MITOCHONDRIAL DYSFUNCTION IN WELL-SUPPRESSED PERINATALLY HIV-INFECTED CHILDREN ON ANTIRETROVIRAL THERAPY

858

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Abstract

Background: Mitochondrial dysfunction is reported in HIV-infected children receiving antiretroviral therapy (ART) as well as those naïve to ART. There are few reports of mitochondrial function in children with sustained viral suppression after initiation of ART in early life. The aim of this study is to evaluate mitochondrial markers in well-suppressed perinatally HIV-infected children initiating ART early in life and age-matched uninfected controls.

Methods: We selected a cross-sectional sample of 120 HIV-infected children with suppressed viral load (<400 copies/mL) and 60 age-matched uninfected controls (22 HIV-exposed uninfected (HEU), 37 HIV-unexposed uninfected (HUU) children) enrolled in a cohort study in Johannesburg, South Africa. Half the HIV-infected children initiated ART <6 months of age, and the other half between 6-24 months. Complex IV (CIV) and citrate synthase (CS) activity were measured by spectrophotometry, and mtDNA content by real-time PCR. CIV/CS ratio was categorized as normal (>0.22 [75th quantile]) or impaired (≤0.22).

Results: Mitochondrial markers (CIV, CS, CIV/CS ratio, lower and higher quantity mtDNA content) are significantly impaired in HIV-infected children compared with uninfected, and most markers remain significant after adjusting for age. Longer duration of ART may partially improve mitochondrial markers in HIV-infected. The increases of mitochondrial markers in children with longer duration of ART still do not reach the normal levels in uninfected controls although some are very close. Children who started ART early do not have more severe mitochondrial deficiency compared with those who started ART later. Mitochondrial markers are impaired in children with lower HAZ (≤-1), and this deficiency is similar in children with stunting (HAZ≤-2). Mitochondrial markers display no difference between HEU and HUU children.

Conclusions: Despite early treatment and prolonged viral suppression, impaired mitochondrial markers are detectable in HIV-infected children particularly among those whose HAZ falls short of expectations. Further study is required to determine if earlier ART initiation and continued treatment will lead to full recovery of mitochondrial function in HIV-infected children.

Introduction

- ART in perinatally HIV-infected children is effective for preventing or reversing immune dysfunction and extending survival.
- Numerous immunologic and metabolic abnormalities, including mitochondrial dysfunction are known to persist despite suppressive ART.
- No study has investigated mitochondrial markers in association with age at ART started and duration of ART (regardless of treatment regimen).
- It is unknown whether mitochondrial markers show full or partial recovery in perinatally HIV-infected children on long-term ART with sustained viral suppression.
- We evaluate mitochondrial markers in perinatally HIV-infected children who initiated ART early in life and achieved sustained long-term viral suppression in CHANGES, a study in Johannesburg, South Africa.

Methods

- CHANGES is an ongoing longitudinal cohort study including 553 perinatally HIV-infected, ART-treated children and 300 HIV-uninfected controls.
- A cross-sectional sample of 120 HIV-infected children with suppressed viremia (VL<400 copies/mL) at the time of enrollment and 60 age-matched uninfected controls were analyzed in the current study.
- Infected children were randomly-selected from those who were suppressed on a ritonavir-boosted lopinavir regimen and half had initiated ART <6 months of age, and the other half between 6-24 months.
- Mitochondrial markers of Complex IV (CIV), citrate synthase (CS) and mtDNA content were measured in blood samples collected at the first 6-month visit after enrollment into the CHANGES study for 120 HIV-infected children and at enrollment for 60 uninfected children.
- Univariate Analysis of Shapiro-Wilk test was performed to describe markers' distribution by HIV status. The means or medians were compared by two-sample test or Wilcoxon Rank-Sum tests.
- Generalized linear model (GLM) or quantile regression to estimate the difference (β) of mean/median adjusted for confounders. Logistic regression model to estimate the crude odds ratio (OR) and 95% confidence interval (CI) of categorical variables.

