

Research Article

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# Hyper-inflammation and Endothelial Activation in HIV Infected Patients with Detectable and Undetectable Viral Load

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## Abstract

Although HIV and antiretroviral therapy (ART) have been linked with an increased cardiovascular risk, the pathological pathways remain unknown. We assessed whether secretory phospholipase A2 (sPLA2), high-sensitivity C-reactive protein (hs-CRP), vascular cell adhesion molecule (VCAM), thiobarbituric acid reactive species (TBARS), Superoxide dismutases (SOD), adiponectin and resistin were elevated in HIV infected patients with detectable and undetectable viral load compared to a control group matched for age, sex and cardiovascular risk factors. We evaluated its correlation with traditional and no traditional cardiovascular risk factors and carotid intima-media thickness. Levels of sPLA2 (median [IQR]) were 8.35 (5.36, 9.6) pg/ml, hsCRP were 3.3 (IQR, 1.27, 5.28) mg/L, VCAM 945.6 (IQR, 655.9, 1404.05) ng/ml and TBARS 1.69 (IQR, 1.39, 1.97) uM/L in HIV infected group compared to levels of sPLA2 1.22 (IQR, 0.69, 2.62) pg/ml ( $p < 0.001$ ), hsCRP 3.05 (IQR, 2.68, 3.27) mg/L ( $p = 0.05$ ), VCAM 678.35 (IQR, 530.39, 831.04) ng/ml ( $p < 0.001$ ) and TBARS 7.47 (IQR, 5.03, 10.4) uM/L ( $p < 0.001$ ) in control group. Levels of VCAM (median [IQR]) were 1047.19 (IQR, 609.06, 1084.1) ng/ml ( $p = 0.015$ ) and sPLA2 were 7.6 (IQR, 5.25, 9.6) pg/ml ( $p < 0.001$ ) in HIV infected patients with undetectable viral load compared to control group. There was a good correlation between all analyzed biomarkers and cardiovascular risk factors. In conclusion, HIV infection induces chronic inflammation and endothelial activation that is not completely suppressed by the treatment.

**Keywords:** Cardiovascular risk; Endothelial activation; Inflammation

## Introduction

HIV-infected patients are at higher risk of developing cardiovascular disease than the general population [1] and factors associated with HIV infection and antiretroviral therapy (ART) have been implicated in the premature development of atherosclerosis and coronary heart disease [2]. Current data are very limited and the mechanism of atherosclerosis and cardiovascular events in these patients remains unknown. HIV can directly or indirectly promote inflammation and damage endothelium, including secretion of proinflammatory cytokines [3], secretion of viral proteins [4], and oxidative stress [5]. On the other hand, it is still not known whether the ART have a beneficial or detrimental effect on subrogate proatherosclerotic biomarkers. Several studies suggest that starting ART reduces inflammation and endothelial dysfunction. Furthermore, withdrawal of ART was associated with an increase of inflammation biomarker and risk of myocardial infarction [6,7]. However, there is an association between time of exposure to ART and increased risk of myocardial infarction [8]. ART may increase lipids and impair glucose metabolism, but classic risk factors do not fully account for the association between ART and an increased risk for cardiovascular disease (CVD) [8], suggesting that additional mechanisms might be involved.

The purpose of our study was to determine whether novels (secretory phospholipase A2, Superoxide dismutases, thiobarbituric acid reactive species, adiponectin and resistin) and known cardiovascular risk biomarkers (hs-C-Reactive protein and vascular cell adhesion molecule) were elevated in HIV-infected patients with and without detectable viral load compared to a control group of healthy subjects matched for age, sex and cardiovascular risk factors. We also investigated the association of biomarkers with other cardiovascular risk and HIV factors as well as intima media thickness (IMT).

