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Preclinical Atherosclerosis in Eastern Africa: Results from a Pediatric Ethiopian Cohort

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Abstract

Background: HIV-infected pediatric patients on antiretroviral therapy (ART) demonstrate an elevated incidence of dyslipidemia, lipodystrophy, and cardiovascular disease risk (CVD). Most studies, however, focus on cohorts from developed countries, with fewer data available for these co-morbidities in sub-Saharan Africa, where the prevalence of HIV-infected youths is much higher.

Methods: Six- to 18-year-old age- and sex-matched HIV-infected subjects who were ART-naïve (n=45), efavirenz (EFV)-treated (n=90), nevirapine (NVP)-treated (n=76), or ritonavir-boosted lopinavir (LPV/r)-treated (n=19) were recruited from Tikur Anbessa (Black Lion) Specialized Referral Hospital in Addis Ababa, Ethiopia. Pulse wave velocity (PWV) was assessed via applanation tonometry, as a measure of arterial stiffness, and carotid intima-media thickness (cIMT) and brachial artery flow-mediated dilation (FMD) were assessed via non-invasive ultrasound as a measure of subclinical atherosclerosis and endothelial function, respectively. Body mass index, waist-to-hip circumference ratio, and skin-fold thickness were used to characterize body composition. CD4+ cell count, fasting glucose, total-, HDL-, and LDL-cholesterol, triglycerides, and complete blood counts were measured. Results are presented as medians (interquartile range). Kruskal-Wallis non-parametric one-way ANOVA was performed to determine statistical significance across groups. Multivariable linear regression models were constructed to examine the association of study parameters with PWV for all ART-treated subjects.

Results: PWV was elevated in LPV/r-treated subjects compared to EFV- and NVP-treated and ART-naïve subjects [5.2 (4.6-5.9) vs. 4.7 (4.3-5.3), 4.6 (4.2-5.2), and 4.6 (4.1-5.0) m/s, respectively, p=0.016]. No differences in cIMT or FMD were observed across groups. LPV/r-treated subjects also exhibited elevated total- and LDL-cholesterol and total:HDL-cholesterol ratio compared to the other groups. EFV- and NVP-treated subjects exhibited elevated total-, LDL- and HDL-cholesterol, compared to ART-naïve subjects; however, no differences were observed in total:HDL-cholesterol ratio between EFV-treated, NVP-treated, and ART-naïve subjects.

Conclusions: LPV/r-treatment was associated with increased arterial stiffness and dyslipidemia in pediatric HIV-infected subjects in Ethiopia. Given the increased use of LPV/r in developing countries, this raises concern regarding the long-term CVD risk in this vulnerable population.

Background

- The massive roll-out of antiretroviral therapy in Ethiopia has resulted in a dramatic increase in the number of HIV+ youth on treatment.
- This population is expected to live well into adulthood.
- The combination of chronic HIV infection and long-term ART use places these youth at risk for increased co-morbidities.
- In developed countries, HIV+ youth have demonstrated an increased risk of CVD, but few studies have examined this risk among HIV+ youth in sub-Saharan Africa.

Objectives

- To determine CVD risk in HIV+ youth on ART in Ethiopia by measuring (i) subclinical atherosclerosis (cIMT), (ii) endothelial function (FMD) and (iii) arterial stiffness (PWV).
- To evaluate differences in CVD risk among HIV+ youth on various first-line and second-line ART regimens
- To investigate traditional and HIV-related risk factors that may be associated with increased CVD risk in this African population

Methods

➢ **SUBJECT SELECTION:**

- HIV+ youth aged 6-18 years old who were ART-naïve or on EFV-, NVP-, or LPV/r-containing regimens for ≥2 months were recruited from Tikur Anbessa Specialized Referral Hospital in Addis Ababa, Ethiopia.
- Subjects were excluded for active AIDS-defining illnesses or diabetes mellitus.

