ROS

RESEARCH ARTICLES

Biomarkers of Oxidative Stress in HIV Seropositive Individuals on Highly Active Antiretroviral Therapy

Augusta Chinyere Nsonwu-Anyanwu, Ehijiagbone Victory Ighodalo, Dianabasi King, Agu Chidozie Elochukwu, Sunday Jeremiah, Offor Tom Solomon, and Chinyere Adanna Opara Usoro

Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, Nigeria

Correspondence: austadechic@yahoo.com (A.C.N-A.)

Nsonwu-Anyanwu AC et al. Reactive Oxygen Species 3(9):197–207, 2017; ©2017 Cell Med Press
http://dx.doi.org/10.20455/ros.2017.827
(Received: January 20, 2017; Revised: February 15, 2017; Accepted: February 16, 2017)

ABSTRACT | Oxidative and nitrosative stress have been implicated in the pathogenesis and progression of human immune deficiency virus (HIV) infection, and seem to be more pronounced with commencement of highly active antiretroviral therapy (HAART). Evaluation of markers of oxidative and nitrosative stress may be useful indices for monitoring progress of infection and development of adverse drug reactions. The total antioxidant capacity (TAC), total plasma peroxides (TPP), oxidative stress index (OSI), nitric oxide (NO), reduced form of glutathione (GSH), malondialdehyde (MDA), and catalase activity were estimated in HIV-infected individuals with or without HAART. A total of 90 consenting subjects comprising of 30 HIV seropositive subjects on HAART, 30 HAART naive and 30 apparently healthy HIV seronegative controls resident in Calabar were recruited into the study. TAC, TPP, NO, GSH, MDA, and catalase activity were determined using colorimetric methods, $CD4^+$ T cell count was done by flow cytometry while body mass index (BMI) and oxidative stress index were determined by calculation. Data was analyzed using ANOVA, LSD post hoc and Pearson's correlation at $p < 0.05$. The results showed that HIV seronegative controls had higher $CD4^+$ T cell count, BMI, NO, and catalase activity and lower TPP compared to HIV seropositive individuals on HAART and HAART naive subjects ($p < 0.05$). Higher levels of NO and catalase and lower $CD4^+$ T cells count, MDA, TPP, and OSI were observed in HIV on HAART compared to HAART naive ($p < 0.05$). OSI correlated positively with TPP ($r = 0.547$, $p = 0.002$) and negatively with TAC ($r = –0.727$, $p = 0.000$) in HIV HAART naive subjects only. In conclusion, HAART may be associated with reduction in lipid peroxidation and restoration of some antioxidants, and their levels may determine initiation of HAART in HIV-infected individuals.

KEYWORDS | Antioxidants; Highly active antiretroviral therapy; Human immune deficiency virus; Lipid peroxidation; Oxidative stress

ABBREVIATIONS | AIDS, acquired immune deficiency syndrome; BMI, body mass index; EDTA, ethylenediaminetetraacetic acid; GSH, reduced form of glutathione; HAART, highly active antiretroviral therapy; HIV, human immune deficiency virus; MDA, malondialdehyde; NO, nitric oxide; OSI, oxidative stress index; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TPP, total plasma peroxide

ROS

RESEARCH ARTICLES

CONTENTS

1. INTRODUCTION

Infection with the human immune deficiency virus (HIV) is associated with progressive loss of cellular immunity which results in life-threatening opportunistic infections and cancers and progressive development of acquired immune deficiency syndrome (AIDS) and ultimately death in the absence of any treatment interventions [1]. HIV infection induces a state of persistent chronic inflammation due to HIV replication and viral activation of macrophages and T cells, leading to increased generation of reactive oxygen and nitrogen species (ROS/RNS). Both ROS and RNS react with many biological molecules, including lipids, proteins, carbohydrates, and nucleic acids, affecting cellular integrity and functions, which often results in oxidative and nitrosative stress [2].

Oxidative stress is a condition characterized by the imbalance between the production of ROS and their neutralization by the antioxidant defenses, leading to the accumulation of ROS and their derived metabolites, with changes in the redox status of the cell [3]. The occurrence of both local and systemic oxidative stress in HIV-1-infection has been implicated in the progressive loss of $CD4^+$ T lymphocytes, and disease progression, as well as in the development of other secondary complications associated with HIV infection [4]. The highly active antiretroviral therapy (HAART) is currently the therapy of choice for HIV-infected patients. However, despite remarkable viral replication suppression and immune response restoration, adverse drug reactions appear to be a major drawback to the success story of long-term HAART use in many patients [5, 6]. HAART regimen have been implicated in increase generation of chemically reactive species in circulation, possibly by producing more oxidized metabolites derived from the interaction between ROS and infected cell biomolecules [7]. This suggests that the HIV-1 infection alone or in combination with the introduction of antiretroviral (ARV)/HAART may induce oxidative stress and further augment HIV-1 pathogenesis [7, 8]. Measurement of oxidative/nitrosative stress molecules could therefore function as a potential surveillance parameter in addition to $CD4^+$ T cell count for estimation of progression of infection and development of adverse drug reactions. This study therefore evaluated some biomarkers of oxidative stress in HIV seropositive individuals with or without HAART.

