Biomarkers of Endothelial Activation in Black South African HIV-Positive Subjects are Associated with Both High Viral Load and Low CD4 Counts

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Abstract

The prevalence of cardiovascular death in the HIV-infected population is higher than in uninfected individuals. Growing evidence suggests that HIV infection itself is directly linked to endothelial activation and dysfunction. Therefore, the aim of this study was to investigate whether endothelial activation is present in African subjects with HIV infection and identify its possible determinants. Eighty HIV-infected treatment-naive cases, categorized into two groups based on CD4 count (38 subjects with CD4 count ≤350 cells/mm^3 and 42 subjects with CD4 count >350 cells/mm^3), were compared with 60 HIV-uninfected controls. A small subgroup of the HIV-infected participants ($n = 13$) were followed up for 18 months following initiation of antiretroviral therapy (ART). Anthropometric data, fasting lipid and glucose levels, viral load, and CD4 counts were measured as were serum levels of intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1, von Willebrand factor (vWF), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8). The HIV-infected low CD4 group had higher levels of ICAM-1 ($p < .05$), VCAM-1 ($p < .0005$), TNF-α ($p < .005$), and vWF ($p < .005$), compared with the controls. In the HIV-infected cohort, VCAM-1 levels were negatively associated with CD4 counts ($\beta = -0.474$; $p < .0005$), whereas vWF levels were positively associated with viral load ($\beta = 0.344$; $p < .01$). Levels of ICAM-1 and VCAM-1 were reduced by ART ($p < .05$ vs. baseline for both), however, levels of IL-6, IL-8, and TNF-α increased ($p < .005$ vs. baseline for all). Endothelial activation and inflammation are evident in African ART-naive HIV-infected patients; the former is attenuated, and the latter is increased after 18 months of ART. In HIV-infected subjects, both immunological dysregulation and viral load are associated with biomarkers of endothelial activation.

Keywords: endothelial activation, HIV, inflammation, cardiovascular disease

Introduction

The rate of new HIV infections is highest in developing countries with sub-Saharan Africa accounting for up to 70% of the total number of reported cases.[1] The high prevalence of HIV infection in this region has led to the large-scale rollout of antiretroviral therapy (ART), especially in South Africa.[2] The development and improvement of ART has resulted in a dramatic increase in the lifespan of HIV patients.[3] However, there are growing concerns in the public health sector regarding the adverse cardiometabolic effects of both HIV infection and long-term ART in an aging population.[4,5]

Systemic inflammation enhances atherosclerosis, and biomarkers indicative of both of these processes are found at higher levels in HIV-infected individuals.[6] Reports from developed countries show the prevalence of cardiovascular disease (CVD) to be higher in the HIV-infected population than in uninfected subjects.[7–9] Little is known about the prevalence of CVD in the sub-Saharan HIV-infected population. Although an increased prevalence of traditional risk factors, such as dyslipidemia, smoking, hypertension, hypercholesterolemia, and diabetes, among HIV-infected patients likely contributes to this increased cardiovascular morbidity, growing evidence suggests that HIV infection increases the likelihood of cardiovascular events independently of these risk factors.[10,11]

One of the possible mechanisms by which HIV may increase CVD risk is through effects on the endothelium. Endothelial dysfunction is regarded as the earliest clinically detectable stage of CVD, and can be assessed by measuring factors either produced by or acting on the endothelium.[12,13] It is characterized by activation of the endothelial cells, induced by reactive oxygen species, and augmented by exposure to CVD risk factors, including inflammation.[14] This leads to the upregulation of endothelial adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin), which promote adherence of leukocytes to the endothelium and the progression of atherosclerotic plaque formation.[15]

Increased adhesion of leukocytes to the aortic endothelium in subjects with HIV infection has been observed in conjunction with increased expression of VCAM-1 and E-selectin.[16] Monocyte chemoattractant protein-1 (MCP-1), also known as CC chemokine ligand 2, is a small proinflammatory chemokine, which recruits monocytes into a compromised endothelium.[17] It is therefore a valuable surrogate marker for foam cell formation and recruitment. The von Willebrand factor (vWF) is a glycoprotein synthesized by the endothelial cells, which plays a role in hemostasis.[18]

Systemic inflammation results in the activation of immune cells, such as macrophages,[19] which in turn express cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α).[20,21] The C-reactive protein (CRP), synthesized in response to acute inflammation, downregulates endothelial nitric oxide synthase messenger RNA expression,[22] and upregulates expression of ICAM-1, VCAM-1, and MCP-1,[23,24] stimulating phagocytosis.[25,26] Expression of CRP is induced by the inflammatory cytokine IL-6.[27] IL-8 also plays a role in the formation of fatty deposits in the arteries, as does IL-6.[20] TNF-α is a transmembrane cell-signaling protein, released by activated macrophages into the inflamed tissue, which induces overexpression of the adhesion molecules, VCAM-1, ICAM-1, and E-selectin.[28,29]

Serum markers of endothelial activation (ICAM-1, VCAM-1, E-Selectin, MCP-1, and vWF) and serum markers of inflammation (CRP, TNF-α, IL-8, and IL-6), are known to be higher in HIV-infected subjects.[30–34] Therefore, it is possible that the endothelial dysfunction observed in HIV-infected subjects is not only a result of the effects of viral peptides but may also be driven by the inflammatory milieu induced by infection.

Most studies investigating endothelial activation and dysfunction in HIV-infected subjects have been conducted outside of the African continent, despite the very high prevalence of infection in sub-Saharan Africa. Therefore, the main aim of this study was to compare serum biomarkers of endothelial activation and systemic inflammation between African HIV-infected, treatment-naïve, and HIV-uninfected populations. Secondary aims were to characterize the main determinants of endothelial activation and to assess the effect of ART on both endothelial function and inflammation. These aims were achieved by measuring a broad array of appropriate serum biomarkers in HIV-infected and control subjects, alongside classical CVD risk factors.

The HIV-infected patients included two groups of subjects: those with a CD4 count ≤350 and those with CD4 count >350 cells/mm^3. These subgroups were used to determine if subjects who were more immunocompromised were at increased risk of endothelial activation. A small longitudinal substudy measured serum biomarkers of endothelial activation and systemic inflammation after initiation of ART.

