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Urine Cell-Free Mitochondrial DNA as a Marker of Weight Loss and Body Composition in Older Adults with HIV

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Abstract

Background: Older adults with HIV (OAH) experience more comorbidities and geriatric syndromes than their HIV-negative peers, perhaps due to chronic inflammation. Cell-free mitochondrial DNA (cfmtDNA) released from cells undergoing necrosis-mediated cell death potentially acts as both a mediator and marker of inflammatory dysregulation. We hypothesized that urinary cfmtDNA would be associated with frailty, body composition and fall history in OAH

Methods: OAH completed frailty testing, a psychosocial survey, body composition assessment, and measurement of urine cfmtDNA and urine albumin:creatinine in this cross-sectional study. Urine cfmtDNA was measured by qPCR and normalized to urinary creatinine.

Results: Across 150 participants, the mean age was 61 years (SD 6 years), half identified as Black, one-third were female, and 93% had HIV-1 viral load <200 copies/ml. Two-thirds met criteria for a pre-frail or frail state. Those with unintentional weight loss had higher urine cfmtDNA concentrations (p=0.03). Higher urine cfmtDNA was inversely associated with skeletal muscle index (SMI) ($\beta = -0.19$, $p<0.01$) and fat mass index (FMI) ($\beta = -0.08$, $p=0.02$) in separate multiple linear regression models adjusted for age, sex, and presence of moderate-severe albuminuria.

Conclusions: In this cross-sectional study of OAH, higher levels of urine cfmtDNA were more common in subjects with less robust physical condition, including unintentional weight loss and less height-scaled body mass of fat and muscle. These findings suggest urine cfmtDNA may reflect pathophysiologic aging processes in OAH, predisposing them to geriatric syndromes. Longitudinal investigation of urine cfmtDNA as a biomarker of geriatric syndromes is warranted.

Background:

HIV-related mortality has decreased in individuals treated with antiretroviral therapy, yet older adults with HIV (OAH) experience more comorbidities and geriatric syndromes than their HIV-negative peers[1,2]. Inflammatory changes with aging are multifactorial and lead to

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the accumulation of detrimental molecular changes within cells, which can result in frailty, disability, and death[3,4].

Mitochondrial dysfunction contributes to inflammation during aging and muscle wasting processes[5,6] and has been proposed as a mechanism driving adverse aging phenotypes in OAH[7,8]. In the setting of cellular stress, mitochondrial dynamics are disrupted which can result in dysregulated mitophagy, redox imbalance, and cell death[9]. Hence, mitochondrial DNA in the circulation- termed cell-free mitochondrial DNA (cfmtDNA)- can serve as a damage-associated molecular pattern, stimulating innate immune pathways and recruiting immune cells to propagate the inflammatory response[9,10].

CfmtDNA can be detected in body fluids, including plasma and urine, and has been proposed as a mediator and marker of inflammation[10–12]. Release of cfmtDNA can lead to activation of innate immunity via multiple pathways, including activation of toll-like receptor 9, cGAS-STING, and inflammasomes, which stimulate pro-inflammatory genes to propagate the innate antiviral response and lytic cell death[12–14]. These inflammatory pathways have widespread physiologic effects, ranging from sepsis to aging phenotypes[2,15], and can offer insight into the pathophysiology of inflammaging. We investigated cfmtDNA in urine as a potential marker of pathophysiologic mechanisms that underlie accelerated or accentuated aging in OAH. We hypothesized that OAH with greater cfmtDNA in urine would have a higher burden of frailty and falls, lower height-scaled muscle mass, and higher levels of conventional serum inflammatory markers.

Methods:

OAH (age 50 and older) were randomly selected from a large urban academic medical center outpatient HIV clinical practice and invited to complete a detailed questionnaire focusing on health status, quality of life, depression screening[16], psychosocial factors, and substance use[17]. Participants age 55 and over who completed the questionnaire were invited to participate in a substudy consisting of frailty testing, bioelectric impedance analysis (BIA) to assess body composition, and blood and urine sample collection. Laboratory data were extracted from the electronic medical record, and HIV-1 viral load was dichotomized as > or ≤200 copies/ml. The Veterans Aging Cohort Study (VACS) Index[18] was calculated. Participants were asked to fast for ≥ 8 hours prior to the study visit. Study participants provided written informed consent, and this study was approved by the Institutional Review Board.