2. MATERIALS AND METHODS

2.1. Study Design

This case control study was carried out at the University of Calabar Teaching Hospital (PEPFAR Clinic), Nigeria. Ethical approval was obtained from the ethical committee and informed consent was obtained from potential subjects. This study was carried out in accordance with the Ethical Principles for Medical Research Involving Human Subjects as outlined in the Helsinki Declaration in 1975 and subsequent revisions. The study population comprised of HIV seropositive subjects on HAART, HIV seropositive subjects not on HAART (HAART naive), and HIV seronegative subjects serving as controls.

2.2. Selection of Subjects

2.2.1. Subjects

A total of 90 subjects of either gender, aged between 18 and 55 years were recruited for this study. There were 60 test subjects (comprised of 30 HAART-treated and 30 HAART- naive HIV-positive persons) and 30 age-matched controls that were apparently healthy, HIV-negative persons residing in Calabar. The HIV seropositive subjects on HAART are subjects who have been on any of the HAART regimens corresponding with the Nigerian National guidelines for HAART [9] for a minimum of six months. Sociodemographic information was obtained through a semi structured questionnaire while anthropometric indices including body weight and height were measured and body mass index (BMI) calculated.

2.2.2. Inclusion and Exclusion Criteria

HIV seropositive individuals on HAART, HIV seropositive individuals not on HAART, and HIV negative apparently healthy controls were included in this study. Individuals with AIDS or any other chronic disease condition were excluded from the study.

2.3. Sample Collection

Five milliliters (5 ml) of venous blood was drawn from each subject. About 2 ml of blood was dispensed into ethylenediaminetetraacetic acid (EDTA)-anticoagulated container for $CD4^+$ cell count and labeled appropriately. The rest of the blood sample was dispensed into a 10 ml plain container, allowed to clot and centrifuged to separate serum from cells. The serum was transferred, using Pasteur pipette, into 5 ml plain containers, labeled, and stored at -20^0C for analysis of some indices of oxidative stress.

2.4. Laboratory Methods

2.4.1. HIV Screening by Immunochromatographic Method

Immunochromatographic test was used for qualitative detection of antibodies to HIV-1 and -2. Samples were added to the pad. As the sample migrated through the conjugate pad, it reconstituted and mixed with selenium colloid-antigen conjugate. The mixture continued to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient site. If HIV-1 and -2 antibodies were present in the sample, the antibodies bound to the antigen selenium colloid and to the antigen at the window forming a red line at the patient window site. If HIV-1 and -2 antibodies were absent, the antigen-selenium colloid flowed past the window, and no red line was found at the patient's site. To ensure assay validity, a protocol control was incorporated in the assay device [10].

2.4.2. HIV Confirmation by Immunochromatographic Method

The Chembio HIV-1 and -2 STAT assay employs a unique combination of a specific antibody-binding pattern which is conjugated to colloidal gold dye particle and HIV-1 and -2 antigen which are bound to the membrane solid phase. The sample was applied to the sample well, followed by the addition of running buffer. The buffer facilitated the lateral flow of the released product and promoted the binding of antibodies to the antigens. If present, the antibodies bound to the gold conjugated antibody-binding protein. In a reactive sample, the dye conjugated immune complex migrated on the nitrocellulose membrane and was capture by the antigen immobilized in the test region producing a pink or purple line. In the absence of HIV antibodies, there was no pink or purple line in the test region. The sample continued to migrate along the membrane and produced a pink or purple line in the control containing

IgG antigens. This procedural control served to demonstrate that the specimen and regent had been applied and had migrated through the device [10].

2.4.3. Enumeration of $CD4^+$ T Cell Count by Flow Cytometry

When fluorescence-labeled cells are passed by a light source, the fluorescent molecules are excited to a higher energy state. Upon returning to their resting states, the fluorochromes emit light energy at higher wavelengths. The use of multiple fluorochromes, each with a similar excitation wavelength and a different emission wavelength allows several cell properties to be measured simultaneously [11]. In this study, $CD4^+$ T cells were quantified by flow cytometry using fluorescence-labelled anti-$CD4^+$ antibody.

2.4.4. Determination of Total Antioxidant Capacity

A standard solution of Fe-EDTA complex reacted with hydrogen peroxide (H_2O_2) by a Fenton type reaction, leading to the formation of hydroxyl radicals. The hydroxyl radicals then degraded benzoate resulting in the release of TBARS (thiobarbituric acid reactive substances). Antioxidants from added sample caused suppression of the production of TBARS. This reaction was measured spectrophotometrically at 532 nm and the inhibition of color development defined as the total antioxidant capacity (TAC) of the sample [12].

2.4.5. Estimation of Total Plasma Peroxide

Total plasma peroxide (TPP) was determined using the reaction of ferrous-butylated hydroxytoluene-xylenol orange complex (FOX-2 reagent) with serum peroxides which yields a colored complex that was measured spectrophotometrically at 560 nm, according to the FOX-2 method. The FOX-2 test system is based on the oxidation of ferrous ions to ferric ions by various types of peroxides present in the serum samples, to produce a colored ferric-xylenol orange complex whose absorbance can be measured [13].