Results

Baseline Characteristics

Table 1. Characteristics of HIV-infected and uninfected children

Characteristics	HIV(-)	HIV(+)	P-value
No. subjects (N)	60	120	
Male, N (%)	34 (56.7)	56 (46.7)	0.207
Age (yrs), mean (SD)	6.4 (1.5)	7.0 (1.4)	0.022
WAZ, mean (SD)	-0.2 (0.9)	-0.7 (0.9)	0.0003
Underweight (WAZ≤-2), N (%)	0 (0.0)	11 (9.2)	0.010*
HAZ, mean (SD)	-0.7 (1.0)	-1.0 (0.9)	0.018
Stunting (HAZ≤-2), N (%)	5 (8.3)	18 (15.0)	0.213
BMI (Kg/m^2), mean (SD)	16.15 (1.87)	15.4 (1.7)	0.009
BAZ, mean (SD)	0.34 (1.01)	-0.1 (1.1)	0.005
Overweight (BAZ>1), N (%)	12 (20.00)	19 (15.8)	0.486

*** Fisher exact test due to zero in one cell**

Table 2. Characteristics of HIV-infected children by age at ART started

Characteristics	HIV(+) ART started <6 mos	HIV(+) ART started 6-24 mos	P-value
No. subjects (N)	60	60	
Male, N (%)	29 (48.3)	27 (45.0)	0.715
Age (yrs), mean (SD)	6.6 (1.2)	7.4 (1.5)	0.001
WAZ, mean (SD)	-0.8 (0.8)	-0.6 (1.1)	0.171
HAZ, mean (SD)	-1.1 (1.0)	-0.9 (0.9)	0.423
BAZ, mean (SD)	-0.2 (0.9)	-0.02 (1.1)	0.278
Age started ART (mos), mean (SD)	3.2 (1.3)	12.3 (5.5)	<0.0001
Duration on ART (yrs), mean (SD)	6.2 (1.2)	6.3 (1.6)	0.835
Initial regimen, N (%)			
LPV/r plus 2 other drugs	56 (93.3)	54 (90.0)	
RTV plus 2 other drugs	4 (6.7)	6 (10.0)	0.512
Pre-ART VL, N (%)			
<750,000 copies/mL	20 (38.5)	21 (45.7)	
≥750,000 copies/mL	32 (61.5)	25 (54.3)	0.472
Pre-ART CD4 counts, N (%)			
<1,000 cells/mL	15 (25.9)	29 (54.7)	
≥1,000 cells/mL	43 (74.1)	24 (45.3)	0.002
Pre-ART CD4 per, N (%)			
<25%	25 (42.4)	33 (61.1)	
≥25%	34 (57.6)	21 (38.9)	0.048

Impaired mitochondrial markers remained detectable in well-suppressed HIV-infected children

- MtDNA was bi-modally distributed in HIV infected and uninfected children and stratified into lower and higher quantity strata.
- Mitochondrial markers are significantly impaired in HIV-infected children compared with uninfected, and most markers remain significant after adjustment for age (Table 3).

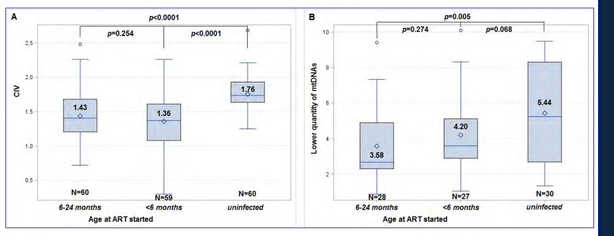
Table 3. Comparison of mitochondrial markers by HIV status

