Current Clinical Pharmacology, 2020, *15*, 110-124

RESEARCH ARTICLE

Mitochondrial and Oxidative Impacts of Short and Long-term Administration of HAART on HIV Patients

Joy E. Ikekpeazu[1], Oliver C. Orji[2], Ikenna K. Uchendu[2,*] and Lawrence U.S. Ezeanyika[3]

[1]*Department of Medical Biochemistry, University of Nigeria Enugu Campus, Nsukka, Nigeria;* [2]*Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Medicine, University of Nigeria Enugu Campus, Enugu State, Nigeria;* [3]*Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.*

ARTICLE HISTORY

Received: March 22, 2019
Revised: April 05, 2019
Accepted: July 11, 2019

DOI:
10.2174/1574884714666190905162237

Abstract: ***Background*:** There may be a possible link between the use of HAART and oxidative stress-related mitochondrial dysfunction in HIV patients. We evaluated the mitochondrial and oxidative impacts of short and long-term administration of HAART on HIV patients attending the Enugu State University Teaching (ESUT) Hospital, Enugu, Nigeria following short and long-term therapy.

***Methods*:** 96 patients categorized into four groups of 24 individuals were recruited for the study. Group 1 comprised of age-matched, apparently healthy, sero-negative individuals (the No HIV group); group 2 consisted of HIV sero-positive individuals who had not started any form of treatment (the Treatment naïve group). Individuals in group 3 were known HIV patients on HAART for less than one year (Short-term treatment group), while group 4 comprised of HIV patients on HAART for more than one year (Long-term treatment group). All patients were aged between 18 to 60 years and attended the HIV clinic at the time of the study. Determination of total antioxidant status (TAS in nmol/l), malondialdehyde (MDA in mmol/l), $CD4^+$ count in cells/µl, and genomic studies were all done using standard operative procedures.

***Results*:** We found that the long-term treatment group had significantly raised the levels of MDA, as well as significantly diminished TAS compared to the Short-term treatment and No HIV groups ($P<0.05$). In addition, there was significantly elevated variation in the copy number of mitochondrial genes (mtDNA: D-loop, ATPase 8, $TRNA_{LEU}^{uur)}$ in the long-term treatment group.

***Conclusion*:** Long-term treatment with HAART increases oxidative stress and causes mitochondrial alterations in HIV patients.

Keywords: HIV/AIDS, HAART, mitochondrial dysfunction, oxidative stress, biochemical alterations, necrosis.

1. BACKGROUND

Human Immunodeficiency Virus (HIV) has been established as the cause of Acquired Immunodeficiency Syndrome (AIDS) which has caused tremendous morbidity and mortality especially in the developing countries. Over the years, relative decline in morbidity and mortality of Human Immunodeficiency Virus (HIV) infection in some countries has been observed due to the use of a potent combined therapy known as Highly Active Antiretroviral Therapies (HAART) [1]. It has led to a decrease in viral load (a measure of the amount of HIV virus in the blood), quantitative and qualitative improvement of immune functions in patients, especially $CD4^+$ T- lymphocytes count, resulting in a decrease of infectious complications and a global clinical improvement [2]. In spite of the positive effects of HAART on the immune and metabolic alterations during HIV infections, it has been reported that the commonly used drugs Zidovudine (AZT), didanosine (ddI) and stavudine (d_4T) are toxic to hepatocytes and other tissues [3].

Recent reports continue to point to the mitochondria as the target for toxicity. This is so because, in addition to impairing the HIV replication machinery (by inhibition of their target-HIV reverse transcriptase), these drugs also inhibit the human polymerase called "gamma polymerase", which is responsible for the replication of mitochondrial DNA (mtDNA) [4]. Decline in mtDNA leads to defects in respiratory chain function because the mtDNA encodes about 13 subunits of the respiratory chain complexes and any alteration in these genes would severely impact tissue respiration,

*Address correspondence to this author at the Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Medicine, University of Nigeria Enugu Campus, Enugu State, Nigeria;
Tel: +2347068199556; E-mail: Ikenna.uchendu@unn.edu.ng

2212-3938/20 $65.00+.00

leading to overdependence on glycolysis as the alternative source of ATP, which is inevitably accompanied with lactic acidosis [1, 5]. A normal respiratory chain function is essential for the synthesis of DNA. Mitochondrial DNA (mtDNA) depletion and thus mitochondrial toxicity in the liver is associated with elevated liver transaminases [6].

Oxidative stress, defined as the disturbance of tissue oxidant/antioxidant equilibrium is characterized by the generation of reactive oxygen species. This is usually due to the oxidative activities of the mitochondria through the functions of the respiratory chain which leaks free radicals that may react with nitric oxide, forming damaging by-products such as peroxynitrite as well as leading to lipid peroxidation [7]. This is because the respiratory chain leaks the superoxide free radical which may react with nitric oxide to form the damaging by-product, peroxynitrite [6]. The mitochondria is thus a major source of Reactive Oxygen Species (ROS) leading to oxidative damage to mitochondrial proteins, membranes, and mtDNA; impairing the ability of mitochondria to synthesize ATP and to carry out other metabolic functions. This contributes to a wide range of pathologies [7, 8].

Cells show a wide range of responses upon exposure to ROS, ranging from increased proliferation, prevention of cell division, senescence, necrosis, apoptosis, cell death mechanisms with features of both. The effects are to some extent cell-type-specific, being influenced by such parameters as the presence of certain cell-surface receptors and signal transduction mechanisms, as well as antioxidant defense levels; (Fig. **1**) [9, 10].

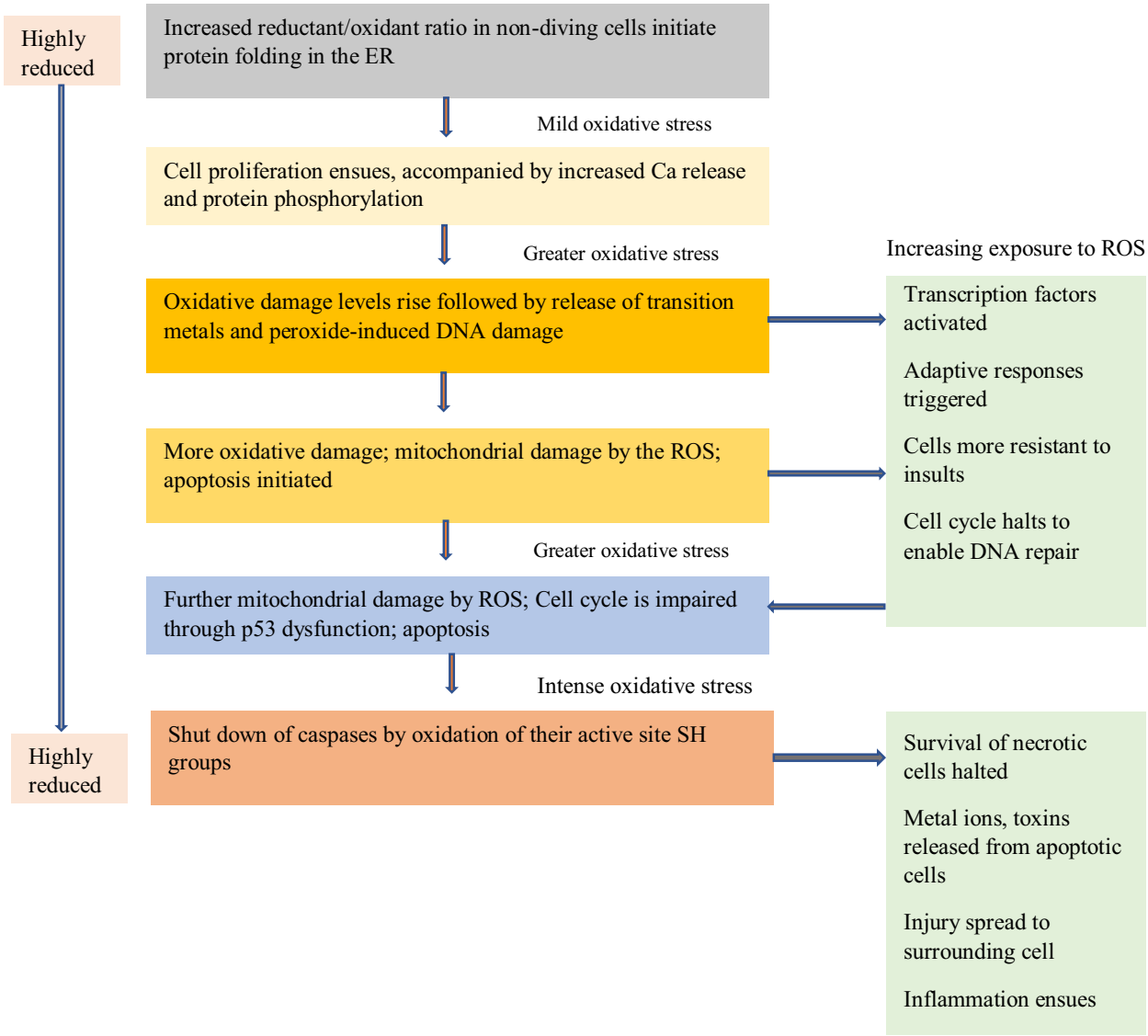


Fig. (1). The cell's response to oxidative stress. Oxidative stress builds up through disturbances in cellular reductive/oxidative equilibrium, with the potential to cause DNA damage, tissue necrosis and mitochondrial destruction. In response, adaptive mechanisms are stimulated through the release of transcription factors, antioxidants, ferritin *etc*. Failure of these responses to protect the cell leads to apoptosis, release of toxins from necrosed cells and induction of inflammation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Diseases of the mitochondria may be inherited or acquired; and the treatment with Highly Active Anti-Retroviral Therapies (HAART) may represent an acquired cause. Interplay of oxidative stress and mitochondrial dysfunction may increase mtDNA mutations. Thus, with increased use of these drugs, mtDNA mutations and dysfunction may become increasingly important, pathophysiologically [7]. In this present study, we aimed to evaluate oxidative stress (by assessment of the Total Antioxidant Status (TAS) and prooxidant biomarker- Malondialdehyde (MDA) levels.); mitochondrial genome analysis and correlate the findings of the above with immune status of the patients (CD_4^+ cell count).

