

Background

There is evidence for mitochondrial (mt) damage from HIV itself and following therapy with nucleoside analogues (NRTIs). NRTI-associated mt toxicity in children has varied clinical presentations but its pathogenesis has been scarcely investigated. We aimed to describe mt respiratory chain (MRC) function, mtDNA and mtRNA levels, and protein subunit content in the peripheral blood mononuclear cells (PBMC) of a series of vertically-HIV-infected pediatric patients and healthy controls.

Methods

A cross-sectional study of mtDNA and mtRNA content, and MRC function in PBMCs was performed in a series of HIV-vertically-infected pediatric patients, either on HAART or off therapy; anonymous blood samples of children who were referred to our laboratory for presurgical routine blood analysis were used as a control group. MtDNA and mtRNA amount were assessed by Real-Time PCR and expressed as the ratio of copies of mtND2 gene to r18s nuclear gene, while MRC complex IV (CIV), CII, G3PDH, CII-III and G3PDH-III function and mitochondrial mass (MM, estimated by citrate synthase (CS) enzymatic activity), were measured by spectrophotometry. Enzymatic activities were expressed in absolute values, as nmols oxidated substrate/minute/mg protein, and relative values by dividing absolute data per MM (e.g. CIV/CS). Subunits COXII and COXIV of complex IV, as well as MM, estimated by voltage-dependent anion channel (VDAC), were assessed by western blot analysis.

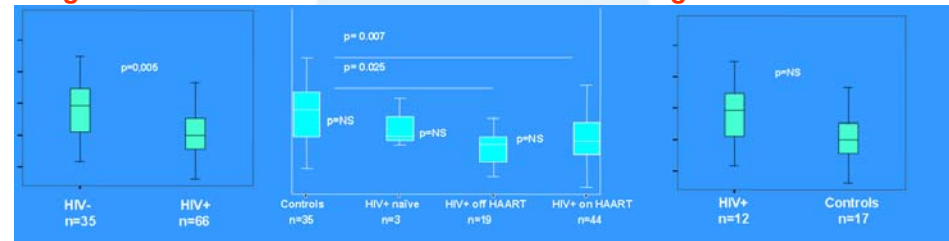
Results

Overall, 66 (34 girls, mean age 13.1 years) HIV-infected pediatric patients and 35 (20 girls, 12.5 years) healthy children were included; among the former, 3 patients were naïve, 44 patients were receiving HAART and 19 had interrupted therapy some time ago (range: 3 months to 7 years) for a variety of reasons. CII and G3PDH (nuclear-encoded) activities (**Figures 1a, 1b**) and MM (**Figure 1f**) showed no differences between groups, as expected. Significantly lower mtDNA values were observed in HIV-infected patients when compared to controls (4.4 vs 5.8; 25%, $p=0.005$; **Figure 2a**). A trend towards higher mtDNA content is observed among naïve patients, when compared to those who had interrupted HAART (**Figure 2b**). This mtDNA impairment did not lead to differences between groups in MRC function parameters (**Figures 1c to 1e**), and subunits COXII and COXIV values (**Figure 3**). No differences were observed in mtRNA levels (17 healthy controls, 0.058 ± 0.006 ; 12 HIV-infected patients, 0.073 ± 0.009 ; $p=0.199$; **Figure 4**).

Among HIV-infected patients, no differences in mtDNA levels or MRC function were observed when the use of HAART was taken into account. Similarly, no relationship was found between current CD4 cell counts, lactate levels or plasma HIV viral load and mtDNA (data not shown).

Figures 2a and 2b

Figure 4



Figures 1a to 1g

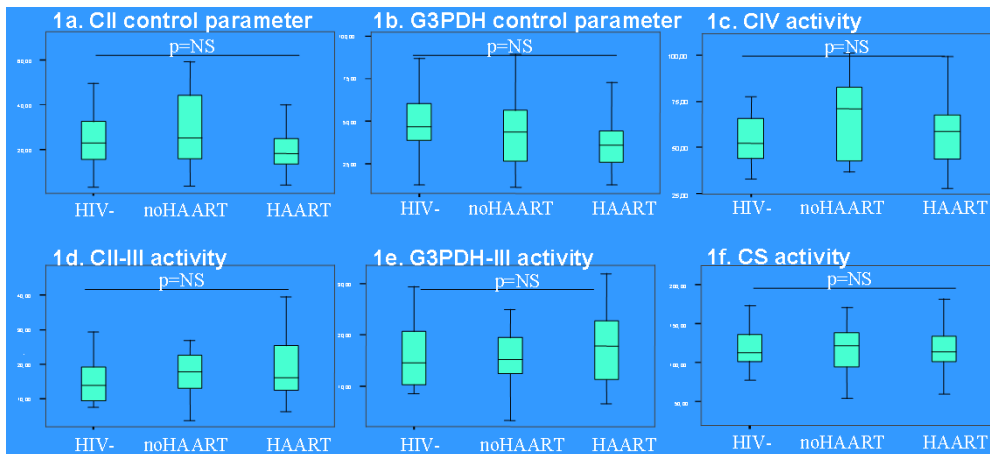
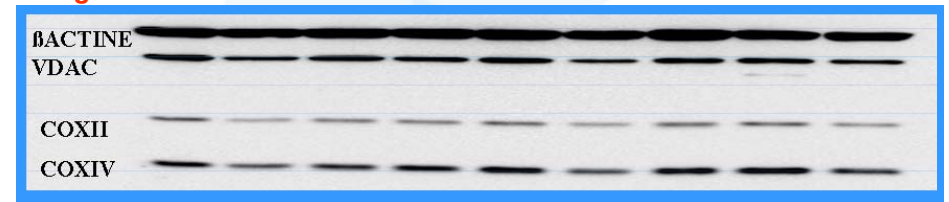


Figure 3



Conclusions

In this cross-sectional study, a reduction in PBMC mtDNA content in HIV-infected children was observed. This depletion did not lead to differences in mtRNA levels or MRC function impairment. Homeostatic compensatory mechanisms at transcription level could explain the lack of correlation between mtDNA depletion and MRC function.

The use of HAART at the time of the study did not seem to interfere with mt parameters, although a trend towards higher mtDNA levels was observed among naïve children. Previous exposition to antiretrovirals in those patients who interrupted therapy probably represents a bias against these findings.