2.4.6. Calculation of Oxidative Stress Index

The ratio of total plasma peroxide (TPP) to total antioxidant capacity (TAC) was calculated as the oxidative stress index (OSI), an indicator of the degree of oxidative stress. OSI (%) = [TPP (μmol/l H_2O_2) × 100] ÷ [TAC μmol/l].

2.4.7. Estimation of Nitric Oxide

The Griess test was used for detecting total levels of nitrite or nitrous acid in samples. The nitric oxide (NO)- derived compounds in the serum combined with alpha-naphthylamine to produce pink azo dye whose absorbance was measured at a wavelength of 540 nm. Total nitrite and nitrate levels were represented as total nitric oxide metabolites (NOx) and measurement of NOx is considered a direct marker of in vivo NO production [14].

2.4.8. Quantification of Reduced Form of Glutathione

Estimation of reduced form of glutathione (GSH) was carried out following the modified standard Ellman's method. The reagent, 5-5′-dithiobis (2-nitrobenzoic acid) (DTNB, also known as Ellman's reagent) reacts with GSH to form the chromophore, 5-thionitrobenzoic acid (TNB), which is measured at 412 nm [15].

2.4.9. Estimation of Catalase Activity by Titrimetric Method

The enzyme catalase is responsible for catalyzing the decomposition of H_2O into H_2O and O_2. The assay of the enzyme was performed based on estimation of residual H_2O_2 by titration with potassium permanganate ($KMnO_4$) to give a faint light pink color [16].

2.4.10. Estimation of Malondialdehyde

Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acid, serves as a convenient index for determining the extent of the peroxidation reaction. MDA, as the product of lipid peroxidation in the sample, reacted with thiobarbituric acid to give a red species absorbing at 532 nm [17].

2.5. Statistical Analysis

Data analysis was done using the statistical package for social sciences (SPSS version 20.0, IBM, USA). Analysis of variance (ANOVA) was used to test sig-

nificance of variations within and among group means and Fisher's least significant difference (LSD) post hoc test was used for comparison of multiple group means. Pearson's correlation was used to determine associations between variables. A probability value $p < 0.05$ was considered statistically significant.

3. RESULTS

The comparison of age, BMI, $CD4^+$ T cell counts, NO, GSH, MDA, TPP, TACy, OSI, and catalase in HIV seropositive subjects on HAART, HAART naive, and sero-negative controls were shown in **Table 1**. Significant variations were observed in the $CD4^+$ T cell count, BMI, NO, MDA, TPP, OSI, and catalase among the three groups studied ($p < 0.05$), while no significant variation was observed in the levels of their GSH and TAC ($p > 0.05$).

The comparison of oxidative stress indices on HAART, HAART naive and seronegative controls using LSD post hoc were depicted in **Table 2**. HIV sero-negative controls had significantly higher $CD4^+$ T cell count, BMI, NO, and lower TPP compared to HIV seropositive subjects on HAART ($p < 0.05$). HIV seronegative controls also had significantly higher $CD4^+$ T cell count, BMI, NO, and catalase compared to the HAART naive subjects ($p < 0.05$). HIV seropositive subjects on HAART recorded significantly higher mean levels of NO and catalase and lower levels of $CD4^+$ T cells count, MDA, TPP and OSI compared to HAART naive seropositive subjects ($p < 0.05$).

Figure 1 shows the correlation plot of TAC against OSI in HIV HAART naive subjects. A significant negative correlation ($r = –0.727$) was observed between TAC and OSI in HIV HAART naive subjects ($p < 0.05$). **Figure 2** shows the correlation plot of TPP against OSI in HIV HAART naive subjects. A significant positive correlation ($r = 0.547$) was observed between TPP and OSI in HIV HAART naive subjects ($p < 0.05$).

4. DISCUSSION

HAART has been the treatment of choice for HIV infection due to its proven effectiveness in suppressing viral load, restoring immune function and decreasing mortality among HIV-infected persons. HAART, however, has been associated with several adverse effects which have become subjects of intense study, one of which is the possible involvement of HAART in HIV-associated oxidative stress. In the present study, TPP, TAC, OSI, MDA, and GSH, which are biomarkers of oxidative stress, were assessed, alongside $CD4^+$ T cell count and BMI in a group of HAART-treated and HAART naive seropositive subjects and apparently healthy HIV negative controls. Our results showed that HIV on HAART and HAART naive had higher levels of MDA and TPP and lower catalase activity compared to HIV seronegative controls. Several studies have demonstrated that HIV infection has been associated with an unbalanced redox system and high oxidative stress profile which is characterized by (1) a depletion of protective system such as GSH, thioredoxin, glutathione peroxidase, superoxide dismutase (SOD); catalase, vitamins A, C , and E, selenium, and zinc; (2) activation of immune signaling molecules (e.g., cytokines and chemokines); (3) increased production of reactive oxygen species (ROS), namely, hydroxyl radicals, superoxide anions, NO, and H_2O_2; and (4) elevated levels of hydroperoxides and MDA in both pediatric and adult HIV seropositive patients [18, 19]. Immunological and biological consequences of this condition include activation of lymphocytes and phagocytizing cells, chronic inflammation, increased polyunsaturated fatty acid concentration, and lipid peroxidation. Alterations in the levels of these oxidative stress indices in HIV infection may therefore be a reflection of degree of cellular damage, immune depression, lipid peroxidation and oxidative stress [20]. Higher levels of ROS and lower antioxidant levels have been reported in HIV subjects on HAART compared to HAART naive seropositive and seronegative individuals. Individuals who strictly adhered to HAART have been shown to display higher oxidative stress than those with intermittent HAART adherence [8]. Consistent with our findings, lower catalase activity has also been reported in HIV HAART naive individuals compared to controls [21]. Increased ROS production and consequent buffering activity by antioxidant enzymes associated with HIV infection and HAART may be responsible for depressed catalase activity. However, the activities of the erythrocyte antioxidant enzyme SOD and catalase were reported to be significantly higher in HIV and AIDS groups compared to HIV-control [18]. Se-

