Vitamin D Deficiency is Associated With the Development of Subclinical Coronary Artery Disease in African Americans With HIV Infection: A Preliminary Study

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Background: Premature coronary artery disease (CAD) is a major concern in human immunodeficiency virus (HIV)-infected African Americans. The objectives of the study were to estimate the incidence of subclinical CAD, defined by the presence of coronary plaque and/or calcification on cardiac computed tomography (CT), and to identify the associated risk factors in this vulnerable population.

Subjects and Methods: Between August 2003 and September 2010, 188 HIV-infected African Americans without known, or symptoms of, CAD underwent cardiac CT. The subset without demonstrable disease underwent a second cardiac CT approximately 2 years later. The incidence of disease over that period and the effects of antiretroviral treatment and other known and hypothesized risk factors were investigated.

Results: Sixty-nine of these 188 African Americans had evidence of subclinical disease on the initial cardiac CT, confirming prior high prevalence reports. A second cardiac CT was performed on 119 African Americans without disease approximately 2 years later. The total person-years of follow-up was 284.4. Subclinical CAD was detected in 14 of these, yielding an overall incidence of 4.92/100 person-years (95% confidence interval, 2.69–8.26). Among the factors investigated, only male sex and vitamin D deficiency were independently associated with the development of subclinical CAD. The study did not find significant associations between CD4 count, HIV viral load, antiretroviral treatment use, or cocaine use and the incidence of subclinical CAD.

Conclusions: The incidence of subclinical CAD in African Americans with HIV infection is provocatively high. Larger studies are warranted to confirm the role of vitamin D deficiency in the development of CAD in HIV-infected African Americans.

Key Words: vitamin D deficiency, subclinical coronary artery disease, HIV infection, African Americans

(*J Investig Med* 2012;60: 801–807)

Survival in persons with human immunodeficiency virus (HIV) infection has dramatically improved after the introduction of highly active antiretroviral therapy. As HIV-infected individuals live longer, some chronic conditions, such as premature coronary artery disease (CAD), have emerged as a long-term concern in this population. The responsible factors are not well-defined. Any chronic infection may increase circulating cytokines and other inflammatory mediators that enhance the development and progression of atherosclerosis. Human immunodeficiency syndrome itself could directly infect, damage, and cause proliferation of human arterial smooth muscle cells.[1] In addition, the antiretroviral therapy (ART) used to treat HIV infection may have adverse effects, including promoting atherosclerosis, although the findings in this regard are not consistent.[2–5]

We have reported cross-sectional data demonstrating high rates of subclinical CAD in HIV-infected African Americans residing in Baltimore, Maryland.[6–8] Because African Americans have the highest overall CAD mortality rate of any ethnic group in the United States,[9] it is critically important to estimate the incidence of subclinical coronary atherosclerosis and investigate whether ART or other factors influence the development of subclinical CAD in HIV-infected African Americans.

The aim of the present study was to estimate the incidence of subclinical CAD and investigate the risk factors for the development of subclinical coronary atherosclerosis in HIV-infected African Americans.

SUBJECTS AND METHODS

Study Participants

Between August 2003 and September 2010, 188 HIV-infected African Americans without known, or symptoms of, coronary artery disease were consecutively enrolled in a prospective study investigating the incidence of and the risk factors for cardiac computed tomography (CT)-defined subclinical CAD in Baltimore, Maryland.

Subclinical CAD was defined as the presence of coronary artery calcium (CAC) and/or coronary plaque by cardiac CT. Inclusion criteria were age between 25 and 60 years, HIV positivity (determined by enzyme-linked immunosorbent assay and confirmed by Western blot test), and African American race (self-designated). Exclusion criteria were the following: (1) any evidence of clinical CAD (All the study participants in this study are patients at the Johns Hopkins HIV clinic. We have access to their medical records, including history of electrocardiographic (ECG) abnormalities and any cardiovascular diagnoses or symptoms, including chest pain or heart failure symptoms. Self-reported information was not used for defining CAD), (2) any symptoms believed to be related to CAD, (3) any evidence of renal insufficiency, (4) known allergy to the contrast used for the CT, and (5) pregnancy. During the baseline visit, each subject was interviewed to obtain information on sociodemographic characteristics, cardiovascular risk (including cigarette smoking and alcohol use),

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Received October 23, 2011, and in revised form January 18, 2012.
Accepted for publication January 19, 2012.
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The study was supported by grants from the National Institute on Drug Abuse, National Institutes of Health (NIH R01DA 12777, DA15020, and DA25524).

