Journal of Antimicrobial Chemotherapy. 2017; 72(11): 3159-3162

Plasma mitochondrial DNA levels are inversely associated with HIV-RNA levels and directly with CD4 counts: potential role as a biomarker of HIV replication

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Abstract

Objectives. To evaluate plasma mitochondrial DNA (mtDNA) levels among HIV-infected patients and its potential role as a biomarker of residual viral replication.

Methods. HIV-infected patients on follow-up at a reference hospital in north-west Spain were selected. DNA was isolated from plasma samples and mtDNA levels were assessed using a quantitative real-time PCR assay. HIV-RNA levels and CD4+ cell counts were evaluated in the same blood samples used for plasma mtDNA quantification. Epidemiological and clinical variables were included for the analysis.

Results. A total of 235 HIV-infected patients were included. Mean plasma mtDNA levels were 217 ± 656 copies/μL for naive (31.9%) and 364 ± 939 copies/μL for HIV-infected patients receiving ART and with suppressed viraemia ($P = 0.043$). Among the latter, mean plasma mtDNA levels were 149 ± 440 copies/μL for those with low-level viraemia (LLV; HIV-RNA 20–200 copies/mL), 265 ± 723 copies/μL for those with detected-not-quantified (DNQ) viraemia (HIV-RNA <20 copies/mL) and 644 ± 1310 copies/μL for those with not-detected (ND) viraemia. Of note, a linear trend ($P = 0.006$) was observed among virologically suppressed (LLV, DNQ and ND) patients. ND patients had higher mtDNA levels compared with LLV patients ($P = 0.057$). Moreover, mtDNA levels were inversely associated with HIV-RNA levels (Spearman's rho −0.191, $P = 0.003$) and directly associated with CD4+ counts (Spearman's rho 0.131, $P = 0.046$).

Conclusions. Increased plasma mtDNA levels are associated with lower HIV-RNA levels and higher CD4+ cell counts. Among ART-suppressed patients, mtDNA levels were significantly higher in those with complete virological suppression (ND) than in those with LLV. These data suggest that plasma mtDNA levels might serve as a biomarker of residual HIV replication.

Topic: hiv, biological markers, cd4 count determination procedure, dna, mitochondrial, plasma, viremia, virus replication, virology, blood hiv rna, hiv infection

Introduction

Current ART combinations effectively suppress HIV replication for extended periods of time and reduce the degree of immune dysfunction. However, the effect of ART seems to be limited in terms of activation, and a chronic inflammatory state persists in most patients on ART, which has been related with poor clinical outcomes, including development of AIDS and non-AIDS defining events and death.[1,2] It remains to be elucidated whether residual viral replication occurring despite ART fuels immune activation and chronic inflammation.

Mitochondria are intracellular organelles involved in a wide range of processes, as they are the main source of cellular energy.[3] Their number and shape constantly change in response to energy demands. In fact, mitochondrial dysfunction has been implicated in ageing and pathological processes, such as carcinogenesis and inflammation.[4,5] This fact implies a possible role of mitochondrial DNA (mtDNA) as a potential biomarker in processes associated with oxidative stress and inflammation. Several studies have described that the metabolic stress derived from HIV replication may result in mitochondrial damage and mtDNA depletion.[6–11]

In this context, the aim of this study was to evaluate the correlation between mtDNA and HIV-RNA levels in HIV-infected patients and to investigate whether mtDNA might be used as a biomarker of residual viral replication among patients on ART resulting in viral suppression.

Materials and methods

Quantification of plasma mtDNA levels was performed in blood samples obtained from an observational cohort of HIV-infected patients in clinical follow-up at Complexo Hospitalario Universitario de A Coruña. Epidemiological and ART characteristics were retrospectively recorded. In addition, HIV-RNA levels (determined using Roche COBAS TaqMan test version 2.0 with a limit of detection of 20 copies/mL) and CD4+ cell counts were assessed in the same blood samples used for plasma mtDNA quantification.