TABLE 1. Comparison of oxidative stress indices in HIV seropositive subjects on HAART, HAART naive, and seronegative controls

Index	HAART (n = 30)	HAART Naive (n = 30)	Controls (n = 30)	F Value	p Value
Age (years)	35.80 ± 8.64	32.53 ± 8.74	34.13 ± 8.94	1.04	0.358
$CD4^+$ (cells/ml)	486.63 ± 300.51	683.13 ± 250.70	1083.70 ± 442.30	23.89	0.000
BMI (kg/m^2)	22.39 ± 1.91	21.62 ± 2.24	23.89 ± 2.59	7.73	0.001
NO (µmol/l)	27.03 ± 10.20	20.83 ± 7.88	39.96 ± 11.17	29.48	0.000
GSH (µmol/l)	11.32 ± 2.15	12.32 ± 15.57	11.96 ± 8.91	0.090	0.914
MDA (nmol/ml)	4.71 ± 1.57	4.87 ± 1.39	3.15 ± 1.87	10.21	0.000
TPP (µmol H_2O_2/l)	1.82 ± 0.29	2.91 ± 0.35	0.64 ± 0.45	264.43	0.000
TAC (mmol/l)	0.82 ± 30.24	0.69 ± 0.30	0.73 ± 0.30	1.64	0.200
OSI (%)	2.68 ± 2.02	5.76 ± 5.10	4.07 ± 2.89	5.57	0.005
Catalase (mU/l)	5.63 ± 0.86	4.73 ± 0.63	5.35 ± 0.69	11.71	0.000

rum catalase has been shown to increase as HIV disease progresses [21].

The GSH levels did not vary among HIV on HAART, HAART naive, and HIV seronegative controls studied. It has been shown that the intracellular levels of either oxidized or total glutathione in $CD8^+$ and $CD19^+$ lymphocytes from HIV-1-infected patients do not differ from those of seronegative controls. HAART has been associated with increased GSH levels and improvement in glutathione-redox status [22]. Contrary to our findings, lower GSH levels have been reported in HIV seropositive individuals compared to their HIV negative counterparts [18, 23]. Mechanisms for lower GSH levels in HIV infection have been elucidated and it has also been shown that HIV infection is able to directly or indirectly inhibit glutathione synthesis [5, 24]. The viral Tat protein induces enhanced ROS production in HIV-infected patients by mitochondrial generation of superoxide anion [25], which in turn may activate nuclear factor κB (NF-κB) [23], thus increasing HIV transcription and progressive generation of more ROS and consequently antioxidant depression especially GSH. Chronic HIV infection leads to excessive production of proinflammatory cytokines such as IL-1, IL-17, and TNF-α. Elevated TGF-β blocks the production of gamma-glutamylcysteine ligase catalytic subunit (GCLC) which reduces the production of new molecules of GSH. Elevated levels of IL-1 will also facilitate the loss of intracellular cysteine, which further reduces the production of new GSH molecules [26]. HAART may increase oxidative stress levels above and beyond levels caused by the virus itself. HAART may increase chemically reactive species in the circulation, possibly by producing more oxidized metabolites derived from the interaction between ROS and infected cell biomolecules [27, 28], leading to GSH depression. HAART may reduce GSH synthesis, enhance GSH utilization, or limit intracellular reduction of its oxidized form (GSSG). GSH reduction modify related functions such as reducing capacity, protein biosynthesis, immune function, accumulations of lipid peroxidation products, and detoxification capacity [5], which may result in disease progression, and poor survival of the HIV-1-infected individuals . Insufficient intake or malabsorption of nutrients, which is common in HIV patients, may exacerbate the depressed GSH status.