ISSN: 1081-5589
DOI: 10.231/JIM.0b013e318250bf99

illicit drug use behaviors, medical history, and all medications used. Cocaine use was defined as chronic use of cocaine by any route for at least 6 months, administered at least 4 times a month. Information about the frequency (how many times a day in the past week, in the past month), patterns/forms of cocaine (speedball, crack, etc.), administration mode (injection, smoking, etc.), and duration of cocaine use was collected. Information about use of other drugs, such as opiates, benzodiazepines, or methamphetamine, was also collected.

A medical chart review was used to confirm information on medical history and medications that was provided by the subjects. Each subject also had a physical examination, and several tests were performed, namely, a fasting lipid profile, vitamin D, high-sensitivity C-reactive protein (hs-CRP) test, 64-slice multidetector CT (MDCT) for CAC, and CT coronary angiography (contrast enhanced). The study participants underwent reexaminations and interviews approximately 2 years later.

Of the 188 participants at baseline, 69 participants had a diagnosis of subclinical CAD and 119 participants were free of subclinical CAD. The 119 participants without subclinical CASD were included in this study.

The Committee on Human Research at the Johns Hopkins School of Medicine approved the study protocol, and all study participants provided written informed consent. All procedures used in this study were in accordance with institutional guidelines.

Blood Pressure Measurement

Sitting systolic and diastolic blood pressures (SBP and DBP) were measured twice with a standard mercury sphygmomanometer. A nurse at the clinic measured the study participants' arm circumference and applied a correctly sized cuff. The participant sat quietly for 5 minutes, and then the nurse obtained the SBP and DBP. A second measurement was obtained 3 minutes later, and the average of the 2 readings is reported.

Measurement of Lipids

Venous blood samples were obtained after an overnight fast from a large antecubital vein. Serum was separated by centrifugation (2000*g* for 15 minutes at 4°C) and stored at −75°C until assayed. Serum lipid variables, including total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein (LDL) cholesterol levels, were directly determined with an analyzer (Hitachi 747 analyzer; Roche, Englewood, NJ).

Vitamin D Measurement

Sera were collected, centrifuged, and stored at −70C until analyzed. Serum 25-OH vitamin D was determined by a direct, competitive chemiluminescence immunoassay (DiaSorin, Stillwater, MN).[10] The level of detection for 25-OH vitamin D was less than 4 ng/mL. This method accurately measures both D2 and D3 together and is reported as a total 25 (OH) vitamin D. The reference range is 32 to 100 ng/mL. This study identifies vitamin D deficiency according to the Framingham Offspring Study as serum 25 (OH) vitamin D less than 10 ng/mL.

Coronary CT Angiography With a 64-Slice SIEMENS MDCT Scanner

A noncontrast MDCT scan was performed on a Sensation 64 cardiac Siemens Medical Solutions scanner (Erlangen, Germany) to determine the CAC score with a sequential scan of 3-mm slices with prospective ECG triggering, 30 × 0.6-mm detector collimation, and 135-mA s tube current at 120 kV. Subsequently, coronary CT angiography was performed on the same equipment using 80 mL of iso-osmolar contrast agent (320 mg of iodine/mL) injected at 4 to 5 mL/s. Imaging was performed with retrospective ECG-gating, 32 × 0.6-mm detector collimation with flying focal spot to give effective detector collimation of 64 × 0.6 mm, 330-millisecond (ms) gantry rotation, 850 mA s, and 120 kV. Subsequently, 0.75-mm-thick axial slices were reconstructed at 0.4-mm intervals with B25 kernel using a half-scan reconstruction algorithm with resulting temporal resolution of 165 to 185 ms. Ten reconstructions were done through the cardiac cycle at 10% increments in the R-R interval. If needed, patients were medicated with metoprolol before the scan to achieve a heart rate of less than 65 beats per minute.

Coronary artery calcium score, volume, and mass were measured on a workstation (Leonardo, Syngo, Siemens Medical Solutions, Malvern, PA). Regions of interest were placed over each of the coronary arteries with a threshold for pixels of greater than 130 Hounsfield units for determining calcified plaque. Coronary vessels were assessed for patency and stenoses using 3-dimensional visualization tools after the axial images were reviewed for determination of anatomy, quality of the study, and appearance of the vessels. The Agatston method was used to signify development of incident CAC.

