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Oxidative damage, total antioxidant capacity and body mass index in Relation to Severity of HIV in Nigerian Patients

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ABSTRACT: *Objectives:* This study was designed to determine the plasma levels of some oxidative damage products and antioxidant capacity; and investigate relationship between body mass index (BMI) and oxidative damage parameters in three severity groups of HIV patients compared with non-HIV-infected controls. *Methods:* Plasma levels of total antioxidant capacity (TAC), malondialdehyde (MDA), protein carbonyl (PCO) and advanced oxidative protein products (AOPP) were estimated spectrophotometrically and BMI measured in controls and patients with CD4 counts of ≥500 cells/µl (group1), 200-499 (group2) and <200 (group 3) *Results*: PCO, AOPP, MDA, increased significantly ($p<0.0001$) while TAC reduced significantly ($p<0.0001$) in all HIV groups compared with control Intra group comparisons of MDA, PCO and AOPP also increased significantly ($p<0.0001$) and TAC reduced significantly ($P<0.001$) when compared with each other. BMI and weight of group 3 was significantly reduced when compared with control ($p<0.05$) but no difference between groups 1 and 2 and control ($P>0.05$). BMI was positively correlated with TAC ($p<0.05$), CD4+ count ($p<0.001$), but negatively correlated with MDA ($p<0.001$), PCO ($p<0.01$) and AOPP ($p> 0.05$). *Conclusion:* Based on the results of this study, we recommend routine BMI or weight measurement and antioxidants supplementation in HIV subjects which is hoped to strengthen the immune system and reduce the oxidative damage. Association of MDA with BMI was strongest followed by PCO while AOPP was least. BMI is a reflection of oxidative damage in HIV infection.

Keywords: Antioxidant capacity, body mass index, oxidative damage, HIV-I.

Introduction

Scientific evidence has shown that HIV infection is caused by a retrovirus, the Human Immunodeficiency Virus (HIV) which is a ribonucleic acid (RNA) virus so designated because of its genome that encodes an unusual enzyme, reverse transcriptase (RT) that enables the virus to make copies of its own genome as deoxyribonucleic acid DNA in its hosts cells (that is, human T4 helper lymphocytes) [1].

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Infection with HIV has a devastating effect on the nutritional status of infected persons [1,2]. It has been noted that weight loss, often profound in magnitude depending on the stage of the infection and associated infections, are known features of HIV infection [2].. HIV-positive patients may lose 30-50% of their body mass before progressing to acquired immunodeficiency syndrome (AIDS) [3] According to different investigators, weight loss can be caused by five mechanisms, namely, inadequate food intake, reduced intestinal absorption, abnormal food utilization, increased excretion of nutrients and host requirements [4,5,6]. It has also been reported that weight loss contributes to the progression of HIV infection to AIDS [7].

Wasting, therefore, is a major complication of human immunodeficiency virus (HIV) infection, and it makes an important contribution to both the morbidity and the mortality of the disease [5]. Weight loss in patients with HIV infection tends to be periodic, occurring particularly in relation to episodes of secondary infection or gastrointestinal disease [8]

Few studies [7, 9] have examined the relationship between HIV infection and anthropometry in Nigeria, but none to the best of our knowledge has examined the association between HIV infection, body mass index and oxidative modifications of lipids and proteins, in this present study, we attempted to determine the plasma levels of carbonyl protein (PCO), advanced oxidative protein products (AOPP), malondialdehyde (MDA) and total antioxidant capacity (TAC); and to ascertain the degree of relationship of each oxidative damage parameters with body mass index (BMI) in HIV 1 adults.

Materials and Methods

Subjects

A total of 84 subjects comprising of 48 females and 36 males were recruited for the study, 58 subjects (33 females and 25 males) were HIV-I infected adults and the remaining 26 subjects (15 females and 11males) were control subjects.