Whole-plasma DNA was isolated from plasma using a DNAeasy Blood and Tissue kit (Qiagen). Briefly, 400 μL of PBS was added to 400 μL of plasma samples and then centrifuged at 16000 **g** for 15 min at 4 °C. Then, 200 μL of supernatant was subsequently processed according to the manufacturer's protocol, and finally 100 μL of elution buffer was added to resolve the isolated plasma DNA.

Plasma mtDNA levels were measured in duplicate by SYBR Green dye-based quantitative real-time PCR assay using a LightCycler II (Roche) thermal cycler. The primer sequences were human 12S ribosomal RNA (mtDNA): forward sequence CCACGGGAAACAGCAGTGAT; reverse sequence CTATTGACTTGGGTTAATCGTGTGA. Total DNA obtained from an immortalized cell line was diluted in 10-fold serial dilutions and used as standard curve. Plasma mtDNA levels were shown in copies of plasma according to the following formula:[12] $C = Q \times V_{DNA}/V_{PCR} \times 1/V_{ext}$, where C is the concentration of plasma mtDNA (copies/μL), Q is the quantity of DNA measured by real-time PCR, V_{DNA} is the total volume of plasma DNA solution obtained from the extraction (100 μL in this study), V_{PCR} is the volume of plasma DNA solution for real-time PCR (5 μL in this study) and V_{ext} is the volume of plasma used for the extraction (400 μL in this study).

The reaction mix consisted of 10 μL of SYBR Green ready reaction mix (Roche®), 0.3 μM forward and reverse primers, 5 μL of plasma DNA and up to 20 μL of double-distilled water. The thermal cycler programme consisted of a first activation cycle at 95 °C for 10 min, followed by an amplification programme of 45 cycles (95 °C for 10 s, 62 °C for 60 s and 72 °C for 5 s). In order to verify the amplification products, a melting cycle was additionally performed (95 °C for 5 s and 65 °C for 60 s, followed by a gradual temperature increase up to 97 °C). Finally, the mix was cooled at 40 °C for 20 s.

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS 19.0, Chicago, IL, USA). Continuous variables were compared by non-parametric Mann–Whitney and Kruskal–Wallis tests, as appropriate. Correlations between mtDNA levels and HIV-related parameters were determined using Spearman's correlation analysis. Polynomial contrasts were used to evaluate linear trends between mtDNA levels and groups of HIV-infected patients receiving ART resulting in virological suppression. A P value <0.05 was considered statistically significant.

Results

A total of 235 HIV-infected patients were included. Of these, 83.3% were male, and their mean age was 44.6 ± 11 years. Main routes of HIV transmission were: MSM (43%), heterosexual (33%) and intravenous drug users (23.5%). CD4 nadir was <200 cells/mm^3 in 40.7% of patients, and 15.3% had an AIDS-defining disease.

Overall, 31.9% were naive (mean HIV-RNA 4.64 ± 0.64 $\log_{10}$ copies/mL) while the remainder were receiving ART: 24.3% had not-detected (ND) HIV-RNA, 23% had <20 copies/mL and detected-not-quantified (DNQ) HIV-RNA and 20.9% had low-level viraemia (LLV) with 20–200 copies/mL of HIV-RNA.

Mean plasma mtDNA levels were 217 ± 656 copies/μL for naive patients and 364 ± 939 copies/μL for HIV-infected patients receiving ART ($P = 0.043$) (Figure 1). According to HIV-RNA quantification among the whole population, mean plasma mtDNA levels were 364 ± 939 copies/μL for patients with HIV-RNA <3 $\log_{10}$ copies/mL; 273 ± 752 copies/μL for 3–5 $\log_{10}$ copies/mL; and 51 ± 45 copies/μL for HIV-RNA >5 $\log_{10}$ copies/mL. Of note, mtDNA levels were inversely associated with HIV-RNA levels (Spearman's rho −0.191, $P = 0.003$) among the whole population (Figure 2).