One reviewer (E.K.F), blinded to the participants' risk factor profiles, independently evaluated the contrast-enhanced MDCT scans by examining the axial slices, curved multiplanar reformations, and thin-slab maximum-intensity projections. The coronary artery tree was segmented according to the modified American Heart Association classification, and the segments were investigated for plaque and luminal narrowing. The coronary arteries were divided into proximal, mid, and distal segments, with each segment investigated for luminal narrowing. Plaques were classified as calcified or noncalcified, and the degree of stenosis was classified as less than, equal to, or greater than 50%-diameter stenosis. Diameter stenosis 50% or greater was defined as significant coronary stenosis. This cutoff has been used in prior studies as well.[11]

Statistical Analysis

Length of follow-up was calculated as the time elapsed from the baseline to the second CT examination. Incidence rate was calculated by dividing the number of newly diagnosed cases by the person-years of follow-up. The 95% confidence interval for the incidence was also calculated. Antiretroviral therapies were categorized based on exposure to 4 classes: nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside RTIs (NNRTIs), protease inhibitors, and other ARTs.

Statistical analysis was performed with SAS (version 9.2, SAS Institute, Cary, NC). All continuous parameters were summarized by medians and interquartile ranges, and all categorical parameters were summarized as proportions. To compare between-group differences, the nonparametric Wilcoxon two-sample test was used for continuous variables and the Fisher's exact test was used for categorical variables. The Framingham risk score was calculated to estimate the CAD risk.[12]

Survival analysis was used to identify the risk factors for the development of subclinical coronary atherosclerosis. The Kaplan-Meier method was used to estimate the survivor functions, and the log-rank test was used to test the equality of survivor functions. Univariate Cox proportional hazards regression models were first fitted to evaluate the crude association between the development of coronary plaques and each individual factor—age, sex, total cholesterol, high-density lipoprotein cholesterol, LDL-C, triglycerides, vitamin D, high-sensitivity C-reactive protein, cigarette smoking, alcohol use, glucose level, history of diabetes, and history of hypertension, SBP, DBP, body mass index, baseline CD4 cell count, baseline HIV RNA quantification, duration of each ART drug use, duration of ART use for each ART class (NRTIs, NNRTIs, protease inhibitors, fusion inhibitors, or

TABLE 1. Characteristics of Study Participants by the Development of Subclinical CAD*

Characteristic	Total (N = 119)	Subclinical CAD		*P*
		No (n = 105)	Yes (n = 14)	
Age, yr	45 (41–49)	45 (41–49)	46 (45–51)	0.32
Male, %	52.1	46.7	92.9	0.001
Family history of CAD, %	21.9	22.9	14.3	0.45
Diabetes, %	7.6	8.6	0.0	0.25
Hypertension, %	19.3	20.0	14.3	0.61
Cocaine use, %	78.2	79.1	71.4	0.52
Cigarette smoking, %	81.5	81.9	78.6	0.76
Alcohol use, %	81.5	81.0	85.7	0.67
Years of HIV infection	15.3 (9.2–19.5)	15.2 (10.0–19.5)	15.7 (7.3–17.8)	0.63
hsCRP ≥2 mg/dL, %	43.7	42.9	50.0	0.61
hsCRP, mg/dL	1.4 (0.7–4.7)	1.4 (0.7–4.6)	1.9 (0.7–5.9)	0.54
Serum 25(OH)D, ng/mL	17 (11–24)	19 (11–26)	12 (9–17)	0.029
Systolic BP, mm Hg	119 (109–130)	119 (109–130)	119 (108–124)	0.80
Diastolic BP, mm Hg	75 (68–83)	76 (68–84)	71 (66–77)	0.06
Glucose, mg/dL	85 (78–94)	85 (78–94)	91 (80–100)	0.52
BMI	25.4 (22.4–29.2)	25.5 (22.4–29.2)	24.8 (22.4–28.4)	0.56
Waist-to hip ratio	0.88 (0.83–0.92)	0.87 (0.83–0.91)	0.91 (0.82–0.93)	0.61
Baseline CD4, cells/mm^3	325 (205–527)	325 (193–528)	297 (222–379)	0.64
Baseline viral load, copies/mL	12,000 (977–66,658)	12,000 (716–97,015)	14,628 (1431–44,000)	0.86
eGFR, mL/min per 1.73 m^2	103 (85–120)	104 (84–120)	100 (90–120)	0.90
Total cholesterol, mg/dL	162 (137–185)	163 (137–185)	160 (137–184)	0.90
LDL-C, mg/dL	80 (62–104)	80 (59–104)	80 (65–113)	0.49
HDL-C, mg/dL	52 (43–64)	51 (43–66)	55 (45–60)	0.87
Triglycerides, mg/dL	98 (78–152)	98 (79–155)	103 (72–120)	0.51
NRTI use, mo	36.0 (0.4–72.0)	36.0 (5.0–72.0)	3.5 (0.0–60.0)	0.09
NNRTI use, mo	0 (0.0–20.9)	0.0 (0.0–17.9)	0.0 (0.0–36.0)	0.73
PI use, mo	24.0 (0.0–70.0)	29.0 (0.0–72.0)	3.9 (0.0–60.0)	0.46
ART use, mo	45.0 (14.0–99.1)	45.0 (17.9–96.0)	54.0 (5.0–101.0)	0.96
Framingham risk score	3.0 (2.0–6.0)	3.0 (2.0–6.0)	4.5 (3.0–8.0)	0.13
Framingham score <10.0, %	91.6	92.4	85.7	0.40

