HDL Cholesterol Efflux Capacity in HIV is Inversely Related to Classical Monocyte Number

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Conference on Retroviruses & Opportunistic Infections 2017
February 13-16, 2017
Abstract Number: 612

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Abstract

Background: HIV represents a chronic inflammatory state with increased incidence of cardiovascular disease (CVD), and circulating monocytes are thought to play a role in CVD. High-density lipoprotein (HDL) cholesterol efflux capacity provides evaluation of the dynamic function of HDL and several studies have shown that HDL cholesterol efflux capacity correlates strongly with CVD outcome events. HDL cholesterol efflux may be inhibited by HIV. This may contribute to increased CVD seen in chronic HIV. Our current study evaluated the relationship between HDL cholesterol efflux capacity and monocyte subtypes.

Methods: HDL cholesterol efflux capacity and monocyte subtypes were measured as part of the Hawaii Aging with HIV Cardiovascular Study. Participants were on stable antiretroviral therapy > 3 months. Monocyte subtypes were determined by differential expression of CD14 and CD16: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+/low}$ $CD16^{++}$) monocytes respectively. Simple and multivariable analyses were performed to evaluate the relationship between HDL cholesterol efflux capacity and monocyte subtypes.

Results: Our study included total of 124 patients: median age 50 years; 86% male; median CD4 count 490 cells/mm3; and 86% undetectable HIV viral load. The HDL cholesterol efflux capacity was negatively associated with classical monocyte numbers (standardized beta = -0.21, P=0.02) even after adjusting for traditional CVD factors, demographics, HIV viral load and body mass index. There were no significant associations between HDL cholesterol efflux capacity and the other monocyte subtypes.

Conclusion: HDL cholesterol efflux capacity is inversely associated with classical monocyte numbers and may suggest an involvement in inflammation and cardiovascular disease in HIV patients.

Background

- Cardiovascular disease (CVD) remains a significant cause of morbidity and mortality in HIV patients despite the advances in early diagnosis and treatment(1)
- Clinical and epidemiological studies have shown a correlation between HDL cholesterol and cardiovascular outcomes(2)
- One of the most important functions of HDL is to promote reverse cholesterol transport from the periphery to the liver and this critical step in reverse cholesterol transport is termed HDL-cholesterol efflux capacity and newer data suggests it is robust indicator for cardiovascular disease compared to HDL itself(3,4)
- Monocytes play a major role in the pathogenesis of atherosclerosis and are subdivided based on surface expression of CD14 and CD16: (classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and nonclassical ($CD14^{low}CD16^{++}$)(5,6)
- We conducted a retrospective study on a well characterized HIV cohort on CVD to determine the correlation between monocyte subset numbers and HDL cholesterol efflux capacity.

Statistical Methods

Pearson and Spearman Correlations were used to assess the relationship between HDL cholesterol efflux capacity and various lipid panel variables.

Simple and multivariable linear regression analyses were used to determine the association between cholesterol efflux capacity and monocyte total and subset counts.

The multivariable linear regression analyses were adjusted using risk factors for CVD including age, HIV viral load, traditional risk factors (DM, HTN, hyperlipidemia, current tobacco use, family history of coronary artery disease) and body mass index (BMI).

Methods

- Data from Hawaii Aging with HIV - Cardiovascular Disease (HAHC-CVD) Study, a study examining the role of oxidative stress and inflammation in HIV cardiovascular risk
- HAHC-CVD enrolled HIV-infected adults ≥ 40 years old on stable ART > 3 months

HDL Cholesterol Efflux Capacity Measurements

- HDL cholesterol efflux capacity was measured at a lipoprotein research laboratory at the National Institute of Health
- Briefly, the J774 cells were plated and radiolabeled with 2 µCi of ^{3}H-cholesterol/mL. ATP-binding cassette transporter A1 (ABCA1) was up-regulated by means of a 16-hour incubation with 0.3 mmol/L 8-(4-chlorophenylthio)-cAMP. We added 2.8% apoB-depleted plasma to the efflux medium for 4 hours
- To quantify the efflux of radioactive cholesterol from the cells, we used liquid scintillation counting. Efflux was calculated by using the following formula: (µCi of ^{3}H-cholesterol in media containing 2.8% apoB-depleted subject plasma-µCi of ^{3}H-cholesterol in plasma-free media / µCi of ^{3}H-cholesterol in media containing 2.8% apoB-depleted pooled control plasma-µCi of ^{3}H-cholesterol in pooled control plasma-free media)
- The pooled plasma was obtained from five healthy volunteers. All assays were performed in duplicate

Monocyte Isolation and Subset Measurement

- Monocyte isolation and subset measurements were done as previously reported by our group(7)
- In brief, the peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and cryopreserved. The cryopreserved PBMCs were thawed in serum-free media containing 10 µg/ml of DNAse and were stained with Live/Dead fixable yellow dead cell stain (YARD), followed by CD3, CD14, CD16, CD56, CD19, CD20, HLA-DR antibodies
- Information was acquired on a custom 4-laser BD LSRFortessa Cell Analyzer and analyses was performed in FlowJo analytical software as previously reported
- Initially the percentages of classical, intermediate, and non-classical monocyte subsets were calculated based on CD14 and CD16 staining and absolute numbers of each subset were calculated from total white blood count and monocyte percent obtained from participants' complete blood count

