Mitochondrial DNA Profiling in A Cohort of Antiretroviral Treated HIV Patients in Lagos, Nigeria: Assessment of Long-Term Effects of cART on Life Quality of PLWH

Azuka Patrick Okwuraiwe, Rahaman A. Ahmed, Disu Tajudeen, Stephnie Nwaiwu, Patrick Irurhe, Rosemary Ajuma Audu, and Chika Kingsley Onwuamah

ABSTRACT

The use of combined antiretroviral therapy (cART) has turned HIV infection to a manageable condition, significantly reducing HIV-related morbidity and mortality globally. Risk of transmission has been substantially reduced in Africa where 67% of global infection is domiciled. However, long-term impact of cART on life quality of HIV patients elicits concern due to possible oxidative stress stimulus and accumulated toxicity. Mitochondrion, responsible for molecular metabolism in eukaryotes has been proposed as a marker for cellular dysfunction and ageing. Metabolic dysfunction due to accumulated oxidative stress may lead to mitochondrial DNA (mtDNA) mutation, protein alteration, and premature apoptosis leading to ageing.

A cohort study comprising of 302 HIV infected persons receiving cART (Tenofovir, Lamivudine, Dolutegravir; TLD) at NIMR HIV reference clinic, and 113 healthy controls. Venous blood was collected in vacutainer tubes and plasma isolated. DNA extraction was done using NIMR-Biotech DNA Extraction kit, and mtDNA levels measured using SYBRGreen dye-based quantitative real-time PCR assay on Quant Studio 5. Primer sequences from human 12S ribosomal RNA with CCACGGAAAAACACGAGTGAT and CTATTGACTTGGGTAAATCTGCTGA as forward and reverse sequences respectively were used to amplify mtDNA locus. Melting curve was performed for every run to confirm successful amplification of targeted region. Total DNA from an immortalized Hela cell line, diluted in 10-fold serial dilutions, was used as standard curve. Plasma mtDNA levels were evaluated and data analyzed using IBM SPSS software (version 24).

Among HIV infected individuals, 185 (88.1%) were female and mean age was 32±0.43 years while 36 (32%) and 27±0.57 years were female and mean age among controls, respectively. Mean CD4 count among HIV subjects was 427±29 cells/µl while 62.4% had less than 50 viral copies/ml. Majority of subjects (74.9%) were on first line cART while mean exposure to ART regimens was 4±0.3 years. No significant difference was observed between mtDNA concentration of HIV subjects (mean = 256±38 copies/µl) and healthy controls (mean: 247±72 copies/µl), neither among only HIV subjects, when stratified based on viral load or CD4 count.

No association was observed between cell free (cf)-mtDNA and cART exposure among HIV patients. Lack of baseline information on initial cf-mtDNA among Africans was challenging in establishing that new ART regimens had enhanced recovery from mitochondrial-DAMP. There is need to continuously assess prolonged effect of cART to ensure good quality of life and healthy ageing for people living with HIV (PLWH).

Keywords: cART, HIV, mitochondrial DNA, PLWH.

Submitted: February 15, 2022
Published: June 23, 2022
ISSN: 2593-8339
DOI: 10.24018/ejmed.2022.4.3.1243

A. P. Okwuraiwe* Centre for Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: apokwuraiwe@nimr.gov.ng)
R. A. Ahmed Centre for Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: ahmedrahman158@gmail.com)
D. Tajudeen Centre for Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: tajudeen.disu@hotmail.com)
S. Nwaiwu Centre for Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: nwaiwustephnie@gmail.com)
P. Irurhe Centre for Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: irurhepatrick@gmail.com)
R. A. Audu Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: rosemaryaudu@yahoo.com)
C. K. Onwuamah Centre for Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. (e-mail: chikaonwuamah@yahoo.com)

*Corresponding Author
I. INTRODUCTION

The use of combination antiretroviral therapy (cART) has substantially reduced HIV-related morbidity and mortality, lessened the rate of HIV transmission, and minimized the health burden of HIV infection [1], [2]. It has successfully changed the paradigm of HIV infection globally from deadly epidemic to manageable chronic condition. All diagnosed HIV patients are now required to initiate cART irrespective of their clinical stage to ensure effective viral suppression and normal immunological function [3]. However, the lifetime exposure to cART poses concern of cumulative toxicity, metabolic dysfunction, organ damage, premature ageing, and eventual poor quality of life for people living with HIV (PLWH) [4], [5]. This concern is enormous in Sub-Saharan Africa where 67% of the global HIV population is found [6].