*Median (interquartile range) for continuous variables, proportion (%) for categorical variables.

BMI indicates body mass index (kg/m^2); CD4, CD4 cell count; eGFR, estimated glomerular filtration rate; Framingham score, Framingham risk score; glucose, fasting glucose; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; PI, protease inhibitor; serum 25(OH)D, 25-hydroxyvitamin D; viral load, HIV RNA quantification.

integrase inhibitors), duration of any ART use, cocaine or other illicit drug use, and the Framingham risk score.

Those factors that were significant at $P \leq 0.20$ level in the univariate models were put into an initial multivariate Cox proportional hazards regression model to identify the factors that were independently associated with the development of subclinical CAD. The variables that ceased to make significant contributions to the models were eliminated in a stagewise manner, yielding a final model. The *P* values reported are 2-sided. $P < 0.05$ indicated statistical significance.

RESULTS

General Characteristics

Among the 119 study participants included in this study, subclinical CAD was detected in 14 during the follow-up visit. Of the 14 patients with incident CAD, 9 patients developed incident CAC, 4 patients developed incident plaque, and one patient developed incident CAC and plaque. The general and

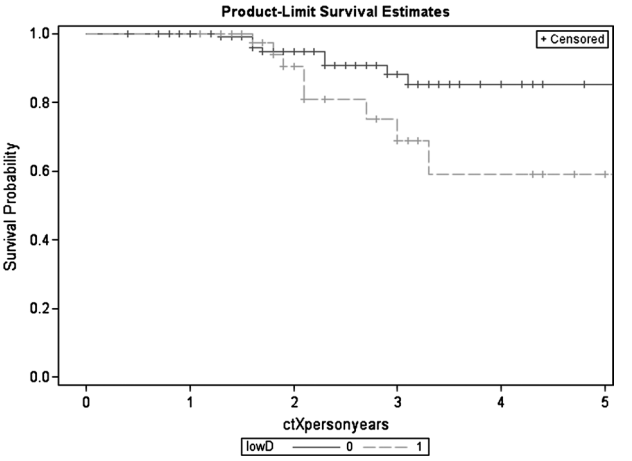

FIGURE 1. Kaplan-Meier subclinical CAD-free survival curve by vitamin D deficiency status. The survival curves by vitamin D deficiency status were statistically different (log-rank test, $P = 0.0245$).

TABLE 2. Demographic, Laboratory, and Clinical Factors in Relation to the Risk of Development of Subclinical CAD, Proportional Hazards Regression Analysis*†