Results

Baseline Characteristics

n	124
Age, yr	50 (45, 56)
Male, n (%)	102 (86.4%)
Caucasian, n (%)	70 (59.3%)
Hypertension, n (%)	36 (30.5%)
Currently Smoking, n (%)	25 (21.2%)
Waist Size, cm	92.4 (87.5, 99.0)
Weight, kg	77.8 (67.4, 87.1)
Height, cm	173.2 (166.9, 179.0)
Waist-Hip Ratio	0.94 (0.91, 0.97)
BMI, kg/m^2	25.75 (23.70, 27.85)
Diabetes Mellitus, n (%)	6 (5.1%)
CRP, µg/mL	0.89 (0.39, 2.66)
History of High Cholesterol, n (%)	60 (50.85%)
Years Living With HIV, yr*	15 (8.25, 20.75)
Plasma HIV-1 RNA (n, % undetectable)	101 (85.59%)
CD4+ T cell count, cells/mm^3	490 (349.75, 639.50)
Anti-Retroviral Therapy	
Total	124 (100.0%)
Nucleoside Reverse Transcriptase Inhibitors	111 (94.1%)
Non-Nucleoside Reverse Transcriptase Inhibitors	61 (51.7%)
Protease Inhibitors	55 (46.6%)
Integrase Inhibitors	15 (12.7%)
Lipid-Lowering Therapy	
Total	42 (35.6%)
Statins	37 (31.4%)
Fibrates	7 (5.9%)
Niacin	4 (3.4%)
Cholesterol Absorption Inhibitors	1 (0.8%)
Omega 3 acid Ethyl Ester	1 (0.8%)
Framingham Risk Score	0.06 (0.02, 0.11)
Metabolic Assessment	-
Fasting Glucose, mg/dL	86 (81, 92)
Fasting Insulin, mg/dL	6.2 (4.08, 10.03)
Matsuda Index^	6.095 (3.794, 9.896)
Lipid Panel	-
Total Cholesterol, mg/dL	178.0 (157.3, 207.0)
LDL-C, mg/dL	108.0 (89.0, 132.0)
HDL-C, mg/dL	44.0 (33.0, 54.8)
Non-HDL-C, mg/dL	134.0 (108.0, 159.8)
Triglycerides, mg/dL	122.5 (92.0, 178.3)
Cholesterol Ratio (Total/HDL-C)	4.1962 (3.4298, 5.0935)
TG/HDL-C Ratio	2.9405 (1.8786, 4.9899)
LDL-C/HDL-C Ratio	2.554 (1.969, 3.228)
Apo AI, mg/dL	74.3 (56.3, 95.9)
Apo AII, mg/dL	21.7 (17.6, 29.6)
Apo B, mg/dL	3.7 (2.5, 6.0)
Apo CII, mg/dL	5.3 (3.5, 7.4)
Apo CIII, mg/dL	12.2 (8.3, 18.9)
Apo E, mg/dL	4.0 (3.1, 5.8)
HDL Function	-
HDL Cholesterol Efflux Capacity	0.975 (0.896, 1.090)
Monocyte Count, cells/L	-
Total Monocyte Count	3.9 x 10^8 (3.2 x 10^8, 5.1 x 10^8)
Classical Monocyte Count	3.1 x 10^8 (2.3 x 10^8, 4.1 x 10^8)
Intermediate Monocyte Count	6.0 x 10^6 (2.5 x 10^6, 1.6 x 10^7)
Non-Classical Monocyte Count	2.3 x 10^7 (1.3 x 10^7, 3.5 x 10^7)

Continuous variables are expressed as median (Q_1, Q_3); Categorical variables are expressed as N (%).
^ N = 101 patients
Abbreviations: CRP: C-reactive protein, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, IDL: Intermediate-density lipoprotein, TG: Triglycerides, Apo: Apolipoprotein

Unadjusted Linear-Regressions Predicting Traditional Lipids and HDL Cholesterol Efflux from Monocyte Subsets

Outcome	Classical	Intermediate	Non-classical
HDL Efflux	**-0.20 (0.03)**	-0.04 (0.09)	-0.01 (0.87)
Total Cholesterol	-4.65 (0.81)	11.8 (0.06)	10.14 (0.38)
Triglycerides	58.2 (0.19)	22.2 (0.12)	25.13 (0.33)
LDL-C	3.97 (0.82)	7.88 (0.15)	8.27 (0.41)
HDL-C	-13.5 (0.06)	3.22 (0.16)	0.31 (0.94)

Multivariable linear regression predicting HDL cholesterol efflux capacity with regards to the various monocyte subsets adjusting for age, HIV viral load, traditional risk factors, and BMI in a step wise fashion

	Classical	Intermediate	Non-classical
	-0.20 (0.03)*	-0.04 (0.09)	-0.01 (0.87)
+ Age	-0.21 (0.02)*	-0.04 (0.09)	-0.01 (0.85)
+ Age and HIV Viral load	-0.21 (0.02)*	-0.04 (0.09)	-0.01 (0.86)
+ Age and BMI	-0.19 (0.04)*	-0.04 (0.13)	-0.004 (0.93)
+ Age, Traditional Risk Factors	-0.22 (0.02)*	-0.04 (0.14)	-0.01 (0.81)
+ Age, HIV Viral load, Traditional Risk Factors, BMI	-0.21 (0.02)*	-0.03 (0.20)	-0.01 (0.90)

Traditional risk factors: Diabetes, hypertension, hyperlipidemia, current tobacco (smoking) use, family history of CAD
Standardized beta estimate (p-value) presented.
* p<.05

Conclusions

HDL cholesterol efflux capacity is inversely associated with classical monocyte numbers.

This may suggest that classical monocytes might be involved in pro-inflammation and CVD in HIV patients on stable ART.

Our findings might suggest that decreasing classical monocyte number numbers and/or their functionality may restore HDL functionality and potentially impact CVD events.

Acknowledgements

We thank our study participants and community physicians for their roles in this study.
This work was supported by NIH grants R01HL095135, U54RR026136, U54RR026136, and R21 N5080656

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