Materials and Methods

Patient population

The study consisted of Black South African urban adults between the ages of 30 and 50 years, with a median age of 37 years, attending selected clinics. Eighty HIV-infected, ART-naive patients, including 38 subjects with CD4 count ≤350 cells/mm^3 (low CD4 group) and 42 subjects with CD4 count >350 cells/mm^3 (high CD4 group), were recruited from the Nthabiseng and Zazi Clinics, Chris Hani Baragwanath Hospital. This cutoff was chosen because at the time of recruitment of subjects into this study, only those with a CD4 count below 350 cells/mm^3 were eligible for ART in the South African ART program.[35,36] A group of 60 HIV-uninfected Black South African adults, 30–48 years of age with a median of 36 years, were recruited from the Zazi Clinic.

The HIV-uninfected group were screened for HIV at the Perinatal HIV Research Unit of the Zazi Clinic using the Bioline HIV 1/2 3.0 Rapid Test Kit (Standard Diagnostics Inc., Gyeonggi-do, Republic of Korea),[36] and HIV status was confirmed using a PCR-based test (see data and sample collection). The groups were matched for age and gender. Informed consent was obtained from all participants. Following initiation of ART, the HIV-infected participants were followed up for 18 months. Serum markers of inflammation and endothelial activation were measured in these subjects at baseline, that is, pre-ART, and at 18 months after initiation of ART.

Ethics

Ethical clearance for patient recruitment and blood sampling was granted by the Human Research Ethics Committee of the University of the Witwatersrand under the ethical clearance number M10408 and M150979.

Exclusion criteria

The exclusion criteria for this study were designed to eliminate subjects who may have received drugs that may influence their glucose, lipid, or blood pressure levels.

Subjects with clinical conditions known to influence endothelial function were also excluded, including a history of diabetes mellitus, CVD; current AIDS-defining illness or opportunistic infection or neoplasm; acute illness in the last 3 months; active drug abuse; patient drug history, for example, statin or antihypertensive therapy; alcohol abuse as defined by the National Institute on Alcohol Abuse and Alcoholism as either a woman who has more than seven drinks per week or more than three drinks per occasion, or a man who has more than 14 drinks per week or more than four drinks per occasion [one drink is defined as one bottle of beer (340 mL) or 150 mL glass of wine or 40 mL of distilled spirits][37]; and smoking defined as ≥10 cigarettes/day in the past year. Pregnant females were also excluded from the study.

Data and sample collection

Blood was collected from all participants into ethylenediaminetetraacetic acid (EDTA) plasma and serum tubes. Blood pressure measurements and anthropometric data, such as height, weight, body mass index (BMI), and waist and hip circumference, were measured at the time of recruitment as described previously.[38]

A fasting morning blood sample was collected from all participants and glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides quantified using the Siemens ADVIA 1800 Chemistry Immunoassay System following standard protocols within the NHLS Chemical Pathology Laboratory of the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). Viral loads were measured from EDTA plasma samples using the Cobas® HIV-1 quantitative nucleic acid test on the Cobas automated 6800 system, whereas CD4 count was measured from EDTA plasma samples by flow cytometry, following standard protocols.[39]

Other relevant data were obtained from patient files and by direct questioning, using a structured questionnaire, in relation to personal history of coronary artery disease, diabetes mellitus, hypertension, cerebrovascular events, recent infections or diagnosed malignancies, drug history, alcohol intake, smoking, and recreational drug use.

Measurement of biomarkers of endothelial activation

Serum levels of ICAM-1, VCAM-1, E-Selectin, MCP-1, TNF-α, IL-6, and IL-8 were measured using a customized Human Magnetic Luminex Screening Assay Kit (R&D Systems, Minneapolis, MN) on the Bio-Plex® Multiplex System (Bio-Rad, Hercules, CA) with Luminex xMAP technology, according to the manufacturer's instructions. The vWF was measured by enzyme-linked immunosorbent assay (ELISA) using a human vWF ELISA Kit (Merck, Darmstadt, Germany) as described by the manufacturer.

Statistical analysis

Statistical analysis was performed using the Statistica software v13.3 (Statsoft Inc., Tulsa, OK). Data that were not normally distributed were either log-transformed or the square root was used to normalize their distribution.

Data were compared across the three groups (HIV-uninfected, HIV-infected high CD4, and HIV-infected low CD4) using one-way analysis of variance and a Tukey's *post hoc* test. This was followed by multivariable linear regression analyses in which endothelial activation markers that were found to differ between the three groups were included as dependent variables, the two CD4 groups included as independent variables, with the HIV-uninfected subjects acting as the reference group. Other variables that differed across the three groups were then added to the models to determine whether they attenuated the relationship of the endothelial activation markers with HIV status.

Data were compared between baseline and follow-up time points using a Student's paired *t* test. Multivariable linear regression analysis was used to find the main determinants of serum biomarkers of endothelial activation (dependent variables) in HIV-infected subjects, particularly noting the input of markers of inflammation, CD4 counts, and viral load.

The models were developed by performing univariate analyses of all appropriate study variables (gender, age, waist, hip, BMI, systolic and diastolic blood pressure, glucose, total cholesterol, LDL-C, HDL-C, triglycerides, TNF-α, IL-6, IL-8, CD4 count, and viral load) against each of the five biomarkers of endothelial activation. Those variables that correlated at $p < .20$ were then included in a multivariable regression model in which backward, stepwise removal of nonsignificant variables was performed until all remaining variables were significant at $p < .05$.

The sample size for this study was determined by infrastructural constraints and data from the literature. At the time of this study, no data were available from South Africa on serum levels of biomarkers of endothelial activation in HIV-infected subjects. Hence, an investigation conducted in Italy by Francisci *et al.*[40] was used to estimate the appropriate sample size. This study reported a significant difference in baseline levels of vWF, VCAM-1, and MCP-1 between HIV-uninfected and HIV-infected subjects using 28 subjects per group. We, therefore, ensured that the sample size for each of our study groups within the cross-sectional analysis was >28, however, we were not able to attain this sample size in the longitudinal analysis, as described later.

