Prevalence and Correlates of Dyslipidemia among HIV-1 Infected and HIV-1 Uninfected Individuals in Nairobi, Kenya

Anne Njoroge

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Carey Farquhar

Brandon L. Guthrie

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Abstract

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Anne Njoroge

Chair of the Supervisory Committee:

Dr. Carey Farquhar, MD, MPH

Department of Medicine, Epidemiology and Global Health,

Background: The burden of cardiovascular disease (CVD) in Sub-Saharan Africa is rising in the background of high prevalence of infectious diseases including HIV. Being HIV infected is associated with lipid imbalance (dyslipidemia), a risk factor for CVD. Whereas the use of certain antiretroviral treatment (ART) drugs has been shown to cause dyslipidemia, little is known about the burden of dyslipidemia in the absence of ART in Sub-Saharan Africa and the factors associated with dyslipidemia, particularly the amount of circulating HIV virus (viral load).

Methods: In the parent study, HIV infected individuals who were not on ART and their HIV-uninfected partners were enrolled for a cohort study. At baseline, socio-demographic data and a detailed medical history were obtained and clinical physical examination performed on participants. Whole blood samples were collected, fractionated and stored frozen at -800C. For this nested study, a random sample of the frozen serum samples were thawed and assayed for total cholesterol and high density lipoprotein cholesterol (HDL). The proportion of participants

with dyslipidemia, characterized by high total cholesterol or low HDL was compared between HIV-infected and HIV-uninfected individuals. Correlates evaluated for association with dyslipidemia included age, gender, CD4 cell count, viral load, body mass index, blood pressure and smoking.

Results: Samples from 196 individuals, collected between 2007 and 2008, were assayed. Median age was 32 years (Interquartile range [IQR] 23-41 years). Of the 99 who were HIV-infected, 47 (47%) were male while 52 (52%) were female and median CD4 count was 393 cells/μL (IQR 57-729). The proportion of individuals with dyslipidemia was high in both groups (>75%) though there was no significant difference between HIV-infected and HIV-uninfected individuals ($p>0.05$).Viral load was an independent risk factor associated with dyslipidemia (OR 6.1, $p=0.028$)

Conclusion: Although there was no significant difference in prevalence of dyslipidemia comparing HIV- infected and HIV-uninfected individuals, the proportion of individuals with dyslipidemia was high irrespective of HIV infection status. Among the HIV-infected, high viral load was associated with increased risk of dyslipidemia. This would be important to consider when choosing the drug regimen during ART initiation.

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To Edwin, my person: “veni, vidi, vici”.Together.

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Dedication

To my family and Edwin, my person.

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Background and Significance

Ischemic heart disease accounts for 25.7% of deaths worldwide annually[1] and is rapidly becoming a leading cause of death in sub-Saharan Africa where HIV and other infectious diseases had previously dominated the landscape. While cardiovascular disease (CVD) is one of the most common causes of death among people aged 40 years and above in North America[2] a similar epidemiologic trend is now observed in sub-Saharan Africa (SSA), with the World Health Organization (WHO) ranking ischemic heart disease as the top 5th cause of death in low-income countries, accounting for 47 deaths per 100,000 people.[1] The increase in cardiovascular disease burden is occurring in the background of a high prevalence of HIV, as 70% of people living with HIV are in SSA.[3]

Being HIV-infected is associated with increased risk of having cardiovascular disease.[4-6] Studies in high-income countries have demonstrated higher rates of myocardial infarction among HIV-infected people compared to HIV uninfected persons.[6, 7] In these developed country settings, HIV populations have increased prevalence of the traditional risk factors for cardiovascular disease, including smoking, obesity and dyslipidemia.[8-10] Elevated lipid levels are an important risk factor for development of CVD as dyslipidemia contributes to cholesterol accumulation in the development of atherosclerosis. There is a complex interplay between HIV infection, cardiovascular disease and dyslipidemia; HIV infection is associated with immune dysfunction, altered coagulation and a chronic pro-inflammatory state; all which favor plaque formation.[11, 12] However, data are scarce on the prevalence of these risk factors and how well they predict CVD risk among HIV infected individuals in SSA, where HIV prevalence is high.