1.1. Specific Research Questions

The specific research questions that this study addressed include:

1 Whether there are mitochondrial dysfunction and oxidative stress in asymptomatic HIV patients and those on HAART?

2 Whether the level of oxidative stress correlates with the mitochondrial dysfunction and indeed with the immune status of these patients?

3 How the oxidative stress status (MDA and TAS) correlates with the genetic variants in terms of mtDNA content and mtDNA deletions and over replication?

4 If there is a possibility that the antioxidants can play a role in the treatment of mitochondrial dysfunction and indeed, the management of patients with HIV/AIDS on HAART?

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Subjects

A total of ninety-six (96) individuals who attended the Antiretroviral (ARV) Clinic at the Enugu State University of Science and Technology (ESUT) Teaching Hospital were used for the study. The individuals consisted of twenty-four (24) apparently healthy sero-negative age-matched individuals (the no-HIV control group), twenty-four (24) sero-positive patients who had not started any form of treatment (the treatment naïve group), another twenty-four (24) sero-positive patients on highly active anti-retroviral therapy (HAART) for less than one year (the group on short-term therapy) and a final twenty-four (24) sero-positive patient on the HAART for greater than one year (group on long-term therapy).

2.1.2. Location of Study

The study was carried out in Enugu State among patients attending the ARV clinic at the ESUT Teaching Hospital, Parklane, Enugu.

2.1.3. Chemicals/Reagents/ Samples

Analytical grade (Analar) reagents were used for the various genomic, biochemical and immunological assays. Commercial kits were products of Northwest Life Sciences NWK-MD-01 UK, Macherey-nagel U.S.A, ABCAM U.K., Teco Diagnostics, U.S.A and Randox, U.S.A.

2.1.4. Instruments/equipment

Equipment and instruments used for this study include the following: Biophotometer (Eppendorf, UK), water bath (Gallenkamp, England), microcentrifuge (Eppendorf, U.K), conical flasks (Pyrex, England), thermocycler (standard PCR machine) (Thermoscientific, U.K), realtime PCR (Applied Biosystems, U.S.A), dry block incubators (Techne, U.K), cyflow cell counter (Partec, U.S.A), pH meter (Hanna, U.S.A), electrophoresis cell (Embi Tec, U.S.A), vortex mixer (Biocot, U.S.A) and pipettes (Fisher Scientific, England).

2.2. Methods

2.2.1. Experimental Design

Ninety-six (96) individuals aged between 18 and 60 years were recruited for the study from ESUT Teaching Hospital, Parklane, Enugu. The criteria for inclusion and exclusion of subjects for the study were based on interview and clinical assessment by the clinician in charge of the ARV clinic. Ethical clearance was obtained from the Ethical Committee of the Hospital. After interviews and clinical assessments/reviews of the patients' medical history, the subjects were divided into four (4) groups, namely:

Group 1: Twenty-four apparently-healthy, HIV sero-negative age-matched individuals served as controls.

Group 2: Twenty-four sero-positive patients not on therapy, also called the treatment naïve group.

Group 3: Twenty- four sero-positive patients on antiretroviral therapy for less than one year.

Group 4: Twenty-four seropositive patients on antiretroviral therapy for more than one year.

The subjects used in groups 3 and 4 were on the combination therapy called ATRIPLA, a combination of three antiretroviral drugs with different mechanisms of action on replicating HIV cycle. ATRIPLA is a combination of Emtricitabine, Tenofovir and Efavirenz. Their formulation has the following concentrations: Tenofovir (300 mg) + Emtricitabine (200 mg) + Efavirenz (600 mg). Vitamins and minerals were introduced into their daily routine drugs for about 3months to serve as supplements. After expressed consent, blood was collected in appropriate containers for biochemical analysis, DNA extraction and $CD4^+$ count.

2.2.2. Collection of Blood Samples

Blood samples of the different groups of individuals were collected *via* venipuncture. The median cubital vein was easily accessible for venous blood collection. Tourniquet was tied to the arm about 12 cm from the puncture site to reveal the vein and provide the needed pressure of blood flow. The skin over the vein in the cubital fossa was cleaned with seventy percent (70%) ethanol. The needle was carefully inserted into the vein and up to 10 ml of blood drawn with moderate suction to avoid frothing and hemolysis. The tourniquet was loosened and the needle withdrawn. The sharp end was destroyed, separated from the syringe and discarded into the waste bin. Blood flow from the punctured site was stopped by suppressing it with dry cotton wool. The collected blood samples (5 ml each) were then dispensed

into EDTA anticoagulated tubes and plain tubes, respectively. The tubes were carefully labeled with the names of the subjects, date and time. The blood in the plane tubes was allowed to clot and when the clots were retracted, they were centrifuged for 5 minutes at 3000 revolutions per minute (rpm) to separate the clotted cells from the serum. The serum samples were then transferred to 2 ml tubes and frozen at -20°C for biochemical analysis. The EDTA anticoagulated blood samples were further divided into two for $CD4^+$ cell count and genomic DNA processing.

2.2.3. Genomic DNA Extraction and Purification

2.2.3.1. Processing Whole Peripheral Blood Down to Guanidium Isothiocyanate Lysate

The extraction and purification of samples were done in the same laboratory using the method described by Voss *et al*. [10]. Briefly, into 15 ml tubes were added the EDTA-whole blood samples and labeled accordingly. Ten milliliters (10 ml) of cold 1X RCL (red cell lysis) buffer was added to each sample and closed. The tubes were properly mixed by inversion and placed in ice for 10 minutes. Samples were centrifuged at 4000 rpm for 7 minutes. Supernatants were carefully decanted, making sure that the cell pellets were not lost. Cold 1X RCL buffer (10 ml) was added to the cell pellets, and mixed by vortexing. When traces of red cells were found, 10 ml of cold RCL buffer was added to the cell pellets, mixed by vortexing and the ice incubation, centrifugation and decantation repeated. Sterile Phosphate-Buffered Saline (PBS) (10 ml) was added to the cell pellets, mixed by vortexing and centrifuged at 4000 rpm for 7 min. The supernatants were again decanted and 5 ml of sterile PBS added into each tube, mixed by vortexing and centrifuged at 4000 rpm for 5 min. The supernatants from the above were decanted (without discarding the pellets) and the 15 ml tubes with samples were each drained on a clean surface. While this was being done, activated Guanidium Isothiocyanate **(**GITC) was prepared by adding 10 µl of β-mercaptoethanol (BME) to 1ml of GITC. The activated GITC (1 ml) was added to the cell pellets in each tube. Using a blunt end 18-gauge needle and 2 ml syringe (needle removed), the GITC lysate was homogenized 18 times in same universal containers where the samples were transferred to. Sterile Pasteur pipettes were used to transfer the GITC lysate into 2 ml vials and labeled accordingly for storage at minus -20°C until they were needed for nucleic acid extraction.

2.2.3.2. Genomic DNA Purification from GITC Lysate

Genomic DNA was extracted from the GITC lysate using the Nucleospin Qiagen DNAeasy Blood kit (Valencia, CA) based on the method described by Voss *et al*. [11] − (A) **Lysing of GITC lysate**: Proteinase K (25 µl) and 200 µl of the GITC lysate were mixed in microcentrifuge tubes. Buffer B3 (200 µL) was added and the mixture was vortexed vigorously for 10-20 seconds (vigorous mixing is important to obtain high yield and pure DNA). Samples were incubated at 79°C for 15 min. **(B) Adjustment of DNA binding conditions:** Twenty micro liters (20 µl) of ethanol (96%) was added to each sample and vortexed again. This procedure helps to achieve appropriate conditions for binding of DNA to the silica membrane. **(C) Binding of DNA:** One nucleospin blood column was taken, placed in a collection tube and loaded with the sample and then centrifuged for 1 min at 11,000 rpm for each preparation. If the samples are not drawn through the matrix completely, the procedure was repeated at higher g-force (< 15,000 rpm). The collection tube was discarded with flow through. **(D) Washing Silica Membrane:** 1st wash: The nucleospin blood column was placed into a new collection tube (2 ml) and 500 µl buffer BW was added and then centrifuged for 1minute at 11,000 rpm. The collection tube was discarded with flow through. 2nd wash: The nucleospin blood column was placed into a new collection tube (2 ml) and 600 µl buffer B5 was added and centrifuged for 1 min at 11,000 rpm. The flow through was discarded and the collection tube reused. **(E) Drying of the Silica Membrane:** The nucleospin blood column was placed back into the collection tube and centrifuged for 1 min at 11,000 rpm. The residual ethanol was removed during this step. **(F) Elution of Highly Pure DNA:** The nucleospin blood column was placed in a 1.5 ml micro-centrifuge tube and 100 µl preheated buffer BE (70°C) was added. The buffer was dispensed directly onto the silica membrane and incubation was allowed at room temperature for 1 min and then centrifuged for 1 min at 11,000 rpm. The use of pre-heated buffer ensures a high elution. About 90–100% of bound nucleic acid can be eluted.

2.2.4. Experimental Analysis

2.2.4.1. Quantification of Mitochondrial DNA Content of Samples

The extracted and purified DNA samples were taken to Safety Molecular Pathology Laboratory, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus for molecular analysis.

2.2.4.2. Primers and Taqman Probes for Mitochondrial DNA Content Quantification.

The mitochondrial DNA content was determined by the method of Bai and Wong [12], using TaqMan Realtime Polymerase Chain Reaction (qPCR). Probes specific for the tRNA leu^{UUR}, ND4, ATP8, and D-loop regions were used.

2.2.4.3. Real Time Quantitative PCR

The method described by Ghatak *et al.* [13] was used. Briefly, the real time qPCR conditions were: 2 minutes at 50°C and 10 minutes at 95°C, followed by 45 cycles; of 15 seconds of denaturation at 95°C and 60 seconds of annealing and/or extension at 60°C. Fluorescent signal intensity was recorded and analyzed on an ABI-Prism 7700 sequence detector (Applied Biosystems, Foster City, CA, U.S.A) using SDS version1.9 software (Applied Biosystems, Foster City, CA, U.S.A).