Results

Comparison of demographic, anthropometric, and cardiometabolic variables between study groups

No significant differences were observed between the three groups for age, anthropometry, blood pressure, glucose, or triglyceride levels (Table 1). However, there were significantly fewer male participants in the HIV-infected high CD4 group compared with the HIV-uninfected group. No difference in total cholesterol was seen between the HIV-uninfected and high CD4 group, however, levels of total cholesterol were significantly lower in the low CD4 group compared with the HIV-uninfected and high CD4 group. Serum levels of LDL-C were significantly higher in the high CD4 group compared with both the HIV-uninfected

group and the low CD4 group. In addition, levels of HDL-C were lower in both the low and high CD4 group compared with the HIV-uninfected group.

Table 1. Demographic, Anthropometric, Cardiometabolic, and Immunological Characteristics of Study Groups

Variables	HIV-uninfected ($n = 60$)	HIV-infected, ART-naive CD4 > 350 cells/mm^3 ($n = 42$)	HIV-infected, ART-naive CD4 ≤ 350 cells/mm^3 ($n = 38$)
Age (years)	36.48 ± 4.80	36.19 ± 6.59	38.89 ± 6.07
Gender, male, *n* (%)	25 (41.67)	5 (11.90)*	10 (26.32)
BMI (kg/m^2)	26.02 ± 5.27	27.52 ± 8.31	25.71 ± 5.31
Waist circumference (cm)	88.20 ± 12.20	87.50 ± 12.34	85.63 ± 10.47
Diastolic BP (mmHg)	80.37 ± 11.25	78.38 ± 12.17	81.92 ± 16.47
Systolic BP (mmHg)	130.22 ± 16.88	130.10 ± 17.84	137.82 ± 23.88
Glucose (mmol/L)	4.62 ± 0.54	4.67 ± 1.94	4.44 ± 0.40
Triglyceride (mmol/L)	0.74 [0.42–0.99]	0.87 [0.61–1.07]	0.64 [0.50–0.86]
Total cholesterol (mmol/L)	3.84 ± 0.85	3.99 ± 0.83	3.21 ± 0.76*,#
LDL-C (mmol/L)	1.58 ± 0.65	2.42 ± 0.58**	1.88 ± 0.63#
HDL-C (mmol/L)	1.64 [1.31–2.38]	1.11 [0.84–1.35]**	0.97 [0.84–1.20]**
CD4+ cell count (cells/mm^3)	—	691.55 ± 232.08	224.18 ± 92.54##
Viral load (copies/mL)	—	37,109.72 ± 71,099.85	81,741.74 ± 90,049.05#

Data given either as mean ± standard deviation or median [lower quartile, upper quartile]; *n* indicates the number of participants.

*$p < .005$, **$p < .0005$ compared with HIV-uninfected; #$p < .005$, ##$p < .0005$ compared with HIV-infected, ART-naive CD4 > 350 cells/mm^3 group.

ART, antiretroviral therapy; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Comparison of markers of endothelial activation and inflammation between study groups

No significant difference was observed across the three groups with regard to plasma levels of IL-8, E-selectin, and MCP-1 (Table 2). In addition, plasma levels of TNF-α, IL-6, ICAM-1, and VCAM-1 did not differ significantly between the HIV-infected high CD4 and HIV-uninfected groups. However, plasma levels of TNF-α, ICAM-1, and VCAM-1 were higher in the HIV-infected low CD4 group compared with the HIV-uninfected group, with levels of vWF being higher in both the low and high CD4 group compared with the HIV-uninfected group. Comparing the HIV-infected, low and high CD4 groups, significant differences between these two groups were observed for plasma levels of TNF-α, IL-6, and VCAM-1, which were significantly higher in the low compared with the high CD4 group.

Table 2. Comparison of Endothelial Dysfunction and Inflammation Between Study Groups

Variables	HIV uninfected ($n = 60$)	HIV-infected, ART-naive CD4 > 350 cells/mm^3 ($n = 42$)	HIV-infected, ART-naive CD4 ≤ 350 cells/mm^3 ($n = 38$)
TNF-α (pg/mL)	4.6 [3.1–7.4]	5.4 [3.6–7.2]	8.2 [6.2–10.4]**,#
IL-6 (pg/mL)	3.4 [2.0–4.6]	2.2 [1.0–3.4]	4.6 [3.8–5.3]##
IL-8 (pg/mL)	7.6 [4.3–20.9]	7.9 [3.1–15.5]	6.3 [4.7–9.3]
ICAM-1 (ng/mL)	335.6 [158.5–575.4]	462.1 [331.9–919.3]	839.4 [419.7–1,042.5]*
VCAM-1 (ng/mL)	464.3 [266.5–609.6]	526.1 [434.9–657.9]	1,163.1 [701.5–1,606.4]***,##
E-selectin (ng/mL)	30.0 [20.9–44.1]	27.9 [19.9–33.9]	30.3 [22.7–40.0]
vWF (μg/mL)	14.1 [9.2–20.7]	22.2 [15.8–36.5]**	21.3 [13.4–43.9]**
MCP-1 (pg/mL)	120.8 [99.3–171.5]	131.2 [102.3–178.6]	139.3 [115.0–208.1]

Data given as median [lower quartile, upper quartile]; *n* indicates the number of participants.

*$p < .05$, **$p < .005$, ***$p < .0005$ versus HIV-uninfected; #$p < .05$, ##$p < .0005$ versus CD4 > 350.

E-selectin, endothelial leukocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, Monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule-1; vWF, von Willebrand factor.