Mitochondrial markers		HIV(-)	HIV(+)	P-value
No		60	120	
CIV (ODs)	Mean (SD)	1.76 (0.27)	1.40 (0.37)	
	Unadjusted β		-0.36	<0.0001
	Age adjusted β		-0.40	<0.0001
CS (ODs)	Median (IQR)	9.29 (5.98, 11.99)	8.45 (7.22, 9.67)	
	Unadjusted β		-0.84	0.135
	Age adjusted β		-0.81	0.153
CIV/CS ratio 75% quantile No (%)				
	> 0.22	19 (31.7)	21 (17.7)	
	≤ 0.22	41 (68.3)	98 (82.3)	
	Unadjusted OR (95%CI)	1.00 (ref)	2.16 (1.05-4.44)	0.036
	Age adjusted OR (95%CI)	1.00 (ref)	3.03 (1.38-6.66)	0.006
Lower quantity mtDNA content (<12 copies/nDNA)		N = 30	N = 56	
	Mean (SD)	5.44 (2.76)	3.88 (2.11)	
	Unadjusted β		-1.55	0.005
	Age adjusted β		-1.43	0.014
Higher quantity mtDNA content (>50 copies/nDNA)		N = 30	N = 64	
	Mean (SD)	1116 (754)	639 (800)	
	Unadjusted β		-477	0.007
	Age adjusted β		-484	0.005

Mitochondrial markers by age at ART started

- No significant differences in any mitochondrial markers between children who started ART early compared with those who started ART later (<6 vs. 6-24 months).
- Compared to uninfected, infected children have lower CIV regardless of age at ART started (Figure 1A).
- There is a gradient favoring earlier ART initiation in those within lower mtDNA quantity regardless of treatment regimen (Figure 1B).

Figure 1. Comparison of mitochondrial markers by age at ART started



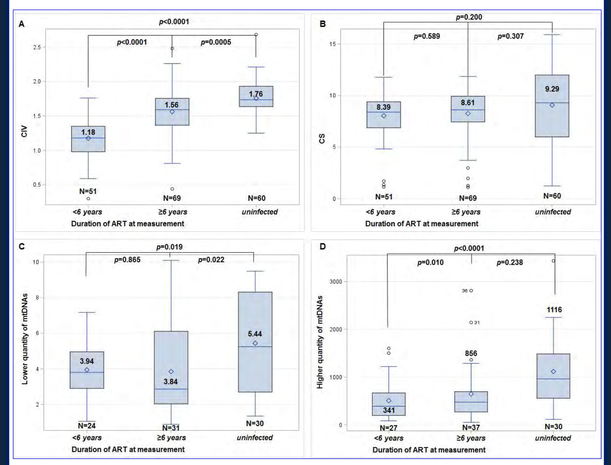
Mitochondrial markers by duration of ART

- Longer duration of ART may partially improve mitochondrial markers (CIV, CS and higher quantity mtDNA content) in HIV-infected children (Figure 2).
- The increases of mitochondrial markers in children with longer duration of ART do not reach the levels in uninfected controls but some are very close (Figure 2B, 2D).

Mitochondrial markers by HAZ and pre-ART VL

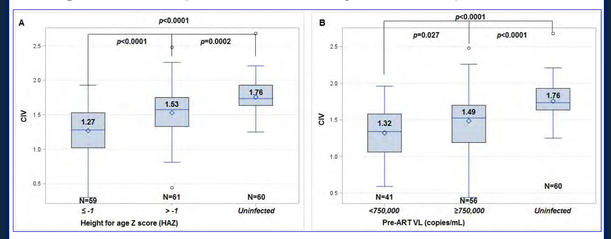
- CIV activity is impaired in HIV-infected children with lower HAZ (≤ -1) compared with normal HAZ, and both are lower than that in uninfected children (Figure 3A).

Figure 2. Comparison of mitochondrial markers by duration of ART



- Unexpectedly, higher pre-ART VL is associated with higher CIV activity compared with low VL (Figure 3B).

Figure 3. Comparison of CIV by HAZ and pre-ART VL



Mitochondrial markers in HIV-uninfected children

- No difference in mitochondrial markers are seen between HEU and HUU children.
- Among HEU infants, mitochondrial markers have no difference by mothers' status of PMTCT (prevention of mother-to-child transmission) interventions during pregnancy.

Conclusions

- Mitochondrial markers are significantly impaired in HIV-infected children even in those with prolonged viral suppression.
- Early age at ART started preserves mitochondrial function when compared to later age at ART started.
- Long-term ART in HIV-infected children may result in partial recovery of mitochondrial function.
- The depletion of mitochondrial markers appears to be more severe in HIV-infected children with stunting or lower HAZ.

Acknowledgements

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