## Material and Methods

A cross-sectional study was conducted at the HIV Outpatient's

Clinic of the University Hospital Reina Sofia, Murcia (Spain) from January to June 2009. It comprised 71 consecutive HIV-infected adults without AIDS-related symptoms who were compared with 71 controls from a cardiovascular clinic at the same hospital over the same period and matched for age, sex and cardiovascular risk factors. The study was approved by the Ethics Committee for Clinical Research and all patients gave informed consent.

Clinical and laboratory data were obtained at the visit. Cardiovascular risk factors were defined according to the National Cholesterol Education Program. Levels of vascular cell adhesion molecule (VCAM), secretory phospholipase A2 (sPLA-2), high sensitivity C-reactive protein (hsCRP), Superoxide dismutases (SOD), thiobarbituric acid reactive species (TBARS), Resistin and Adiponectin were determined and plasma concentrations of VCAM and sPLA-2 were measured using commercially available ELISA kits (Quantikine: R&D Systems Europe Ltd, Abingdon, UK). Plasma levels of hsCRP were measured using the IMMULITE 2000 Analyzer (Siemens, Los Angeles, CA, USA). Plasma levels of adiponectin and resistin were measured using a commercially available enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA). SOD was measured using

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colorimetric method (EnzyChrom, US) and TBARS was measured using colorimetric method (BioAssay System, USA).

For determining the carotid IMT, B-mode high-resolution ultrasound was used following a standard procedure as described previously [9]. To quantify the degree of carotid artery wall thickening, the mean of six measures performed in the posterior wall of the left common and bulb carotid artery was taken. IMT was considered elevated whether was higher than 0.8 mm in common or higher than 1 mm in bulb carotid artery.

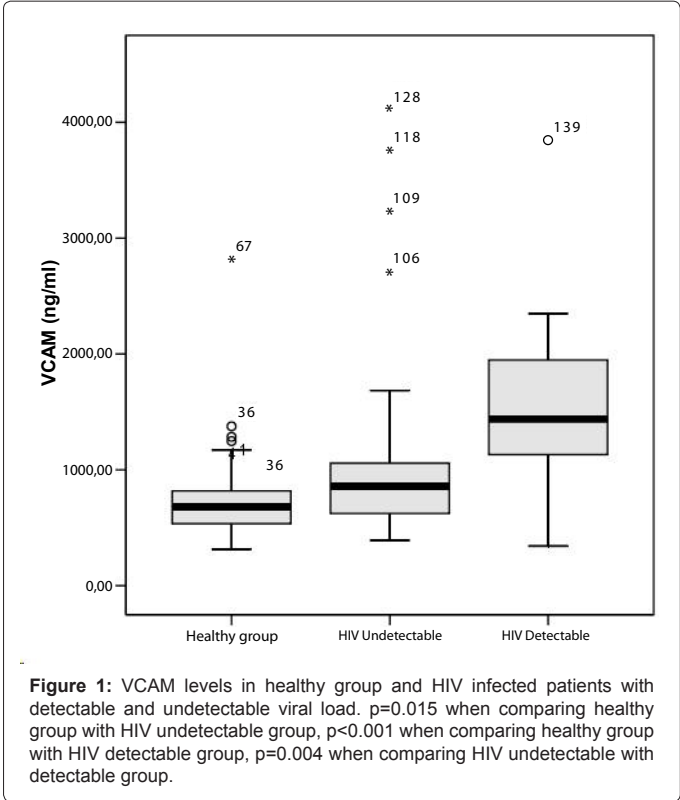
Statistical Analysis

Descriptive analysis of the baseline characteristics of patients was performed using frequency distributions for categorical variables and median and IQR for continuous variables, respectively. Differences in demographic and clinical characteristics between HIV-infected patients and healthy control group were assessed using the  $\chi^2$  or Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables.

Spearman's correlation coefficient was used to determine the association between levels of biomarkers and continuous variables. Differences in serum biomarkers between clinical characteristics and HIV-related factors were assessed using t-Student test unless otherwise indicated.

Binary logistic regression analysis was carried out using forward regression models to obtain an adjusted measure of the effect of biomarkers on HIV status. A p-value of <0.05 was considered to be statistically significant.