Age is a known unmodifiable risk factor for CVD and dyslipidemia. Gender is also associated with dyslipidemia, as males are at a higher risk of developing an atherogenic lipid profile compared to women. This association with gender has been shown to vary in the context of HIV infection. Many studies in SSA that have shown an association between immune suppression and dyslipidemia, indicated by an association between low CD4 cell counts

(≤200cells/μl) and increased risk of dyslipidemia. CD4 cell count is used as a marker for immune dysfunction, but is also inversely correlated with viral load.[13] It remains unclear whether dyslipemia in HIV-infected individuals is driven primarily by immune dysfunction or whether high viral loads have a direct role. While higher viral load is usually associated with lower CD4 counts, this relationship does not hold in all individuals.[14] To our knowledge, no studies have described associations between viral load patterns and dyslipidemia in HIV, particularly in SSA, where viral load testing is not done routinely in HIV care settings.

The risk of CVD among HIV-infected individuals is only bound to increase, given that increased access to antiretroviral therapy (ART) is associated with prolonged survival hence HIV-infected individuals are at increased risk of CVD associated with age like everyone else and in addition, most of the antiretroviral drugs commonly used in SSA are associated with dyslipidemia. Our study sought to estimate the prevalence of dyslipidemia comparing HIV-infected ART naïve and HIV-uninfected individuals by assessment of total cholesterol (TC) and high density cholesterol (HDL) in stored non-fasting plasma samples and to assess the risk factors associated with dyslipidemia including age, gender and viral load.

Methods

Study design

Nested cross-sectional study: The parent prospective cohort study (R01 AI068431) was designed to assess the specificity, function and durability of HIV-1 specific immune responses over time and their association with reduced risk of HIV-1 infection.[15]

Study population.

The parent study enrolled 938 individuals from 469 HIV-1 sero-discordant relationships from recruited from voluntary counseling centers (VCT) in Nairobi, Kenya from September 2007 to December 2009. HIV-1-infected participants with a history of clinical AIDS (WHO stage IV) and currently on ART were excluded. In this study, all couples for whom baseline plasma

samples were available were divided into 2 groups: those in whom the male was HIV-infected and those with an HIV-infected female. We randomly selected 50 couples from each group were selected. Of the individuals selected, 1 HIV infected 3 HIV uninfected individuals were excluded with to inadequate plasma samples.

Study Procedures

The study procedures for the larger study have been described elsewhere in detail.[15] At enrollment, clinic staff administered questionnaires collecting socio-demographic data, a detailed medical history and performed clinical physical examination on participants. Participants were tested for HIV-1 by two rapid tests conducted in parallel using a Determine HIV-1/2 rapid test (Abbott Laboratories, Tokyo, Japan) and the Bioline HIV 1/2 rapid test (Standard Diagnostics Inc., Suwon, South Korea). Positive results were confirmed using an enzyme-linked immunosorbent assay (ELISA). Participants were eligible for enrollment as index participants if they had confirmed HIV positive results. Participants were eligible as partner participants if they had concordant HIV negative rapid test results and reported vaginal sex ≥3 times in the 3 months prior to enrollment and planned to remain together for the duration of the study. Plasma from HIV-1-infected partners collected at enrollment was assayed for HIV-1 RNA load using Gen-Probe Transcription Mediated Amplification (Gen-Probe, San Diego, CA). This assay detects the prevalent HIV-1 subtypes in Kenya, subtypes A, C, and D and could detect as low as ~10-25 copies/ml. CD4 cell counts was measured using FACs Caliber system (Becton Dickinson). Whole blood samples were fractionated and plasma samples were stored frozen at -80^{0}C. They were transported while frozen in liquid nitrogen from Nairobi Kenya to Seattle Washington. For the nested study; socio-demographic, behavioral and clinical physical examination data obtained during enrollment in the parent study was utilized.

Laboratory assays for nested study

Frozen plasma samples were retrieved and thawed. Quantitative determination of total cholesterol and HDL was done using the UniCel DxC 800 (Beckman Coulter Inc.) spectrophotometry auto analyzer.

