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COMPETITIVE PCR-ANALYSIS OF SUBCUTANEOUS ADIPOSE TISSUE MITOCHONDRIAL DNA FROM INDIVIDUALS WITH HIGHLY ACTIVE ANTIRETROVIRAL THERAPY -ASSOCIATED LIPODYSTROPHY

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BACKGROUND: Semi-quantitative analysis for mitochondrial DNA (mtDNA) has previously been reported from individuals with highly active antiretroviral therapy (HAART)-associated lipodystrophy. Because the aetiology of HIV-associated lipodystrophy is unknown and studies have shown that mitochondrial toxicity may be secondary to HAART, a more quantitative mtDNA assessment of affected tissue may help in determining the aetiology of fat redistribution in this setting.

OBJECTIVES: The objective was to use double competitive PCR to determine the number of copies of mtDNA per cell from biopsy specimens from HIV-infected individuals with lipodystrophy.

DESIGN: Double quantitative-competitive PCR (QC-PCR) was performed using a competitive plasmid containing a 180 bp fragment of the mitochondrial gene encoding for the subunit VI of FOF1 ATPase and a 259 bp fragment of the CD95L gene. The mtDNA and genomic fragments in the competitive plasmid are both 20 bp shorter than wild-type mtDNA and genomic DNA (gDNA), which can be distinguished from the unknown specimen DNA. Both mtDNA and gDNA double QC-PCR were performed on specimens previously obtained from individuals with lipodystrophy. The number of copies of mtDNA per cell was calculated.

RESULTS: Nineteen of 23 biopsies from six of eight individuals with lipodystrophy and three of 20 specimens from one of seven non-HIV-infected individual had complete analysis by QC-PCR. The mtDNA to gDNA ratio from the specimens obtained from the lipodystrophic individuals ranged from <1 to 20 copies of mtDNA per cell. This was compared to >200 copies of mtDNA per cell from the specimens obtained from the non-HIV-infected individual. Completion of the QC-PCR of all of the specimens will help determine the significance of quantitative PCR analysis of mtDNA in this setting.

CONCLUSIONS: Quantitative decrease in the copy number of mtDNA per cell from subcutaneous adipose tissue was detected from HIV-infected individuals with lipodystrophy, which is consistent with mitochondrial toxicity. Additional analysis and standardization of mtDNA analysis of tissue-specific pathology are needed to determine if mtDNA toxicity is important in HAART-associated lipodystrophy.

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