Fifty-eight HIV-positive patients classified according to the Centre for Disease Control and Prevention (CDC) criteria; [Group 1 (CD4<200), n = .20], [Group 2 (CD4: 200-499), n = .20] and [Group 3 (CD4>500), n = 22] and 24 age- and sex-matched healthy HIV-I seronegative adults with a mean age of 33 years (range: 22–49 years) diagnosed as HIV-1 infected patients (using DETERMINE® immunoassay (Japan)), were recruited from the HIV Clinic run by the Institute of Human virology, Nigera (IHVN) at the Olabisi Onabanjo University Teaching Hospital, Sagamu before the commencement of antiretroviral therapy. All subjects underwent an initial screening that included a detailed history (medical, smoking, diet, and alcohol and supplemental vitamin intakes) and anthropometric (weight and height) and biochemical (complete blood count, glucose, cholesterol, triglyceride, creatinine, urea, and liver enzymes) measurements. Patients were eligible if they had no acute opportunistic infection. Exclusion criteria included use of alcoholic beverages, smoking, initiation of antioxidant vitamin and antiretroviral drug therapies before the study, hyperlipidemia, hypertension, clinical tuberculosis, diabetes, kidney or liver dysfunction, intractable diarrhoea (more than six liquid stools per day), vomiting, or evidence of gastrointestinal bleeding. Informed consent was obtained from participants and the entire experimental protocol was approved by institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki Declaration of 1964 [10]. Blood was collected by venipuncture into 5 ml plain vacuutainer and 5ml EDTA vacuutainer tubes. CD4+ were estimated by Partec Cyflow (Germany).

Assay of plasma advanced protein oxidation products (AOPP) and protein carbonyl (PCO) levels

Spectrophotometric determination of AOPP levels was performed by modification of Witko's method [11]. Absorbances were measured at 340nm and concentrations of AOPP were calculated by using the extinction coefficient of 26 M^{-1} x cm^{-1}**.** Plasma PCO levels were measured spectrophotometrically by using the method of Rednecks et al [12]. Absorbances were measured at 360nm, using molar extinction coefficient of DNPH, $\varepsilon = 2.2 \times 10^4$ M^{-1} x cm^{-1}. Protein content were determined on the HCl blank pellets spectrophotomtrically by Biuret method using bovine serum albumin (BSA) as standard [13]

Total antioxidant capacity measurement

Total antioxidant capacity was measured by ferric reducing antioxidant power (FRAP) assay according to the method of Benzie and Strain [14]. At low pH, when a ferric tripyridyltriazine (Fe III-TPTZ) complex is reduced to the ferrous (Fe II) form, an intense blue colour with an absorption maximum at 593 nm develops.

Lipid peroxidation assay

Lipid peroxidation was estimated spectrophotometrically by the thiobarbituric acid reactive substance (TBARS) method as described by Varshney and Kales [15] and Malondialdehyde (MDA) was quantitated by using $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$[16].

Results

A total of 84 subjects comprising of 48 females and 36 males were recruited for the study, 58 subjects (33 females and 25 males) were HIV-I infected adults and the remaining 26 subjects (15 females and 11males) were control subjects.

In Table 1, there was no significant difference in the ages of HIV groups and control, but significant difference was observed in height ($p>0.05$), weight ($p>0.0001$) and BMI ($p<0.01$) reflecting changes in weight and BMI as the disease progresses. Significant decrease in antioxidant capacity ($p<0.0001$) and CD4+ ($p<0.0001$) were noticed in HIV groups when compared with control (Table 1) indicating deteriorating immune function coupled with depletion of antioxidant. Significant increase in MDA, AOPP and PCO ($p<0.0001$) in HIV groups compared with controls was observed which confirmed appreciable oxidative damage in HIV groups.

Table 2 shows no significant difference in the ages and heights among HIV groups when compared with each other and each HIV group and control, but significant difference was observed in weight and BMI of HIV G_3 and control ($p<0.05$), G_3 and G_1 ($p<0.05$) and G_2 and G_3 ($p<0.05$) implying that G_3 was the most affected of the HIV groups. Significant difference in the intra group comparison of immune, antioxidant capacity and oxidative damage among HIV groups when compared with each other and each HIV group and control ($p<0.0001$) were observed. This implied that immune status is a probable determinant of antioxidant capacity and oxidative damage in HIV-infected individuals.