2.2.4.4. Measurement of Serum Total Antioxidant Capacity

This was measured using the total antioxidant capacity assay kit provided by Abcam according to the method described by Suresh *et al*. [14].

2.2.4.5. Measurement of Serum Malondialdehyde

The assay was carried out using the reagent kit provided by the North West Life Science Specialities according to the method described by Mihara and Uchiyama [15].

2.2.4.6. Measurement of the CD4$^+$ Cells in the Subjects

The CD4$^+$ cell count was determined using flow cytometry using the Partec^R Cyflow machine according to the method described by Yar'zever *et al*. [16]. Briefly, well mixed blood in EDTA (20 µl) was pipetted into the Partec test tube and mixed briefly. The mixture was then incubated for 15 minutes at room temperature in the dark. This was finally taken to the CyFlow® counter and run. The results were directly displayed in terms of absolute cell number per microliter blood sample.

2.2.4.7. Statistical Analysis

All data were entered into statistical products and service solutions version 17. Analysis of Variance (ANOVA) and independent sample t-test of the data was determined. Pearson's correlation coefficients between the parameters were also determined. Results were expressed in mean ± Standard Error of Mean (SEM) and $p < 0.05$ was considered significant.

3. RESULTS

3.1. Total Antioxidant Capacity across the Different Groups of Subjects

Table **1** shows the Total Antioxidant Capacity (TAC) for the four (4) groups studied. Patients on HAART for less than 1 year (group 3) had the highest TAS value at 1208.21 ± 12.56 mmol/l, followed by group 1 (No HIV control group) at 1172.67 ± 20.42 mmol/l, greater than 1year on HAART at 500.88 ± 6.13 mmol/l and Naïve HIV group at 402.17 ± 5.53 mmol/l. The TAC of group 3 subjects (subjects less than 1 year on HAART) was significantly ($p < 0.05$) higher than that of group 1 subjects (No HIV subjects), group 2 subjects (Naïve HIV subjects) and group 4 (subjects greater than 1 year on HAART). Subjects in group 1 had a significantly ($p < 0.05$) higher TAC when compared to those in groups 2 and 4, while subjects in group 4 had a significantly higher TAC compared to subjects in group 2.

Tables **2** and **3** show the Total Antioxidant Capacity (TAC) for the four (4) groups studied in male and female subjects, respectively. Patients on HAART for less than 1 year (group 3) had the highest TAS value at 1166.00 ± 36.22 mmol/l; 1225.59 ± 7.28 mmol/l, followed by group 1 (No HIV control group) at 1187.00 ± 20.55 mmol/l; 1158.33 ± 35.84 mmol/l, greater than 1year on HAART at 488.00 ± 15.37 mmol/l; 505.17 ± 6.34 mmol/l and Naïve HIV group at 399.88 ± 11.39 mmol/l; 403.31± 6.31 mmol/l, respectively across the groups. In the male subjects, the TAC of group 3 (subjects less than 1 year on HAART) was significantly ($p < 0.05$) higher than that of group 2 subjects (Naïve HIV subjects) and group 4 (subjects greater than 1 year on HAART).

Table 1. Statistical comparison of oxidative stress parameters, gene copy numbers and CD4$^+$ cell count across the different groups for all subjects.

Parameters	Group 1 (No HIV)	Group 2 (Naive HIV)	Group 3 (< 1 Year HAART	Group 4 (> 1 Year HAART)
TAC(mmol/l)	1172.67 ± 20.42^b	402.17 ± 5.53^d	1208.21 ± 12.56^a	500.88 ± 6.13^c
MDA(mmol/l)	1.63 ± 0.35^c	25.33 ± 0.38^a	12.29 ± 0.20^b	14.72 ± 0.78^b
MT-104-NCN (D-loop gene)	0.33 ± 0.16^b	5.46 ± 2.69^a	0.58 ± 0.58^b	0.67 ± 0.34^b
MT-141-NCN (ATPase 8 gene)	0.08 ± 0.07^d	10.85 ± 0.80^a	2.45 ± 0.43^c	5.38 ± 0.98^b
MT-108-NCN (tRNAleu^{UUR} gene)	0.02 ± 0.01^d	540.32 ± 16.14^a	9.06 ± 1.70^c	123.63 ± 10.11^b
MT-78-NCN (ND_4 gene)	0.56 ± 0.54^a	0.06 ± 0.05^a	0.09 ± 0.07^a	0.22 ± 0.19^a
CD4$^+$ cell count (cell/µl)	748.04 ± 25.26^a	258.54 ± 54.11^d	422.42 ± 30.08^c	680.83 ± 48.41^b

Data are reported as mean ± standard error of mean (SEM). Mean values different alphabets across the group are considered statistically significant at $p < 0.05$; while mean values the same alphabets across the group are considered statistically non-significant at $p > 0.05$. HAART = highly active antiretroviral therapy.

Table 2. Statistical comparison of oxidative stress parameters and CD4$^+$ cell count across the different groups for male subjects.

Parameters	Group 1 (No HIV)	Group 2 (Naive HIV)	Group 3 (< 1 Year HAART	Group 4 (> 1 Year HAART)
TAC(mmol/l)	1187.00 ± 20.55^a	399.88 ± 11.39^b	1166.00 ± 36.22^a	488.00 ± 15.37^c
MDA(mmol/l)	10.89 ± 0.36^a	13.71 ± 1.03^b	11.53 ± 0.66^a	9.86 ± 0.17^c
CD4$^+$ cell count (cell/µl)	287.33 ± 42.10^a	759.50 ± 110.12^b	289.43 ± 44.20^a	820.33 ± 110.08^b
Age	28.83 ± 0.82^a	39.38 ± 3.39^b	45.29 ± 4.38^b	45.50 ± 4.26^b

Data are reported as mean ± standard error of mean (SEM). Mean values different alphabets across the group are considered statistically significant at $p < 0.05$; while mean values the same alphabets across the group are considered statistically non-significant at $p > 0.05$. HAART = highly active antiretroviral therapy.

Table 3. Statistical comparison of oxidative stress parameters and $CD4^+$ cell count across the different groups for female subjects.

Parameters	Group 1 (No HIV)	Group 2 (Naive HIV)	Group 3 (< 1 Year HAART	Group 4 (> 1 Year HAART)
TAC(mmol/l)	1158.33 ± 35.84^b	403.31 ± 6.31^d	1225.59 ± 7.28^a	505.17 ± 6.34^c
MDA(mmol/l)	1.19 ± 0.59^b	25.31 ± 0.41^a	2.32 ± 0.20^b	15.62 ± 0.64^c
$CD4^+$ cell count (cell/µl)	208.75 ± 24.82^b	758.06 ± 62.49^a	336.00 ± 38.67^b	634.33 ± 50.31^a
Age	29.58 ± 2.09^a	31.88 ± 2.21^a	33.24 ± 2.69^a	31.33 ± 1.67^a

Data are reported as mean ± standard error of mean (SEM). Mean values different alphabets across the group are considered statistically significant at $p < 0.05$; while mean values the same alphabets across the group are considered statistically non-significant at $p > 0.05$. HAART = highly active antiretroviral therapy.

Subjects in group 1 had a significantly ($p < 0.05$) higher TAC when compared to those in groups 2 and 4, while subjects in group 4 had a non-significantly higher TAC compared to subjects in group 2. Meanwhile in female subjects, the TAC of group 3 subjects (subjects less than 1 year on HAART) was significantly ($p < 0.05$) higher than that of group 1 subjects (No HIV subjects), group 2 subjects (Naïve HIV subjects) and group 4 (subjects greater than 1 year on HAART). Subjects in group 1 had a significantly ($p < 0.05$) higher TAC when compared to those in groups 2 and 4, while subjects in group 4 had a significantly higher TAC compared to subjects in group 2.

3.2. Malondialdehyde Concentration across the Different Groups of Subjects

Malondialdehyde (MDA) concentrations for the four (4) groups of all subjects studied are shown in Table **1**. Subjects in group 2 (Naïve HIV group) had a significantly ($p < 0.05$) higher malondialdehyde concentration at 25.33 ± 0.38 mmol/l than subjects in group 1 (the No HIV control group) at 1.63 ± 0.35 mmol/l, subjects in group 3 (less than 1year on HAART) at 12.29 ± 0.20 mmol/l and subjects in group 4 (greater than 1 year on HAART) at 14.72 ± 0.78 mmol/l. The MDA concentration of groups 3 and 4 were significantly ($p < 0.05$) higher than that in group 1 subjects. Meanwhile, there is no significant ($p < 0.05$) difference between the MDA concentrations of groups 3 and 4 subjects.

Malondialdehyde (MDA) concentrations for the four (4) groups of male and female subjects studied separately are shown in Tables **2** and **3**, respectively. Subjects in group 2 (Naïve HIV group) had a significantly ($p < 0.05$) higher malondialdehyde concentration at 13.71 ± 1.03mmol/l; 25.31 ± 0.41 mmol/l than subjects in group 1 (the No HIV control group) at 10.89 ± 0.36 mmol/l; 1.19 ± 0.59 mmol/l, subjects in group 3 (less than 1year on HAART) at 11.53 ± 0.66 mmol/l; 2.32 ± 0.20 mmol/l and subjects in group 4 (greater than 1 year on HAART) at 9.86 ± 0.17 mmol/l; 15.62 ± 0.64 mmol/l, respectively across the groups. The MDA concentration of groups 3 and 4 were significantly ($p < 0.05$) higher than that in group 1 subjects in both genders.

3.3. House-Keeping (Control) Genes Used as Normalizers

Fig. **2** shows the housekeeping genes nr 134, nr 86 and nr 116 which codes for AIBI (amplified in breast cancer 1), β2-microglobulin and β-actin respectively.