Higher NO levels were seen in HIV seronegative subjects compared to HAART and HAART naive subjects studied. NO has been implicated in several biological functions, which vary under physiological and pathological conditions. Physiologically, NO has low reactive properties, and participates in the maintenance of vascular homeostasis, neurotransmission, and in some posttranslational modifications of signaling cascades that modulate normal cell functioning [29]. On the other hand, under pathological conditions, especially chronic inflammation such as HIV infection, NO is quickly consumed in a reaction with superoxide anion, yielding peroxynitrite and other highly reactive NO-derived species, which

TABLE 2. Comparison of oxidative stress indices in HIV seropositive subjects on HAART, HAART naive, and seronegative controls using LCD post hoc analysis

Parameter	Groups		Mean Difference	p Value
	HAART (n = 30)	**Controls (n = 30)**		
$CD4^+$ (cells/ml)	486.63 ± 300.51	1083.70 ± 442.30	–597.1 ± 88.00	0.000
BMI (kg/m^2)	22.39 ± 1.91	23.89 ± 2.59	–1.49 ± 0.58	0.012
NO (µmol/l)	27.03 ± 10.20	39.96 ± 11.17	–12.93 ± 2.54	0.000
MDA (nmol/ml)	4.71 ± 1.57	3.15 ± 1.87	1.55 ± 0.42	0.000
TPP (µmolH_2O_2/l)	1.82 ± 0.29	0.64 ± 0.45	1.18 ± 0.09	0.000
	HAART Naive (n = 30)	**Controls (n = 30)**		
CD_4^+ (cells/ml)	683.13 ± 250.70	1083.70 ± 442.30	–400.6 ± 88.00	0.000
BMI (kg/m^2)	21.63 ± 2.23	23.89 ± 2.59	–2.26 ± 0.58	0.000
NO (µmol/l)	20.83 ± 7.88	39.96 ± 11.17	–19.13 ± 2.54	0.000
Catalase (mU/l)	4.74 ± 0.63	5.35 ± 0.69	–0.62 ± 0.19	0.002
TPP (µmolH_2O_2/l)	2.91 ± 0.34	0.64 ± 0.45	2.27 ± 0.17	0.000
	HAART (n = 30)	**HAART Naive (n = 30)**		
$CD4^+$ (cells/ml)	486.63 ± 300.51	683.13 ± 250.70	–196.5 ± 88.00	0.028
NO (µmol/l)	27.03 ± 10.20	20.83 ± 7.88	6.20 ± 2.54	0.007
MDA (nmol/ml)	4.71 ± 1.57	4.87 ± 1.39	–1.72 ± 0.42	0.000
TPP (µmolH_2O_2/l)	1.82 ± 0.29	2.91 ± 0.34	–2.09 ± 0.09	0.000
OSI (%)	2.68 ± 2.01	5.76 ± 5.10	–3.08 ± 0.92	0.001
Catalase (mU/l)	5.63 ± 30.86	4.74 ± 0.63	0.90 ± 0.19	0.000

mainly mediate cellular injury and microbe killing. Therefore, NO is a pivotal component during the generation of innate immune response against intracellular pathogens, such as HIV-1 [30]. Reduced NO in HIV-1-infected patients could lead to proliferative signals in lymphocytes. Reduced endothelial NO production has also been reported in individuals undergoing indinavir-based antiretroviral treatment, suggesting that HAART itself may potentiate NO reduction in these patients. This process has been shown to be mediated by the HIV-1 glycoprotein gp120, which could reduce the expression of the constitutive isoform of NO synthase in some tissues, such as the brain [31]. Contrary to our findings, clinically, higher NO in serum was reported in asymptomatic HIV-1 individuals and patients with AIDS having opportunistic infections. Association of increased NO with lower $CD4^+$ T cell counts has been reported [30].

HIV on HAART subjects had significantly lower levels of TPP, MDA, and OSI compared to HAART naive individuals. This shows that HAART naive subjects experience the highest oxidative stress. Higher levels of oxidative stress markers have been observed in untreated than HAART-treated HIV positive subjects [23]. HAART results in suppression of viral replication and dramatic improvement in clinical and immunological status. Several pre-HAART studies have reported that both asymptomatic HIV-infected individuals and AIDS patients had higher levels of oxidative stress, as indicated by increased plasma metabolites of lipid peroxidation and/or reduced antioxidant levels, compared with healthy controls [18]. Though there is evidence supporting redox imbalance due to HAART drugs [32], these findings may indicate that HIV is a faster generator of free radicals than drugs as evidenced in those untreated subjects who are dependent on the body's immunity alone for defense against infection. It suggests that the chronic inflammatory state of HAART naive HIV subjects may enhance their state of oxidative stress since viral replication proceeds more freely and inflammatory cells increase their activity to compensate for this and generate more ROS in the