Table 3 shows nutritional status as assessed by body mass index (BMI) in the study population. Prevalence of severe malnutrition in HIV-I patients with CD4+ <200 lymphocytes/mm^3 was 36.4%, moderate was 9.1% while those with normal weight and over weight were 50% and 0.5% respectively Patients with CD4+ between 200-499 lymphocytes/mm^3 had 6.3% moderate malnutrition and 93.7% normal weight; those with CD4 >500 lymphocytes/mm^3 were normal weight 72.7% and overweight 37.3% In the controls, prevalence of normal weight was 80%, overweight and obesity were 10% respectively. Body mass index was related to severity of disease in HIV-I positive patients.

Table 4 shows correlation of BMI and weight, height, CD4+ count, MDA, PCO, AOPP and TAC of HIV-I positive patients and control subjects. BMI was significantly correlated with weight ($r = +0.787$, $p<0.0001$), CD4+ count ($r = + 0.476$, $p<0.001$), but negatively correlated with MDA ($r = -0.384$, $p<0.001$), PCO ($r = -0.364$, $p<0.01$) and AOPP ($r = -0.177$, $p> 0.05$). Association between oxidative damage parameters analysed and BMI was strongest with MDA followed by PCO, while AOPP had least association with BMI in HIV-infected adults studied.

Table1: Biophysical data and serum concentrations of MDA, PCO, AOPP and TAC of HIV-I positive patients and control subjects of HIV-I positive patients and control subjects

Parameters	Control (n=26)	G_1 (n = 20)	G_2 (n = 22)	G_3 (n = 22)	F-value	P-value
Age (Years)	30.97 ± 8.90	38.45 ± 10.95	33.87 ± 6.74	35.22 ± 10.91	2.27	0.086
Weight (kg)	60.73 ± 8.80	66.13 ± 16.36	59.46 ± 14.39	48.72 ± 12.14	7.06	0.000
Height (m)	1.62 ± 0.79	1.58 ± 0.79	1.62 ± 0.60	1.56 ± 0.76	3.37	0.022
BMI (kg/m^2)	22.92 ± 3.18	26.03 ± 5.16	23.58 ± 6.51	19.89 ± 5.36	4.65	0.005
CD4+‡	904.1 ± 138.0	689.1 ± 158.0	325.0 ± 83.50	141.5 ± 54.3	9.39	0.000
MDA (nmol/ml)	1.71 ± 0.86	1.95 ± 1.16	3.10 ± 1.35	3.90 ± 1.35	4.56	0.000
PCO[#]	0.31 ± 0.14	0.46 ± 0.14	0.75 ± 0.39	1.48 ± 0.43	11.40	0.000
AOPP[#]	0.49 ± 0.44	0.68 ± 1.00	1.87 ± 1.42	2.10 ± 1.13	15.97	0.000
TAC†	1048.2 ± 138.6	903.3 ± 162.4	741.1 ± 135.3	682.8 ± 83.2	28.04	0.000

Values are expressed as mean ± standard deviation (SD). †Levels expressed as µmol/l #Levels expressed as nmol/mg protein, ‡Count expressed as lymphocytes/mm^3.
G_1 =HIV infected subjects having a CD4 T lymphocyte counts > 500 cell/mm^3
G_2= HIV infected subjects having a CD4 T lymphocyte counts between 200-499 cell/mm^3
G_3= HIV infected subjects having a CD4 T lymphocyte counts <200 cell/mm^3

Table 2: Intra group comparison of biophysical parameters, CD4+ count, MDA, PCO, AOPP and TAC of HIV-I positive AIDS patients and control subjects