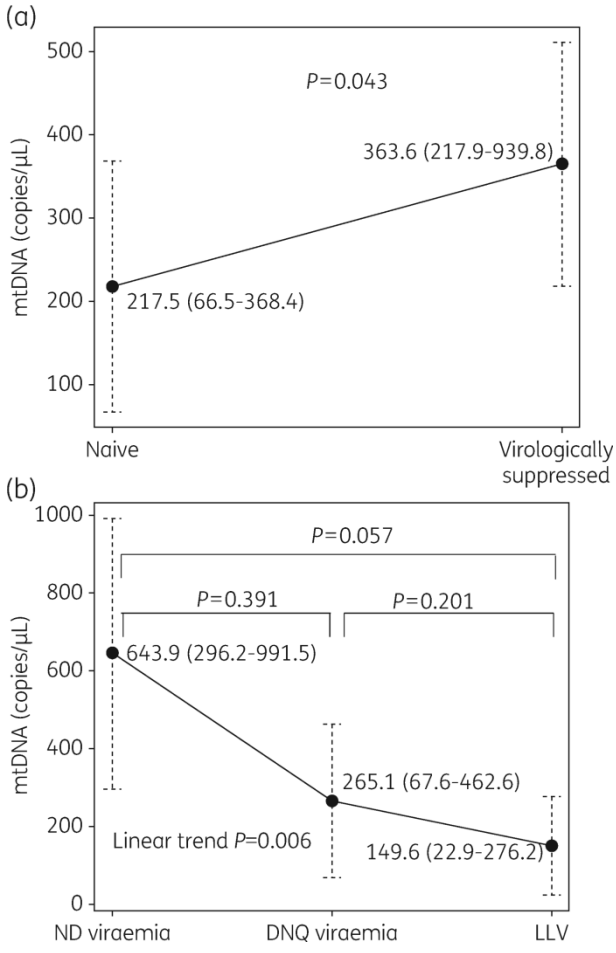

Figure 1. Comparison of mtDNA levels expressed as mean (95% CI) among HIV patients. (a) Naive versus HIV-infected patients with virological suppression (HIV-RNA <200 copies/mL) receiving ART. (b) HIV-infected patients with ND viraemia, DNQ viraemia (HIV-RNA <20 copies/mL) and LLV (20–200 copies/mL) receiving ART.