Mitochondrion is the cellular organelle responsible for oxidative phosphorylation and regulation of cellular metabolism in eukaryotic cells. These functions are regulated by thirteen proteins which are encoded in the mitochondrial DNA [7]. Exogenous stressors such as long-term exposure to certain drugs leading to susceptibility of mtDNA to genetic mutation, abnormal mtDNA replication, protein alteration, elevated respiratory oxidative species (ROS) production, and eventual mitochondrial metabolic dysfunction [8], [9]. Therefore, mtDNA profiling, through DNA mutation or copy number assessment, has been identified as a biomarker of mitochondrial dysfunction following exposure to certain diseases or chemical therapies [10]-[14].

Cellular molecules can be released into the extra-cellular spaces due to necrosis or apoptosis, enhanced by disease conditions, or exposure to other endogenous or exogenous stressors, and are regarded as damage associated molecular patterns (DAMPs) [15], [16]. Mitochondrial DAMP causes fragmentation of mtDNA and their release into the extracytoplasmic fluid due to conditions such as ill health and exposure to highly active drugs that cause impairment to mitochondrial functions [17]-[20]. Level of mitochondrial DAMP can be measured by assessing mtDNA concentration in plasma, a process known as cell free mtDNA (cf-mtDNA) copy number assessment [18]-[21].

Nucleoside reverse transcription inhibitors (NRTIs) can inhibit the activity of polymerase gamma which is responsible for mtDNA replication, thereby causing impaired mitochondrial activities [22]. Protein inhibitors (PIs) have been reported to promote type-2 diabetes and insulin resistance, comorbidities which contribute to high production of respiratory oxidative species (ROS) leading to oxidative DNA damage and imbalance mtDNA concentration [23]-[25]. Although the new antiretroviral regimens have been reported to have better safety profile than the old ones - such as ART combinations with stavudine or zidovudine with vast reports of toxicity [26], [27], the prolonged effect in people living with HIV (PLWH) still need to be re-assessed across different populations of diverse genetic variation, heterogenous metabolic capability, and varied environmental exposures.

Level of cf-mtDNA correlated with immune inflammation in ART-treated HIV patients [28] while two studies found elevated cf-mtDNA in cART-treated HIV patients as compared to healthy controls [29], [30]. Pernas and colleagues also reported higher cf-mtDNA in cART-treated patients compared to ART-naive patients, [31], [32] they found high cf-mtDNA in ART-treated patients exhibiting lipodystrophy as compared to ART-treated without lipodystrophy. However, [33] did not find significant variation of cf-mtDNA level between ART-treated patients and healthy control. There have been studies that investigated mtDNA concentration in peripheral mononuclear cells or whole blood of PLWH who were on cART treatment or in children who received ART as prophylactics in Africa [34]-[40], but little or no report has been documented on cf-mtDNA in HIV patients from any part of Africa regions.

Due to possible variation in the dynamics of mitochondrial responses to endogenous and exogenous environments in different populations, this study aimed at assessing the level of cf-mtDNA in a cohort of cART-treated HIV patients in Nigeria.

II. MATERIALS AND METHODS

A. Ethical Consideration

Ethical approval (IRB/21/001) for this study was obtained from the Nigerian Institute of Medical Research (NIMR), and signed informed consent was obtained from all participants recruited for this investigation. Participation in this study was entirely voluntary and handling of subjects conformed with the Declaration of Helsinki. The minimum age of participants for inclusion in this study was 18 years, while HIV patients with co-infections of hepatitis B or hepatitis C were excluded from the study.

B. Study Design

This was a cross-sectional cohort study of HIV patients who receive treatment at the HIV reference clinic of and healthy controls, who reside within Lagos state, Nigeria. Patient recruitment was carried out between November 2020 and April 2021.

C. Sample Population and Size

Blood samples were collected from 302 HIV infected persons receiving cART (Tenofovir, Lamivudine, Dolutegravir (TLD)) at NIMR HIV reference clinic, and 113 healthy controls. The sample size was determined using:

\[
\text{Sample size} = (Z^2 \times \text{SD} (1-\text{Sd}))^2 \mid 41, \]

where \(Z\) = Normal deviation of type-I error, \(Sd\) = Standard deviation, \(d\) = level of precision

D. Data Collection

A simple questionnaire was administered to the participants to ascertain age, long have they had been diagnosed for HIV infection, etc.