3.4. mt-104 Gene across the Different Groups of Subjects

Table **1** and Fig. **3** showed the distribution of the copy numbers of the mitochondrial gene, mt-104 (D-loop gene) across the studied groups and in both genders respectively. D-loop gene was most expressed in group 2 (Naïve HIV) with a copy number of 5.46 ± 2.69. The group 1 (No HIV subjects), group 3 (subjects on HAART for less than a year) and group 4 (subjects on HAART for greater than a year) each had a copy number of 0.33 ± 0.16, 0.58 ± 0.58 and 0.67 ± 0.34 respectively. This also shows that the D-loop gene expression of the mitochondria was affected mostly in group 2 (Naïve HIV subjects), and group 3 (less than 1 year on HAART), group 4 (greater than 1 year on HAART) and was least affected in group 1 (No HIV subjects).

3.5. mt-141 Gene across the Different Groups of Subjects

Table **1** shows the distribution of the copy numbers of the mitochondrial gene, mt-141 (ATPase 8 gene) across the studied groups. ATPase 8 was mostly expressed in group 2 (Naïve HIV subjects) with a copy number of 10.85 ± 0.80. The group 1 (No HIV), group 3 (subjects on HAART for less than a year) and group 4 (subjects on HAART for greater than a year) each had copy numbers of 0.08 ± 0.07, 2.45 ± 0.43 and 5.38 ± 0.98, respectively. The ATPase 8 gene was expressed significantly ($p < 0.05$) higher in group 2 compared to groups 1, 3 and 4. Similarly, the ATPase 8 gene was expressed significantly ($p < 0.05$) higher in group 4 compared to groups 1, and 3. This also shows that the ATPase 8 activity of the mitochondria was affected mostly in group 2 (Naïve HIV), and group 4 (greater than 1 year on HAART) and was least affected in group 1 (No HIV).

3.6. mt-108 Gene across the Different Groups of Subjects

Table **1** and Fig. **3** showed the distribution of the copy numbers of the mitochondrial gene, mt-108 ($tRNAleu^{UUR}$ gene) across the studied groups and in both genders respectively. The $tRNAleu^{UUR}$ gene was significantly ($p < 0.05$) expressed higher in group 2 (Naïve HIV subjects) with a copy number of 540.32 ± 16.14 than in other studied groups. Group 1 (No HIV), group 3 (subjects on HAART for less than a year) and group 4 (subjects on HAART for greater than a year) each had copy numbers of 0.02 ± 0.01, 9.06 ± 1.70 and 123.63 ± 10.11 respectively. The $tRNAleu^{UUR}$ gene was significantly ($p < 0.05$) expressed higher in group 2 compared to groups 1, 3 and 4. Meanwhile, the $tRNAleu^{UUR}$ gene was significantly ($p < 0.05$)

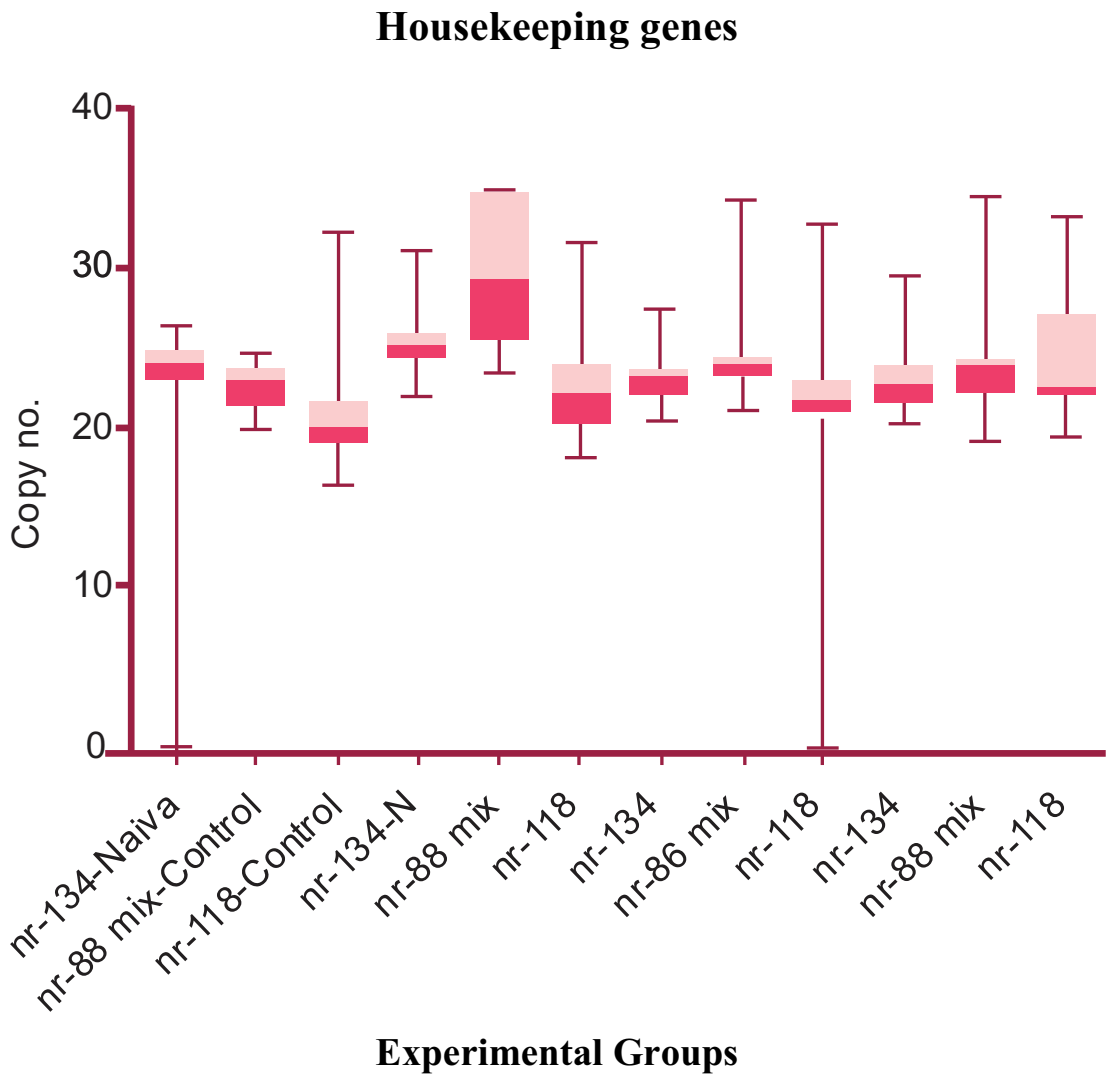


Fig. (2). The Control Gene (housekeeping genes). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

expressed higher in group 4 subjects when compared to groups 1 and 3 subjects. Thus, this shows that the $tRNAleu^{UUR}$ gene of the mitochondria was affected mostly in groups 2 and 4 ((Naïve HIV and greater than 1 year on HAART respectively), and least affected in group 3 (less than 1 year on HAART).

3.7. mt-78 Gene across the Different Groups of Subjects

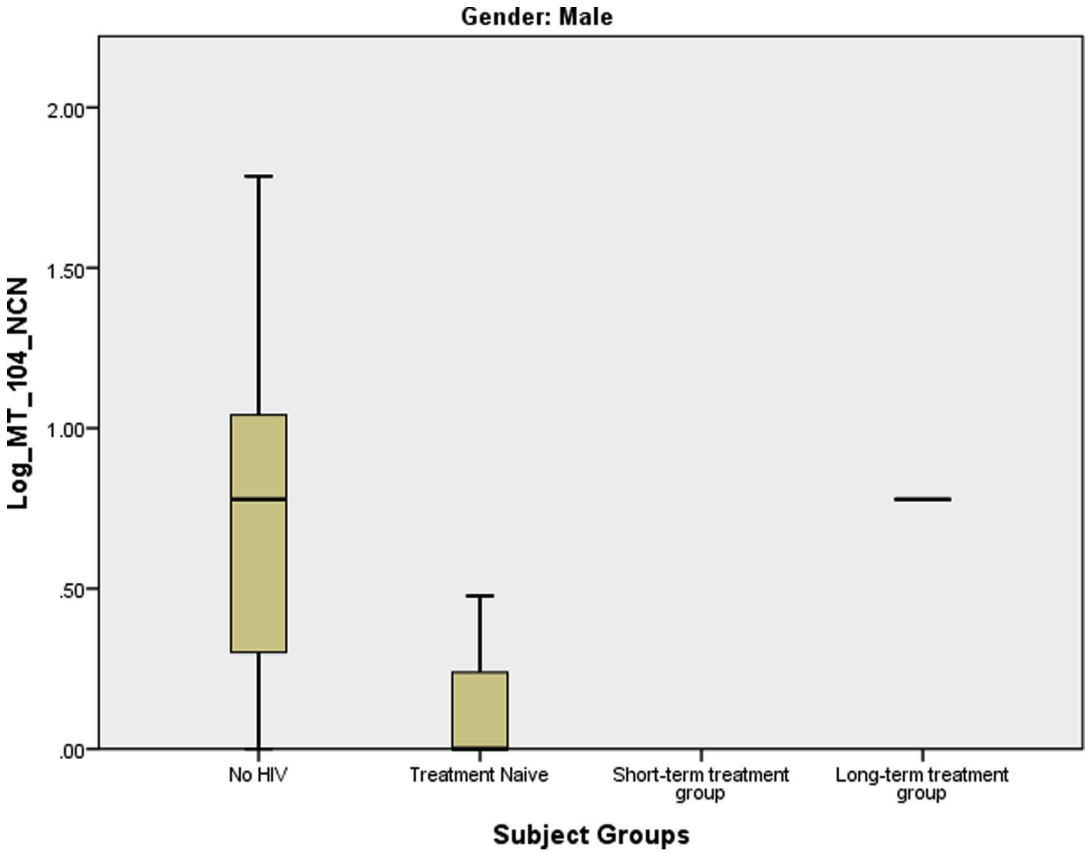
Table **1** and Fig. **3** showed the distribution of the copy numbers of the mitochondrial gene, mt-78 (ND_4 gene) across the studied groups and in both genders respectively. The ND_4 gene was significantly ($p < 0.05$) expressed higher in group 1 (No HIV subjects) with a copy number of 0.56 ± 0.54, than in other studied groups. The group 2 (Naïve HIV), group 3 (subjects on HAART for less than a year) and group 4 (subjects on HAART for greater than a year) each had copy numbers of 0.06 ± 0.05, 0.09 ± 0.07 and 0.22 ± 0.19 respectively. There was no significant ($p < 0.05$) difference between the expression of the ND_4 gene in the four groups of subjects studied.