Statistical Methods

Variable definition

The primary outcome was dyslipidemia, as defined by the National Cholesterol Education Program Adult Treatment Panel. (ATP)III[16]: Total cholesterol (TC) ≥200mg/dl or HDL <40mg/dl. For CVD risk prediction the TC:HDL ratio was calculated and categorized according to the American Heart Association: Low risk ≤3.5, intermediate 3.6 – 4.9, high risk ≥5.[17]

Demographic, behavioral, clinical and laboratory characteristics of participants at enrollment were summarized. Age was grouped in 10 year categories starting from 25years. Height and weight were measured using standardized procedures.[18] Standard Body Mass Index (BMI) categories were used.[16] Blood pressure cut-offs as defined by the European Society of Cardiology were used.[19] Socio-economic status (SES) was categorized as monthly below or above the median monthly income per couple (Kenyan shillings).

CD4 cell count categories were defined using a cut-off of 350 cells/μl, as this was the WHO recommended cut-off for initiation of ART at the time of the study.[20]

Viral load was categorized as above or below 1000 copies/ml. based on the WHO threshold defining virologic failure.[20]

Statistical Analysis

We calculated counts and proportions (%) for categorical variables as well as median and interquartile range (IQR) for continuous variables. We compared the prevalence rates of each outcome among HIV-infected individuals versus HIV-uninfected individuals using

Pearson's chi-square test. A 2 sample Student's t-test with unequal variance was used to compare the mean total cholesterol, HDL and TC: HDL ratio between HIV-infected and HIV-uninfected individuals. Logistic regression models with robust standard error estimates were used to evaluate the association between dyslipidemia and potential predictors. All potential correlates of dyslipidemia were evaluated adjusted for age and gender as *a priori* potential confounders.[21] The assessments of CD4 cell count and viral load were restricted to HIV-infected individuals. All statistical tests were evaluated using a 2-sided test using a p-value <0.05 to define statistical significance. Statistical analyses were conducted using Stata version IC 13 (StataCorp Inc., College Station, TX, USA).

Results

Description of Baseline Characteristics

A total of 99 HIV-infected and 97 HIV-uninfected individuals were included in this analysis. Of those who were HIV-infected, 47 (47%) were male and of the HIV-uninfected, 48 (49%) were male (Table 1). The median age among the HIV infected was 31.5 years (interquartile range [IQR], 23.5 to 39.5) years and among the HIV uninfected was 32 years (IQR 22.5 to 41.5). Of the 196 individuals in the study, 46.7% had a normal BMI (between 18.5 to <25) while 20.5% were classified as obese. (BMI≥30). Although only 17 individuals (9%) had essential hypertension as defined by a resting systolic blood pressure ≥130mmHg and diastolic blood pressure ≥85 mmHg[19], an additional 40 individuals (20%) had elevated systolic blood pressure (≥130mmHg) only and 10 (5%) had elevated diastolic blood pressure (≥85 mmHg) only. The median couple income was 12,000 Kenya Shillings per month. The HIV infected and HIV uninfected groups were generally well-balanced with respect to the baseline characteristics. Specific to the HIV infected group, the median CD4 count was 393 cells/μl (IQR, 57 to 729) and the median plasma viral load was 4.5 $\log_{10}$ copies/ml (IQR, 3.2 to 5.8). They were all ART-naïve and 92 (92%) were on Cotrimoxazole prophylaxis.

Plasma lipid levels and Dyslipidemia.

