Immune Activation Biomarkers, Inflammatory Markers, and Subclinical Atherosclerosis among People Living with HIV in Kenya

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Abstract

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Introduction: People living with HIV (PLWH) are at a high risk of developing cardiovascular disease (CVD). Interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and high-sensitivity C-reactive protein (hsCRP) have been associated with the onset of subclinical atherosclerosis. We assessed the association between immune biomarkers and carotid intima media thickness (CIMT), an indicator of subclinical atherosclerosis, among HIV-negative individuals and PLWH in Kisumu, Kenya.
Methods: In a cross-sectional study among adults seeking care at Kisumu Hospital in Kenya, we measured CIMT using ultrasound and analyzed concentrations of IL-1β, IL-6, TNF-α, and hsCRP using multiplex immunoassay. CIMT and concentrations of immune markers between the two groups were compared using Kruskal-Wallis test and multivariate linear regression was used to assess the associations between CIMT and immune markers after adjusting for traditional CVD risk factors.
Results: 117 HIV-negative individuals and 145 PLWH had CIMT and immune marker data available and were included in the analyses. Participants had a mean CIMT of 0.42 mm (SD: 0.12) and there was a trend for PLWH to have lower CIMT than HIV-negative participants (0.41 mm; SD: 0.11 vs 0.44 mm; SD: 0.14; p = .056). Differences in the concentrations of IL-1β, IL-6, TNF-α, and hsCRP by HIV status were not statistically significant. There was an association between IL-1β an CIMT: a 1 pg/ml increase in IL-1β was associated with a decrease in CIMT of 0.0397 mm (-0.0794, -0.0001; p = .05). IL-6, TNF-α, and

hsCRP concentrations were not significantly associated with CIMT. Higher serum levels of IL-1β were associated with lower CIMT measurements among HIV-negative persons, but not among PLWH.

Conclusion: We found that higher plasma levels of IL-1β, IL-6, TNF-α, and hsCRP were not associated with higher CIMT, however an increase in IL-1β was consistently associated with lesser CIMT values. There was no significant association between IL-1β and CIMT among PLWH.

INTRODUCTION

People living with HIV (PLWH) are at an elevated risk of cardiovascular disease (CVD), mainly due to the atherosclerotic disease. Evidence shows that the relative risk of acute myocardial infarction and coronary heart disease in PLWH is nearly twice that of HIV- negative individuals.[1] These findings suggest that HIV infection may explain the elevated risk, which has been demonstrated in multiple studies.[2–4] To elucidate bimolecular mechanisms linking HIV infection and increased CVD risk, several studies have been conducted examining the role of immune activation on cardiovascular disease. Evidence shows that PLWH have higher levels of systemic inflammatory biomarkers, with or without HIV viral load suppression.[5–7] Further evidence suggests that the high burden of CVD among PLWH may in part be due to adaptive and innate immunity abnormalities and, potentially, monocyte/macrophage-mediated endothelial dysfunction, contributing to atherosclerotic lesions and thrombotic events.[8,9] These biomolecular pathways may explain excess CVD risk, which is not fully explained by the traditional risk factors, such as dyslipidemia, hypertension, age, cigarette smoking, diabetes mellitus, obesity, and physical inactivity.[10,11] One of the inflammatory biomarkers associated with increased risk of cardiovascular disease in the general population and PLWH is C-reactive protein (CRP).[12–14] CVD risk has also been shown to increase with increasing levels of immune activation biomarkers, such as IL-1β, IL-6, and TNF-α.[15,16]

Subclinical atherosclerosis is an early sign of atherosclerotic disease.[17] Therefore, early detection of subclinical atherosclerosis is crucial to slowing down progression to overt cardiovascular and preventing CVD. Carotid intima-media thickness, measured in B-mode ultrasound images of the carotid arteries, is considered an important indicator of atherosclerosis, a strong predictor of future cardiovascular events, and a surrogate endpoint in interventional studies to assess the efficacy of pharmacotherapy.[18,19] Additionally, measuring CIMT is safe, non-invasive, and cost-effective[20]—making it an ideal choice for assessing subclinical atherosclerosis in low-resource settings like sub-Saharan Africa (SSA).

Studies linking immunological markers and subclinical atherosclerosis are overwhelmingly from high-income countries—characterized by a low burden of HIV. Additionally, these studies have shown inconsistent findings on the association between markers of inflammation, such as CRP and IL-6, and

cardiovascular events.[21,22] Limited cross-sectional studies from SSA countries did not find an association between CRP and CIMT.[23–26] However, one prospective, longitudinal study of PLWH conducted in Uganda found that IL-6 levels six months after ART initiation were associated with higher CIMT values. Studies assessing the association between TNF-alpha and IL-1-beta with CIMT are limited. The high HIV burden and improved life expectancy of PLWH have increased the CVD burden in SSA. The prevalence of HIV/AIDS in Kenya is still high, and advances in ART treatment have prolonged life among people living with HIV, making this population more vulnerable to cardiovascular disease.[27]

We investigated the association between subclinical atherosclerosis and IL-1β, IL-6, TNF-α, and hsCRP and determined whether this association is different between PLWH and HIV-negative persons. We hypothesized that higher concentrations of the immune markers would be associated with higher CIMT values and that these associations will be different by HIV status.