Table 3. Comparison of Endothelial Dysfunction Between HIV-Infected and HIV-Uninfected Groups Using Regression Analysis Without and With Adjustment for Possible Confounding Variables

		Independent variables with standardized β-coefficient (*p* value)[a]			
Model number	Dependent variables	Without adjustment		With adjustment[b]	
1	ICAM-1[c]	CD4 > 350	0.123 (.206)	CD4 > 350	0.110 (.374)
		CD4 ≤ 350	0.229 (.019)	CD4 ≤ 350	0.223 (.059)
2	VCAM-1[c]	CD4 > 350	0.012 (.890)	CD4 > 350	0.006 (.955)
		CD4 ≤ 350	0.429 (<.0005)	CD4 ≤ 350	0.376 (.0006)
3	vWF[d]	CD4 > 350	0.324 (.002)	CD4 > 350	0.325 (.012)
		CD4 ≤ 350	0.342 (.001)	CD4 ≤ 350	0.278 (.026)

[a]The β-coefficients and *p* values for the CD4 > 350 and the CD4 ≤ 350 groups are shown in reference to HIV-uninfected subjects.

[b]Adjusted for gender, LDL-C, HDL-C, TNF-α, and IL-6.

[c]Square root.

[d]Logged values used.

Determinants of systemic inflammation and endothelial activation

It is possible that significant differences noted in ICAM-1, VCAM-1, and vWF between the HIV-infected groups and the HIV-uninfected group are due to confounding by other variables that also differ across these groups, that is, gender, total cholesterol, LDL-C, HDL-C (Table 1), TNF-α, and IL-6 (Table 2). Therefore, multivariable regression models were set up for each of these three markers of endothelial activation with the two CD4 groups as independent variables and with the HIV-uninfected group as the reference. The models were run with and without adjustment for gender, LDL-C, HDL-C, TNF-α, and IL-6. Total cholesterol was not included due to high collinearity with LDL-C. The models are shown in Table 3. In the unadjusted models, the differences noted in Table 2 for the CD4 groups with the HIV-uninfected group were repeated, as would be expected.

After adjustment for the possible confounding variables, there was some attenuation of the β-coefficients noted in the unadjusted models, but all *p* values remained significant, except for the ICAM-1 levels in the CD4 ≤ 350 cells/mm^3 group, which fell from $p = .019$ in the unadjusted model to $p = .059$ in the adjusted model.

Table 4 shows the results of regression models for each of the serum biomarkers of endothelial activation in the HIV-infected population (both CD4 groups combined). These models were generated to isolate the main determinants of endothelial activation in this population, and to observe the effects of inflammation, CD4 counts, and viral load. Regression analyses show that the only significant determinant of VCAM-1 serum levels was CD4 count (inverse relationship; Table 4, model 1), whereas male gender and diastolic blood pressure were both positively associated with E-selectin levels (model 2). Viral load was a strong positive determinant of vWF levels (model 3), whereas age correlated positively with MCP-1 (model 4). After backward regression analysis, no significant associations were observed for ICAM-1 serum levels.

Table 4. Multivariable Backward Stepwise Linear Regression Models for Serum Biomarkers of Endothelial Dysfunction in HIV-Infected Subjects

Model number	Dependent variable	Independent variable with standardized β (*p* value)		Adjusted R^2 (*p* value) for full model
1	VCAM-1[a]	CD4 counts	−0.474 (<.0005)	0.214 (<.0005)
2	E-selectin	Male[c]	0.367 (.002)	0.172 (.001)
		Diastolic BP	0.247 (.036)	
3	vWF[b]	Viral load	0.344 (.007)	0.103 (.007)
4	MCP-1[a]	Age	0.357 (.002)	0.115 (<.002)

Models were built by performing univariate analyses of all appropriate study variables (gender, age, waist, hip, BMI, systolic and diastolic blood pressure, glucose, total cholesterol, LDL-C, HDL-C, triglycerides, TNF-α, IL-6, IL-8, CD4 count, and viral load) against each of the dependent variables, that is, E-selectin, ICAM-1, VCAM-1, MCP-1, and vWF. Those variables that correlated at $p < .20$ were included in a multivariable regression model in which backward, stepwise removal of nonsignificant variables was performed until all remaining variables were significant at $p < .05$. These final models are shown in the table above.

[a]Square root.

[b]Logged values used.

[c]Compared with females.

It was interesting to note from Table 4 that none of the markers of inflammation, that is, TNF-α, IL-8, and IL-6, appeared as determinants of any of the biomarkers of endothelial activation. In the initial univariate regression models that were performed to identify factors that were associated with these biomarkers and for inclusion in the multivariable regression models, IL-6 and TNF-α were found to be significantly associated with VCAM-1 ($p = .010$) and MCP-1 ($p = .042$), respectively. However, when IL-6 was included in the multivariable regression model for VCAM-1 and TNF-α in the model for MCP-1, the significant associations observed in the univariate models became nonsignificant ($p = .183$ and $p = .322$, respectively).

Changes in endothelial activation and inflammation following initiation of ART

Seventy-four subjects were initiated onto ART and followed up 18 months later, however, 61 were lost to follow-up leaving only 13 subjects for whom blood samples were available at 18 months. Of note, 8 out of the 13 subjects (61.5%) had a CD4+ cell count of ≤350 cells/mm^3 at baseline. These subjects were receiving a first-line ART regimen of tenofovir–lamivudine–efavirenz or a second-line regimen that included darunavir or lopinavir, both boosted with ritonavir. Data obtained from these follow-up participants are reported in Table 5. No difference was observed between baseline and follow-up measures with regard to BMI and blood pressure.