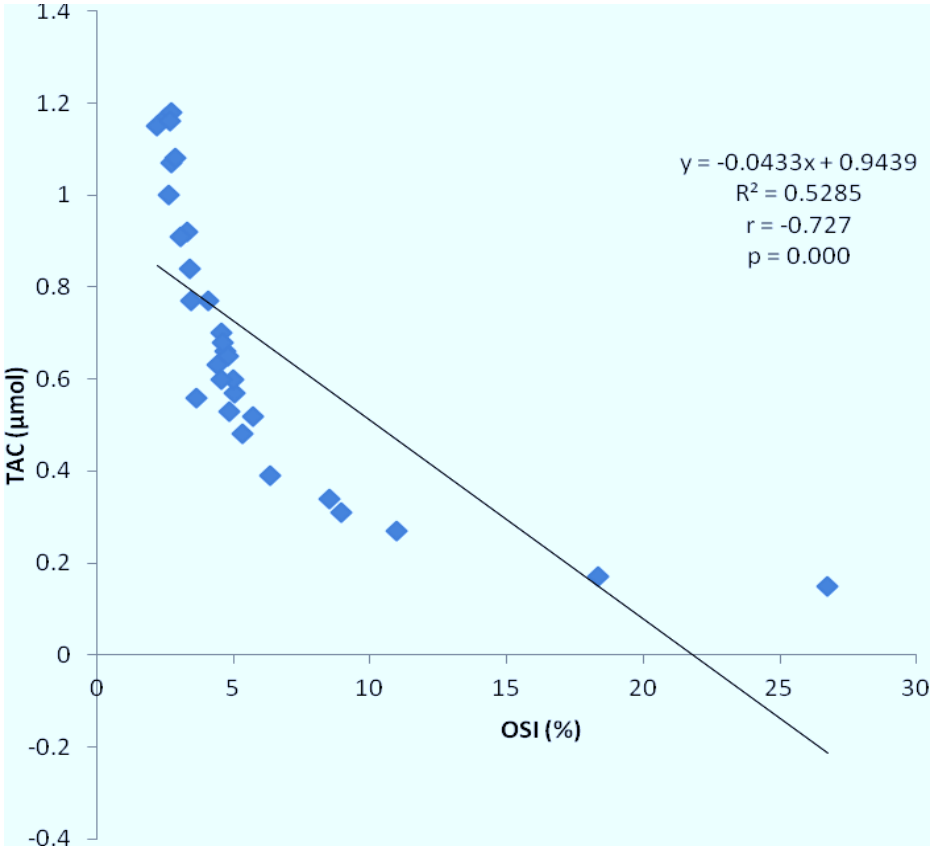

FIGURE 1. Correlation plot of TAC against OSI in HIV HAART naive subjects. As shown, a significant negative correlation (r = –0.727) was observed between TAC and OSI in HIV HAART naive subjects ($p < 0.05$).

process. Antiretroviral treatment during infection has been shown to play a major role in the reduction of ROS associated with HIV infection, hence restoring the antioxidant defense system in HIV-infected patients [21].

Higher catalase activity was seen in HIV on HAART compared to HAART naive individuals. The observed decrease in catalase activity in the HAART naive subjects could be attributed to rapid depletion of the endogenous enzyme by high level of circulating chemically reactive species associated with the HIV untreated patients [33]. The decrease in catalase activity in untreated HIV patients accounts for the production of high ROS in these patients. Increased oxidative stress has been attributed to the deficiency of antioxidant defense system [34]. Increase in catalase activity has been reported with commencement of HAART in HIV infection [21]. Contrary to our findings, some studies have reported higher levels of oxidative stress indices in HIV on HAART compared to HAART naive individuals [23]. The level of production of free radical species in HIV-1 infected individuals receiving HAART was reported to be higher than that in those who harbor HIV-1 infection without receiving any treatment or normal and healthy subjects. These observations suggest that the HIV-1 infection alone or in combination with introduction of ARV/HAART may induce oxidative stress and further augment HIV-1 pathogenesis [35]. The differences in oxidative stress as well as in the plasma concentrations of antioxidants between pre-HAART and HAART patients may be explained by HAART influences. Although HAART results in suppression of viral replication

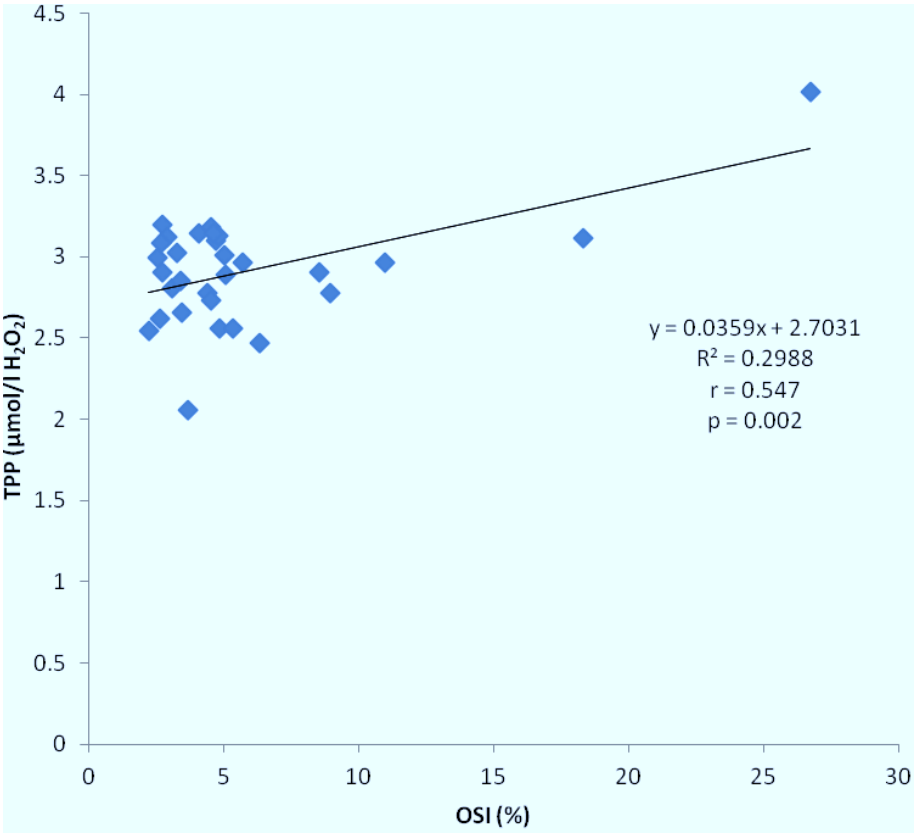

FIGURE 2. Correlation plot of TPP against OSI in HIV HAART naive subjects. As shown, a significant positive correlation (r = 0.547) was observed between TPP and OSI in HIV HAART naive subjects ($p < 0.05$).