METHODS

Study Design and Setting

A cross-sectional study was conducted between September 2017 and May 2018. This is a secondary analysis of the data collected. This study included 300 PLWH and 300 HIV-negative individuals recruited at Kisumu County Hospital, a tertiary, public county referral facility in Western Kenya. HIV prevalence in the region is high (17.5%).[28] Being at least 30 years old and living within a 50-kilometer radius of the hospital were the criteria for participation in the primary study. Participants were only included if they had been taking ART for at least 6 months and attended a HIV Comprehensive Care Clinic (CCC). Study subjects that met the eligibility criteria and provided informed consent were enrolled by a study nurse from the HIV clinic. From HIV testing centers, participants with HIV-negative status were recruited until the sample size was reached. Informed consent was obtained from all participants prior to any study procedures or data collection. Ethical and scientific review committee approval was obtained from Kenyatta National Hospital/University of Nairobi as well as the University of Washington Institutional Review Board. The primary study had 80% power to detect a mean change in CIMT of 0.04 mm, with a minimum necessary sample size of 110 participants in both groups.

Study Procedures

A detailed description of the procedures for this cross-sectional study has been published elsewhere.[29] In summary, participant demographic and health data were collected using validated WHO STEPS questionnaires modified to fit the Kenyan context.[30] In addition to physical examinations, which included anthropometric measurements, laboratory investigations and medical chart abstraction were conducted. Following the initial visit, participants were requested to return for a carotid ultrasound or blood draw after fasting for 8 hours, if they had not done so already.

CIMT Measurement

Radiologic technologists from Kisumu Hospital received training in imaging technique, using a Sonosite M Turbo ultrasound machine (FUJIFILM, Sonosite Inc., Bothell) equipped with a HFL38X/13-6 MHZ transducer, as well as interpretation of sonographic images. A radiologist experienced in performing carotid ultrasounds and measuring CIMT for research purposes provided training and quality assurance for radiographers.[23] This study was conducted in accordance with the standards of the American Society of Echocardiography (ASE) for measuring CIMT.[31] Images were acquired and reported using the Sonosite Software program (SonoCalc V5.0.0.12).

The participant was positioned in a supine position, with the head slightly tilted upward and the neck rotated opposite to the side being examined to screen for changes in wall thickness and plaques in the carotid arteries. The probe was then adjusted and advanced slowly to examine both near and far walls. The probe was subsequently oriented to acquire the maximum diameter of the arterial lumen in the transverse plane. If narrowing was determined, pre- and post-stenosis blood flow velocities was recorded. Following the plaque screening and description, longitudinal images of the common carotid artery (CCA) from three planes (optimal angle of incidence and two complementary angles at anterior, lateral, and posterior angles)—10 mm distal to the CCA—were obtained.[32] A mean value for each portion of the blood vessel was calculated and the average of all readings was reported as overall CIMT for the CCA.[31]

A record of the ultrasound images was created, stored, and transmitted off-site after each ultrasound examination. The research radiologist then reported the findings, and a formal report was prepared using SonoCalc V50.0.12. To maximize inter-rater reliability, all reports were interpreted by a single research

radiologist. A program assistant at the International AIDS Research and Training Program at the University of Washington was responsible for managing the study data.[32]

Laboratory Procedures

As part of the study, blood samples were collected at least eight hours after fasting to measure lipids (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides), glucose, inflammatory markers, CD4 counts, and viral loads for PLWH. Blood samples were then processed to obtain serum, which was then stored at -80 degrees Celsius at the Kenya Medical Research Institute (KEMRI). Absolute CD4+ T-cell count was performed at the KEMRI laboratory in Kisumu, along with HIV-1 RNA viral load testing. HIV RNA viral load titres below 50 copies per milliliter were considered undetectable. Flow cytometry was used to determine absolute CD4+ cell count. The samples were then batched and sent to the University of Washington, Seattle, for analysis of lipids, glucose, and inflammatory markers. Using an automated Beckman Coulter, Inc. Brea, CA AU5812, serum lipid profile, glucose, and hsCRP lab investigations were performed at the University of Washington Research Testing Services. In accordance with the manufacturer's instructions, all samples were tested in duplicate.[5]

Cytokine Measurements

Using a Mesoscale Discovery, Rockville, Maryland (MSD) VPlex Proinflammatory Panel 1 human kit, serum samples were analyzed for levels of IL-1β, IL-6, and TNF-α. A reanalysis was conducted if the coefficient of variation of the samples was greater than 0.3. For duplicate samples with a lower coefficient of variation, a mean was obtained. The lower limit was used if a biomarker level fell below the lower threshold of detection. Lower thresholds of detection concentrations were 0.2 mg/L, 0.01 pg/mL, 0.05 pg/mL, and 0.01 pg/mL for hsCRP, IL-1β, IL-6, and TNF-α, respectively.[5]

Primary Outcomes and Independent Variables

The primary outcome of this study was carotid intima-media thickness (CIMT). Our analyses treated CIMT as a continuous variable with a normal distribution. The predictors included concentrations of hsCRP, IL-1, IL-6, and TNF-α. Multivariable models were adjusted for age, sex, hypertension, diabetes, tobacco smoking, alcohol consumption, BMI, and lipids (LDL, total cholesterol, triglycerides, and HDL), and HIV

status. Several of these confounding factors were identified a priori and are supported by existing research. The study assessed HIV status as a potential effect modifier.