The mean plasma lipids were similar between the HIV-infected and HIV-uninfected groups (Table 2). The mean total cholesterol was 96.6 mg/dl (64.4-128.8) in the HIV-infected group and 95.3 mg/dl (65.6-125) in the HIV-uninfected group. (p=0.69) The mean HDL was 30.9 mg/dl (18.9-42.9) in the HIV-infected group and 31.6 mg/dl (21.6-41.6) in the HIV-uninfected group (p=0.77). The average TC: HDL ratio was 3.2 (2.4-4.0) among the HIV-infected and 3.1(2.4-3.8) among the uninfected (p=0.22). The prevalence of dyslipidemia, defined as either TC>200mg/dl or HDL <40 mg/dl, was 83.8% among HIV-infected individuals compared to 78.4% among HIV-uninfected individuals (OR=1.4, 95% CI: 0.7-2.9, p=0.3). The TC: HDL ratio was optimal in 68 (68.7%) of the HIV-infected and 72 (74.2%) of the uninfected, while the TC: HDL ratio was intermediate risk in 29 (29.3%) of the infected and 25 (25.8%) of the uninfected and high risk in 2 (2%) infected and 0 uninfected. The odds of a non-optimal TC: HDL risk was 1.31 times higher in the HIV-infected than the uninfected. (95%CI: 0.7-2.4, p=0.4)

Correlates

In univariate analysis, age showed a trend towards association with dyslipidemia (odds ratio [OR] =1.5; 95%Confidence Interval [CI]: 0.9, 2.4; p=0.1) while the association between female gender and dyslipidemia was non-significant (OR=0.7; 95%CI: 0.4, 1.5; p=0.4). None of the other correlates investigated, including BMI, hypertension (including systolic and diastolic hypertension), smoking and socio-economic status, were associated with dyslipidemia (Table 3). There was a non-significant 1.6-fold increased odds of having dyslipidemia among the HIV infected compared to the HIV-uninfected, adjusting for age and gender (OR=1.6; 95% CI: 0.8, 3.4; p=0.2).

Viral load was independently associated with dyslipidemia. Among the HIV-infected, those with a viral load ≥1000 copies/ml had a 6.1-fold higher odds of dyslipidemia compared to those whose viral load was <1000 copies/ml, adjusted for age and gender (OR=6.1; 95% CI: 1.2, 30.9; p=0.028)

CD4 cell count was associated with dyslipidemia, with those having a CD4 cell count <350 cells/μl having a 10.7 fold increased odds of having dyslipidemia compared to those with CD4 cell count >350cells/μl (95% CI: 1.3, 86.3, p=0.026). However, the strength of this association was markedly reduced upon adjusting for age and gender. (HR 7.1, 95%CI: 0.8, 61.7, p=0.075)

Discussion

Although there was no significant difference in prevalence of dyslipidemia in this non-fasting cohort comparing ART naïve HIV-infected individuals to HIV-uninfected individuals, the proportion of individuals with dyslipidemia was high irrespective of HIV infection status. However, among the HIV-infected, a high viral load was significantly associated with risk of dyslipidemia, even after adjusting for age and gender. A low CD4 cell count was associated with increased risk of dyslipidemia, although the strength of this relationship was reduced when age and gender were considered.

Although there was no significant difference in prevalence of dyslipidemia comparing HIV-infected and HIV-uninfected groups, there was a slightly higher proportion of HIV-infected with low HDL (83%) compared to HIV-uninfected (76%). The total cholesterol was also lower among the HIV-infected relative to the HIV-uninfected, although this could be due to chance (p>0.05). This is in keeping with other studies among similar ART naïve populations in SSA[13, 22-24]

The interplay between HIV infection, cardiovascular disease and dyslipidemia is complex and not completely understood. The HIV virus is associated with vascular structural and

functional alterations.[25] One molecular theory of HIV-induced endothelial dysfunction is that HIV synthesizes Tat, a transcriptional protein secreted by HIV-infected cells that increases the expression of adhesion molecules and induces apoptosis of endothelial cells, which allows the penetration of plasma lipids (low density lipoproteins) into the sub-endothelial space where they undergo oxidation. The adhesive proteins and inflammatory cytokines promote the recruitment of monocytes into the intima here they transform into macrophages and foam cells by engulfing the accumulated lipids.[26] These lipid-rich macrophages upon undergoing apoptotic death contribute toward the formation of the necrotic lipid core that is the hallmark of advanced atherosclerotic lesions[12, 26, 27] It is thus expected that HIV-infected individuals would have a higher risk of developing dyslipidemia compared to HIV-uninfected individuals. This process may be limited in an immune competent HIV-infected individual and thus quite similar to a HIV-uninfected individual. An explanation as to the lack of difference in dyslipidemia between the HIV-infected ART naïve and the HIV uninfected among our population could thus be the high-functioning immune system in our population. While our median CD4 count was 393 cells/μl, most of the ART naïve cohorts demonstrating significant difference between HIV-infected and uninfected have had low CD4 counts (≤200cells/μl) on average among the HIV uninfected.[13, 22, 28]