Frailty Testing:

The frailty phenotype was assessed using criteria from Fried et al[19]. Participants completed a timed 4-meter walk, dominant-hand grip strength assessments via dynamometer, and self-report questions regarding exhaustion, activity level, and unintentional weight loss of 10 lbs or greater. Individual frailty score components were dichotomized as previously described as frail/pre-frail or non-frail[19,20].

Urine Measurements:

Spot urine samples were collected, immediately placed on ice, and centrifuged at 1,000g for 12 minutes at 4°C within 3 hours of collection. CfmtDNA levels were measured by SYBR Green dye-based qPCR assay using a PRISM 7500 sequence detection system (Applied Biosystems) with primer sequences specific for human NADH dehydrogenase 1 gene, as previously described[10,11]. Urine albumin and creatinine were measured from frozen urine samples, and the estimated glomerular filtration rate (eGFR) was calculated based on serum creatinine with the CKD-Epi equation[21].

Body Composition Assessment:

Body composition was assessed with tetra polar bioelectrical impedance analysis (BIA, Quantum IV, RJL Systems, Inc., Clinton Township, MI) by trained study personnel. Raw data were analyzed and reported using the RJL Systems software (BC4 version 4.2.0). Skeletal muscle index and fat mass index were calculated by dividing by height (in meters) squared[22].

Inflammatory Biomarker Methods:

Serum samples were stored at −80° C prior to assaying. Interleukin-6 (IL-6), interferon gamma (IFNγ), and tumor necrosis factor alpha (TNFα) were assayed using a multiplex kit (K15052G), and C-reactive protein (CRP) using a singleplex kit (K151STG) from MesoScale Discovery (Rockville, MD). TNF Receptor 1 (TNFR1) was assayed using a Quantikine ELISA kit (DRT100) from R&D Systems (Minneapolis, MN). Assays were run in duplicate with quality controls; 10% were repeated for confirmation. The intra-assay coefficient of variation (CV) for CRP, IL-6, IFN-γ, and TNF-α ranged from 2.4% to 6.4%, and inter-assay CVs ranged from 5.0%−10.2%. The detection limits of CRP, IL-6, IFN-γ, TNF-α, and TNFR1 are 0.01 ng/mL, 0.10 pg/mL, 0.40 pg/mL, 0.20 pg/mL, and 0.80 pg/ml respectively.

Statistical Methods:

Statistical analysis was conducted using Stata/IC version 15.1 (StataCorp LLC, College Station, Texas). Comparisons between groups were conducted by t-test or Wilcoxon rank-sum test. Relationships between urine cfmtDNA levels and clinical variables were explored using backwards stepwise regression models, with a threshold of $P<0.10$ set as the inclusion threshold. Ordinal logistic and linear regression models were used to assess for relationships between frailty state, frailty components, body composition measurements, and urine cfmtDNA level with adjustment for potential confounders determined a priori or via backwards stepwise regression. Reported p-values are 2-sided.

Results:

There were 164 participants in this study, and 151 provided urine samples. One male participant with a complicated urologic history provided a grossly bloody sample, and was excluded from analysis. Participant demographics are summarized in Table 1. The median VACS index score was 28 (Q1, Q3: 18, 39), which corresponds to a 10.8% risk of mortality in 5 years[18].

Urine cfmtDNA levels were natural log transformed due to rightward skew; geometric mean cfmtDNA level in urine was 2.4×10^8 copies/gram of urine creatinine (95%CI: 2.0×10^8-3.1×10^8). Median eGFR was 77.4 mL/min/1.73 m^2 (Q1, Q3: 62.7, 91.4). The median urine albumin:creatinine ratio (ACR) was 8.6 mg/g urine creatinine (Q1, Q3: 4.1, 27.7), consistent with 94 (64%) meeting criteria for normal to mildly increased albuminuria (ACR<30), 46 (31%) for moderately increased albuminuria (ACR 30–300), and 8 (5%) for severely increased albuminuria (ACR>300) [Table 1].