Statistical Analysis

Normally distributed continuous variables were summarized using means and standard deviations. Continuous variables with a non-normal distribution were summarized using medians and interquartile ranges (IQR). Proportions were used to summarize categorical variables. The Kruskal-Wallis test was used to compare continuous demographic and clinical characteristics, after determining that all variables, except CD4+ count, were not normally distributed. Categorical characteristics were compared using the chi-square test.

A linear regression was used to assess the association between CIMT and inflammatory and immune activation markers. A number of univariate linear regression models were developed to assess the association between CIMT and immune markers, as well as other variables (see Appendix 2). In order to assess the association between CIMT and immune markers, several unadjusted and adjusted multivariate linear regression models were constructed (see Tables 2 and 3). Final adjusted multivariate linear regression model was built after dropping variables alcohol consumption and triglycerides levels. To determine whether HIV status modified the association between CIMT and interleukin 1, we added an interaction term between IL-1β and HIV status to the model. The final mode adjusted for HIV status, age, sex, hypertension, diabetes, tobacco smoking, BMI, total cholesterol, and HDL. Subclinical atherosclerosis was defined using a cut-off value of CIMT (≥0.78 mm), as one study found that, on average, a healthy adult reaches a CIMT of 0.78 mm at the age of 76 years.[33] Participants with missing data were excluded from the analysis.

The regression coefficients were reported along with their 95% confidence intervals and p-values. A significance level of 0.05 was used in all analyses. The analyses were conducted using R (version 4.2.0, R foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of Study Participants

The study recruited 600 participants. However, after validation, only 262 of them were eligible, with similar proportions of female and male participants, 51.5 and 48.5 percent respectively. Of these, 117 were HIV-negative and 145 were HIV positive (Table 1). Female participants represented more than half of the PLWH group (55.9% vs. 44.1%). The median age was lower among PLWH (54 years; Interquartile range [IQR] = 44, 58) vs. 57 years; IQR = 44, 67, p-value = .003).

Less than 25 percent (n = 64) had elevated blood pressure—defined as either systolic ≥140mmHg and/or diastolic ≥90mmHg. Compared to HIV-negative participants, PLWH group had lower prevalence of hypertension (p-value < .001). On the other hand, only 2.4 percent of the participants were diabetic. 21.0 (n = 55) and 12.6 (n = 33) percent of the participants were overweight and obese, respectively. 43.6% of the study subjects were abdominally obese, defined as a waist to hip ratio above 0.90 in males and 0.85 in females.[34] Only five percent were smokers. 34 percent of the participants had consumed alcohol in the past 12 months. None of the participants followed the recommended healthy diet—defined as consuming at least five servings of fruits and vegetables per day. There were no significant differences in tobacco smoking, diet, physical activity, alcohol consumption in the past 12 months between HIV-negative individuals and persons living with HIV.

Overall, most of the participants had normal total cholesterol (77.2%), LDL cholesterol (81.7%), and triglycerides (90.7%), and HDL (71.5%) serum levels. Persons living with HIV had higher triglycerides levels, and this difference was statistically significant. Also, PLWH had lower serum HDL cholesterol levels compared to HIV-negative participants, but this difference was borderline significant. Differences in total and LDL cholesterol between the two groups were not significant.

PLWH had been on antiretroviral treatment for a median of 9 years (IQR = 4, 11). All 139 HIV-positive participants were virally suppressed—defined as viral load <1000 copies/ml—and had a mean CD4+ count of 504 cells/mm3 (SD = 235).

The study participants had median (IQR) IL-1β, IL-6, TNF-α, and hsCRP levels of 0.08 pg/mL (0.05, 0.22), 1.01 pg/mL (0.52, 1.95), 2.98 pg/mL (1.97, 4.16), and 1.90 mg/mL (0.80, 4.40), respectively. PLWH

had higher median IL-1β, IL-6, and hsCRP levels compared to HIV-negative participants. (Figure 1) On the other hand, this group had lower TNF-α levels than the HIV-negative group. However, these differences were not statistically significant.

Overall, the study subjects had a mean CIMT of 0.42 mm (SD: 0.12). PLWH had a lower mean carotid intima-media thickness compared to the HIV-negative participants; 0.41 mm (SD: 0.11) vs. 0.44 mm (0.14). This difference did not reach significance but there was a trend noted (p-value = 0.056). Only four of the participants had subclinical atherosclerosis—defined as CIMT ≥0.78 mm. All the subjects with subclinical atherosclerosis were HIV-negative.