Variable	Subclinical CAD	
	Crude HR (95% CI)	Adjusted HR (95% CI)
Age, yr	1.03 (0.93–1.14)	
Sex		
Female	1.00	1.00
Male	9.30 (1.21–71.3)	18.1 (2.18–149.9)
Cigarette Smoking		
Never	1.00	
Ever	0.55 (0.15–2.03)	
Alcohol Use		
No	1.00	
Yes	3.86 (0.23–1.54)	
Cocaine Use		
Never	1.00	
Ever	0.80 (0.24–2.61)	
Diabetes		
No	1.00	
Yes	0.00 (0.00—not estimable)	
Hypertension		
No	1.00	
Yes	0.44 (0.10–2.03)	
Years of HIV Infection		
<15	1.00	
≥15	0.71 (0.24–2.14)	
eGFR, mL/min per 1.73 m^2	0.99 (0.98–1.02)	
hsCRP, mg/dL	1.05 (0.97–1.14)	
Serum 25(OH)D, ng/mL	0.87 (0.77–0.98)	
Vitamin D Deficiency	3.66 (1.11–12.1)	9.37 (2.23–39.4)
Systolic BP, mm Hg	1.00 (0.97–1.04)	
Diastolic BP, mm Hg	0.98 (0.94–1.03)	
Glucose, mg/dL	0.99 (0.97–1.02)	
BMI, kg/m^2	0.97 (0.89–1.06)	
Waist-to-Hip Ratio	4.20 (0.05–357.4)	
Baseline CD4 count, $cells/mm^3$	1.00 (0.99–1.00)	
Baseline Viral Load, copies/mL	0.90 (0.72–1.12)	
Total Cholesterol, mg/dL	1.12 (1.00–1.03)	
LDL-C, mg/dL	1.02 (1.00–1.03)	
HDL-C, mg/dL	1.00 (0.97–1.04)	
Triglycerides, mg/dL	1.00 (0.99–1.01)	
NRTI Use, mo		
Combivir	0.76 (0.17–3.45)	
Epzicom	0.49 (0.06–3.82)	
Trizivir	2.43 (0.29–20.0)	
Zerit	0.55 (0.07–4.26)	
Truvada	0.46 (0.14–1.50)	
NNRTI Use, mo		
Sustiva	1.46 (0.45–4.73)	
PI Use, mo		
Kaletra	0.86 (0.19–3.95)	
Lexiva	0.93 (012–7.22)	
Norvir	0.69 (0.23–2.09)	
Reyataz	0.51 (0.13–1.93)	
Use of Other ARTs, mo		
Isentress	0.50 (006–3.86)	
Duration of NRTI Use, mo	0.99 (0.98–1.00)	
Duration of NNRTI Use, mo	1.00 (0.99–1.01)	
Duration of PI Use, mo	1.00 (0.99–1.01)	
Duration of ART Use, mo	1.00 (0.99–1.01)	
Framingham Score	1.17 (1.02–1.34)	

*Vitamin D deficiency, serum 25(OH)D <10 ng/mL.

†The initial multivariate proportional hazards model included age, sex, cigarette smoking, serum 25(OH)D, vitamin D deficiency, SBP, DBP, glucose, LDL, triglycerides, total cholesterol, and Framingham risk score.

clinical characteristics of the study participants with or without incident subclinical CAD are presented in Table 1. Compared with those who remained free of subclinical CAD, those who developed subclinical CAD were more likely to have been male ($P = 0.001$) and have a lower vitamin D (serum 25[OH] D) level ($P = 0.029$). None of the participants experienced a cardiovascular event during the follow-up period.

According to the Framingham risk score algorithm, 109 (91.6%) of the 119 participants (54 of the 62 men and 55 of the 57 women) had a low risk of CAD; 12 (85.7%) of the 14 participants who developed subclinical CAD had a low risk of CAD.[12]

Incidence of Subclinical CAD

The total sum of person-years (PYs) of follow-up was 284.4. The mean (SD) follow-up time was 2.39 (1.26) years. Subclinical CAD was detected in 14 of the 119 patients on the second cardiac CT, yielding an overall incidence of 4.92 per 100 PYs (95% confidence interval [CI], 2.69–8.26).

Association Between Vitamin D Deficiency and Time to the Development of Subclinical CAD

Kaplan-Meier curves of time to the development of subclinical CAD by vitamin D deficiency status are presented in Figure 1. According to the log-rank test, the time to the development of subclinical CAD in those who were vitamin D deficient was significantly shorter than that in those who were not vitamin D deficient ($P = 0.024$).

Factors Associated With the Development of Subclinical CAD

By univariate Cox proportional hazards regression analyses, male sex, serum 25(OH)D level, vitamin D deficiency, baseline CD4 count, total serum cholesterol, LDL cholesterol, and Framingham risk score were associated with the development of subclinical CAD at 0.20 level or less.