3.8. $CD4^+$ Count across the Different Groups of Subjects

$CD4^+$ count for the four (4) groups of subjects studied is shown in Table **1**. Subjects in group 1 (the No HIV control group) had a significantly ($p < 0.05$) higher $CD4^+$ count at 748.04 ± 25.26 cell/µl than subjects in group 2 (Naïve HIV group) at 258.54 ± 54.11 cell/µl, subjects in group 3 (less than 1year on HAART) at 422.42 ± 30.08 cell/µl and subjects in group 4 (greater than 1 year on HAART) at 680.83 ± 48.41 cell/µl. The $CD4^+$ count of subjects in group 3 was significantly ($p < 0.05$) higher compared to that in group 2, while the $CD4^+$ count of subjects in group 4 was significantly ($p < 0.05$) higher compared to that in group 3.

3.9. Correlation of $CD4^+$ Count and Other Parameters Studied

The correlation of $CD4^+$ and other parameters studied is shown in Table **4** below. There was a significant ($p < 0.001$) negative correlation and positive correlation between $CD4^+$ in subjects studied and TAS and MDA ($r = 0.304$, $p = -0.714$; $r = 0.709$, $p = 0.000$; respectively). However, there


Gender: Male
Log_MT_104_NCN
2.00
1.50
1.00
.50
.00
No HIV
Treatment Naive
Short-term treatment group
Long-term treatment group
Subject Groups

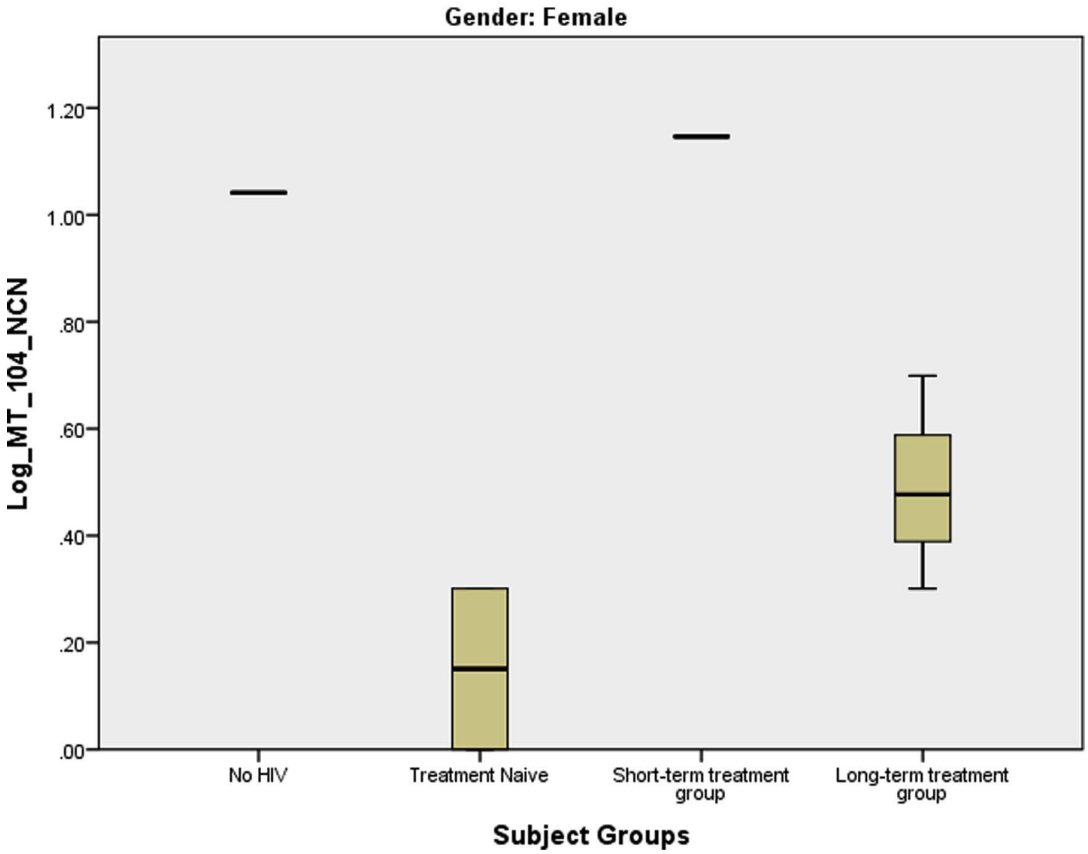
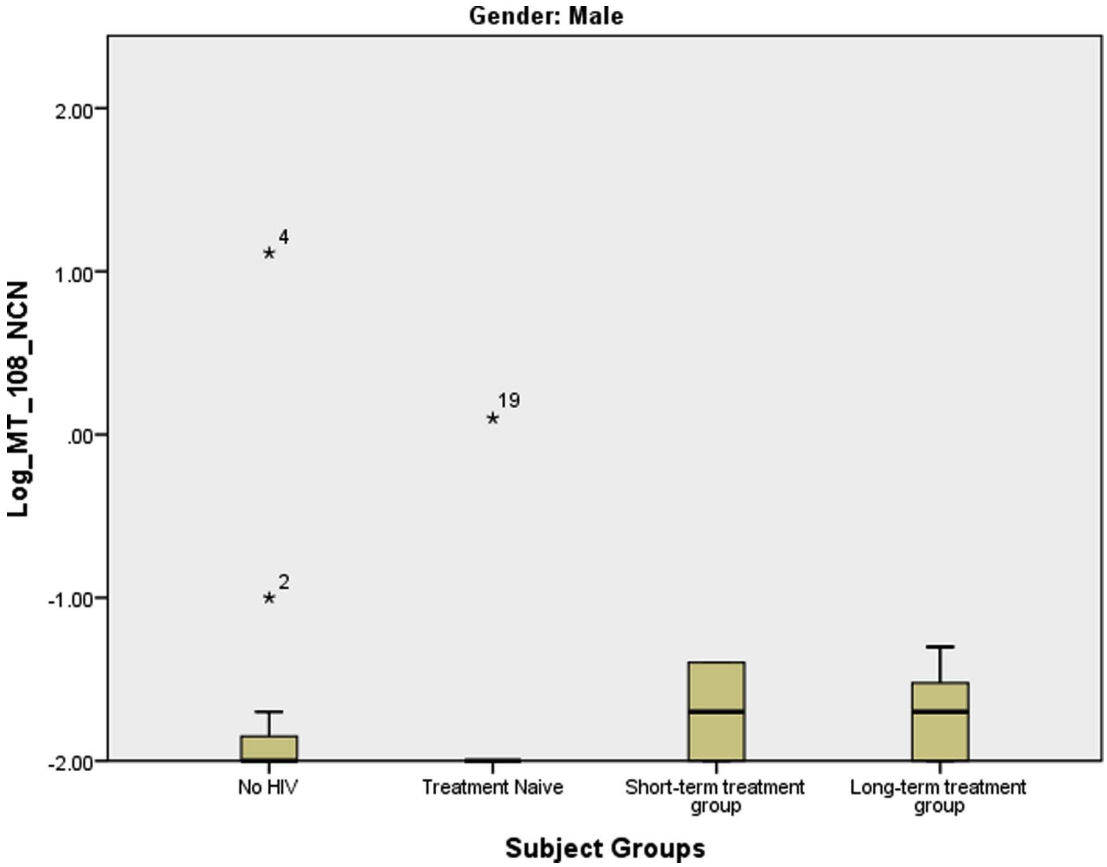

Gender: Female
Log_MT_104_NCN
1.20
1.00
.80
.60
.40
.20
.00
No HIV
Treatment Naive
Short-term treatment group
Long-term treatment group
Subject Groups

Fig. (3) contd….


Gender: Male
Log_MT_108_NCN
2.00
1.00
.00
-1.00
-2.00
4
19
2
No HIV
Treatment Naive
Short-term treatment group
Long-term treatment group
Subject Groups

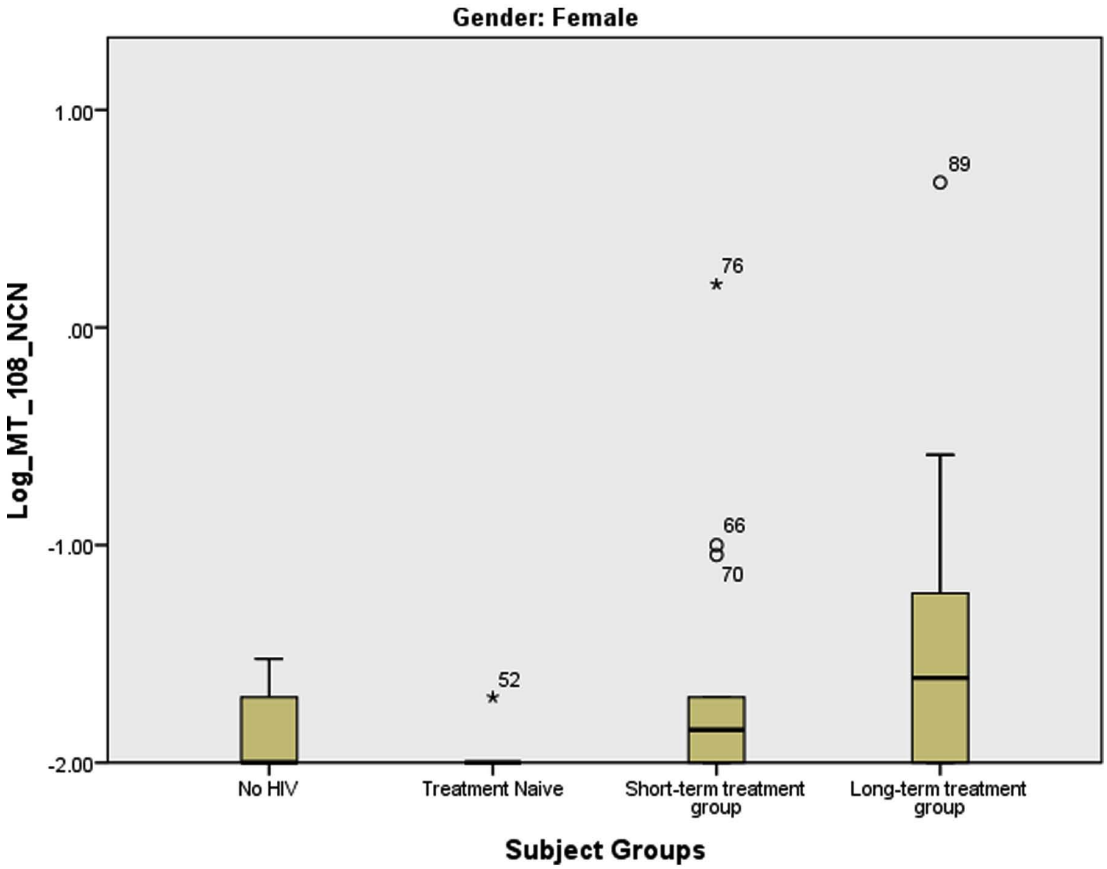

Gender: Female
Log_MT_108_NCN
1.00
.00
-1.00
-2.00
89
76
66
70
52
No HIV
Treatment Naive
Short-term treatment group
Long-term treatment group
Subject Groups

Fig. (3) contd….