Table 1. Characteristics of 262 Study Participants Stratified by HIV Status

	Total (N=262)	**HIV-negative (N=117)**	**HIV-positive (N=145)**	**p-value**
	Mean (SD), Median (IQR) or Number (%)			
Sociodemographic characteristics				
Age	54 (44, 62)	57 (44, 67)	53 (44, 58)	**0.003**
Sex				0.118
Female	135 (51.5%)	54 (46.2%)	81 (55.9%)	
Male	127 (48.5%)	63 (53.8%)	64 (44.1%)	
Carotid intima-media thickness				
CIMT	0.42 (0.36, 0.48)	0.43 (0.37, 0.52)	0.42 (0.36, 0.47)	0.056
Subclinical Atherosclerosis (CIMT > 0.78 mm)				**0.025**
Normal	258 (98.5%)	113 (96.6%)	145 (100.0%)	
Subclinical Atherosclerosis	4 (1.5%)	4 (3.4%)	0 (0.0%)	
Immunological biomarkers				
Diabetes				0.246
Nondiabetic	240 (97.6%)	103 (96.3%)	137 (98.6%)	
Diabetic	6 (2.4%)	4 (3.7%)	2 (1.4%)	
Hypertension				**0.015**
Non hypertensive	198 (75.6%)	80 (68.4%)	118 (81.4%)	
Hypertensive	64 (24.4%)	37 (31.6%)	27 (18.6%)	
BMI	23.03 (20.12, 26.33)	23.22 (21.03, 26.37)	22.88 (19.86, 26.32)	0.216
Obesity				0.415
Healthy weight	148 (56.5%)	64 (54.7%)	84 (57.9%)	
Underweight	26 (9.9%)	12 (10.3%)	14 (9.7%)	
Overweight	55 (21.0%)	22 (18.8%)	33 (22.8%)	
Obese	33 (12.6%)	19 (16.2%)	14 (9.7%)	
Current Smoking History				0.209
No	249 (95.0%)	109 (93.2%)	140 (96.6%)	
Yes	13 (5.0%)	8 (6.8%)	5 (3.4%)	

Serum Lipids				
Total Cholesterol Levels				0.844
Normal Total Cholesterol	190 (77.2%)	82 (76.6%)	108 (77.7%)	
Elevated Total Cholesterol	56 (22.8%)	25 (23.4%)	31 (22.3%)	
Triglycerides Levels				**0.027**
Normal Triglycerides	223 (90.7%)	102 (95.3%)	121 (87.1%)	
Elevated Triglycerides	23 (9.3%)	5 (4.7%)	18 (12.9%)	
HDL Levels				0.062
Normal HDL	176 (71.5%)	70 (65.4%)	106 (76.3%)	
Low HDL	70 (28.5%)	37 (34.6%)	33 (23.7%)	
LDL Levels				0.254
Normal LDL	201 (81.7%)	84 (78.5%)	117 (84.2%)	
Elevated LDL	45 (18.3%)	23 (21.5%)	22 (15.8%)	
Characteristics of PLWH				
Nadir CD4 count	503.50 (234.55)	NA	503.50 (234.55)	
Virally suppressed				**< 0.001**
Yes	139 (100.0%)	NA	139 (100.0%)	
Time on ARV Treatment	108 (48, 132)	NA	108 (48, 132)	

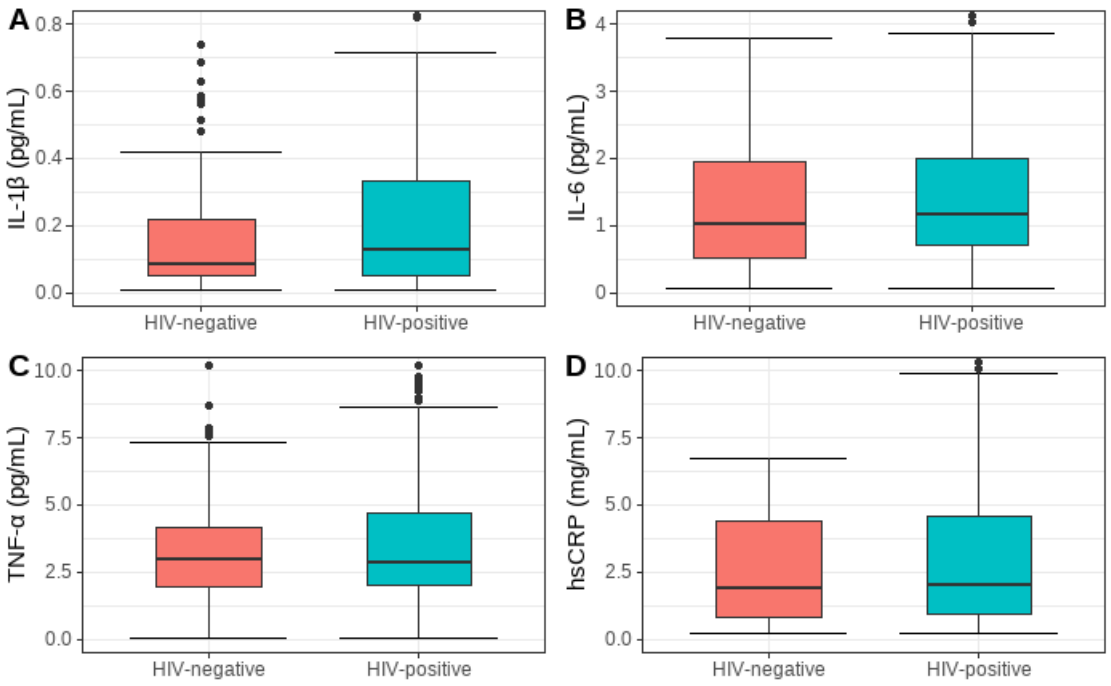

Figure 1. Immune activation biomarkers and inflammatory markers in HIV-negative and HIV-positive study participants. Abbreviations: IL-1β: interleukin 1 beta; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; hsCRP: high-sensitivity C-reactive protein