➢ **STUDY ASSESSMENTS:**

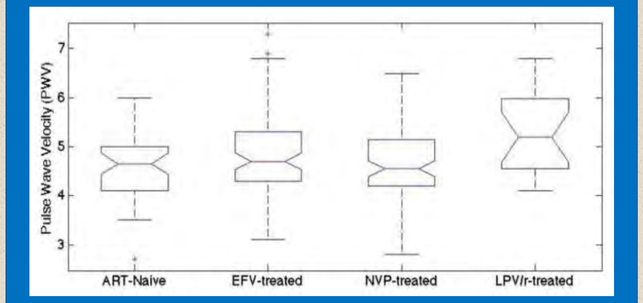
- Clinical: Demographics and clinical information were collected by an extensive review of each subject's medical records. Blood pressure (BP) was measured with a calibrated, automated Sphygmomanometer after a 10-minute rest.
- cIMT: Measurements were collected using a Sonocyte Micromax portable ultrasound device with vascular software for 2-D imaging. Right and left common carotid arteries (CCA) were examined. cIMT was reported in systole and diastole as the average of the near and far wall measurements from three separate clips from each side.
- FMD: Following a 10-minute rest, a sphygmomanometer was placed on the widest part of the proximal right forearm. Prior to inflation, three 6-second clips of the brachial artery image and EKG tracings were stored for baseline. The sphygmomanometer was inflated ≥50 mmHg above the subjects' systolic BP. Five minutes after occlusion, the cuff was deflated and a 6-second clip of the brachial artery image and EKG tracing was stored at 30, 45, 60, 75, and 90 seconds.
- PWV: Measurements were taken with SphygmoCor CPV Clinical System following manufacturer protocol after a 10 minute rest. Measurements meeting the manufacturer quality control criteria were collected in triplicate and averaged.
- Laboratory measurements: Complete blood count with differential (CBC/d), CD4+ cell count, glucose, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol were measured.

➢ **STATISTICAL ANALYSIS:**

- A one-way analysis of variance (ANOVA) and Kruskal-Wallis non-parametric one-way ANOVA were performed on continuous variables that did and did not show normality, respectively, to determine differences across groups. Categorical variables were analyzed with X^2.
- For continuous and categorical variables with significance across groups (P<0.05), a pairwise Wilcoxon rank sum test and Marascuillo procedure were used, respectively, to detect differences between individual groups
- Stepwise-fit multivariable linear regression models were used to investigate associations with PWV. Variables with Spearman Rank Correlation Coefficients of P<0.50 were chosen for inclusion in the initial models, but only those with P<0.05 were included in the final model.

Results

PWV Values Described by ART Regimen



*P<0.05 for LPV/r vs. ART-naïve and EFV-treated; *P<0.005 for LPV/r vs. NVP-treated

Multivariable Regression Model for Associations with PWV

	β	SE(β)	P
Current LPV/r Use	0.5720	0.1614	0.0005
Age (Years)	0.0822	0.0205	0.0001
BMI (kg/m^2)	0.0609	0.0264	0.0225
Heart Rate (BPM)	0.0159	0.0038	0.0001
FMD (%)	-2.0337	0.9418	0.0322
	Adjusted R^2= 0.2691		

Variables evaluated but excluded from the final model include current EFV or NVP use, current CD4+ count, years on ART, systolic BP, cIMT, CBC/d, lipoprotein profile, and skinfold thickness.