The lack of difference in dyslipidemia prevalence could also be attributed to a relatively high prevalence of dyslipidemia among the general Kenyan population. Recently, the Africa Middle East Cardiovascular Epidemiological (ACE) Study assessing cardiovascular risk factors in the general population found dyslipidemia prevalence at 70% in Kenya,[29] mostly marked by low HDL levels, compared to 54% in Cameroon where 2 studies had previously found significantly higher dyslipidemia in ART naïve HIV-infected individuals relative to HIV- uninfected.[13, 30]

The significant association between viral load and risk of dyslipidemia, independent of age and gender, is in keeping with the proposed causal mechanism described above, as it supports a dose-response relationship. Several studies that have shown LDL as a major

modifiable cardiovascular risk factor have at the same time shown an inverse association between HDL levels and cardiovascular events.[31] Our findings demonstrate that increased viral load is also associated with dyslipidemia characterized by low HDL levels, although the molecular signaling mechanism remains unclear. This is also significant because though the risk of dyslipidemia has been shown to be associated with reduced CD4 cell counts, high CD4 cell count does not always correlate well with viral suppression.[14] Furthermore, the significance of the association between low CD4 cell count and increased risk of dyslipidemia seemed to weaken upon adjustment for age and gender in our population.

Even in a non-fasting state, our demonstration of a higher proportion of HIV-infected individuals with a non-optimal TC: HDL ratio risk compared to the HIV-uninfected in this population (31% vs 26% respectively) though statistically insignificant is in keeping with previous findings among fasting individuals.[22, 28, 32] This is relevant in terms of screening because the TC: HDL ratio assessment is direct, has insignificant variation with fasting and is thus convenient for use in routine clinical settings to screen for cardiovascular risk among this at-risk population.

Overall, our mean lipid levels were low compared to similar populations previously described. In Tanzania, Liu et al found an average TC of 152 mg/dl and HDL of 38 mg/dl among a cohort of ART naïve patients.[24] Our low levels could be art factual due to the extended period of storage (up to 7 years) prior to lipid measurement. Using the rate of degradation described elsewhere at 1.2% - 2% for every year stored,[33] the mean plasma lipid level and prevalence of dyslipidemia did not vary upon adjustment for storage. The degree to which storage truly affected the lipid levels in our sample remains unclear and would affect the reported prevalence of dyslipidemia. However, the rate of degradation would be expected to be similar among the HIV-infected and the HIV- uninfected, hence the difference between the HIV-infected and the HIV-uninfected would not be affected.

Our study population is unique in that these were HIV sero-discordant couples and thus presumed to have similar dietary and environmental exposures e.g. access to exercise facilities that would affect their lipid levels. The use of a viral load cut-off of 1000 copies/ml and CD4 cell count of 350 cells/μl for association with dyslipidemia is relevant because of their clinical relevance in defining significant viremia and eligibility for ART initiation respectively in primary HIV care settings in SSA.

Long term use of ART is associated with an atherogenic profile characterized by increase in triglyceride, total cholesterol and low density lipoprotein (LDL) cholesterol levels with a modest rise in HDL.[26] This is significant because the TC: HDL ratio is bound to increase with exposure to ART.[31, 34] Using the current Kenya HIV treatment guidelines, 34% of this study population would have been eligible for ART initiation, increasing their probability of having a high ratio thus increased cardiovascular risk. Our study shows that screening for dyslipidemia in ART naïve individuals, even in a non-fasting state, is still important as it would identify HIV-infected individuals at high risk of developing an even higher TC:HDL ratio upon ART initiation and may help inform the choice of their ART drug regimen.