Parameters	Control vs G_1	Control vs G_2	Control vs G_3	G_1 vs G_2	G_1 vs G_3	G_2 vs G_3
Age (Years)	NS	NS	NS	NS	NS	NS
Weight (kg)	NS	NS	$p < 0.05$	NS	$p < 0.05$	$p < 0.05$
Height (m)	NS	NS	NS	NS	NS	NS
BMI (kg/m^2)	NS	NS	$p < 0.05$	NS	$p < 0.05$	$p < 0.05$
CD4+‡	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
MDA (nmol/ml)	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
PCO[#]	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
AOPP[#]	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
TAC†	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

†Levels expressed as µmol/l #Levels expressed as nmol/mg protein, ¶Count expressed as lymphocytes/mm^3.
Con = Control, NS=Not significant
G_1 =HIV infected subjects having a CD4 T lymphocyte counts > 500 cell/mm^3
G_2= HIV infected subjects having a CD4 T lymphocyte counts between 200-499 cell/mm^3
G_3= HIV infected subjects having a CD4 T lymphocyte counts <200 cell/mm^3

Table 3: Nutritional status assessed by Body mass index (BMI) in the study population

Category	BMI	Controls (n=26)	G_1 (n = 20)	G_2 (n = 22)	G_3 (n = 22)
Malnutrition					
Severe	> 16.0	0%	0%	0%	8 (36.4%)
Moderate	10.0 – 16.9	0%	0%	1 (6.3%)	2 (9.1%)
Mild	17.0 – 18.5	–	–	–	–
Others					
Normal weight	18.5 – 24.9	33 (80%)	8 (72.7%)	15 (93.7%)	11 (50%)
Overweight	25.0 – 29.9	4 (10%)	4 (37.3%)	0%	1 (0.5%)
Obesity	> 30	4 (10%)	0%	0%	0%

Exact numbers and percent in parenthesis (rounded).
G_1 =HIV infected subjects having a CD4 T lymphocyte counts > 500 cell/mm^3
G_2= HIV infected subjects having a CD4 T lymphocyte counts between 200-499 cell/mm^3
G_3= HIV infected subjects having a CD4 T lymphocyte counts <200 cell/mm^3

Table 4: Correlation of BMI weight, height, CD4+ count, MDA, PCO, AOPP and TAC of HIV-I positive patients and control subjects

Parameters	HIV-1 Patients		Controls	
	r	p	r	p
BMI (kg/m^2)	1	–	1	–
Weight (kg)	0.787***	0.000	0.747***	0.000
Height (m)	0.045	0.760	–0.241	0.128
CD4+ lymphocytes/mm^3	0.476**	0.001	0.076	0.638
MDA (nmol/ml)	–0.384*	0.006	–0.154	0.338
PCO (nmol/mg protein)	–0.364*	0.010	–0.150	0.351
AOPP (nmol/mg protein)	–0.177	0.229	0.201	0.213
TAC (µmol/l)	0.328*	0.021	0.216	0.252

**p>0.01, *p>0.05

Discussion

Altogether 72.4% of newly diagnosed HIV-infected patients who were not in advanced phase of the disease and 27.6% who were in advanced phase were evaluated and found to have some degree of oxidative damage with concomitant depletion of total antioxidant. capacity which affect body mass index differently. There was significantly higher oxidative stress in the group of HIV-positive subjects, than

seronegative control subjects as determined from plasma protein and lipid peroxidation products' concentrations.

Lipid peroxidation results from release of free radicals that can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes to form malondialdehyde (MDA). The concentration of serum MDA which is used for monitoring lipid peroxidation in biological samples was found to be increased in HIV-1 seropositive patients with CD4+ count < 200 lymphocytes/mm^3 and that of 200-499lym/mm^3 but there was no significant changes in those with CD4>500lym/mm^3 compared to the control. This finding is similar to the result obtained in the study of Ogunro et al [17] but is in contrast to the studies of Allard, and his co-workers [18]