Subject Characteristics by Study Group

Median (interquartile range) unless otherwise specified	ART-Naïve (n = 45)		EFV-Treated (n = 90)		NVP-Treated (n = 80)		LPV/r-Treated (n = 19)	
Demographics								
Age (Years)	11	(9 - 14)	13	(10 - 15)	11	(9 - 14)	13	(11 - 15)
Male Sex [# (%)]	20	(44%)	41	(46%)	40	(50%)	12	(63%)
Body Composition								
Body Mass Index (kg/m^2)	16	(15 - 18)	16	(14 - 18)	16	(15 - 18)	16	(15 - 18)
HIV / ART History								
Current CD4+ count	456	(378 - 608)	575	(412 - 744) A	639	(460 - 902) A,B	554	(336 - 776)
Total Years on ART	--		4.5	(3.0 - 5.7)	3.4	(1.8 - 5.5) B	6.1	(5.5 - 6.3) B,C
Years on Current ART Regimen	--		4.2	(2.3 - 5.4)	3.1	(1.7 - 5.5)	3.1	(2.0 - 3.6) B
Current NRTI backbone								
d4T+3TC [# (%)]	--		33	(37%)	31	(39%)	2	(11%)
AZT+3TC [# (%)]	--		51	(57%)	48	(60%)	1	(5%)
ABC+DDI [# (%)]			0	(0%)	0	(0%)	15	(79%)
Other [# (%)]	--		6	(7%)	1	(1%)	1	(5%)
Cardiovascular Metrics								
Heart Rate (BPM)	81	(71 - 89)	81	(73 - 87)	82	(74 - 91)	69	(65 - 87)
Systolic BP (mmHg)	103	(96 - 108)	100	(93 - 108)	100	(94 - 107)	105	(99 - 111)
Diastolic BP (mmHg)	62	(58 - 71)	61	(56 - 67)	63	(59 - 68)	63	(57 - 73)
FMD (%)	8.6	(5.4 - 13.6)	6.4	(4.1 - 10.0)	7.9	(4.1 - 12.1)	9.6	(5.0 - 12.2)
cIMT (microns)	45	(42 - 47)	45	(43 - 48)	46	(42 - 50)	47	(45 - 51)
Laboratory Analysis								
Glucose (mg/dL)	83	(76 - 87)	86	(79 - 92) A	82	(77 - 87) B	81	(74 - 86)
TC (mg/dL)	128	(110 - 157)	161	(134 - 186) A	157	(135 - 183) A	199	(173 - 244) A,B,C
TG (mg/dL)	90	(71 - 135)	104	(76 - 143)	99	(80 - 127)	146	(120 - 249) A,B,C
HDL Cholesterol (mg/dL)	37	(33 - 42)	45	(40 - 52) A	48	(43 - 55) A	40	(33 - 50) C
LDL Cholesterol (mg/dL)	70	(59 - 94)	97	(70 - 111) A	91	(75 - 115) A	144	(98 - 174) A,B,C
TC:HDL Ratio	3.8	(3.1 - 4.1)	3.5	(3.1 - 4.3)	3.2	(2.7 - 4.1)	4.8	(3.7 - 6.1) A,B,C

*P<0.05 vs. ART-naïve (A), EFV (B), and NVP (C), respectively

Summary of Results

- PWV was significantly higher in HIV+ youth on LPV/r compared to those on NVP or EFV and those not on ART.
- There were no statistically significant differences in cIMT or FMD across groups regardless of ART status or regimen.
- TC and LDL cholesterol were elevated in LPV/r-treated subjects compared to those on EFV and NVP, but all three groups had higher levels compared to ART-naïve.
- TC:HDL ratio and TG were elevated in LPV/r-treated subjects compared to all other groups.
- Multivariable regression showed that PWV was associated with current LPV/r use, age, BMI, heart rate, and FMD.

Limitations

- The sample size of the LPV/r-treated group was much smaller than the other groups.
- Cross-sectional design does not allow for a causal relationship between specific drugs and markers studied.
- Plasma HIV-1 RNA levels were not obtained.
- cIMT images were taken from a single profile view rather than the average of several orthogonal views.
- Subjects on LPV/r had a longer cumulative ART duration compared to those on EFV or NVP which may have contributed to increased PWV; however, ART duration was not significant in the regression analysis.

Conclusions

- Current LPV/r use may contribute to CVD risk in HIV+ youth in Ethiopia.
- The dyslipidemia associated with LPV/r use did not explain the increased arterial stiffness.
- PWV appears to hold promise as a reliable measure of CVD risk in HIV+ youth
- Given the increased use of LPV/r in developing countries as a 2nd-line regimen, longitudinal studies are needed to further evaluate its CVD risk.

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