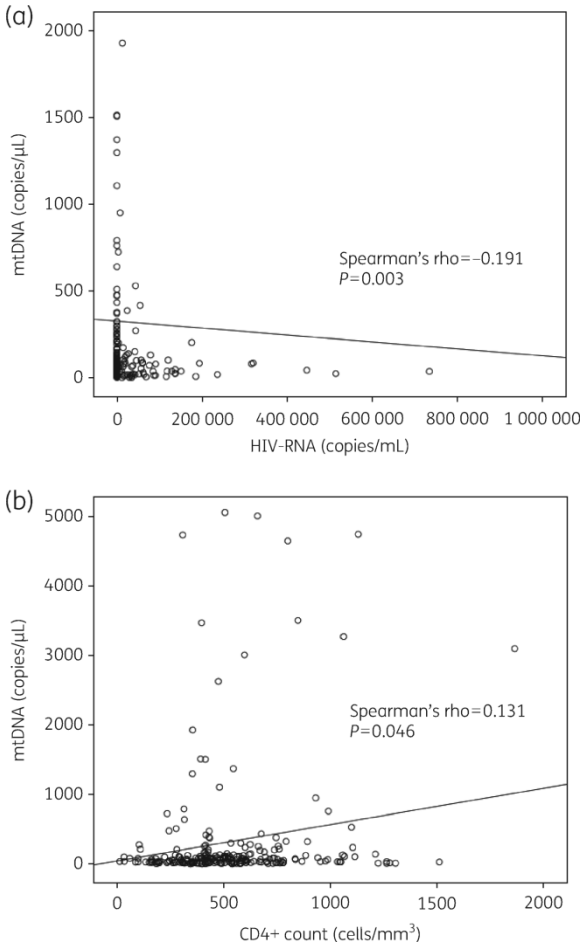

Figure 2. Correlation between HIV-RNA (copies/mL) (a) or CD4 count (cells/mm^3) (b) and mitochondrial DNA (mtDNA) levels (copies/μL) according to Spearman's rho analysis among the whole population.

In addition, mean plasma mtDNA levels were 149 ± 440 copies/μL for those with LLV, 265 ± 723 copies/μL for DNQ viraemia and 644 ± 1310 copies/μL for those with ND viraemia. Interestingly, among patients receiving ART, those with LLV had lower levels of mtDNA than those with ND viraemia ($P = 0.057$). Although no differences in mtDNA levels were observed between ND and DNQ patients, the trend analysis showed that mtDNA levels decrease among virologically suppressed HIV-infected patients (ND, DNQ and LLV) in a linear way (Figure 1). The *F*-ratio for the linear trend was 7.81 ($P = 0.006$). In addition, significant differences were observed between naive and patients receiving ART with ND viraemia (217 ± 656 versus 643 ± 1310 copies/μL, $P = 0.010$). Among HIV-infected patients receiving ART, no differences in plasma mtDNA levels were observed according to the different ART regimens used (NNRTI-, PI- or integrase inhibitor-based combinations) ($P = 0.808$).

In regard to the immunological status, mean mtDNA levels were directly associated with CD4+ cell counts among the whole population: ≤200 cells/mm^3, 60 ± 64 copies/μL; 201–350 cells/mm^3, 270 ± 803 copies/μL; 351–500 cells/mm^3, 278 ± 622 copies/μL and >500 cells/mm^3, 417 + 1075 copies/μL, respectively (Spearman's rho 0.131, $P = 0.046$) (Figure 2).

Moreover, a negative correlation between mtDNA levels and HIV-RNA (Spearman's rho −0.246, $P = 0.011$) was found for the subgroup of patients with CD4+ cell count >500 cells/mm^3, and a positive correlation between mtDNA levels and CD4+ cell count (Spearman's rho 0.296, $P = 0.028$) was also found for the subgroup of ND viraemia. Interestingly, multivariate lineal regression found a linear regression coefficient of 0.636 for CD4+ cell count ($P = 0.010$) and −4.171 for HIV-RNA levels ($P = 0.043$) among virologically suppressed HIV-infected patients receiving ART.

Discussion

This study evaluated the potential role of plasma mtDNA levels as a biomarker of residual viral replication. Of 235 HIV-infected patients, 31.9% were naive and the rest of patients were receiving ART with different levels of HIV-RNA quantification. Herein, to our knowledge, we provide the first clinical evidence that mtDNA levels showed a linear trend among virologically suppressed HIV-infected patients receiving ART. In fact, those patients with complete virological suppression (HIV-RNA ND) have a trend towards higher mtDNA levels than HIV-infected patients with LLV (20–200 copies/mL) receiving ART. Of note, increased plasma mtDNA levels were associated with lower HIV-RNA levels and higher CD4+ cell counts.

These data suggest that plasma mtDNA levels could be a biomarker of residual viral replication beyond HIV-RNA quantification associated with mitochondrial damage, as a consequence of an inadequate control of HIV replication.