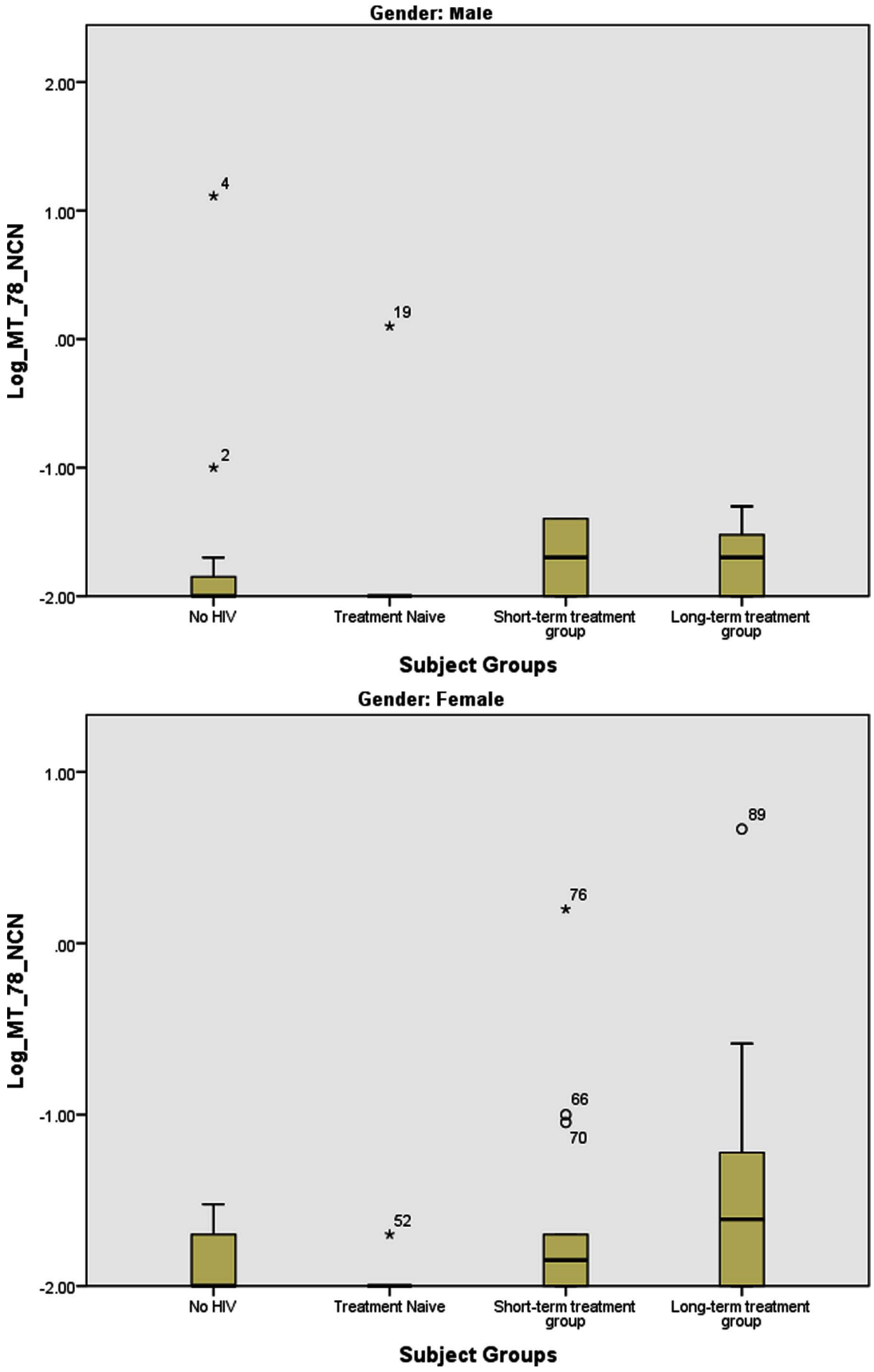


Fig. (3). Plots of Gene copy numbers across the groups for male and female subjects. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 4. Correlation of $CD4^+$ and other parameters studied.

Parameters	Correlation Coefficient (r)	p-value
$CD4^+$ vs TAS	-0.714*	0.000
$CD4^+$ v. MDA	0.709*	0.000
$CD4^+$ vs mt-104-NCN	0.009**	0.928
$CD4^+$ vs mt-141-NCN	0.056**	0.587
$CD4^+$ vs mt-108-NCN	-0.180**	0.080
$CD4^+$ vs mt-78-NCN	-0.051**	0.618

Correlation coefficients with "*" as superscript are considered statistically significant at $p < 0.01$ while correlation coefficients with "**" as superscript are considered statistically non-significant at $p > 0.05$.

was non-significant ($p > 0.01$) positive correlation between $CD4^+$ in subjects studied and the following parameters: mt-104-NCN and mt-141-NCN ($r = 0.009$, $p = 0.928$; $r = 0.056$, $p = 0.587$ respectively). $CD4^+$ showed significantly ($p < 0.01$) negative correlation with TAS ($r = -0.714$, $p= 0.000$) and non-significant ($p > 0.05$) negative correlation with mt-108-NCN and mt-78-NCN ($r = -0.002$, $p= 0.986$; $r = -0.180$, $p= 0.080$; $r = -0.051$, $p= 0.618$ respectively).

4. DISCUSSION

The treatment of HIV patients with HAART, especially the NRTI, inhibits the human polymerase γ required for mtDNA replication. This compromises oxidative synthesis of ATP leading to overdependence on cytosolic glycolysis for energy generation [17]. Oxidative stress is thought to be an important factor in mitochondrial dysfunction because the respiratory chain continually leaks the free radical superoxide which interacts with nitric oxide to form damaging by-product peroxynitrite that damage cellular membranes and all mitochondrial components [18].

The present study was therefore aimed at evaluating oxidative stress (by assessment of the total antioxidant status and the pro-oxidant biomarker- malondialdehyde concentrations); qualitative and quantitative analysis of some mtDNA genes and correlation of the findings with immune status (CD_4^+ cell count) of South East Nigerian HIV patients on ATRIPLA as the Highly Active Antiretroviral Therapy (HAART) provides evidence on the status of these patients with regard to mitochondrial dysfunction and its relationship with the biochemical findings.

In the genomic studies, the average normalized copy number for the mt104 (D-loop of the mitochondria) of 5.46 ± 2.69 in Group 2 subjects is significantly ($p < 0.05$) higher compared to copy numbers of groups 1, 3 and 4 (0.33 ± 0.16, 0.58 ± 0.58 and 0.67 ± 0.34 respectively). Thus the amplification was highest in the naïve HIV group which indicates dysfunction. The amplification is a compensatory mechanism to increase mtDNA replication as a result of its decreased number due to the burden of untreated HIV.

For mt141 (ATPase 8) the naïve population has a copy number of 10.85 ± 0.80 which is significantly ($p < 0.05$) higher than that of subjects in control group with copy number 0.08 ± 0.07. Subjects on HAART for <1year had a copy number of 2.45 ± 0.43, whereas those on HAART for >1year had a copy number of 5.38 ± 0.98. Thus, the normalized copy number of mt141 gene showed that the gene which codes for ATPase 8 in the mitochondria was amplified the most in the naïve HIV group followed by the subjects on HAART for > 1 year. The result suggests that the ATPase 8 gene were mostly affected in these two groups of subjects, possibly as a result of mitochondrial dysfunction. These genes have been known to be involved in energy generation by mitochondria, a function that is mostly affected by mitochondrial dysfunction. Mitochondrial dysfunction causes cell damage and death by compromising ATP production, disrupting calcium homeostasis and increasing oxidative stress. The result also shows that both long duration of HAART intake and untreated HIV infection affect the mitochondrial ATPases, with the greatest effect seen in untreated patients.

The mt108 gene which codes for transfer RNA (tRNA leu ^{UUR}) showed a significantly ($p < 0.05$) higher amplification in the naïve HIV subjects with copy number 540.32 ± 16.14 compared to subjects in group 4 (subjects on HAART for greater than 1 year) with copy number 123.63 ± 10.11. Meanwhile, subjects in group 4 have significantly ($p < 0.05$) higher copy number compared to that of subjects on HAART for less than 1 year with copy number 9.06 ± 1.70. The result also shows that both long duration of HAART intake and untreated HIV infection affect the mitochondrial RNA (tRNA leu ^{UUR}) gene, with the greatest effect seen in untreated patients. No significant amplification was observed across the groups for mt78 (ND4 gene).