Oxidative damage to protein, lipids or DNA may all be seriously deleterious and may be concomitant. However, proteins are possibly the most immediate vehicle for inflicting oxidative damage on cells because they are often catalysts rather than stoichiometric mediators; hence, the effect of damage to one molecule is greater than stoichiometric [19] Protein perxidation can occur by various mechanisms: namely, an increase in the production of ROS, decrease in the rate of scavenging of ROS, an increase susceptibility of the protein to oxidation and a decrease in the rate of removal of oxidized species [20]

ROS leading to OPD include radical species such as superoxide, hydroxyl, perxyl, alkoxyl, hydroperoxyl and non-radical species such as hydrogen peroxide, hypochlorus acid, ozone, singlet oxygen and peroxynitrite [19] Considerable evidence indicates that the maintenance of protein redox status is of fundamental importance for cell function, whereas structural changes in proteins are considered to be among the molecular mechanisms leading to disease complication. PCO content is usually the most general indicator and by far the most commonly used marker of OPD [19]. Among the various oxidative modifications of amino acids in proteins, PCO formation may be an early marker for protein oxidation [21]. PCO have a major advantage over lipid peroxidation products as markers of oxidative stress because oxidized proteins are generally more stable, PCO form early and circulate in the blood for longer periods compared with other parameters of oxidative stress [22]. We found that plasma PCO and AOPP levels were increased significantly compared with controls. Dalle-Donne et al [19] have reported that PCO groups may be introduced into proteins by secondary reaction of the nucleophilic side chains of cysteine, histidine and lysine residues, and aldehydes (4-hydroxy-2-nonenal, malondialdehyde, 2-propenal acrolein) produced during lipid peroxidation.

Lipid peroxides condense with the side chain of protein amino acids to result in carbonyl groups in proteins [23] Increased lipid peroxide in the form of malondialdehyde accumulates in patients with HIV-I infection. In the present study, the concentration of serum PCO was found to increase in HIV-1 seropositive patients with CD4+ count < 200 lymphocytes/mm^3 and that of 200-499lym/mm^3 but there was no significant changes in those with CD4>500lym/mm^3 compared to the control. Increase in plasma PCO levels observed in HIV-I patients studied may be due to the increase in lipid peroxidation due to increase formation or decrease degradation and clearance.

Recently, AOPP a new marker of oxidative protein damage (OPD) has begun to attract the attention of various investigators [24] AOPP are defined as dityrosine containing cross-linked protein products [25]. AOPP levels correlated with concentrations of dityrosine and advanced glycation end products-pentosidine as indices of oxidant-mediated protein damage [25]. AOPP are considered as reliable markers to estimate the degree of oxidant-mediated protein damage [24]. The concentration of serum AOPP and carbonyl protein which are markers of protein peroxidation were found to be increased in HIV-1 seropositive patients with CD4+ count < 200 lymphocytes/mm^3 and that of 200-499lym/mm^3 but there was no significant changes in those with CD4>500lym/mm^3 compared to the control, this agrees with the study of Ngondi et al [26].

The increase in lipid peroxidation and protein oxidative parameters was also associated with lower plasma total antioxidant capacity. There was marked reduction in the serum total antioxidant of HIV-I seropositive individual in this study, the degree of reduction was related to the degree in the reduction in the CD4+ which determines the level of immunity in HIV-I.

Although it was reported that there was no correlation between body weight loss and level of immunosuppression [27], our findings showed significant reverse relationship between severity of weight loss (as assessed by body mass index) and serum CD4+ lymphocyte count that is comparable with the findings of Rivera et al [28].

The BMI and weight for HIV-I seropositive patients with CD4+ count of <200lymphocytes/mm^3 were significantly reduced when compared with the control ($p<0.05$) BMI was significantly correlated with weight, CD4+, but negatively correlated with MDA, PCO and AOPP. .Lipid peroxidation seems to occur

first, followed by protein carbonyl formation while advance oxidative protein products are produced much later.

Conclusion

Significant oxidative damage and depletion of TAC in relation to severity of HIV infection when compared with the controls was demonstrated. Association of MDA with BMI was highest followed by PCO while AOPP was least

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