levy, 2005). Lipids are also known sources of transport for vitamins and essential fatty acids serve as hormones or hormone precursors, aids digestion and act as functional and structural components in biomembranes (Mayes, 2000; Third Report of the National Cholesterol Education, 2001; Merz and Wickner, 2004; Randox cholesterol enzymatic endpoint method Manual, 2007). HDL-c is known as 'good lipid' because it helps to remove cholesterol from the body while LDL-c is regarded as 'bad cholesterol' as it carriers cholesterol to cells throughout the body.

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HIV infection affects the way the body deals with lipids. The disease caused a rise in total cholesterol, triglycerides, Low Density Lipoprotein (LDL) and a decrease in the level of High Density Lipoprotein (HDL) (Kotler, 2000; Aznar *et al*., 2000; Aberg *et al*., 2004; Dube´ *et al*., 2003; Wanke *et al*., 2005; Buchacz *et al*., 2008). This action resulted in lipid peroxidation which is linked with free radicals produced during peroxide formation from fatty acid containing methylene-interrupted double bonds. Mayes (2000) and Porter *et al*. (1995) observed that HIV infection can be linked with malnutrition which accompanies chronic weight loss, decreased immune cells, loss of immune functions and inflammatory response that occur due to free radicals causing cell apoptosis.

The human body can be protected against damaging effects of reactive oxygen species by a variety of systems (Van Haaften *et al*., 2003). One of such lines of defense is formed by antioxidants like vitamins A, C and E (Van Haaften *et al*., 2003; Gil *et al*, 2005; Olaniyia and Arinolab, 2007). Antioxidants are compounds that can donate electrons to electron-seeking (oxidizing) compounds. This function reduces the destructive nature of oxidizing compounds and save the lives of cells in the cell membranes. Antioxidants therefore are used to control and reduce lipid peroxidation. Common sources of antioxidants are fruits and vegetables. Previous studies (Pasupathi *et al*., 2009; Gil, 2005; Gil *et al*., 2005; Levy, 2005; Allard *et al*., 1998; McDermid *et al*., 2002; Pace and Leaf, 1995; Sundaram *et al*., 2008; Carter, 2006) have confirmed the relationship between deficient antioxidants and oxidative stress in HIV-infected persons; low levels of antioxidants like vitamins A, C and E, zinc, selenium and elevated levels of Superoxide Dismutase (SOD), Malondialdehyde (MDA) (Niedernhofer *et al*., 2003) and uric acid among infected persons were associated with oxidative stress. To counter the effect of oxidative stress in cells, it was suggested that plasma levels of antioxidants of people living with HIV be improved with routine assessment and appropriate supplementation (Olaniyia and Arinolab, 2007; Gil, 2005; Pasupathi *et al*., 2009).

The aim of this study was to examine the lipid and antioxidants profiles in relation to oxidative stress of HIV infected persons.

MATERIALS AND METHODS

This study was carried out in Heart-to Heart Clinic, General Hospital Calabar, where HIV-infected persons are counseled, tested and treated. The study population consisted of 128 subjects divided into 3 groups; 50 HIV-sero-positive on antiretroviral therapy (ART), 50 HIV sero-positives Not on Antiretroviral Therapy (NART) and 28 HIV sero-negative control group. The subjects were between 25-55 years of age who attended Heart-To Heart Clinic in General Hospital Calabar. Informed consent was obtained from all clients and the study protocol was approved by the Research Ethics Committee, Centre for Clinical Governance, Research and Training, Ministry of Health Cross River State, Nigeria.

Blood samples were collected from the subjects by brachial venous puncture using sterile needle and syringe. A portion of the blood was put into plain sample tubes for serum samples and the remainder in Ethylene di-amino-tetra acetic acid (EDTA) treated sample tubes as whole blood. Serum was obtained from the clotted blood in plain sample tubes which were allowed to stand for 2 h at room temperature before centrifugation at 3000 rpm for 10 min using bench top centrifuge. Sera from the samples were carefully removed using Pasteur pipettes and put into respective dry labeled plastic specimen bottles and kept frozen in a refrigerator until it was used for analysis. The whole blood collected into the sample tubes was immediately corked and shaken gently to allow the blood mix with anti coagulant to prevent clotting of red blood cells and haemolysis of cells. Superoxide Dismutase (SOD), Malondialdehyde (MDA), beta-carotene, ascorbic acid and Uric acid (antioxidant profile) were determined. Cayman's superoxide dismutase (Malstrom *et al*., 1975; Sandstrom *et al*., 1994) and Cayman's lipid hydroperoxide (Cross *et al*., 1987; Porter *et al*., 1995) assay kits were used to measure SOD and MDA. Beta-carotene plasma levels were determined in haemolysis-free plasma by using Bessey *et al*. (1946) method and results compared with reference standards for vitamin A (National Institute of Standards, 2002). Vitamin C (ascorbic acid) was determined by redox titration method (Thompson, 1990; Brody, 1994). Uric acid was determined by Randox enzymatic-colorimetric method (Fossati *et al*., 1980).