and dramatic improvement in clinical and immunological status [36], weight loss and wasting may still be observed in some HAART patients. Certain aspects of HAART toxicity may be associated with oxidative stress, thereby increasing the body's demand for certain antioxidants [37]. Another study reported that HAART may increase oxidative stress levels above and beyond levels caused by the virus itself. HAART may induce an increase in oxidant generation, a decrease in antioxidant protection, or a failure to repair oxidative damage [19].

A significant positive correlation was observed between OSI and TPP in HIV HAART naive subjects and a negative correlation between OSI and TAC in the same subject group. Increased generation of free radicals increases the scavenging action of antioxidants leading to depletion of the antioxidants [5]. Thus, as oxidative stress increases, TPP (a product of oxidant damage) increases, and TAC falls. HAART-treated and HAART naive subjects had significantly lower BMI and $CD4^+$ T cell count when compared to apparently healthy controls. The lower $CD4^+$ T cell count in both test groups is consistent with the pathogenesis of HIV infection, the hallmark of which is massive destruction of $CD4^+$ T cell populations leading to failure of immune functions [37]. BMI is a function of body weight. Lipodystrophy accompanied by weight loss is one of the findings in HIV positive persons in the presence or absence of HAART [38].

In summary, the findings of this work suggests that HIV infection is associated with increased lipid peroxidation and depression of NO and catalase activity, while HAART reduces plasma peroxidation with restoration of antioxidants. Evaluation of the levels of oxidative stress indices in HIV infection may

therefore be useful surveillance markers for monitoring progress of infection, and initiation and dosing of HAART.

ACKNOWLEDGEMENTS

Authors declare no conflicts of interest.

REFERENCES

1. Annam V, Suresh DR. Correlation between oxidative stress indices and CD4$^+$ T cell counts in HIV infected patients. *BMC Infect Dis* 2012; 12(Suppl 1):71 (abstract). doi: 10.1186/1471-2334-12-S1-P71.
2. Teto G, Kanmogne DG, Ntorimiro J. Very high lipid peroxidation indices in highly active antiretroviral (HAART)-naïve hiv-positive patients in Cameroon. *Health Sci Dis* 2011; 12(4)1–4.
3. Colado Simao AN, Victorino VJ, Morimoto HK, Reiche EM, Panis C. Redox-driven events in the human immunodeficiency virus type 1 (HIV-1) infection and their clinical implications. *Curr HIV Res* 2015; 13(2):143–50.
4. Simao AN, Lehmann MF, Alfieri DF, Meloni MZ, Flauzino T, Scavuzzi BM, et al. Metabolic syndrome increases oxidative stress but does not influence disability and short-time outcome in acute ischemic stroke patients. *Metab Brain Dis* 2015; 30(6):1409–16. doi: 10.1007/s11011-015-9720-y.
5. Mandas A, Iorio EL, Congiu MG, Balestrieri C, Mereu A, Cau D, et al. Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. *J Biomed Biotechnol* 2009; 2009:749575. doi: 10.1155/2009/749575.
6. Fisher JD, Fisher WA, Cornman DH, Amico RK, Bryan A, Friedland GH. Clinician-delivered intervention during routine clinical care reduces unprotected sexual behavior among HIV-infected patients. *J Acquir Immune Defic Syndr* 2006; 41(1):44–52.
7. Hulgan T, Donahue JP, Hawkins C, Unutmaz D, D'Aquila RT, Raffanti S, et al. Implications of T-cell P-glycoprotein activity during HIV-1 infection and its therapy. *J Acquir Immune Defic Syndr* 2003; 34(2):119–26.
8. Sharma A, Slaughter A, Jena N, Feng L, Kessl JJ, Fadel HJ, et al. A new class of multimerization selective inhibitors of HIV-1 integrase. *PLoS Pathog* 2014; 10(5):e1004171. doi: 10.1371/journal.ppat.1004171.
9. WHO. Global update on HIV treatment 2013: results, impact and opportunities. World Health Organization.
10. Arai H, Petchclai B, Khupulsup K, Kurimura T, Takeda K. Evaluation of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus. *J Clin Microbiol* 1999; 37(2):367–70.
11. Mandy FF, Bergeron M, Minkus T. Principles of flow cytometry. *Transfus Sci* 1995; 16(4):303–14.
12. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 2001; 54(5):356–61.
13. Miyazawa T. Determination of phospholipid hydroperoxides in human blood plasma by a chemiluminescence-HPLC assay. *Free Radic Biol Med* 1989; 7(2):209–17.
14. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 2001; 5(1):62–71. doi: 10.1006/niox.2000.0319.
15. Bulaj G, Kortemme T, Goldenberg DP. Determination of sulfhydryl groups. *Biochemistry* 1998; 37:8965–72.
16. Goldblith SA, Proctor BE. Photometric determination of catalase activity. *J Biol Chem* 1950; 187(2):705–9.
17. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978; 52:302–10.
18. Pasupathi P, Ramchandran T, Sindhu PJ, Saranavan G, Bakthavathsalam G. Enhanced oxidative stress markers and antioxidant imbalance in HIV infection and AIDS patients. *J Sci Res* 2009; 1(2):370–80.
19. G.H. R, Ivon GB, Alicia T. Antiretroviral concentration, redox indexes and progression markers in HIV-infected patient's blood samples follow-up during six month. *Oxid Antioxid Med Sci* 2014; 3(2):99–108.
20. Niki E. Biomarkers of lipid peroxidation in clinical material. *Biochim Biophys Acta* 2014;