The final Cox regression model indicated that male sex (adjusted hazard ratio [HR], 18.1 [95% CI, 2.18–149.9]) and vitamin D deficiency (adjusted HR, 9.37 [95% CI, 2.23–39.4]) were independently associated with subclinical CAD (Table 2).

DISCUSSION

Ours may be the first study to investigate the incidence of and the risk factors for subclinical CAD in HIV-infected African

Americans. The key strength of this report is that the study participants were cardiac CT-proved subclinical CAD-free participants. The first objective of this study was to estimate the incidence rate of subclinical CAD in HIV-infected African Americans. Although the prevalence of subclinical coronary atherosclerosis has been reported in HIV-infected African Americans, the incidence of subclinical CAD has not. Our findings demonstrate that the overall incidence of subclinical CAD in a population of HIV-infected African Americans is provocatively high—4.92 per 100 PYs (95% CI, 2.69–8.26)—despite that the participants in this study were at low risk according to the Framingham risk score.

The National Heart Lung and Blood Institute sponsored several studies in which the incidence of clinical CAD was estimated in the United States.[13] For example, the Atherosclerosis Risk in Communities study reported that the mean age-adjusted CAD incidence rates per 1000 PYs were 1.06 and .51 in African American men and women aged 45 to 64 years, respectively.[13] Nevertheless, the incidence of subclinical CAD in African American population has not been reported. Because African Americans have the highest overall CAD mortality rate of any ethnic group in the United States and acute myocardial infarction or sudden cardiac death is often the first clinical manifestation of CAD in up to 50% of patients, early detection of subclinical disease, before the clinical CAD occurs, and the early identification of the risk factors of subclinical CAD may be critical in this population.[9,14,15]

The second objective of this study was to identify the risk factors of subclinical CAD in HIV-infected African Americans. We found that male sex was independently associated with development of subclinical CAD. Although it has been reported that males are at a higher risk of developing CAD in general populations,[16] this study suggests that the risk associated with the development of subclinical CAD in HIV-infected African American men is far greater than that in HIV-infected African American women: the male sex was associated with an 18-fold increased risk of developing subclinical CAD compared with the female sex. Further studies are needed to examine the magnitude of relative risk of developing subclinical CAD of men and women. Although one's sex is not a modifiable risk factor, this finding is useful in identifying those who might benefit from intensive primary prevention/intervention.

The present study also suggests that vitamin D deficiency is significantly associated with the development of subclinical CAD in HIV-infected African Americans. The effects of vitamin D deficiency on clinical CAD in general populations have been widely investigated. A recent published study analyzed a large electronic medical records database to determine the prevalence of vitamin D deficiency and the relation of vitamin D levels to prevalent and incident CV risk factors and diseases in a general health care population.[17] This study revealed that vitamin D deficiency was significantly associated with increases in the prevalence of diabetes, hypertension, hyperlipidemia, and peripheral vascular disease, and that vitamin D levels were also highly associated with coronary artery disease, myocardial infarction, heart failure, and stroke, as well as with incident death, heart failure, coronary artery disease/myocardial infarction, and stroke.[17] The Framingham Offspring Study, which evaluated 1739 study participants without prior cardiovascular disease, reported that vitamin D deficiency (defined as 25[OH] vitamin D <10 ng/mL) was associated with an increased risk of developing a first cardiovascular event after 5 years of follow-up compared with subjects with 25(OH) vitamin D levels greater than 15 ng/mL (HR, 1.80; 95% CI, 1.05–3.08).[18] A recent study of ours suggests that vitamin D deficiency (adjusted odds ratio, 2.18; 95% CI, 1.07–4.43) is independently associated with the presence of significant coronary stenosis after controlling for traditional risk factors in cocaine users.[19]

According to the present study, vitamin D deficiency is associated with a 9-fold increased risk of developing subclinical CAD. This finding has not been reported before and suggests new approaches to prevention of CAD and opportunities to investigate the mechanisms responsible for the development of subclinical CAD.

The mechanisms explaining the association between vitamin D deficiency and the development of subclinical coronary disease are unclear. It has been reported that low circulating 25(OH) D levels are associated with several risk factors for CAD, such as obesity, diabetes, hypertension and dyslipidemia, and inflammatory markers, such as C-reactive protein and IL-6.[20–23] A large cohort study of 3258 patients scheduled for coronary angiography with a median follow-up period of 7.7 years found that low 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels are independently associated with all-cause and cardiovascular mortality.[24] Nevertheless, elevating these risk factors by low vitamin D levels may not be sufficient to explain why vitamin D deficiency is associated with the rapid development of coronary atherosclerosis.