Likewise, some studies have shown mtDNA depletion in HIV-infected patients compared with HIV-negative controls.[6–11] Different results were found in other studies, likely due to the heterogeneity of the study populations and/or the methodology used. For example, no impact on mtDNA levels has been described among untreated HIV-infected patients, elite controllers, ART-suppressed or HIV-negative subjects.[1] However, higher mtDNA plasma levels have been described in acute HIV infection and late presenters compared with long-term non-progressors and healthy individuals.[13] Correlation of mitochondrial toxicity with CD4 counts or HIV-RNA levels was not reported from previous studies conducted in HIV-infected patients.[6–11] As an exception, Miura *et al.*[14] found that mtDNA levels were positively correlated with CD4+ cell counts and inversely correlated with plasma HIV-RNA in naive patients. Similarly, this study showed a positive correlation between mtDNA levels and CD4+ cell counts, while a negative correlation was found with HIV-RNA levels. The mechanisms underlying these findings are not yet elucidated and different viral and host factors might be influencing mitochondrial function. Based on the results obtained, we speculate that among treatment-naive patients, continuous antigenic exposure might lead to T cell exhaustion and a decrease in mitochondrial biogenesis. Conversely, low levels of ongoing HIV replication and ultimately the complete suppression of HIV-1 replication by ART might restore T cell proliferation and mitochondrial biogenesis.[15] Our data also suggest that even in the context of LLV replication or DNQ viraemia, persistent antigen exposure might still have a negative effect on the mitochondria, although lower than in the naive population. Moreover, this explanation would fit with the positive correlation found between mtDNA levels and CD4+ cell count.

Nowadays, most HIV-infected patients receiving ART exhibit suppressed viraemia for long periods of time. Although LLV is frequently detected in these patients, its clinical impact is far from clear. We have recently described an association of persistent residual viraemia (at least three consecutive determinations of HIV-RNA <20 copies/mL and DNQ) with virological failure.[16] However, HIV treatment guidelines consider that transient LLV has no clinical relevance. Indeed, the Department of Health and Human Services guidelines consider virological failure as HIV-RNA >200 copies/mL.[17] Interestingly, we found that lower levels of mtDNA were observed in patients with LLV (HIV-RNA 20–200 copies/mL) compared with HIV-infected patients with ND viraemia. Although no significant differences were observed between ND and DNQ patients, a linear trend was found according to the level of viraemia among suppressed patients receiving ART (LLV, DNQ and ND).

It is tempting to speculate that HIV-1 infection itself may be responsible for a decrease in mtDNA although the mechanisms underlying this conjecture need to be established. In the context of HIV replication even at low levels, chronic antigen exposure might impair mitochondria biogenesis. In this context, mitochondrial metabolism might be reprogramming toward fatty acid oxidation, increasing the generation of reactive oxygen species that might further affect mtDNA integrity as has been described in the context of viral infections.[15,18] In addition, certain HIV proteins, such as Vpr, have been shown to adversely affect mitochondrial integrity.[19]

This study has some limitations. Comparisons with an HIV- negative population were not made. However, a large number of HIV-infected patients with different HIV-RNA levels were included, allowing a detailed evaluation regarding the impact of HIV replication on mtDNA levels. On the other hand, the techniques used to measure mtDNA levels are not standardized among published studies. This fact might explain differences in the results found in the literature.

In conclusion, our results are consistent with the concept that HIV replication may be associated with mtDNA depletion. Among patients receiving ART, higher mtDNA levels were found in patients with ND viraemia compared with those with LLV and DNQ, suggesting a potential role of mtDNA level as a biomarker of residual viral replication. Therefore, mtDNA level might be considered as a surrogate marker in studies addressing viral eradication.

Acknowledgements

We are thankful for the collaboration of Fina Baliñas (HIV and Viral Hepatitis Unit, Complexo Hospitalario Universitario de A Coruña) and Biobanco of A Coruña.

Funding

This work was supported in part by grants from Fondo de Investigación Sanitaria (CPII14/00014, PI10/02166, PI13/02266, FI14/00557, CM15/00233, PI16/02159, MV16/02159 PTA2013-8277-I), and Fundación Profesor Novoa Santos, A Coruña.

Transparency declarations

None to declare.

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