E. Blood Sample Collection and Processing

Whole blood sample was collected by trained phlebotomists into EDTA vacutainer tubes to profile CD4 count, HIV viral load, and plasma mitochondrial concentration. Within six hours of blood collection, the blood sample for viral load and plasma mtDNA analysis were centrifuged at 3000 rpm for 10 minutes, and plasma samples
were aliquoted into three cryovials. For the HIV+ cohort, CD4 count was assessed immediately from the EDTA-collected blood using the CY-S-3022 CyFlow® Counter instrument and reagents (Sysmex Partec GmbH, Gorlitz, Germany) while the plasma samples were cryopreserved at -80°C prior to further processing. Plasma samples from serologically confirmed HIV negative participants were also preserved at -80°C for cf-mtDNA profiling. HIV-1 RNA viral load determination was carried out using the COBAS 6800/8800 HIV-1 Quantitative Test kits (Roche Diagnostics, Branchburg, USA) at the Center for Human Virology and Genomics (CHVG) NIMR, an ISO accredited (ISO 15189:2012) and World Health Organization (WHO) national HIV drug resistance laboratory for HIV drug resistance surveillance in Nigeria.

F. Data Mining

Retrospective data of HIV patients were assessed through a secure processing channel at the NIMR HIV reference clinic, following appropriate consent from participants and Institute’s approval. Personal identities were removed from the data and securely encrypted.

G. MiDNA Profiling

A volume of 200 μl of phosphate buffered saline (PBS) was added to 200 μl of plasma samples, briefly vortexed and centrifuged at 16000g for 15mins. One hundred microliter (100 μl) supernatant of the mixture was used to extract DNA from plasma. DNA extraction was done using an inhouse DNA extraction kit (NIMR BioTech, Lagos, Nigeria) and DNA was eluted in 50 μl eluent buffer.

Cf-mtDNA concentration was measured using 5× HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) which was assayed on QuantStudio 5 (Thermofisher) qPCR machine. A volume of 3 μl plasma DNA was added to 4 μl of the 5× HOT FIREPol EvaGreen qPCR Mix Plus, 0.2 μl of each of forward and reverse primers (10 μM each) and 12.6 μl of molecular grade water to make 20 μl reaction mix. The primer set (which comprises of CTCGCGGAAACACGACGTGAT and CTATTGACTTGGTAAATCGTGTGA as forward and reverse sequences) targeted the 12S ribosomal RNA region of the human mitochondrial genome [21]. The PCR condition involved an initial denaturation step at 95°C for 15 minutes followed by 40 cycles of amplification processes, comprising 15 seconds of denaturation step at 95°C, 20 seconds of annealing at 60°C and 20 seconds elongation at 72°C. Every sample was run in replicate while melting curve was performed for every set of qPCR runs to confirm true amplification of the targeted region. DNA was also extracted from an immortalized Hela cell line, quantified using Qubit fluorometer and diluted in 10-fold serial levels. The 10-fold serial dilutions were used as standard curve for the real-time PCR setup. The concentration of cf-mtDNA was evaluated using the following formula:

\[ C_{mt} = Q \times V_{DNA}/V_{PCR} \times 1/V_{EXT} \]

Where \( C_{mt} \) = concentration of cf-mtDNA (copies/μl)  
\( Q \) = quantity of DNA measured by real-time PCR  
\( V_{DNA} \) = total volume of plasma DNA obtained from extraction  
\( V_{PCR} \) = volume of plasma DNA used for real-time PCR  
\( V_{EXT} \) = volume of plasma used for extraction [42]

H. Statistical Analysis

The mean cf-mtDNA concentration among the HIV positive (+) and HIV negative (-) cohort were evaluated, and the cf-mtDNA concentration based on matched age group were further analyzed, to reduce natural bias of DAMP’s phenomenon due to normal ageing process. To investigate putative association of cf-mtDNA concentration with clinical phenotypes among HIV patients, pre-ART records and recent medical data were utilized in the investigation. HIV+ cohort were stratified according to clinical phenotypes using viral load, CD4 count, duration of exposure to ART drugs, and present line of treatment. Mean of variables were expressed with their respective standard deviation while difference in mean cf-mtDNA concentration between categories were analyzed using t-test or pairwise t-test depending on the number of categories involved in a group. Pearson correlation test was also performed to identify the relationship of clinical parameters with the level of cf-mtDNA. Statistical inferences in this study were made at 5% level of significance. All analyses and charts were done using codes and software packages in R (version 4.0.2).