In a backwards stepwise linear regression model, urine cfmtDNA as the outcome was associated with greater urine albumin measured per gram of urine creatinine as a continuous variable (β=0.002, p=0.005), greater age (β=0.04, p=0.03), greater CD4 T-cell count (β=0.0007, p=0.034), whereas neither eGFR, diabetes, hypertension, smoking, HIV-1 viral load >200 copies/ml, years living with HIV, nor CD4 T-cell nadir remained in the model. Use of an integrase inhibitor, tenofovir disoproxil fumarate, tenofovir alafenamide, or protease inhibitor, angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) medications did not meet criteria to remain in the model.

Frailty:

Overall, 46 (31%) were nonfrail, 85 (56%) were prefrail, and 19 (13%) were frail. In a univariate ordinal logistic regression model, overall frailty state was related to age; for every 5-year increase in age the odds of greater frailty state increased by 1.5 (95% CI:1.2–2.1, p=0.003). Frailty state, however, was not related to urine cfmtDNA level (p=0.46) in a separate model. The mean urine cfmtDNA level, but not urine ACR, was higher in participants who met frailty criteria for unintentional weight loss (n=8) (p=0.03, p=0.71, respectively) by t-test compared to those with stable weight (n=142) [Figure 1]. The mean urine cfmtDNA level did not differ statistically by other dichotomized frailty components (i.e. slow walk, weak grip, exhaustion, and low physical activity; p=0.46, p=0.53, p=0.23, p=0.68, respectively).

In a backwards stepwise logistic regression model examining unintentional weight loss as the outcome that included urine cfmtDNA, age, moderate-severe albuminuria, drug use, smoking, COPD and depression, only urine cfmtDNA met criteria to remain in the model, and was statistically related to unintentional weight loss with an odds ratio of 2.1 [95% CI: 1.1– 4.0] for every one unit increase in log urine cfmtDNA (p=0.02). In a logistic regression model with unintentional weight loss as the outcome and urine cfmtDNA, age, and urine albumin per gram of creatinine as predictor variables, the relationship between urine cfmtDNA and unintentional weight loss remained statistically significant with an odds ratio of 2.3 (95%CI: 1.2–4.5, p=0.02).

Body Composition:

Median body mass index was in the overweight range (27 kg/m^2, Q1, Q3: 25, 31). BIA data were available for 125 participants. In separate multiple linear regression models adjusted for age, sex, and presence of moderate-severe albuminuria, higher urine cfmtDNA was inversely associated with skeletal muscle index (SMI) (β =−0.19, p<0.01) as well as fat mass index (FMI) (β =−0.08, p=0.02). In a backwards stepwise linear regression model

exploring the relationship between SMI as the outcome variable and log urine cfmtDNA, albuminuria, eGFR, age, sex, race, CD4 T-cell count, HIV viral load, current smoking, and use of integrase inhibitors, protease inhibitors, TDF or TAF, only log urine cfmtDNA (β= −0.27, p=0.02), female sex (β= −1.8, p<0.01), and current smoking (β= −0.93, p=0.02) met criteria to remain in the model.

Falls:

Falls within the past 6 months were reported by 22% of participants. The proportion of falls was higher with more advanced frailty state (p=0.04) by Chi-squared test. Six-month falls history was not significantly associated with urine cfmtDNA level, unintentional weight loss, SMI, or FMI.

Inflammatory Markers:

CRP, IL-6, IFN-γ, TNF-α, and TNFR1 did not statistically differ between the group with unintentional weight loss and those without by Wilcoxon Rank-Sum test (data not shown). Urine cfmtDNA level was correlated with serum TNFα level (Spearman rho=0.22, p<0.01), had a trend towards correlation with TNFαR1 (rho=0.14, p=0.09), but was not significantly correlated with IL-6, IFNγ nor CRP (data not shown).