Table 5. Characteristics of HIV-Infected Art-Naive Participants at Baseline and After 18 Months of Treatment

Variables	HIV-infected, ART-naive at baseline ($n = 13$)	HIV-infected after 18 months of ART ($n = 13$)
BMI (kg/m^2)	25.7 ± 6.3	26.3 ± 5.9
Waist circumference (cm)	82.6 ± 10.2	87.7 ± 11.6**
Diastolic BP (mmHg)	80.3 ± 16.8	81.7 ± 12.3
Systolic BP (mmHg)	128.7 ± 19.0	132.5 ± 17.2
Glucose (mmol/L)	4.6 ± 0.5	4.3 ± 0.5*
Triglyceride (mmol/L)	0.7 [0.5–1.0]	0.7 [0.6–1.0]
Total cholesterol (mmol/L)	3.2 ± 0.9	4.0 ± 1.1*
LDL-C (mmol/L)	1.9 ± 0.6	2.5 ± 0.9*
HDL-C (mmol/L)	1.0 [0.8–1.0]	1.0 [0.8–1.0]
CD4+ cell count (cells/mm^3)	433.7 ± 291.6	495.5 ± 157.8
TNF-α (pg/mL)	7.7 [5.9–9.0]	19.1 [13.9–22]**
IL-6 (pg/mL)	3.4 [2.7–5.0]	11.2 [7.7–12.0]**
IL-8 (pg/mL)	7.9 [4.9–13.0]	34.7 [20.1–43.0]**
ICAM-1 (ng/mL)	425.2 [355.4–899.8]	149.8 [107.5–205.0]*
VCAM-1 (ng/mL)	676.4 [607.3–1,203.0]	270.7 [151.9–343.5]*
E-selectin (ng/mL)	36.1 [23.4–37.8]	23.7 [22.2–28.2]
vWF (μg/mL)	24.4 [19.8–41.8]	23.4 [20.9–29.5]
MCP-1 (pg/mL)	175.4 [133.6–225.0]	203.6 [153.8–506.0]

Data given either as mean ± standard deviation or median [lower quartile, upper quartile]. *n* indicates the number of participants.

*$p < .05$, **$p < .005$ versus baseline.

The waist circumference, total cholesterol, and LDL-C levels were significantly higher in the follow-up group compared with baseline; however, glucose level was lower in the follow-up group but still within the normal reference range (<5.6 mmol/L).

Comparing markers of endothelial activation and inflammation in the HIV-infected individuals at baseline and following 18 months of treatment, serum levels of TNF-α, IL-6, and IL-8 significantly increased in the 18-month follow-up group compared with baseline. However, levels of ICAM-1 and VCAM-1 significantly decreased. No significant difference in plasma levels of E-selectin, vWF, and MCP-1 were observed after ART initiation. Due to the low sample size ($n = 13$), we performed *post hoc* sample size calculations to determine the sample size required to show significant differences (at $p < .05$ and power of 80%) between the baseline and follow-up time points for those serum endothelial biomarkers that showed no significant differences, that is, E-selectin, MCP-1, and vWF. These calculations provided sample sizes of 41, 28, and 1,284, respectively.

Discussion

In this study, HIV-infected participants with CD4 counts >350 cells/mm^3 had higher LDL and total cholesterol levels than subjects with CD4 counts ≤350 cells/mm^3 and higher LDL levels than HIV-uninfected subjects, whilst HDL levels were lower in the high CD4 group compared with the HIV-negative subjects. Triglyceride levels were not significantly different between the groups. Serum levels of the inflammatory markers, TNF-α and IL6, and endothelial activation markers ICAM-1, VCAM-1, and vWF, were highest in HIV-infected subjects with low CD4 counts. In HIV-infected subjects,high VCAM-1 and vWF serum levels were not associated with markers of inflammation but with low CD4 counts and high viral load, respectively.

Following 18 months of ART in a small subgroup of participants, serum levels of ICAM-1 and VCAM-1 were lower, whereas levels of TNF-α, IL-6, and IL-8 were higher than that observed before treatment.

The serum levels of HDL were found to be lower in the HIV-infected compared with the HIV-uninfected group. Similar data have been reported in previous studies.[33,41] Total cholesterol levels were lower in subjects with low CD4 counts compared with those with high CD4 counts and HIV-uninfected subjects, while LDL-C showed a similar difference but only between the two CD4 groups. It is known that untreated HIV infection is associated with lower total cholesterol levels.[42] The current data suggest that this effect is more prominent in those with low CD4 counts and high viral loads. The mechanism by which the virus causes hypocholesterolemia in infected subjects is not known, however, it has been suggested that cholesterol malabsorption[43] could potentially account for the observed phenomenon.

Data from the current study demonstrate that the inflammatory cytokines, IL-6 and TNF-α, were elevated in the HIV-infected cohort, particularly in those with low CD4 counts. This persisted even after 18 months of administration of ART. Thus, there is a continuous inflammatory response in the HIV-infected population despite treatment. Other studies also show that inflammation is maintained in HIV-infected subjects even after ART initiation.[44–46] Thus, serum levels of sCD14 and CRP remained at pre-ART levels in HIV-1-infected participants in the study conducted by Macatangay *et al.*[45] even after 9 months of therapy with lopinavir, tenofovir, and emtricitabine.

This chronic inflammation may lead to endothelial dysfunction as characterized by increased platelet aggregation, vascular smooth muscle cell proliferation, greater monocyte adhesion and transmigration, and oxidative stress, which promote atherosclerosis.[47] In addition, endothelial progenitor cells decrease in number and lose their capacity to repair endothelial damage.[48] These inflammation-induced changes in endothelial function may be one of the reasons for the increased prevalence of CVDs observed in HIV-infected subjects receiving ART.[6–8] It must be noted that in the current study, although we did observe high levels of inflammatory cytokines 18 months after initiation of ART, these observations were from a small number of subjects ($n = 13$) and must be confirmed using a larger sample size.

In this study, IL-8 levels did not differ between the HIV-infected ART-naive study groups and the uninfected cohort. This finding is contrary to a report from De Pablo-Bernal *et al.*[32] in which serum levels of IL-8 were significantly higher in the HIV-infected ART-naive group, 27–37 years of age, when compared with age-matched uninfected controls, but significantly lower when compared with uninfected subjects, >64 years of age.[12] However, it must be noted that this study was performed in a Caucasian population of HIV-infected subjects that were younger and with a much higher proportion of male participants (82.0% vs. 18.7%) when compared with the current study.

Serum markers of endothelial activation, that is, VCAM-1, ICAM-1, and vWF, were higher in HIV-infected subjects when compared with HIV-uninfected subjects. These data are supported by several other studies.[30,32,33,41,49] However, serum levels of E-selectin were not elevated in the HIV-infected subjects. A previous study from South Africa also reported no difference in E-selectin levels between HIV-uninfected and HIV-infected, ART-naive subjects.[50] Increased expression of VCAM-1 and ICAM-1 on endothelial cells would augment the infiltration of leukocytes into the vessel walls, thus, promoting atherosclerosis by enhancing plaque formation in the arterial wall.[15] Serum levels of MCP-1 were higher in both the HIV-infected groups when compared with the controls, but this difference was not statistically significant.