Total Cholesterol (TC) was determined by Randox enzymatic-colorimetric method (Third Report of the National Cholesterol Education Programme, 2001); Randox precipitant method was used to determine High Density Lipoprotein (HDL) (Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, 2001) while triglycerides concentrations were determined using GPO-PAD method (Tietz, 1990).

Low Density Lipoprotein (LDL) was calculated with Chawla (1999) equation: LDL cholesterol = total cholesterol-triglycerides/5- High Density Lipoprotein Cholesterol (HDL). Reference values were taken from Third Report of the National cholesterol Education, Evaluation Programme (NCEP) (2001).

Statistical analysis: The statistical significance was evaluated by ANOVA followed by a post hoc (LSD) test using SPSS 15.0 Version. A p-value of less than 0.05 was considered statistically significant. Data were expressed as Mean±SEM.

Table 1: Lipid profile of respondents

Group	Cholesterol	HDL-c	Triglyceride	LDL-c
Control	112.11±9.71	12.67±1.85	82.71±22.65	78.12±11.45
NART	161.05±9.58[a]	18.06±1.50	132.56±7.37[a]	116.66±8.66[a]
ART	93.53±6.41[b]	18.46±2.45	75.49±7.09[b]	59.66±5.76[b]

Mean±SEM, n = 128

Table 2: Antioxidant profile of respondents

SOD	MDA	B-carotene	Vitamin C	Uric acid
228.19±53.88	1457.58±119.11	3.44±0.46	1.40±0.10	6.63±0.51
641.27±75.13	1628.11±111.56	5.17±0.67	2.30±0.20[a]	5.83±0.37
166.07±75.31[a,b]	2403.73±310.99[a,b]	7.53±1.83[a]	1.58±0.10[b]	4.12±0.25[a,b]

Mean±SEM, n = 128

RESULTS

Lipid peroxidationindex, Malondialdehyde (MDA) and some antioxidant indeces including Superoxide Dismutase (SOD) activity, β carotene (provitamin A), ascorbic acid (vitamin C) and uric acid were measured in serum of respondents in the study and results presented in Tables 1 and 2.

Lipid profile of subjects (Table 1) showed higher levels of total cholesterol, triglyceride and LDL-c in blood samples of NART subjects than the control and ART groups at $p<0.05$. There was however no difference in the HDL-c level among groups. Table 2 shows the antioxidant profile of the subjects. Plasma levels of superoxide dismutase, ascorbic acid and uric acid were significantly higher among HIV positive untreated subjects (NART) than the control group and HIV-positive treated subjects (ART). Malondialdehyde plasma levels in ART subjects were significantly higher than control and NART subjects at $p<0.05$.

DISCUSSION

Several evidences exist suggesting that HIV-infected persons are under chronic oxidative stress due to lipid peroxidation (Pasupathi *et al*., 2009; Wu and Cederbaum, 2003; Ridder *et al*., 2003; Shevitz *et al*., 2001; Dobmeyer *et al*., 1997; Pace and Leaf, 1995; Block and Langseth, 1994). Lipid peroxidation is the oxidative degradation of lipids in cell membranes which results to cell damage. In this study, the total cholesterol plasma level of NART subjects was significantly higher than the control and ART groups. Lipids are stored in the adipose tissues and the cell membranes of the body. When there is fuel deprivation in the body, the stored lipids in the adipose tissues is withdrawn and used. In pathological conditions like HIV infection, there was increased oxidation (peroxidation) of the lipids in the cell membranes hence the increase concentration of total cholesterol in the blood (Kotler, 2000; Aznar *et al*., 2000; Aberg *et al*., 2004). In this study, we observed increased plasma levels of triglycerides and Low density Lipoprotein in NART subjects compared with the control group. The TG and LDL-c levels of ART subjects were also much ($p<0.05$) lower than those of the NART group. This observation was also reported in Pace and Leaf (1995), Allard *et al*. (1998), Carter (2006), Pasupathi *et al*. (2009). Increased plasma levels of cholesterol, triglyceride and LDL-c are major risk factors for heart attack or stroke; triglycerides levels <150 mg/dl had adverse effects on individuals infected with HIV virus while HDL-c levels of ≥40, LDL-c <100 mg/dl and total cholesterol levels <200 mg/dl were favourable to the body (Aberg *et al*., 2004; Ridder *et al*., 2003; Dube´ *et al*., 2003; Stein, 2005). Some of the drugs used to treat HIV had effects on the lipids levels of people living with the disease (Ridder *et al*., 2003; Polsky *et al*., 2002; Piwoz and Preble, 2000; Carr and Cooper, 2000).

