Increased mtDNA Content in Neuron-Derived Extracellular Vesicles from African Americans with HIV

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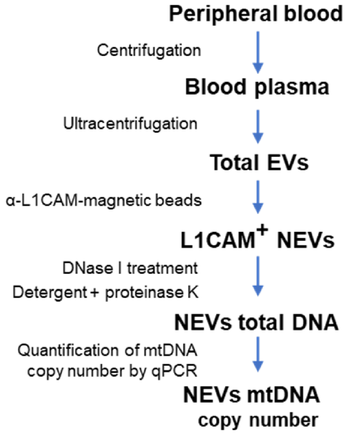
Vladimir Berthaud, Waldemar Popik, Tarik Smith, Derek Wilus, Franklin Nouvet, Venkateswara Rao Amara
Meharry Medical College, Nashville, Tennessee, USA

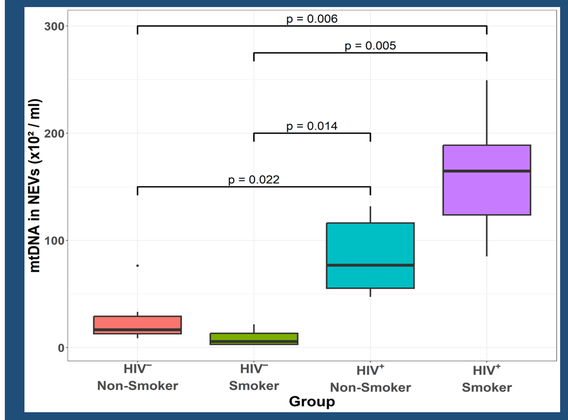
BACKGROUND

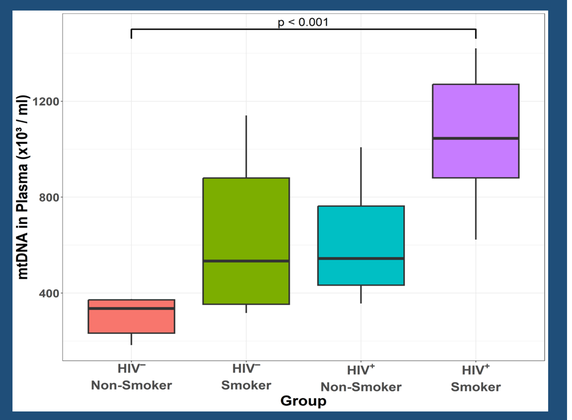
Cigarette smoking induces mitochondrial damage and is more prevalent among PWH. Mitochondrial DNA (mtDNA) content reduction often occurs before neuronal degeneration. Our objective was to determine the effect of smoking on mtDNA content in neuron-derived extracellular vesicles (NEVs) isolated from the peripheral blood of African Americans (AAs), according to smoking and HIV status. We hypothesized that smoking exacerbates neuronal mtDNA damage in virally suppressed AA PWH, leading to increased release of mtDNA in NEVs.

METHODS

Isolation of Neuron-Derived Extracellular Vesicles (NEVs) from Blood Plasma. Four ml of plasma was thawed, centrifuged (30 min, 3000 x g, 4°C), and 1 ml was saved for total plasma mtDNA analysis. The rest was mixed with 8 ml ice-cold PBS, ultracentrifuged (3 h, 100,000 x g, 4°C), and the EV pellet was resuspended in 200 µl PBS with inhibitors and incubated O/N with 2 µg L1CAM-biotinylated antibody, followed by 4 h incubation with Dynabeads-streptavidin. NEVs were isolated using magnetic beads, washed, and treated with DNase I (37°C, 30 min). After stopping DNase I with EDTA, the NEVs were heated (80°C, 10 min) to inactivate DNase I, lysed (1 h, 37°C) using 0.05% detergent mix (Triton X-100 and SDS) with proteinase K to release intra-NEVs DNA. After proteinase K inactivation (56°C, 15 min), NEVs DNA was separated from beads and stored at -80°C for mtDNA analysis.







The mtDNA content in neuron-derived extracellular vesicles and in plasma was significantly higher in HIV-positive smokers as compared to non-smokers and participants without HIV. The study found significant interaction between HIV and smoking status (P-Value = 0.011).

RESULTS

Twenty-four African American male participants, aged 45-64, were recruited into four groups of six: HIV-negative non-smokers, HIV-negative smokers, HIV-positive non-smokers, and HIV-positive smokers. NEVs mtDNA content was heteroscedastic (P-Value < 0.001). Significant pairwise difference was found between HIV-negative non-smokers and HIV-positive non-smokers (P-Value = 0.022)
HIV-negative smokers and HIV-positive non-smokers (P-Value = 0.014)
HIV-negative non-smokers and HIV-positive smokers (P-Value = 0.006)
HIV-negative smokers and HIV-positive smokers (P-value = 0.006)

Smoking and HIV Status	Mean Age	Median mtDNA in NEVs ($x10^2$/ml)	Median mtDNA in plasma ($x10^3$/ml)
Non-Smoker	56.67	47.88	375.05
Smoker	52.83	53.41	902.00
HIV Negative	53.00	13.65	368.25
HIV Positive	56.50	121.29	830.00

STATISTICAL ANALYSIS

Welch's One-way ANOVA was applied to determine differences in mean mtDNA content and multiple comparisons done with Games-Howell test, which considers heteroscedasticity. We used multiple linear regression and tested for interaction to determine the association between HIV and mtDNA content, adjusting for smoking status.

CONCLUSIONS

Our findings indicate that alterations in plasma mtDNA content could serve as an early biomarker for mitochondrial dysfunction, potentially heralding subsequent neuronal damage in PWH who have achieved viral suppression. The exacerbating effect of cigarette smoking on mtDNA integrity within neuron-derived extracellular vesicles and plasma underscores the importance of lifestyle factors in the management of mitochondrial health in this population. Further elucidation of the underlying mechanisms through larger, targeted clinical trials is essential to validate mtDNA content as a reliable indicator and to explore its potential clinical applications in mitigating neuronal damage. Recognizing the limitations of our study, including the need for larger sample sizes, including women, and the exclusion of potentially confounding variables, we advocate for continued research into the complex interplay between viral load suppression, mitochondrial function, and neurodegenerative processes in PWH.

ADDITIONAL KEY INFORMATION

Author Contact Information:
615 327 5753 vberthaud@mmc.edu

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