Previous studies have shown that MCP-1 levels are higher in HIV-infected subjects, but these studies were conducted in mixed populations (Hispanic, African American, and White) of children or in White subjects only.[26,40] A study conducted in Tanzania has also shown that MCP-1 levels were higher in HIV-infected compared with HIV-uninfected subjects before initiation of ART.[51] This study had a higher sample size for HIV-infected subjects than the current study and it is therefore possible that our study was not sufficiently powered to detect significant differences in MCP-1 levels within the context of an African population.

The current study demonstrated that biomarkers of endothelial activation that were elevated in HIV-infected, compared with HIV-uninfected subjects, fell significantly after 18 months of ART. This observation is in agreement with other studies involving individuals on ART for 12–24 months.[52,53]

Some studies, however, have shown that endothelial activation is not alleviated after long-term (8–12 years) ART use.[30,46] It is also possible that different ART regimens may have differential effects on the endothelium. One of few studies directly comparing the effects of different antiretroviral regimens on endothelial activation was conducted in Denmark. This study analyzed flow-mediated dilatation (FMD) and serum markers of endothelial activation in HIV-positive subjects first receiving a protease inhibitor-based regimen, which was switched to a non-nucleoside reverse transcriptase inhibitor-based therapy, both lasting 3

months. This study showed that both regimens were characterized by increased FMD and reduced serum levels of the markers of endothelial activation.[52]

Multiple regression analyses demonstrated that differences in the level of serum markers of endothelial dysfunction, particularly VCAM-1 and vWF, between the HIV-infected and HIV-uninfected groups were specifically associated with viral infection, and were not due to confounding or moderation from gender, lipid, or cytokine levels. These findings are supported by previous studies conducted at both African sites and elsewhere.[12,30,33]

The current data are novel in that the panel of endothelial activation biomarkers includes serum vWF, which has not previously been studied in the context of HIV infection and CVD risk factors in African populations. Earlier African studies have shown elevated vWF levels in HIV-infected compared with HIV-uninfected subjects, but these studies were performed in the context of stroke, malaria, and sepsis and coagulation markers and included no adjustment for possible confounding from CVD-related variables.[32–34]

The present study demonstrates that elevated serum levels of VCAM-1 and vWF in HIV-infected subjects were related to low CD4 counts and high viral load, respectively. This finding suggests that endothelial activation in HIV infection is due to direct effects of the virus on the endothelium and to virus-associated immune dysregulation, as demonstrated by previous studies. Thus, it is known from *in vitro* studies that viral proteins, such as Nef, Tat, and gp120 can induce endothelial activation,[12] whereas CD4+ T cell suppression by the virus may lead to immune activation, which in turn has been associated with endothelial dysfunction.[54] It is interesting to note that elevated serum levels of VCAM-1 have been associated with activated macrophages in subjects with HIV infection.[55] However, no studies have investigated the effect of HIV viral proteins on endothelial vWF production.

An important finding of the current study was that inflammation was not associated with serum biomarkers of endothelial dysfunction and that ART attenuated the level of these biomarkers but not the level of the inflammatory cytokines. Despite this lack of association between cytokines and endothelial activation, it is possible that the cytokines are able to modulate aspects of endothelial function other than those involving VCAM-1, ICAM-1, MCP-1, E-selectin, and vWF and also to effect other factors related to vascular function. Thus, inflammation is also able to cause impairment of cholesterol metabolism, activation, and differentiation of monocytes and reduce endothelial repair mechanisms.[19,56] However, it is possible that the lack of association of cytokines with the serum biomarkers of endothelial dysfunction observed in the present study may be due to low power.

If this were the case, then a larger sample size would uncover significant associations but this would in turn suggest that cytokines are weaker determinants of endothelial activation than the factors shown to be significantly associated with endothelial activation in the regression models in Table 4. It should also be noted that significant associations of IL-6 and TNF-α with VCAM-1 and MCP-1, respectively, in univariate regressions were attenuated to nonsignificance in multivariable models demonstrating that the initial significant univariate associations were due to confounding.

The main limitation of this study was the relatively small sample size, particularly that of the longitudinal analysis, where *post hoc* sample size calculations showed that increasing subject numbers from 13 to 41 or 28 would be sufficient to show possible significant differences (at $p < .05$ and 80% power) in E-selectin and MCP-1, respectively. This suggests that the

longitudinal data for these analytes should be interpreted with caution. With respect to vWF, the required sample size to show significant differences was 1,284, suggesting that any change in vWF may be small and of little clinical significance. However, these are theoretical interpretations and do need to be confirmed in a longitudinal study with a larger sample size.

Despite these limitations, significant differences were observed across the study groups and these findings were supported by data from other studies.[30,32,33,41] Also, regression analyses were performed using cross-sectional data, which does not allow us to identify causation, but only to observe associations. In addition, for HIV-infected subjects, the duration of infection was not known.

In this study, we hypothesized that the negative association of CD4 cell count with vWF may be mediated by immune activation, however, we did not measure indicators of immune activation such as the soluble biomarkers sCD163 and sCD14.[54] Lastly, we lacked a clinical measure of endothelial function such as flow-mediated dilation or pulse wave velocity, but endothelial activation was assessed using the serum biomarkers ICAM-1, VCAM-1, vWF, MCP-1, and E-selectin. We are therefore unable to draw any conclusions on the functional integrity of the endothelium but are able to state that endothelial activation is greater in the HIV-positive subjects.