III. RESULTS

A. Study Demography

A total of 302 HIV positive (+) and 113 HIV negative (-) persons were recruited for this study. Among HIV+ subjects, 202 (66.9%) were female and the mean age was 47.5±9.9 years while among HIV- subjects, 52 (46.0%) were female and the mean age was 38.4±8.2 years.

B. Clinical Characteristics of HIV Infected Participants

Fig. 1-3 describes the clinical phenotype in the HIV positive cohort. The median viral load prior to initiating cART was 41,405 (4.62 log) RNA copies/ml, while the mean CD4 count before ART treatment was 271±208 cells/μl. At the time of this investigation, all HIV patients were on cART treatment. The mean duration of exposure to cART was 9.81±4.19 years, and 23.2% of patients were on second line treatment. Furthermore, during the time point of this study, 68.5% had undetectable viral load and the mean CD4+ count was 536±236 cells/μl.

Fig. 1a shows the first CD4 count of patients at NIMR clinic before commencing treatment. 60.6% (183/302) of the patients were on first line treatment) had CD4 count below 300 cells/μl, and 23.2% of patients were on second line treatment. Additionally, 45.5% (133/294) of patients had CD4 count below 200 cells/μl. At the first CD4 count, 82.8% had below 500 cells/μl, while 68.5% had undetectable viral load and the mean CD4+ count was 536±236 cells/μl.

Before treatment with cART, Fig. 2a shows that most of the patients (52.7%) had more than 4 log (equivalent to 10,000) viral RNA copies/ml of blood while Fig. 2b revealed that following initiation of cART treatment, 82.8% had below 3 log or 1000 RNA copies/ml, out of which 68.5% had undetectable viral copies in the blood plasma during the time point of this study.
The mean duration of exposure to cART among HIV patients was 9.8±4.2 years (mean expressed as $\bar{x} \pm SD$). The mean duration of exposure among male and female patients were 9.8±4.45 years and 9.8±4.1 years respectively. There was no significant difference in the duration of exposure to cART between male and female HIV patients.

C. Cf-mtDNA Concentration in the Study Cohort

The mean cf-mtDNA concentration among HIV non-infected group (560±611 copies/μl) was higher when compared with HIV infected group (440.8±384 copies/μl). However, there was no significant difference ($p=0.06$) in the mean cf-mtDNA concentration between the two groups and there were no significant variations when matched according to age groups (Table I). Among HIV patients, the mean cf-mtDNA concentration in individuals with present viral load of “<1000 copies/ml” was 456±382 copies/μl while among those with ‘>10000 copies/ml’, the mean cf-mtDNA concentration was 346±389 copies/μl. The mean cf-mtDNA concentration of HIV patients with CD4 count of at least 500 cells/μl and those below 300 cells/μl were 428±363 and 485±415 respectively. There were no significant differences in the mean cf-mtDNA concentration of HIV patients based on stratifications using the pre-treatment and recent records of viral load and CD4 count. Furthermore, considering exposure to ARV regimens such as Zidovudine or Stavudine that had been discontinued due to their toxicity, which may had enhanced mitochondrial DAMP in exposed patients who initiated cART at least 6 years ago at the NIMR clinic, we considered duration of exposure based on years of exposure to stratify those with and without exposure to the old regimens. Patients whose exposure to cART drugs was below 3 years had a mean cf-mtDNA concentration of 443±467 copies/μl while those with above 7 years exposure had 442±377 copies/μl. There was no significant correlation between cf-mtDNA concentration and duration of exposure to cART in the HIV cohort.