Subclinical Atherosclerosis and Immune Markers

Overall, higher values of CIMT were associated with lower levels of interleukin-1, TNF- α, and hsCRP (Appendix 2). This association was the strongest between CIMT and interleukin 1-beta, where the increase in IL-1β by one pg/ml was associated with a reduction of the carotid intima-media thickness by 0.04 mm (95% CI: -0.079, 0.003). However, none of these associations were significant, except for the association between CIMT and IL-1β, which was borderline significant (p-value = .068). On the other hand, interleukin-6 was associated with an increase in CIMT by 0.003 mm. This association was not statistically significant.

In the initial unadjusted multivariate linear regression model of the entire study cohort, which included the immunological markers as independent variables, IL-1β, TNF- α, and hsCRP were associated with a decrease in the CIMT. The association was again the strongest between CIMT and interleukin 1-beta, where one pg/ml increase in IL-1β was associated with a decrease in the carotid intima-media thickness by 0.04 mm (95% CI: -0.0812, 0.0028). This association was borderline significant (p-value = .067). Conversely, interleukin-6 was associated with an increase in the carotid intima-media thickness. When comparing individuals that differed in IL-6 by one pg/ml, those with higher values had carotid intima-media 0.005 mm (95% CI: 0.0005, 0.0101) thicker. This association was statistically significant (p-value = .032).

After adjusting for the traditional CVD risk factors (age, sex, hypertension, diabetes, tobacco smoking, total cholesterol), BMI, HDL, triglycerides, and alcohol consumption in past 12 months, and dropping the association between CIMT and interleukin 1-beta was statistically significant, where CIMT values decreased by 0.042 mm (95% CI: -0.08329, -0.00074) with one pg/ml increase in IL-1β. On the contrary, increase in interleukin-6 by one pg/ml was associated with a 0.002 mm (95% CI: -0.00261, 0.00671) increase in CIMT. However, this association was not statistically significant (p-value = .386).

In the final adjusted model, an increase in interleukin-1-beta was significantly associated with a decrease in CIMT. One pg/ml increase in IL-1β decreased CIMT by 0.04 mm (95% CI: -0.0794, -0.0001; p-value = .05).

Table 2. Association between CIMT and immune markers

	Unadjusted model		Adjusted model*		Adjusted model**	
Variable	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Interleukin-1β	-0.0392 (-0.0812, 0.0028)	.067	-0.04201 (-0.08329, -0.00074)	**.046**	-0.0397 (-0.0794, -0.0001)	**.05**
Interleukin-6	0.0053 (0.0005, 0.0101)	**.032**	0.00205 (-0.00261, 0.00671)	.386		
TNF-alpha	-0.0038 (-0.0096, 0.0019)	.192				
hsCRP	-0.0005 (-0.0017, 0.0008)	.469				
Adjusted R^2	0.0252		0.1191		0.173	

*CIMT and Interleukin-1 and Interleukin-6, adjusting for the traditional CVD risk factors, triglycerides, and alcohol consumption
**CIMT and Interleukin-1, adjusting for the traditional CVD risk factors

Subclinical Atherosclerosis and Immune Markers among People Living with HIV

Overall, CIMT in PLWH was 0.036 mm lower (95% CI: -0.067, -0.006). This difference was statistically significant (p-value = .019). Nadir CD4 count was associated with increase in CIMT. Increase in the duration on ART treatment was associated with the decrease in CIMT. (Table 3). However, this association was not statistically significant.

When adjusting for HIV status and the traditional CVD risk factors, increase in IL-1β, hsCRP, and TNF-α is associated with a decrease in carotid intima-media thickness (Table 3). Of the three immune markers, interleukin-1 had the strongest association with CIMT. An increase in interleukin-1 by one unit was associated with a decrease in CIMT by 0.0371 mm (95% CI: -0.0790, 0.0048). This association was borderline significant (p-value = .082), while the other two associations were not significant. On the other hand, increase in IL-6 by one unit significantly increased CIMT values by 0.005 mm (95% CI: 0.0001, 0.0098); p-value = .044.

When adjusting for HIV status, age, sex, hypertension, diabetes, tobacco smoking, total cholesterol, BMI, and HDL, increase in interleukin-1-beta is associated with a significant decrease in CIMT by 0.0649 mm (95% CI: -0.1248, -0.0050, p-value = .034).