1840(2):809–17. doi: 10.1016/j.bbagen.2013.03.020.
21. Ibeh BO, Habu JB, Eze SC. Discordant levels of superoxide dismutase and catalase observed in ART naïve and experienced HIV patients in Southeastern Nigeria. *J Infect Dis Ther* 2013; 1:8–16.
22. Aukrust P, Muller F, Svardal AM, Ueland T, Berge RK, Froland SS. Disturbed glutathione metabolism and decreased antioxidant levels in human immunodeficiency virus-infected patients during highly active antiretroviral therapy: potential immunomodulatory effects of antioxidants. *J Infect Dis* 2003; 188(2):232–8. doi: 10.1086/376459.
23. Awodele O, Olayemi SO, Nwite JA, Adeyemo TA. Investigation of the levels of oxidative stress parameters in HIV and HIV-TB co-infected patients. *J Infect Dev Ctries* 2012; 6(1):79–85.
24. Martin JA, Sastre J, de la Asuncion JG, Pallardo FV, Vina J. Hepatic gamma-cystathionase deficiency in patients with AIDS. *JAMA* 2001; 285(11):1444–5.
25. Kruman, II, Nath A, Mattson MP. HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress. *Exp Neurol* 1998; 154(2):276–88. doi: 10.1006/exnr.1998.6958.
26. Overbaugh J, Morris L. The antibody response against HIV-1. *Cold Spring Harb Perspect Med* 2012; 2(1):a007039. doi: 10.1101/cshperspect.a007039.
27. Cossarizza A, Moyle G. Antiretroviral nucleoside and nucleotide analogues and mitochondria. *AIDS* 2004; 18(2):137–51.
28. Lewis W. Mitochondrial dysfunction and nucleoside reverse transcriptase inhibitor therapy: experimental clarifications and persistent clinical questions. *Antiviral Res* 2003; 58(3):189–97.
29. Halliwell B, Zhao K, Whiteman M. Nitric oxide and peroxynitrite. The ugly, the uglier and the not so good: a personal view of recent controversies. *Free Radic Res* 1999; 31(6):651–69.
30. Mannick JB. The antiviral role of nitric oxide. *Res Immunol* 1995; 146(9):693–7.
31. Bagetta G, Corasaniti MT, Costa N, Berliocchi L, Finazzi-Agro A, Nistico G. The human immunodeficiency virus type 1 (HIV-1) glycoprotein gp120 reduces the expression of neuronal nitric oxide synthase in the hippocampus but not in the cerebral cortex and medial septal nucleus of rat. *Neurosci Lett* 1997; 224(2):75–8.
32. Smith MK, Powers KA, Muessig KE, Miller WC, Cohen MS. HIV treatment as prevention: the utility and limitations of ecological observation. *PLoS Med* 2012; 9(7):e1001260. doi: 10.1371/journal.pmed.1001260.
33. Gil L, Tarinas A, Hernandez D, Riveron BV, Perez D, Tapanes R, et al. WITHDRAWN: Altered oxidative stress indexes related to disease progression marker in human immunodeficiency virus infected patients with antiretroviral therapy. *Biomed Pharmacother* 2010. doi: 10.1016/j.biopha.2010.09.009.
34. Lizette GD, Hernández RG, Ávila JP. Oxidative stress associated to disease progression and toxicity during antiretroviral therapy in human immunodeficiency virus infection. *J Virol Microbiol* 2013:ID 279685.
35. Sharma B. Oxidative stress in HIV patients receiving antiretroviral therapy. *Curr HIV Res* 2014; 12(1):13–21.
36. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J* 2012; 5(1):9–19. doi: 10.1097/WOX.0b013e3182439613.
37. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunol Rev* 2013; 254(1):54–64. doi: 10.1111/imr.12066.
38. da Cunha J, Maselli LM, Stern AC, Spada C, Bydlowski SP. Impact of antiretroviral therapy on lipid metabolism of human immunodeficiency virus-infected patients: Old and new drugs. *World J Virol* 2015; 4(2):56–77. doi: 10.5501/wjv.v4.i2.56.