Mitochondrial dysfunction with mtDNA as a target may lead to increase or inhibition of mtDNA replication, its increased deletion, mutation and depletion. Therefore, heterogenous clinical expression of mitochondrial DNA (mtDNA) disorders depends on both qualitative and quantitative changes in mtDNA [18]. Molecular diagnosis of mitochondrial DNA disorders is usually focused on point mutations and large deletions. In the absence of these, abnormal amounts of mtDNA, either depletion or elevation can be indicative of mitochondrial dysfunction [19, 20]. The prediction of phenotypic expression of mitochondrial diseases cannot be solely based on the proportion of mutant load in the affected tissue. The total amount of mtDNA is also a deter-

mining factor. Mitochondrial proliferation and thus the amount of mtDNA increase can be a cellular response to mitochondrial dysfunction [12].

The mechanisms by which HIV drugs induce mitochondrial dysfunction and cell death are not clearly established. However, HAART may compete with endogenous deoxyribonucleotides for incorporation into nascent DNA chains, and thereby inhibit DNA polymerase g (Polg) [21]. Polg is required for the replication of mtDNA and therefore inhibition of Polg results in mtDNA depletion, altered mitochondrial oxidative phosphorylation enzyme activities and changes in mitochondrial morphology, thereby initiating the apoptotic cell death process [21].

The TAS level was statistically ($p < 0.05$) lower in the naïve group (402.17 ± 5.53 mmol/l) compared to subjects in groups 1, 3 and 4 (1172.67 ± 20.42 mmol/l, 1208.21 ± 12.56 mmol/l and 500.88 ± 6.13 mmol/l respectively). The body contains antioxidant system which protects it against the effect of reactive oxygen species (oxidative free radicals) produced during metabolic processes or produced as a result of increased activation of polymorphor nuclear leucocytes during HIV or other infections. HAART is a combination of drugs that must be metabolized. This process of metabolism apparently generated oxidative free radicals that need to be scavenged by the body's antioxidant defence system, hence, reducing the total antioxidant status of the system [22]. HIV infection leads to increased production of reactive oxygen species. However, the subject that initiated treatment had higher TAS compared to HIV naïve subjects and subjects on HAART for greater than 1 year. This may be due to change in lifestyle, such as increased consumption of antioxidant-rich food and supplements by newly-infected and subject newly-initiated on ART.

The increase in levels of MDA and decrease in TAS, as observed from the present study indicate oxidative stress which is significantly ($p < 0.05$) higher in the HIV naïve subjects and those on treatment for greater than 1 year than subjects in groups 1 and 3. Earlier researchers showed an increase in oxidative stress markers such as malondialdehyde in HIV infected individuals taking drugs and naïve subjects [23, 24]. It was observed that inbalances in MDA and TAS result in oxidative stress [25]. HIV thrives in a highly oxidized environment and with its attack on the body's $CD4^+$ cells, an abundance of free radicals are produced with the mitochondria as a possible target. In HIV infection, oxidative stress may be caused by both overproduction of reactive oxygen species and other free radicals and a simultaneous deficiency of antioxidant defense. Moreover, free radicals production increases in people with HIV and metabolism of anti-HIV drugs also increases ROS generation.

The higher serum MDA level observed in patients on HAART is an evidence of higher lipid peroxidation in these patients as compared to controls. Since the antiretroviral drugs were not designed to discriminate nuclear (their target) from mitochondrial DNA, it is probable that mitochondrial DNA is attacked secondary to their actions. Mitochondrial DNA polymerase gamma (DNA pol-γ) is the enzyme that replicates and maintains mitochondrial DNA [26]. The proteins encoded by mitochondrial DNA participate in electron transport complexes of oxidative phosphorylation and so, inhibition of γ-DNA results in mitochondrial DNA depletion and altered oxidative phosphorylation, energy deprivation, all of which contribute to accumulation of free radicals [26], due to blockage of key reactions of the electron transport chain especially those of complexes I and III [27].

Studies reported that HIV infection is associated with oxidative stress caused by reactive oxygen species which promotes the progression of HIV to AIDS [28-30]. Also, Deresz *et al*. [29] and Wang *et al.* [31] reported that antiretroviral drugs (ARVs) increase the oxidative stress. These two factors lead to unhealthy situation in people living with HIV/AIDS which can be further aggravated by factors like diarrhea, loss of appetite, poor absorption of nutrients and low dietary intake which are all associated to both HIV and ARVs [32].

The findings in the present study are consistent with a study conducted by others, Gil *et al*. [33] and Lizette *et al*. [26] who reported an increase in MDA level of HIV positive patients who are naive and those on HAART compared with control subject. High level of MDA observed in this study may be due to deficiencies of micronutrients and immune system degradation which generate ROS, inflammation and release of cytokines to initiate neutrophils and macrophages to produce free radicals which subsequently enhances lipid peroxidation often leading to oxidative stress and cell apoptosis [34, 35]. The free radicals produced as a result of HIV infection are accompanied with weight loss, decreased immune cells, extensive loss of immune function which subsequently results in massive peroxidation of polyunsaturated lipid [36, 37]. The significantly higher level of MDA in the HIV positive non-treated group compared to the control group may be due to increase in the activities of lipoxygenase which is mediated by cytokine levels [38]. Different cells of the immune system such as neutrophils and leucocytes contained lipoxygenase enzymes which are the principal enzymes involved in the catabolism of phospholipid from cell membrane [39].

Studies by Suresh *et al.* [14] and Wanchu *et al*. [30] showed increased lipid peroxidation, measured by MDA concentrations, in HIV-1 sero-positive patients and decreased concentrations of individual antioxidants and total antioxidant capacity which was evident from HIV-1 asymptomatic stage itself. Thus, the possibility of counteracting oxidative stress by a pool of proper antioxidants plus an appropriate diet, mainly in patients whose blood antioxidant deficiencies can be easily rebalanced, may have real health benefit and represent a promising way of inhibiting the progression of disease. Furthermore, the significant ($p < 0.05$) negative correlation between TAS and MDA ($r = -0.714$) showed that TAS may be useful as an early marker of oxidative stress to monitor and optimize antioxidant therapy as an adjunct in the management of HIV-1 infected patients.

The naïve group had a non-significantly ($p > 0.05$) higher lactate concentration compared to those on HAART for <1 and >1 year as well as the control group. The higher concentration of lactate may be attributed to increased viral load and increased cytokines production during HIV infection as well as immune reconstitution; since mitochondria is a path-

way through which these cytokines act and dysfunctional mitochondria may lead to lactic acidosis. Moreover, several clinical conditions have been associated with impaired clearance of lactate, and liver dysfunction for instance, has been shown to impair lactate clearance. This, however, is in contrast with the study by McComsey *et al.* [40], who reported higher serum lactate concentration in the HIV-treated subjects compared to the HIV naïve subjects. This variation in result may be due to differences in the length of time in which the subjects are exposed to treatment. Meanwhile, the observed increase in lactate concentration during HIV infection as seen in group 2 compared to that in group 1 further confirmed the possibility of mitochondrial respiratory chain disorder.

The No HIV group has significantly ($p < 0.05$) higher $CD4^+$ cell count followed by the HAART-treated patients, while the Naïve group gave the least value. Infection with HIV has been established to induce CD^+ T lymphocyte depletion [41]. The HAART treatment of HIV patients leads to the decrease in viral load and an increase in $CD4^+$ lymphocytes [2]. A similar trend was obtained by [42]. It, therefore, implies that $CD4^+$ count continues to increase with the length of time of treatment.

CONCLUSION

- The present study showed that HIV infection increases oxidative stress status and this becomes prominent after long-term use of HAART.
- The use of two or more NRTIs (more of the ones with less toxic effect) may attenuate the mitochondrial toxicities experienced in several tissues.
- Recommendation of supplementation with vitamin cocktails is therefore made in this regard.
- The information derived from this study justifies the use of ATRIPLA (with supplement) for the management of patients with HIV infection but long term follow-up plan will be needed especially with regards to mitochondrial dysfunction.
- Biochemical variables raised, which are consistent with mitochondrial disease/dysfunction may not be used to predict the onset of mitochondrial dysfunction following HAART therapy.
- The inclusion of antioxidants in the therapeutic approach in managing HIV-seropositive patients and those on HAART will prevent the additional damage that free radicals could cause to such patients.

RECOMMENDATIONS

- This work has shown that drug choice plays an important role in the avoidance of mitochondrial toxicity at least in the short term. Hence, one important future goal is to prevent or attenuate the side effects so that improved efficacy is achieved. Therefore, additional international collaborative studies are required to look at interventions that may be used such as the inclusion of enough antioxidants in the therapeutic approach in managing patients (both naïve and treated) to enable individuals to remain on specific therapies of choice but with a diminished risk of mitochondrial toxicity.
- Recommendation of supplementation with vitamin cocktails is therefore made in this regard.
- More extensive studies of mtDNA mutation at the single molecular level are required to correlate mitochondrial dysfunction with NRTI-caused molecular defects.
- This whole analysis may mean then that increased production of ROS by the consumption of HAART may indirectly encourage the perpetuation of the disease, while trying to ameliorate it. We then recommend that in the treatment of HIV/AIDS with HAART, antioxidant preparations, for example, N-acetyl cysteine (NAC), a prodrug of GSH (reduced glutathione), should be included, to help replenish antioxidants and improve survival of HIV patients [43].

LIST OF ABBREVIATION

AIDS	=	Acquired Immune Deficiency Syndrome
HAART	=	Highly Active Antiretroviral therapy
HIV	=	Human Immunodeficiency Virus
NAC	=	N-acetyl cysteine
ROS	=	Reactive Oxygen Species
GITC	=	Guanidium Isothiocyanate

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research ethical approval was obtained from the Enugu State University Teaching Hospital Ethics Committee. Enrollment into the study was voluntary and informed consent was obtained for all participants.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

An informed consent was obtained from the volunteers.

AVAILABILITY OF DATA AND MATERIALS

Not Applicable

FUNDING

This study did not receive any funding from any grant agency or organization.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We would like to thank our study participants for spending their time with us during data collection period. Second, we would like to thank especially the management and staff of HIV clinic in Enugu State University Teaching Hospital, (ESUT), Parklane, Enugu State, Nigeria.