Discussion:

In this study of OAH, a substantial portion of participants exhibited physical vulnerabilities, as two-thirds met criteria for a pre-frail or frail state and over one-fifth had experienced a fall in the prior 6 months. Higher levels of urine cfmtDNA were associated with unintentional weight loss, as well as lower SMI and FMI. Urine cfmtDNA was not related to falls, slow gait, weak grip, exhaustion, or low physical activity. Over one-third (36%) of participants exhibited moderate to severe albuminuria, a marker of renal dysfunction, which was related to urine cfmtDNA levels. Overall, these data support mitochondrial dysfunction as a potential contributing factor to less successful aging outcomes, including “shrinking” with unintentional weight loss and lower height-scaled body mass of fat and muscle. Higher CD4 T-cell count was associated with higher urine cfmtDNA levels, which was an unexpected and interesting finding that warrants further investigation.

The pathogenesis of geriatric syndromes in OAH is complex and remains incompletely understood, although it is likely due to multiple factors including chronic inflammation[23,24], mitochondrial dysfunction[7,25], and cellular death pathways associated with stimulating ongoing inflammation[26]. Recent data suggest the presence of cfmtDNA may stimulate pathways of immune activation that can lead to autoimmune disease processes[27], and mtDNA haplogroup H has been associated with frailty in OAH[28]. Despite suppression of HIV-1 viremia ongoing inflammation could stimulate cellular metabolic changes which may be reflected by higher cfmtDNA levels, and could be investigated in future studies with radiolabeled tracers[29]. Urine cfmtDNA has been associated with poor longitudinal renal function, and maintaining healthy mitochondria has been suggested as a key pathway in maintaining kidney health[30]. As such, understanding the physiologic mechanisms of cfmtDNA may provide insight to the complex process of aging with HIV.

Our study is unique in that it focused on urine cfmtDNA as a novel biomarker in OAH, and to our knowledge, is the first study to evaluate urine cfmtDNA in relation to frailty or body composition. A strength of our study is the diverse participant population, with just over half identifying as Black, and over one-third women. Our study also assessed a broad range of psychosocial, functional, and biometric variables, which allowed for consideration of potential confounding factors in analyses of frailty, weight loss and body composition.

Our study has several limitations. Firstly, participants were recruited from a single academic medical center clinic in New York City, and it is possible that those who chose to participate may have been more health-conscious. We attempted to reduce such selection bias by randomly selecting individuals from the entire patient pool to invite to the study. Secondly, our data are limited by a lack of HIV-negative controls, which would help to determine whether cfmtDNA shows similar links among those without HIV. Thirdly, we relied on BIA to assess body composition and could not evaluate muscle quality or fat distribution[31]. We collected cross-sectional data from participants at the time of study enrollment and do not have historical data of prior ARV regimens, nor prior smoking history. Finally, the absolute difference in urine cfmtDNA levels between the group with unintentional weight loss and the group without was small, albeit statistically significant, and lacks a longitudinal assessment to measure body composition change and frailty transitions. Future studies could include evaluation of matched HIV-negative controls and longitudinal timepoints to assess how body composition, frailty, and renal function evolve over time in OAH in relation to urine cfmtDNA levels.

In summary, our data suggest that urine cfmtDNA may provide insight into the complex process of accelerated/accentuated aging in OAH, as higher levels of urine cfmtDNA were more common in participants with less robust physical condition. Hence, urine cfmtDNA has potential to serve as a biomarker of pathophysiologic aging processes that predispose OAH to geriatric syndromes, and longitudinal studies are warranted.