Table 3. Association between CIMT and immune markers in the presence of HIV infection

	Unadjusted model		**Adjusted model***		**Adjusted model****	
Variable	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Interleukin-1β	-0.0371 (-0.0790, 0.0048)	.082	-0.0386 (-0.0797, 0.0025)	**.065**	-0.0649 (-0.1248, -0.0050)	**.034**
Interleukin-6	0.0050 (0.0001, 0.0098)	**.044**	0.0020 (-0.0027, 0.0066)	.402		
TNF-alpha	-0.0029 (-0.0087, 0.0030)	.332				
hsCRP	-0.0005 (-0.0018, 0.0007)	.413				
HIV-positive			-0.0352 (-0.0716, 0.0011)	.057	-0.042 (-0.0817, -0.0022)	**.039**
Adjusted R^2	0.0335		0.1322		0.1837	

*CIMT and Interleukin-1 and Interleukin-6, adjusting for HIV and the traditional CVD risk factors, triglycerides, and alcohol consumption
**CIMT and Interleukin-1, adjusting for HIV and the traditional CVD risk factors

Among the HIV-negative individuals, CIMT was significantly associated with IL-1β. One-unit increase in interleukin-1 was associated with a decrease in CIMT by 0.065 mm (p-value = .046) (Table 4). In PLWH, none of the immune markers were significantly associated with CIMT.

Table 4. Association between CIMT and immune markers in PLWH and HIV- persons

	HIV-negative			**HIV-positive**		
	Unadjusted model	Adjusted model*	Adjusted model**	Unadjusted model	Adjusted model*	Adjusted model**
	Coefficient (p-value)			Coefficient (p-value)		
Interleukin 1-β	-0.060 (.089)	-0.065 (.063)	-0.065 (**.046**)	-0.008 (.782)	-0.012 (.678)	-0.014 (0.573)
Interleukin 6	0.005 (.050)	0.003 (.236)		-0.003 (.763)	-0.0001 (.990)	
TNF-α	-0.011 (.152)	-0.012 (.157)		0.0005 (.905)	-0.002 (.679)	
hsCRP	-0.001 (.286)	-0.001 (.444)		0.0004 (.800)	0.00003 (.986)	
Adjusted R^2	0.057	0.235	0.295	-0.038	0.013	0.052

*A multiple linear regression model between CIMT and Interleukin-1 and Interleukin-6, adjusting for the traditional CVD risk factors triglycerides, and alcohol consumption
**A multiple linear regression model between CIMT and Interleukin-1, adjusting for the traditional CVD risk factors

DISCUSSION

In this cross-section study conducted in Kenya, we found that higher levels of immune activation biomarkers and inflammatory markers were not associated with higher values of carotid intima-media thickness. Interestingly, one of immune activation biomarkers, interleukin-1β, was negatively associated with the CIMT values. This association persisted even after adjusting for traditional risk factors for CVD, namely age, sex, hypertension, diabetes, obesity, smoking, and dyslipidemia, and HIV status. Upon stratifying the study participants by HIV status, all the immune markers were not significantly associated

with CIMT, except for interleukin-1β, which was negatively associated with CIMT measurements among HIV-negative individuals only.

Several studies conducted in Sub-Saharan Africa on the association between CIMT and C-reactive protein and interleukin-6 have yielded similar findings. The SABPA Study in South Africa examining the role of inflammation on the development of atherosclerosis among HIV-negative individuals did not find association between both C-reactive protein and interleukin-6 and carotid intima-media thickness.[35] Ssinabulya et al. found no association between high sensitivity C-reactive protein and subclinical atherosclerosis among PLWH in Uganda.[23] Another study in Uganda found that IL-6 was not significantly associated with subclinical atherosclerosis among HIV patients receiving suppressive ART treatment.[26] To the best of our knowledge, no single study in SSA has assessed the association between subclinical atherosclerosis and TNF-α. Similar findings on the association between CIMT and hsCRP, TNF-α, and IL-6 were also observed in studies conducted in high-income countries (HICs). In two studies conducted in Italy, CIMT measurements were not found to be significantly associated with IL-6, TNF-α, and hsCRP.[36,37] In the Netherlands, a large prospective population-based cohort study of 7,983 participants above 55 years of age found that IL-6 was not independently associated with CIMT.[38] Several studies in the United States did not find significant association between CIMT and IL-6 and hsCRP.[39–41] Ross et al. found that TNF-α was associated with CIMT in internal carotid artery, but not in the common carotid artery.[40]

An unexpected finding in our study is that increased concentrations of serum IL-1β were associated with the decrease in carotid intima-media thickness. This is not consistent with existing evidence, where IL-1β—as a pro-inflammatory cytokine—has consistently been associated with the onset of atherosclerosis and subsequent cardiovascular disease.[42–44] The difference in the association when comparing PLWH and HIV-negative participants may be explained by the distribution of the concentrations of IL-1β and CIMT measurements in this groups. HIV-negative study subjects had higher CIMT values and lower serum IL-1β levels, leading to negative correlation and association. Possible explanation for higher CIMT values in the HIV-negative persons is poor CVD risk management compared to the PLWH, who are more likely to access care and receive counseling which can, subsequently, lead to behavioral changes associated with healthy lifestyles. In this study, access to quality care and better CVD risk management

may be reflected by the observed 100 percent viral suppression rate and less prevalence of CVD risk factors among PLWH. On the other hand, higher plasma IL-1β levels among PLWH may be explained by increased interleukin-1β synthesis induced by HIV infection.[45,46] Lastly, disproportionate number of missing IL-1β among PLWH in our study may have led to the observed distribution. (Appendix 2). Population studies assessing the association between CIMT and IL-1β are scarce.