In this context, a recent study from South Africa has shown that baseline serum levels of ICAM-1 and VCAM-1 were not correlated with carotid–femoral pulse wave velocity or carotid intima-media thickness at 5 years of follow-up and that these markers of vascular function and structure were not different between subjects with or without HIV infection.[57] However, it should be noted that at baseline, 44.1% of the HIV-positive subjects were receiving ART and this rose to 81.4% at follow-up and ART is known to attenuate endothelial activation.[57]

The main advantage of this study was the analysis of a broad range of serum markers of endothelial activation and inflammation in an understudied population, in which HIV infection is highly prevalent. Furthermore, this is one of the very few studies to measure the levels of such biomarkers after the initiation of ART, and to investigate the interaction of these biomarkers with each other and with classical CVD risk factors.

Conclusion

Our data show evidence of endothelial activation in the Black South African ART-naive HIV-infected population. Commencement of HIV treatment was associated with attenuation of endothelial activation, but markers of inflammation continued to increase. This study suggests that endothelial activation is not due to inflammation but may arise from direct effects of the virus on the endothelium and from immune dysregulation.

Authors' Contributions

The protocol was conceptualized and designed by N.J.C. and N.L. Anthropometric data, patient samples, and information were collected by N.L., while experiments were performed by G.M. who equally analyzed the data and wrote the article. C.W. and M.G. assisted with data acquisition, and the final article was read and approved by all contributing authors.

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Author Disclosure Statement

The authors declare no competing interests.

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References

1. UNAIDS. UNAIDS Data 2019. Available at https://www.unaids.org/en/resources/documents/2019/2019-UNAIDS-data (2019), accessed August 28, 2019.

2. Johnson LF: Access to antiretroviral treatment in South Africa, 2004–2011. South Afr J HIV Med 2012;13:a156.

3. Grinsztejn B, Luz PM, Pacheco AG, et al: Changing mortality profile among HIV-infected patients in Rio de Janeiro, Brazil: Shifting from AIDS to non-AIDS related conditions in the HAART era. PLoS One 2013;8:e59768.

4. Chow FC, Regan S, Feske S, Meigs JB, Grinspoon SK, Triant VA: Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US health care system. J AIDS (1999) 2012;60:351.

5. Jerico C, Knobel H, Montero M, et al: Metabolic syndrome among HIV-infected patients: Prevalence, characteristics, and related factors. Diabetes Care 2005;28:132–137.

6. van Leuven SI, Sankatsing RR, Vermeulen JN, Kastelein JJ, Reiss P, Stroes ES: Atherosclerotic vascular disease in HIV: It is not just antiretroviral therapy that hurts the heart! Curr Opin HIV AIDS 2007;2:324–331.

7. Al-Kindi SG, ElAmm C, Ginwalla M, et al: Heart failure in patients with human immunodeficiency virus infection: Epidemiology and management disparities. Int J Cardiol 2016;218:43–46.

8. Alonso A, Barnes AE, Guest JL, Shah A, Shao IY, Marconi V: HIV infection and incidence of cardiovascular diseases: An analysis of a large healthcare database. J Am Heart Assoc 2019;8:e012241.

9. Touloumi G, Kalpourtzi N, Papastamopoulos V, et al: Cardiovascular risk factors in HIV infected individuals: Comparison with general adult control population in Greece. PLoS One 2020;15:e0230730.

10. Freiberg MS, Chang C-CH, Kuller LH, et al: HIV infection and the risk of acute myocardial infarction. JAMA Intern Med 2013;173:614–622.

11. Pinto DSM, da Silva M: Cardiovascular disease in the setting of human immunodeficiency virus infection. Curr Cardiol Rev 2018;14:25–41.

12. Mezoh G, Crowther NJ: Deciphering endothelial dysfunction in the HIV-infected population. Adv Exp Med Biol 2019;1134:193–215.

13. Mayne ES, Louw S: Good fences make good neighbors: Human immunodeficiency virus and vascular disease. Open Forum Infect Dis 2019;6:ofz303.

14. Deanfield JE, Halcox JP, Rabelink TJ: Endothelial function and dysfunction: Testing and clinical relevance. Circulation 2007;115:1285–1295.

15. Čejková S, Králová-Lesná I, Poledne R: Monocyte adhesion to the endothelium is an initial stage of atherosclerosis development. Cor et Vasa 2016;58:e419–e425.

16. Zietz C, Hotz B, Stürzl M, Rauch E, Penning R, Löhrs U: Aortic endothelium in HIV-1 infection: Chronic injury, activation, and increased leukocyte adherence. Am J Pathol 1996;149:1887.

17. Xia M, Sui Z: Recent developments in CCR2 antagonists. Expert Opin Therapeut Patents 2009;19:295–303.

18. Sadler JE: Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem 1998;67:395–424.

19. Mazzuca P, Caruso A, Caccuri F: HIV-1 infection, microenvironment and endothelial cell dysfunction. New Microbiol 2016;39:163–173.

20. Sprague AH, Khalil RA: Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol 2009;78:539–552.

21. Hedges JC, Singer CA, Gerthoffer WT: Mitogen-activated protein kinases regulate cytokine gene expression in human airway myocytes. Am J Respir Cell Mol Biol 2000;23:86–94.

22. Verma S, Anderson TJ: Fundamentals of endothelial function for the clinical cardiologist. Circulation 2002;105:546–549.

23. Deshmane SL, Kremlev S, Amini S, Sawaya BE: Monocyte chemoattractant protein-1 (MCP-1): An overview. J Interf Cytok Res 2009;29:313–326.

24. Verma S, Buchanan MR, Anderson TJ: Endothelial function testing as a biomarker of vascular disease. Circulation 2003;108:2054–2059.

25. Black S, Kushner I, Samols D: C-reactive protein. J Biol Chem 2004;279:48487–48490.

26. Pepys MB, Hirschfield GM: C-reactive protein: A critical update. J Clin Invest 2003;111:1805–1812.

27. Kushner I, Jiang SL, Zhang D, Lozanski G, Samols D: Do post-transcriptional mechanisms participate in induction of C-reactive protein and serum amyloid A by IL-6 and IL-I? Ann N Y Acad Sci 1995;762:102–107.

28. Wang Y, Wang L, Ai X, et al: Nicotine could augment adhesion molecule expression in human endothelial cells through macrophages secreting TNF-alpha, IL-1beta. Int Immunopharmacol 2004;4:1675–1686.