Table 1: Baseline characteristics of HIV-infected & HIV-uninfected individuals

Characteristic	HIV Infected Median(IQR) or N(%) n=99	HIV uninfected Median(IQR) or N(%) n=97
Age(years)		
<25	10 (10)	11 (11)
25 - 34	58 (58)	47 (48)
35 - 44	24 (24)	29 (30)
≥45	7 (7)	10 (10)
Sex (Female)	51 (51)	48 (49)
BMI(mg/kg2)		
<18.5 (underweight)	0	2 (2)
18.5 - <25 (normal)	44 (44)	44 (45)
25 - <30 (overweight)	33 (33)	28 (29)
≥30 (obese)	19 (19)	21 (22)
Blood pressure (mmHg)		
Normotensive (<130/85)	66 (66)	63 (65)
Elevated BP (≥130/≥85)	10 (10)	7 (7)
Systolic HTN (>130)	16 (16)	24 (25)
Diastolic HTN (>85)	7 (7)	3 (3)
Smoking		
Never	67 (67)	77 (79)
Past	22 (22)	9 (9)
Current	10 (10)	11 (11)
Social Economic Status		
Low (<Median=Kshs. 12,000)	47 (47)	46 (47)
High (>Median=Kshs. 12,000)	48 (48)	49 (51)
CD4 Cell count (cells/ml)	393(57-729)	-
< 200	10 (10)	-
200 - 349	24 (24)	-
350 - 499	23 (23)	-
≥ 500	30 (30)	-
Viral Load (>1000copies/ml)	90 (90)	-
$\log_{10}$ VL	4.53(3.2 - 5.8)	-

Table 2: Mean plasma lipid values (non-fasting)

Lipid parameter (mg/dl)	HIV infected Mean (95% CI)	HIV uninfected Mean (95% CI)	p-value
TC	96.56 (90.33, 102.78)	95.30 (89.31, 101.29)	0.773
Plasma-HDL	30.93 (28.55, 33.31)	31.56 (29.54, 33.58)	0.691
TC:HDL ratio	3.24 (3.08, 3.40)	3.11 (2.97, 3.25)	0.217

Table 3: Analysis of risk factors associated with Dyslipidemia

Characteristic	Total N=196	Dyslipidemia n=159	Prevalence %	aOR	95%CI	p-value
Age(years)						
<25	21	14	7	1.00	Ref	*0.13*
25 - 34	105	86	44	2.26	0.79 - 6.46	
35 - 44	53	44	22	2.44	0.75 - 7.98	
≥45	17	15	8	3.75	0.61 – 23.00	
Gender						
Male	97	81	41	1.00	Ref	0.40
Female	99	78	40	0.73	0.36– 1.51	
*BMI(mg/kg2)**						
<18.5 (underweight)	2	1	1	0.32	0.03 – 3.54	
18.5 - <25 (normal)	88	69	36	1.00	Ref	0.79
25 - <30 (overweight)	61	52	27	1.58	0.63 -3.95	
≥30 (obese)	40	33	17	1.39	0.47 -4.12	
Blood Pressure						
Normotensive	129	104	53	1.00	Ref	0.7
Elevated BP (≥130/≥85)	17	13	7	0.78	0.21-2.89	
systolic HTN (>130)	40	34	17	1.08	0.38 – 3.11	
diastolic HTN (>85)	10	8	4	1.01	0.19 – 5.23	
Smoking						
Never	144	117	60	1.00	Ref	0.8
Past	31	28	14	0.56	0.27-3.12	
Current	21	14	7	0.91	0.16 – 1.80	
*SES (KSh12000)***						
Above median(High)	97	78	41	1.00	Ref	0.81
Below median (Low)	93	76	40	1.14	0.55 -2.39	
HIV status						
HIV-	97	76	39	1.00	Ref	0.32
HIV+	99	83	42	1.61	0.77 – 3.38	
Viral Load						
<1000 copies/ml	9	5	5	1.00	Ref	**0.028**
(>1000copies/ml)	90	78	79	6.14	1.22 – 30.99	
CD4 Cell count (cells/ml)¥						
>350	53	40	46	1.00	Ref	*0.075*
< 350	34	33	38	7.10	0.82 – 61.72	

*N=191
**N=190
¥N=91

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