The mean cf-mtDNA of HIV patients, age 26-35yrs, was the highest from both groups while patients of above 45 years had the lowest mean cf-mtDNA concentration. There was no significant variation in the mean cf-mtDNA concentration in any of the age category between HIV+ and HIV- groups.
TABLE I: CF-MTDNA CONCENTRATION IN AGE MATCHED HIV POSITIVE AND HIV NEGATIVE GROUPS

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean cf-mtDNA conc. (x±SD)</th>
<th>HIV+ group</th>
<th>HIV – group</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 25</td>
<td>626±441</td>
<td>1531±1292</td>
<td>0.259</td>
<td></td>
</tr>
<tr>
<td>26-35</td>
<td>1906±459</td>
<td>480±470</td>
<td>0.983</td>
<td></td>
</tr>
<tr>
<td>36-45</td>
<td>451±370</td>
<td>609±614</td>
<td>0.117</td>
<td></td>
</tr>
<tr>
<td>≥45</td>
<td>435±387</td>
<td>440±560</td>
<td>0.972</td>
<td></td>
</tr>
</tbody>
</table>

The mean cf-mtDNA concentration among HIV patients (mean and standard deviation inputted at the top of each whisker) with viral load "<1000 copies/ml" before cART treatment was lower compared to those who had viral load of "1000-10000 copies/ml" but there were no significant variations in the mean cf-mtDNA among the three viral load categories (Fig. 4a). In the recent viral load categories, the mean cf-mtDNA concentration of the “1000-10000 copies/ml” group was the highest while those with “>10000 copies/ml” had the lowest mean cf-mtDNA concentration. There was no significant variation in cf-mtDNA concentration across the three recent viral load categories (Fig. 4b).

Using individual cf-mtDNA concentration, the correlation coefficient (r) of pre-treatment viral load and cf-mtDNA concentration was 0.015 (p = 0.703, Fig. 5a) while the coefficient with recent viral load was -0.063 (p = 0.483, Fig. 5b). cf-mtDNA did not correlate with pre-ART or the most recent viral load.
Fig. 6a shows cf-mtDNA in the HIV+ cohort stratified according to categories of CD4 count before initiating cART. There was a decreased trend in the mean cf-mtDNA concentration from the “<300 cells/µl” to the “≥500 cells/µl” of the pre-treatment record but no significant difference was identified. Fig. 6b shows the distribution of cf-mtDNA in the HIV+ cohort stratified according to recent CD4 count. There were no significant differences in the mean cf-mtDNA concentration of HIV patients based on recent CD4 count, although a reduced trend in the mean mtDNA value was observed from “<300 cell/µl” through “≥500 cell/µl” categories.

Generally, there was a negative correlation between cf-mtDNA concentration and CD4+ T-cells count. The correlation coefficient between the cf-mtDNA concentration and pre-treatment CD4 count was -0.083 ($p = 0.148$, Fig. 7a) while the correlation with the recent CD4 count was -0.037 ($p = 0.088$, Fig. 7b). cf-mtDNA concentration did not significantly correlate with CD4 count.

The correlation coefficient between cf-mtDNA concentration and duration of exposure to ARV was 0.0019 ($p=0.974$, Fig. 8a). The mean cf-mtDNA concentration among HIV patients whose cART treatment were below 3 years was 443±467 while those whose treatment were above 7 years had a mean cf-mtDNA concentration of 442±377 (Fig. 8b). There was no significant correlation between duration of exposure to cART and cf-mtDNA concentration, and there were no significant differences in the mean cf-mtDNA concentration when patients were grouped according to duration of exposure to ARV drugs.

Fig. 8. A) Relationship between duration of exposure to cART and cf-mtDNA concentration. B) Relationship between duration of exposure to cART and mean cf-mtDNA concentration.

IV. DISCUSSION

This study did not find significant difference in the cf-mtDNA concentration of ART treated HIV+ patients compared with healthy controls, even when they were matched to their respective age groups. Some of the previous studies reported significant difference of mtDNA concentration between healthy individuals and ART-treated HIV infected patients or ART-prophylactic treated children [36]-[39] and [35] also reported a significant depletion of mtDNA copy number among ART treated HIV patients compared to healthy controls in Enugu, a South-eastern part of Nigeria. However, [32] did not find marked variation in a study involving a cohort of HIV infected and non-infected children. Likewise, [43] found no such variation between ART-treated patients (age ≤ 50 years with suppressed viral load) and healthy controls. All these studies aimed at identifying the level of depletion of mtDNA concentration in functional cells such as PBMC which were expected to be lower due to mitochondrial dysfunction induced by disease condition and exposure to cART, as compared to healthy controls [40]-[43]. However, studies targeting DAMP-enhanced plasma mtDNA (known as cf-mtDNA) concentration as the case in this study, would hypothesize elevated mtDNA concentration among ART-treated HIV patients due to possible high oxidative damage and impairment in mitochondrial function, supposedly enhanced.
by exposure to ART drugs. This will facilitate fragmentation of mtDNA content and their release into the extracellular fluid. Therefore, the target source for assessment of mtDNA concentration in this study is different from what was utilized in those mentioned reports.