29. Signorelli SS, Mazzarino MC, Di Pino L, et al: High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test. Vasc Med 2003;8:15–19.

30. Mosepele M, Mohammed T, Mupfumi L, et al: HIV disease is associated with increased biomarkers of endothelial dysfunction despite viral suppression on long-term antiretroviral therapy in Botswana. Cardiovasc J Africa 2018;29:1–7.

31. Borges ÁH, O'connor JL, Phillips AN, et al: Factors associated with plasma IL-6 levels during HIV infection. J Infect Dis 2015;212:585–595.

32. De Pablo-Bernal RS, Ruiz-Mateos E, Rosado I, et al: TNF-alpha levels in HIV-infected patients after long-term suppressive cART persist as high as in elderly, HIV-uninfected subjects. J Antimicrob Chemother 2014;69:3041–3046.

33. Fourie C, van Rooyen J, Pieters M, Conradie K, Hoekstra T, Schutte A: Is HIV-1 infection associated with endothelial dysfunction in a population of African ancestry in South Africa? Cardiovasc J Afr 2011;22:134–140.

34. Neuhaus J, Jacobs DRJr., Baker JV, et al: Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis 2010;201:1788–1795.

35. Moorhouse M, Conradie F, Venter F: What is the role of CD4 count in a large public health antiretroviral programme? South Afr J HIV Med 2016;17:1–3.

36. Govender S, Otwombe K, Essien T, et al: CD4 counts and viral loads of newly diagnosed HIV-infected individuals: Implications for treatment as prevention. PLoS One 2014;9:e90754.

37. U.S. Department of Health and Human Services and U.S. Department of Agriculture: 2015–2020 Dietary Guidelines for Americans, 8th Edition. https://health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015 (2015), accessed December 2015.

38. Crowther NJ, Norris SA: The current waist circumference cut point used for the diagnosis of metabolic syndrome in Sub-Saharan African women is not appropriate. PLoS One 2012;7:e48883.

39. Glencross DK, Janossy G, Coetzee LM, et al: Large-scale affordable PanLeucogated CD4+ testing with proactive internal and external quality assessment: In support of the South African national comprehensive care, treatment and management programme for HIV and AIDS. Cytometry B Clin Cytom 2008;74(Suppl. 1):S40–S51.

40. Francisci D, Giannini S, Baldelli F, et al: HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. AIDS 2009;23:589–596.

41. Fourie CM, Schutte AE, Smith W, Kruger A, van Rooyen JM: Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. Atherosclerosis 2015;240:154–160.

42. Riddler SA, Smit E, Cole SR, et al: Impact of HIV infection and HAART on serum lipids in men. JAMA 2003;289:2978–2982.

43. Weerakkody M, Buddhakorala K, Somasundaram N. Diabetes and metabolic complications among patients with HIV and AIDS. Sri Lanka J Diabetes Endocrinol Metab 2014;3:68–75.

44. Kiefer E, Hoover DR, Shi Q, Dusingize J, Sinayobye J, Anastos K: Longitudinal evaluation of markers of inflammation in HIV-positive and HIV-negative Rwandan women. HIV Med 2018;19:734–744.

45. Macatangay BJ, Yang M, Sun X, et al: Brief Report: Changes in levels of inflammation after antiretroviral treatment during early HIV infection in AIDS clinical trials group study A5217. J Acquir Immune Defic Syndr 2017;75:137–141.

46. Rönsholt FF, Ullum H, Katzenstein TL, Gerstoft J, Ostrowski SR: Persistent inflammation and endothelial activation in HIV-1 infected patients after 12 years of antiretroviral therapy. PLoS One 2013;8:e65182.

47. Davignon J, Ganz P: Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109(Suppl. 1):Iii27–Iii32.

48. Seang S, Kelesidis T, Huynh D, et al: Low levels of endothelial progenitor cells and their association with systemic inflammation and monocyte activation in older HIV-infected men. AIDS Res Hum Retroviruses 2018;34:39–45.

49. van den Dries LW, Gruters RA, Hovels-van der Borden SB, et al: von Willebrand Factor is elevated in HIV patients with a history of thrombosis. Front Microbiol 2015;6:180.

50. Hoffman M, Ipp H, Phatlhane DV, Erasmus RT, Zemlin AE: E-Selectin and markers of HIV disease severity, inflammation and coagulation in HIV-infected treatment-naïve individuals. Afr Health Sci 2018;18:1066–1075.

51. Haissman JM, Vestergaard LS, Sembuche S, et al: Plasma cytokine levels in Tanzanian HIV-1-infected adults and the effect of antiretroviral treatment. J Acquir Immune Defic Syndr 2009;52:493–497.

52. Arildsen H, Sorensen KE, Ingerslev JM, Ostergaard LJ, Laursen AL: Endothelial dysfunction, increased inflammation, and activated coagulation in HIV-infected patients improve after initiation of highly active antiretroviral therapy. HIV Med 2013;14:1–9.

53. van Vonderen MG, Hassink EA, van Agtmael MA, et al: Increase in carotid artery intima-media thickness and arterial stiffness but improvement in several markers of endothelial function after initiation of antiretroviral therapy. J Infect Dis 2009;199:1186–1194.

54. Beltrán LM, Rubio-Navarro A, Amaro-Villalobos JM, Egido J, García-Puig J, Moreno JA: Influence of immune activation and inflammatory response on cardiovascular risk associated with the human immunodeficiency virus. Vasc Health Risk Manag 2015;11:35.

55. Grome HN, Barnett L, Hagar CC, Harrison DG, Kalams SA, Koethe JR: Association of T cell and macrophage activation with arterial vascular health in HIV. AIDS Res Hum Retrov 2017;33:181–186.

56. Kleinbongard P, Heusch G, Schulz R: TNFα in atherosclerosis, myocardial ischemia/reperfusion and heart failure. Pharmacol Therapeut 2010;127:295–314.

57. Phalane E, Fourie C, Schutte AE, Kruger IM, Mels C: Arterial structure and function in Africans with HIV for >5years: Longitudinal relationship with endothelial activation and cardiovascular risk markers. HIV Med 2021;22:650–661.