Our study has several strengths. We included both HIV-negative and HIV-positive individuals. PLWH had been on ART treatment for at least 6 months, and all had suppressed viral load. We analyzed several immune markers to determine associations with CIMT. Lastly, we adjusted for traditional CVD risk factors as well as other potential confounders. There are two limitations to this study. This is a cross-sectional study; thus, we cannot establish a cause-and-effect relationship on the observed associations. A follow up longitudinal study may help address that limitation. We also had instances of missing data which were not imputed. This may have impacted the observed results negatively.

CONCLUSION

Our cross-sectional study conducted among HIV-negative and HIV-positive individuals in Kisumu, Kenya, found that higher plasma levels of IL-1β, IL-6, TNF-α, and hsCRP were not associated with higher carotid intima-media thickness. On the contrary, increase in IL-1β was consistently associated with the decrease in the thickness, even when we adjusted for HIV status and the traditional cardiovascular risk factors. Among HIV-negative participants, higher serum levels of IL-1β were associated with lower CIMT measurements. Overall, the prevalence of subclinical atherosclerosis in our study sample was very low, which may be attributed to the characteristics of our sample population and better management of cardiovascular risk factors. A subsequent longitudinal study with a larger study sample may further elucidate the relationship between immune markers and subclinical atherosclerosis.

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APPENDICES

Appendix 1: Missing data

Variable	Total (N=262)	HIV-Negative (N=117)	HIV-Positive (N=145)
Interleukin-1β	69	25	44
Interleukin 6	17	11	6
TNF-α	16	10	6
hsCRP	16	10	6
Blood Glucose	16	10	6
Diabetes	16	10	6
Recommended Physical Activity	226	102	124
Recommended Healthy Diet	8	7	1
Total Cholesterol	16	10	6
Triglycerides	16	10	6
HDL	16	10	6
LDL	16	10	6
Virally Suppressed	6	NA	6

Appendix 2: Univariate linear regression models

Variable	N	Coefficient	95% CI[1]	p-value
Interleukin-1	193	-0.03824	-0.07936, 0.00289	0.068
Interleukin-6	245	0.00304	-0.00143, 0.00751	0.182
TNF-alpha	246	-0.00366	-0.00878, 0.00145	0.159
hsCRP	246	-0.00016	-0.00134, 0.00102	0.786
Age	262	0.00175	0.00057, 0.00292	**0.004**
Sex	262			
Female		—	—	
Male		-0.01823	-0.04855, 0.01208	0.237
Diabetes	246			
Nondiabetic		—	—	
Diabetic		0.12292	0.02161, 0.22424	**0.018**
Hypertension	262			
Non hypertensive		—	—	
Hypertensive		0.04993	0.01511, 0.08476	**0.005**
BMI	262	-0.0022	-0.00455, 0.00015	0.066
Waist-to-hip ratio	262	-0.10052	-0.27114, 0.07011	0.247
Current Smoking History	262			
No		—	—	
Yes		-0.01108	-0.08102, 0.05886	0.755
Past smoking history	262			
No		—	—	
Yes		0.03683	-0.00998, 0.08365	0.123
Alcohol consumption	262			
No		—	—	

Yes		-0.02131	-0.05328, 0.01066	0.191
Total cholesterol	246	0.00043	0.00005, 0.00082	**0.027**
Triglycerides	246	0.00009	-0.00019, 0.00037	0.522
HDL	246	-0.00037	-0.00142, 0.00069	0.495
LDL	246	0.00071	0.00023, 0.00119	**0.004**
HIV status	262			
HIV-negative		—	—	
HIV-positive		-0.0363	-0.06653, -0.00607	**0.019**
Nadir CD4 count	145	0.00003	-0.00005, 0.00011	0.475
Duration on ART	145	-0.00002	-0.00036, 0.00032	0.899
[1]CI = Confidence Interval				

Appendix 3: Multivariate linear regression models corresponding to Aim 1

	Model 1*		**Model 2****		**Model 3*****	
Variable	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Interleukin-1	-0.0392 (-0.0812, 0.0028)	**0.067**	-0.04201 (-0.08329, -0.00074)	**0.046**	-0.0397 (-0.0794, -0.0001)	**0.05**
Interleukin-6	0.0053 (0.0005, 0.0101)	**0.032**	0.00205 (-0.00261, 0.00671)	0.386		
TNF-alpha	-0.0038 (-0.0096, 0.0019)	0.192				
hsCRP	-0.0005 (-0.0017, 0.0008)	0.469				
Age			0.00184 (0.00029, 0.00340)	**0.021**	0.002 (0.0005, 0.0035)	**0.008**
Sex						
Female						
Male			-0.0619 (-0.10210, -0.02169)	**0.003**	-0.0635 (-0.1017, -0.0254)	**0.001**
Hypertension						
Non hypertensive						
Hypertensive			0.04062 (-0.00508, 0.08632)	**0.081**	0.0256 (-0.0178, 0.0691)	0.246
Diabetes						
Nondiabetic						
Diabetic			0.11297 (0.00813, 0.21780)	**0.035**	0.1288 (0.0310, 0.2266)	**0.01**
Tobacco Smoking						
No						
Yes			-0.02014 (-0.09722, 0.05694)	0.607	-0.031 (-0.1061, 0.0440)	0.415
Alcohol						