The mechanisms that contribute to vitamin D deficiency and early atherosclerosis remain unclear. Several studies demonstrate that vitamin D modulates the immune system through multiple mechanisms. In fact, some of these mechanisms, such as dendritic cell activation, induction of T-cell responses, and cytokine production, are associated with early stages of atherosclerosis.[25,26] Therefore, the severely depressed levels of vitamin D in HIV patients with subclinical CAD noted in our study may be a reflection of an up-regulation of immune responses and inflammation.

Activated vitamin D ($1,25(OH)_2$ D) and its analogs have been reported to inhibit proliferation of mitogen-activated vascular smooth muscle cell proliferation in vitro[27–30] and to inhibit ET-induced activation of Cdk2 activity in neonatal rat vascular smooth muscle cells.[30] Recently, it was shown that 1,25 dihydroxyvitamin D ($1,25[OH]_2D$) inhibits vascular smooth muscle cell proliferation through a cell division cycle 25 homolog A-dependent mechanism, suggesting that this hormone may prove useful in the management of disorders characterized by aberrant proliferation of vascular smooth muscle cells in the vascular wall.[31] Further studies are certainly warranted.

This study found no evidence suggesting CD4 count, HIV viral load, ART use, or cocaine use were significantly associated with subclinical CAD. A study with a larger sample size is needed to examine the effects of CD4 count, HIV vital load, and cocaine use on the development of subclinical CAD.

According to the Framingham risk score algorithm, 109 (91.6%) of the 119 participants (54 of the 62 men and 55 of the 57 women) in this study were at low risk of CAD, and 12 (85.7%) of the 14 participants who developed subclinical CAD were at low risk of CAD. Thus, the conventional preventive strategies to reduce traditional risk factors may not be effective in this population.

To our best knowledge, there are only 2 published studies evaluating the effect of vitamin D supplementation on surrogate markers of CAD. One examined the effect of 16-week vitamin D supplementation on flow-mediated dilation in overweight African American adults.[32] The other trial was performed in African American adolescents aged 14 to 18. The outcome variables were adiposity and arterial stiffness.[33]

We recognize several limitations to the present study. First, the participants were not a random sample of African Americans with HIV infection; and therefore, the results may not be generalizable to other populations. Second, because most of the

participants were cigarette smokers and/or cocaine users, the effects of cigarette smoking or cocaine use on subclinical CAD or the joint effects of vitamin D deficiency and cigarette smoking or cocaine use on subclinical CAD could not be fully evaluated, either individually or combined. Third, the sample size was small and the follow-up time was short. Thus, the confidence intervals of the HRs are quite wide, indicating great uncertainty in the point estimate of HRs. Owing to the small sample size and the low number of end-points, the generalizability of the findings is quite limited. The study was underpowered to fully evaluate risk factors that may be associated with the development of incident CAD. Fourth, since this study was performed in African Americans living in inner-city Baltimore, the effects of some socioeconomic variables on subclinical CAD could not be completely controlled for. Fifth, we only had one reviewer for the CAC results. In addition, because 8 of 9 participants developed CAC of less than 10, our study may suffer from interscan variation in CAC. It has been reported that the largest score variation in CAC occurred in the left main coronary artery.[34] No CAC in our study was identified in the left main coronary artery. Lastly, because the baseline data in this prospective study were not collected at the time of diagnosis of HIV infection, the effect of HIV infection and ART use on vitamin D levels could not be evaluated.

Despite its limitations, this study provides a disturbing estimate of the incidence of subclinical CAD in HIV-infected African Americans. This high incidence of subclinical CAD may highlight the urgent need for an identification of high-risk groups in African Americans with HIV infection.

Although this study suggests that vitamin D deficiency is an independent risk factor for subclinical CAD, further studies with a larger sample size should be conducted to validate our findings. Because there are no adequate data to support vitamin D supplementation to reduce cardiovascular risk in HIV-infected individuals, well-designed randomized clinical trials may be urgently needed to examine the safety and efficacy of vitamin D supplementation in HIV-infected persons who are vitamin D deficient.

ACKNOWLEDGMENTS

The authors thank the study participants for their contributions.

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