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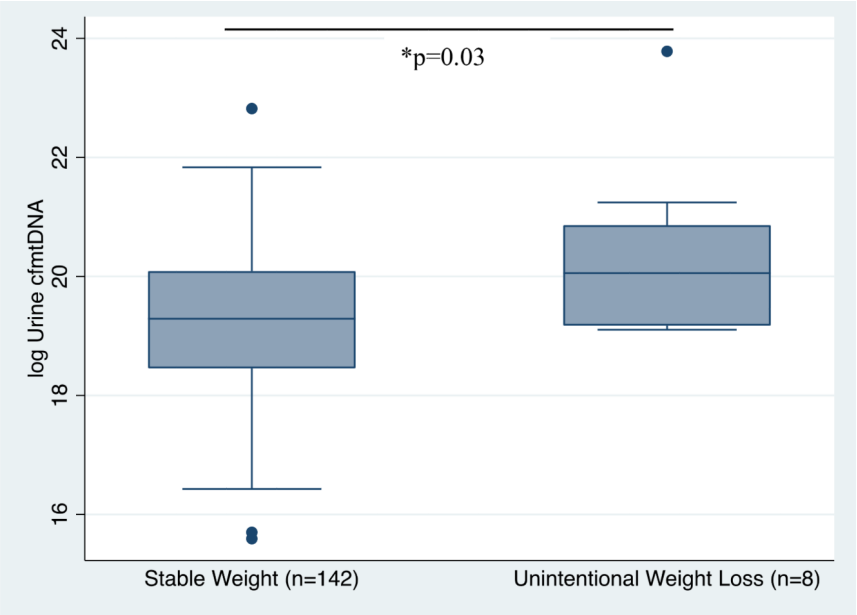

Figure 1: Higher Urine CfmtDNA Levels in OAH with Unintentional Weight Loss

The boxplots above indicate the minimum, Q1, median, Q3, and maximum log-transformed urine cfmtDNA measurements for the group without unintentional weight loss and the group with unintentional weight loss of 10 pounds or greater.

Table 1.

Participant Demographics

Measure	Result (N=150)
Age (years)	61 (SD:6)
Female sex	48 (32%)
Race: Black White Other Declined	 75 (50%) 45 (30%) 27 (18%) 3 (2%)
Ethnicity: Hispanic/Latinx Non-Hispanic/Latinx Declined	 37 (25%) 91 (61%) 21 (14%)
Years with HIV	25 (22, 29)
HIV-1 Viral load <200 copies/ml	93%
CD4 Count	594 cells/mm^3 (104, 1,397)
Veteran's Aging Cohort Study (VACS) Index	28 (18, 39)
HCV Antibody Positive HCV RNA Detectable	13 (9%) 0 (0%)
Body Mass Index (kg/m^2)	27 (25, 31)
Elevated depressive symptoms	73 (49%)
CKD-Epi GFR (mL/min/1.73m^2) GFR Stages: - G1 (GFR>90) - G2 (GFR 60–90) - G3 (GFR 30–59) - G4 (GFR 15–29) - G5 (GFR <15)	77 (63, 91) 41 (27%) 77 (51%) 30 (20%) 1 (1%) 1 (1%)
Drug Use: Crack, Cocaine, Crystal Methamphetamine and/or Heroin	11 (7%)
Tobacco Smoking (current)	26 (17%)
COPD	19 (13%)
Urine Data:	
Log Urine cfmtDNA[§]	2.4x10^8 copies/gram urine Cr
Albumin: Creatinine Ratio Albuminuria Stages: - Normal/Mildly Increased Albuminuria - Moderately Increased Albuminuria -Severely Increased Albuminuria	8.6mg/g (4.1, 27.7) 94 (64%) 46 (31%) 8 (5%)
Skeletal Muscle Index (kg/m^2)	8.9 (7.5, 9.9)
Fat Mass Index (kg/m^2)	7.6 (6.1, 10.3)
Frailty Testing Non-Frail Pre-Frail Frail Frailty Components: Unintentional Weight Loss Slow Walk Weak Grip Exhaustion Low Physical Activity	 46 (31%) 85 (56%) 19 (13%) 8 (5%) 28(19%) 29 (20%) 50 (34%) 43 (29%)

Continuous data are expressed as mean (SD) or median (quartile(Q)1, Q3) Abbreviations: CKD-Epi GFR = Glomerular Filtration Rate as calculated by the CKD-Epi equation (mL/min). COPD= Chronic Obstructive Pulmonary Disease. Depression was measured by the CES-D 10 (Center for Epidemiological Studies Depression) 10 question Screen; scores range 0–30, scores above 10 indicated elevated depressive symptoms[16]. HCV= Hepatitis C Virus.

[§]Urine cfmtDNA is expressed as the natural-log transformed geometric mean with 95%CI: 2.0×10^8–3.1×10^8