Low levels of antioxidants adversely affect the immune system of HIV positive persons resulting in oxidative stress. Our data showed a significantly higher level of superoxide dismutase in NART subjects than ART subjects and were consistent with those of Pace and Leaf (1995) and Pasupathi *et al*. (2009). Superoxide dismutase is an enzyme that acts as both antioxidant and anti-inflammation in the body. It helps the body use zinc; copper and manganese. There are two types of SOD (Vitaminstuff.com, 2009): Copper/zinc (Cu/Zn) SOD and Manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. The Cu/Zn SOD protects the cells' cytoplasm while the Mn SOD protects their mitochondria from free radicals damage. This enzyme is found in the dermis and epidermis of the skin and is essential in the production of fibroblasts (skin-building cells) (Vitaminstuff.com, retrieved 30/7/2009). In a disease state, it becomes abundant in the blood, acting to reduce and repair damage done to cells. The enzyme depends on vitamin C and copper for its availability in the body.

Malondialdehyde is an antioxidant index that occurs naturally as a product of lipid peroxidation which is mutagenic in human cells. High plasma levels of MDA suggest oxidative stress. Our data showed that the plasma levels of MDA were significantly higher in the ART subjects than the control and the NART groups (Table 2). This difference may be attributed to the medications used for treatment (Levy, 2005; Carr and Cooper, 2000) or may be due to the fact that the vitamin

C levels of the ART subjects were lower. Niedernhofer *et al*. (2003) and Pace and Leaf (1995) found elevated serum levels of MDA in HIV naive infected patients. Gil (2005) however noticed no change in MDA in his subjects but a lower CD38+/CD8+.

Vitamin A is an antioxidant that improves or affects the immune system of HIV positive persons. Plasma levels of vitamin A in this study (Table 2) were much higher in ART subjects than the control. These high levels could be due to antiretroviral treatment and the fact that the people were advised to take food containing high vitamins to improve their immune status. This finding agrees with (Pace and Leaf, 1995; Allard *et al*., 1998; Gil, 2005; Olaniyia and Arinolab, 2007; Pasupathi *et al*., 2009) who opine that Supplementation with vitamins improves the immune status of HIV infected persons thereby reducing oxidative stress. Vitamin A is a fat soluble vitamin that is absorbed in the intestines in the presence of dietary fat and bile. It is needed for transport of vitamins between different compartments of cell (Stump *et al*., 1991; Brody, 1994). It is also essential for healthy skin, normal growth and development as well as prevention of infection. Its deficiency results in lowered resistance to infection among other disorders. HIV infection is documented to affect the digestive system which in turn affects the absorption and metabolism of this vitamin. Low levels of the vitamin increases SOD levels which have anti-inflammatory role of protecting the cell cytoplasm and their mitochondria from free radicals. Several investigators have suggested that low vitamin C levels are associated with increased oxidative stress (Pasupathi *et al*., 2009; Gil *et al*., 2005; Levy, 2005; McDermid *et al*., 2002). In our study, the HIV infected subjects not on antiretroviral therapy showed much higher plasma levels than the control and ART subjects (Table 2). These findings differ from other reports which indicated a decrease in plasma vitamin C concentration in untreated HIV patients (Pasupathi *et al*., 2009; Gil *et al*., 2005; Levy, 2005; McDermid *et al*., 2002; Allard *et al*., 1998; Pace and Leaf, 1995). This water soluble vitamin performs a variety of important cell functions such as preserving the integrity of artery walls and strengthening cardiovascular tissues. Vitamin C is an independent antioxidant that protects cells against death from oxidative stress (Guaiquil *et al*., 2001) and acts as a nonspecific reducing agent vitamin that donates electrons to metal ions thus reducing oxidative stress. Vitamin C works with vitamin E as a pair of free radical scavengers and protects folate by stabilizing the coenzyme in its reduced state and is required in the formation of collagen (Wardlaw, 1999).

Our ART subjects showed lower uric acid plasma levels than control and NART groups as reported in Pasupathi *et al*. (2009), Olaniyia and Arinolab (2007) and Carter (2006). Uric acid is the end-product of purine catabolism in man. Normal serum level is 0.025-0.080 mg/ml in males and 0.015-0.060 mg/ml in females. A level greater than 0.070 mg/ml is associated with an increased risk for gout. Gout is a metabolic disease associated with painful inflammation of joints-a common condition complained by HIV positive naïve persons. A high plasma uric acid level was associated with neutrophil release of toxic forms of oxygen in response to pain and inflammation. This reaction results in oxidative stress (Brody, 1994).

We therefore conclude that HIV infection is associated with increased levels of TC, TG, LDL-c, SOD and uric acid levels but decreased vitamin A and MDA levels which were reversed following treatment with anti-retroviral drugs. Antiretroviral drugs therefore reduce the risk of cardiovascular incidence and oxidative stress in HIV infected persons.

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