REFERENCES

[1] Maartens G, Celum C, Lewin SR. HIV infection: Epidemiology, pathogenesis, treatment, and prevention. Lancet 2014; 384(9939): 258-71. http://dx.doi.org/10.1016/S0140-6736(14)60164-1 PMID: 24907868

[2] Klatt NR, Silvestri G. Perspective HIV CD4+ T cells and HIV: A paradoxical pas de Deux. Sci Transl Med 2012; 4(123): 123ps4. http://dx.doi.org/10.1126/scitranslmed.3003862 PMID: 22378922

[3] Heil EL, Townsend ML, Shipp K, *et al.* Incidence of severe hepatotoxicity related to antiretroviral therapy in HIV /HCV co-infected patients In: Aids Res Treat-ment. 2010.

[4] Fraser C, Lythgoe K, Leventhal GE, *et al.* Virulence and pathogenesis of HIV-1 infection: An evolutionary perspective. Science 2014; 343(6177): 1243727. http://dx.doi.org/10.1126/science.1243727 PMID: 24653038

[5] Apostolova N, Blas-García A, Esplugues JV. Mitochondrial interference by anti-HIV drugs: Mechanisms beyond Pol-γ inhibition. Trends Pharmacol Sci 2011; 32(12): 715-25. http://dx.doi.org/10.1016/j.tips.2011.07.007 PMID: 21899897

[6] Koczor CA, Lewis W. Nucleoside reverse transcriptase inhibitor toxicity and mitochondrial DNA. Expert Opin Drug Metab Toxicol 2010; 6(12): 1493-504. http://dx.doi.org/10.1517/17425255.2010.526602 PMID: 20929279

[7] Malik AN, Czajka A. Is mitochondrial DNA content a potential biomarker of mitochondrial dysfunction? Mitochondrion 2013; 13(5): 481-92. http://dx.doi.org/10.1016/j.mito.2012.10.011 PMID: 23085537

[8] Murphy MP. How mitochondria produce reactive oxygen species. Biochem J 2009; 417(1): 1-13. http://dx.doi.org/10.1042/BJ20081386 PMID: 19061483

[9] Alfadda AA, Sallam RM. Reactive oxygen species in health and diseaseJ Biomed Bio-tech. 2012.

[10] Halliwell BH, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press 2007; pp. 55-9.

[11] Voss JG, Raju R, Logun C, *et al.* A focused microarray to study human mitochondrial and nuclear gene expression. Biol Res Nurs 2008; 9(4): 272-9. http://dx.doi.org/10.1177/1099800408315160 PMID: 18398222

[12] Bai RK, Wong LJ. Simultaneous detection and quantification of mitochondrial DNA deletion(s), depletion, and over-replication in patients with mitochondrial disease. J Mol Diagn 2005; 7(5): 613-22. http://dx.doi.org/10.1016/S1525-1578(10)60595-8 PMID: 16258160

[13] Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech 2013; 24(4): 224-31. http://dx.doi.org/10.7171/jbt.13-2404-001 PMID: 24294115

[14] Suresh DR, Annam V, Pratibha K, Prasad BV. Total antioxidant capacity--a novel early bio-chemical marker of oxidative stress in HIV infected individuals. J Biomed Sci 2009; 16: 61-4. http://dx.doi.org/10.1186/1423-0127-16-61 PMID: 19583866

[15] Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86(1): 271-8. http://dx.doi.org/10.1016/0003-2697(78)90342-1 PMID: 655387

[16] Yar'zever IS, Abubakar U, Toriola AL, *et al.* Effects of 12 weeks cycle exercise programme on CD4 count and viral load in HIV sero-positive patients in Kano, Nigeria. J AIDS HIV Res 2013; 5(11): 415-21.

[17] Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol 2012; 2012: 645460. http://dx.doi.org/10.1155/2012/645460 PMID: 22919381

[18] Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010; 107(9): 1058-70. http://dx.doi.org/10.1161/CIRCRESAHA.110.223545 PMID: 21030723

[19] Tanaka N, Goto Y, Akanuma J, *et al.* Mitochondrial DNA variants in a Japanese population of patients with Alzheimer's disease. Mitochondrion 2010; 10(1): 32-7. http://dx.doi.org/10.1016/j.mito.2009.08.008 PMID: 19703591

[20] Jitratkosol MH, Sattha B, Maan E, *et al.* Blood mitochondrial DNA mutations in HIV-infected women and their infants exposed to highly active antiretroviral therapy during pregnancy. AIDS 2012; 26: 1-9. http://dx.doi.org/10.1097/QAD.0b013e32835142eb

[21] Pinti M, Salomoni P, Cossarizza A. Anti-HIV drugs and the mitochondria. Biochim Biophys Acta 2006; 1757(5-6): 700-7. http://dx.doi.org/10.1016/j.bbabio.2006.05.001 PMID: 16782042

[22] Aniagolu MO, Ezeanyika LUS, Parker J, *et al.* Changes in some antioxidants in HIV-positive patients on highly active antiretroviral therapy (case study of Nsukka, South East Nigeria). Asian Journal of Science and Technology 2015; 6(4): 1289-92.

[23] Mgbekem MA, John ME, Umoh IB, *et al.* Plasma antioxidant micronutrients and oxidative stress in people living with HIV. Pak J Nutr 2011; 10(3): 214-9. http://dx.doi.org/10.3923/pjn.2011.214.219

[24] Akiibinu MO, Adeshiyan AA, Olalekan AO. Micronutrients and markers of oxidative stress in symptomatic HIV-positive/AIDS Nigerians: A call for adjuvant micronutrient therapy. IIOAB 2012; 3(2): 7-11.

[25] Halliwell B, Whiteman M. Measuring reactive species and oxidative damage *in vivo* and in cell culture: How should you do it and what do the results mean? Br J Pharmacol 2004; 142(2): 231-55. http://dx.doi.org/10.1038/sj.bjp.0705776 PMID: 15155533

[26] Lizette GV, Rosario GH, Jorge PÁ. Oxidative stress associated to disease progression and toxicity during antiretroviral therapy in human immunodeficiency virus infection. J Virol and Microbiol 2013; 80: 27-35.

[27] Jiang B, Khandelwal AR, Rogers LK, *et al.* Antiretrovirals induce endothelial dysfunction *via* an oxidant-dependent pathway and promote neointimal hyperplasia. Toxicol Sci 2010; 117(2): 524-36. http://dx.doi.org/10.1093/toxsci/kfq213 PMID: 20621964

[28] Kashou AH, Agarwal A. Oxidants and antioxidants in pathogenesis of HIV/AIDS. Open Reprod Sci J 2011; 3: 154-61. http://dx.doi.org/10.2174/1874255601103010154

[29] Deresz LF, Sprinz E, Kramer AS, *et al.* Regulation of oxidative stress in response to acute aerobic and resistance exercise in HIV-infected subjects: A case-control study. AIDS Care 2010; 22(11): 1410-7. http://dx.doi.org/10.1080/09540121003758549 PMID: 20640951

[30] Wanchu A, Rana SV, Pallikkuth S, Sachdeva RK. Short communication: Oxidative stress in HIV-infected individuals: A cross-sectional study. AIDS Res Hum Retroviruses 2009; 25(12): 1307-11. http://dx.doi.org/10.1089/aid.2009.0062 PMID: 20001519

[31] Wang X, Chai H, Yao Q, Chen C. Molecular mechanisms of HIV protease inhibitor-induced endothelial dysfunction. J Acquir Immune Defic Syndr 2007; 44(5): 493-9. http://dx.doi.org/10.1097/QAI.0b013e3180322542 PMID: 17245228

[32] Drain PK, Kupka R, Mugusi F, Fawzi WW. Micronutrients in HIV-positive persons receiving highly active antiretroviral therapy. Am J Clin Nutr 2007; 85(2): 333-45. http://dx.doi.org/10.1093/ajcn/85.2.333 PMID: 17284727

[33] Gil L, Tarinas A, Hernandez D, *et al.* Altered oxidative stress indexes related to disease progression marker in human immunodeficiency virus infected patients with antirretroviral therapy. Biomed Aging Pathol 2011; 1(1): 8-15. http://dx.doi.org/10.1016/j.biomag.2010.09.001

[34] Saikat S, Raja C. The role of antioxidants in human health educational society's college of pharmacy, Kurnool, Andhra. Eur J Exp Biol 2011; 1(2): 53-61.

[35] Teto G, Kanmogne DG, Torimiro J, *et al.* Very high lipid peroxidation indices in HAART-Naïve HIV-positive patients in Cameroon. Health Sci Dis 2011; 12(4): 1-4.

[36] Abduljalil MM, Liman HM, Umar RA, *et al.* Effect of HIV and HAART on antioxidants markers in HIV positive patients in Sokoto State, Nigeria. Int J Sci Eng Res 2015; 6(2): 1069-74.

[37] Asiimwe S. Nutri-medicinal plants used in the management of HIV/AIDS opportunistic infections in Western Uganda: Documentation, phytochemistry and bioactivity evaluation 2015.

[38] Kovacic P, Somanathan R. Broad overview of oxidative stress and its complications in human health. Open J Prev Med 2013; 3(1): 32-41. http://dx.doi.org/10.4236/ojpm.2013.31005

[39] Sharma B. Oxidative stress in HIV patients receiving antiretroviral therapy. Curr HIV Res 2014; 12(1): 13-21. http://dx.doi.org/10.2174/1570162X12666140402100959 PMID: 24694264

[40] McComsey GA, Walker UA. Role of mitochondria in HIV lipoatrophy: Insight into patho-genesis and potential therapies Mitochondrion 2005; 4(2,3): 111-8.

[41] Alimonti JB, Ball TB, Fowke KR. Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. J Gen Virol 2003; 84(Pt 7): 1649-61. http://dx.doi.org/10.1099/vir.0.19110-0 PMID: 12810858

[42] Soghoian DZ, Jessen H, Flanders M, *et al.* HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Sci Transl Med 2012; 4(123): 123ra25. http://dx.doi.org/10.1126/scitranslmed.3003165 PMID: 22378925

[43] Herzenberg LA, De Rosa SC, Dubs JG, *et al.* Glutathione deficiency is associated with impaired survival in HIV disease. Proc Natl Acad Sci USA 1997; 94(5): 1967-72. http://dx.doi.org/10.1073/pnas.94.5.1967 PMID: 9050888