Reference [21] reported a higher cf-mtDNA concentration among ART-treated compared to ART-naive HIV patients, but the study did not include healthy controls. In this study however, all HIV+ participants were on cART treatment, in line with WHO recommendation. Initiating cART for every diagnosed HIV patient irrespective of stage of infection [3]. Reference [31] and [30] also found elevated cf-mtDNA concentration compared to healthy controls. However, our finding is consistent with that of [33] who found no significant variation in cf-mtDNA concentration between ART treated HIV patients and healthy individuals. The previous studies that evaluated cf-mtDNA concentration among HIV patients were carried out during the use of the old ART regimens including Zidovudine or stavudine or at very early stage of the new ART regimens (Tenofovir, Lamivudine, Dolutegravir; TLD) [30]-[33]. However, the new PIs and NRTIs have been reported to have better safety profile and may not cause marked oxidative stress that could enhance mitochondrial-DAMP or they will only promote DAMP after very prolonged exposure [23]. The new ART regimens are about 5 years of use in Nigeria, so the level of their cumulative oxidative stress may need continuous evaluation to be comprehensively elucidated.

Due to low magnitude of cf-mtDNA concentration in the HIV+ cohort (although not significantly established) compared with the healthy persons, we hypothesized that different clinical categories in the HIV cohort may have complementary effects on others, some may manifest high level cf-mtDNA phenotype while another may express low level phenotype, thereby balancing the cumulative level of cf-mtDNA concentration. We therefore considered individual and group data using clinical parameters including viral load, CD4 count, and current line of ART. We also defined the groups based on duration of exposure to cART, so that we could identify those that have been on previous ART drugs. Those within 5 years of exposure to cART would have been exposed only to the new ART regimens which have been reported safer than the previous ARV drugs. However, we did not find significant correlation in the cf-mtDNA concentration based on clinical parameters assessed in the HIV+ cohort. Reference [21] and [30] found viral suppression and CD4 count associated with cf-mtDNA, but other studies found no association between cf-mtDNA and clinical phenotypes of patients [29]-[33].

Although [23] mentioned that initiation of cART can enhance recovery from mitochondrial dysfunction, while [44] found that a switch to less toxic NRTIs can salvage mitochondrial toxicity, no initial cf-mtDNA study had been carried out on this cohort or from any close population in Africa. Therefore, we could not establish if the new ART regimens had enhanced recovery from mitochondrial-DAMP for patients, who at one time were on the old regimens. Or if mitochondrial-DAMP had not even been markedly expressed despite being exposed to the old NRTIs or PIs. Therefore, this study only established that at the time-point of this investigation, there was neither significant variation in the cf-mtDNA concentration between healthy persons and cART treated HIV patients, nor within the HIV positive cohorts when stratified based on clinical phenotype before or after initiation of cART. However, this report will be followed up in our prospective investigation to continually assess the impact of cART on the quality of life of HIV patients living in Sub-Saharan Africa.

In conclusion, this study shows that there was no association between cf-mtDNA and cART exposure among HIV patients in Lagos, Nigeria. The lack of baseline information on initial cf-mtDNA in African population, made it a challenge to establish if the new ART regimens had enhanced recovery from mitochondrial-DAMP for patients who were previously on the old regimen, or had not been expressed despite being exposed to the regimen. There is need to continuously assess the prolonged effect of cART to ensure good quality of life and healthy ageing process for PLHIV. This report will serve as a basis for further investigation of cell free assessment of mitochondrial toxicity among PLHIV in Nigeria and other Sub-Saharan countries.

ACKNOWLEDMENT

We acknowledge all the patients that consented to participate in this study.

FUNDING

This project was supported financially with NIMR funding; with grant number 3NM-JRGT-20-0049.

V. CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES


Koczor CA, Lewis W. Nucleoside reverse transcriptase inhibitor toxicity and mitochondrial DNA. Expert Opinion on Drug Metabolism and Toxicology. 2010; 6(12): 1493–1504.