consumption					
No					
Yes		-0.00707 (-0.04564, 0.03151)	0.718		
LDL cholesterol					
Normal LDL					
Elevated LDL		0.00653 (-0.04242, 0.05549)	0.793	0.0737 (-0.0071, 0.1545)	**0.073**
Obesity					
Healthy weight					
Underweight		0.05574 (-0.00309, 0.11457)	**0.063**	0.0652 (0.0067, 0.1238)	**0.029**
Overweight		-0.02071 (-0.06820, 0.02679)	0.391	0.0126 (-0.0366, 0.0617)	0.615
Obese		-0.05212 (-0.10722, 0.00297)	**0.064**	-0.0231 (-0.0852, 0.0391)	0.465
HDL cholesterol					
Normal HDL					
Low HDL		-0.01438 (-0.05542, 0.02665)	0.49	-0.0101 (-0.0493, 0.0290)	0.61
Triglycerides					
Normal Triglycerides					
Elevated Triglycerides		-0.00149 (-0.06554, 0.06256)	0.963		
Adjusted R-squared	0.0252	0.1191		0.1730	
*Model 1 is a multiple linear regression model between CIMT and inflammatory and immunological biomarkers **Model 2 is a multiple linear regression model between CIMT and Interleukin-1 and Interleukin-6, adjusting for age, sex, hypertension, diabetes, tobacco smoking, total cholesterol, BMI, HDL, triglycerides, and alcohol consumption ***Model 3 is a multiple linear regression model between CIMT and Interleukin-1 and Interleukin-6, adjusting for age, sex, hypertension, diabetes, tobacco smoking, total cholesterol, BMI, and HDL					

Appendix 4: Multivariate linear regression models corresponding to Aim 2

	Model 1*		**Model 2****		**Model 3*****	
Variable	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Interleukin-1	-0.0371 (-0.0790, 0.0048)	**0.082**	-0.0386 (-0.0797, 0.0025)	**0.065**	-0.0649 (-0.1248, -0.0050)	**0.034**
Interleukin-6	0.0050 (0.0001, 0.0098)	**0.044**	0.0020 (-0.0027, 0.0066)	0.402		
TNF-alpha	-0.0029 (-0.0087, 0.0030)	0.332				
hsCRP	-0.0005 (-0.0018, 0.0007)	0.413				

HIV status						
HIV-negative	—		—		—	
HIV-positive	-0.0303 (-0.0672, 0.0067)	0.108	-0.0352 (-0.0716, 0.0011)	**0.057**	-0.042 (-0.0817, -0.0022)	**0.039**
Age			0.0018 (0.0002, 0.0033)	**0.025**	0.0018 (0.0004, 0.0033)	**0.014**
Sex						
Female			—			
Male			-0.0674 (-0.1077, -0.0271)	**0.001**	-0.0624 (-0.1002, -0.0245)	**0.001**
Hypertension						
Non hypertensive			—			
Hypertensive			0.0382 (-0.0072, 0.0836)	**0.099**	0.0248 (-0.0184, 0.0681)	0.258
Diabetes						
Nondiabetic			—		—	
Diabetic			0.0988 (-0.0063, 0.2038)	**0.065**	0.1283 (0.0297, 0.2268)	**0.011**
Tobacco Smoking						
No			—		—	
Yes			-0.0228 (-0.0994, 0.0537)	0.557	-0.0306 (-0.1053, 0.0441)	0.42
Alcohol consumption						
No			—		—	
Yes			-0.0062 (-0.0445, 0.0321)	0.751		
LDL cholesterol						
Normal LDL						
Elevated LDL			0.0038 (-0.0449, 0.0525)	0.878	0.0889 (0.0172, 0.1606)	**0.015**
Obesity						
Healthy weight						
Underweight			0.0611 (0.0024, 0.1197)	**0.041**	0.0658 (0.0075, 0.1242)	**0.027**
Overweight			-0.0187 (-0.0659, 0.0285)	0.435	0.0099 (-0.0392, 0.0589)	0.692
Obese			-0.0582 (-0.1132, -0.0032)	**0.038**	-0.0251 (-0.0869, 0.0367)	0.425
HDL						
Normal HDL						
Low HDL			-0.0196 (-0.0607, 0.0215)	0.348	-0.0163 (-0.0555, 0.0230)	0.414
Triglycerides						
Normal Triglycerides						
Elevated			0.0116 (-0.0534,	0.726	—	

Triglycerides		0.0765)	
Adjusted R-squared	0.0335	0.1322	0.1837

*Model 1 is a multiple linear regression model between CIMT and inflammatory and immunological biomarkers, adjusting for HIV status

**Model 2 is a multiple linear regression model between CIMT and Interleukin-1 and Interleukin-6, adjusting for HIV status, age, sex, hypertension, diabetes, tobacco smoking, total cholesterol, BMI, HDL, triglycerides, and alcohol consumption

***Model 3 is a multiple linear regression model between CIMT and Interleukin-1, adjusting for HIV status, age, sex, hypertension, diabetes, tobacco smoking, total cholesterol, BMI, and HDL