Anova one way test and Bonferroni correction was used to investigate the association between biomarkers and HIV with undetectable and detectable viral load and healthy group. Statistical



analyses were performed using SPSS, Version 17.0 (Chicago, Illinois).

Results

We included 71 HIV infected patients and 71 healthy subjects matched for age, sex and cardiovascular risk factors.

The main baseline characteristics [mean (SD) unless otherwise indicated] of the 71 HIV infected patients were: age, 44.49 (9.36) years; sex male [n (%)], 59 (83.09); current antiretroviral therapy [n (%)], 62 (87.3), 24 (33.8) with PI, 33 (44.5) with NNRTI; 39 (54.9) had previous PI exposure; time of exposure to antiretroviral therapy, 7.42 (6.5) years; HIV-RNA viral load less than 50 copies/ml [n (%)], 51 (71.8); CD4 cell count, 539.59 (303) cells/ml; lipodystrophy [n (%)], 11 (15.49); body mass index, 25.85 (10.03) kg/m<sup>2</sup>; LDL cholesterol, 107.59 (34.81) mg/dl; HDL cholesterol, 45.93 (14.68) mg/dl; current smoking [n (%)], 38 (53.52); hypertension [n (%)], 29 (40.8), dyslipidemia [n (%)], 39 (54.9) and type 2 diabetes [n (%)], 5 (7.04).

Clinical characteristics, fasting metabolic parameters and biomarkers of HIV infected patients with detectable and undetectable viral load and Healthy control group are shown in Table 1.

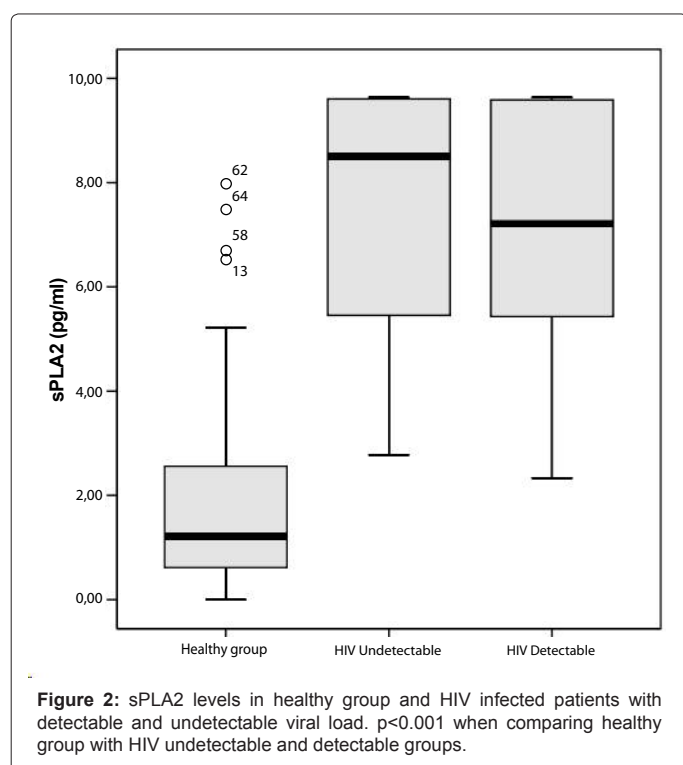
To investigate the effect of biomarkers on viral load and healthy group, the population was divided into three groups (1. HIV infected patients with detectable viral load, 2. HIV infected patients with undetectable viral load, and 3. Healthy group). The results are shown in Figures 1 and 2. HIV infected patients with detectable and undetectable viral load had high levels of VCAM and sPLA2. There were no differences in clinical, cardiovascular risk factors and the others biomarkers between the three groups.

Binary logistic regression analysis was performed to identify

	HIV-infected patients, n=71	Non-HIV controls n=71	p
Age, years	45 (40, 49)	45 (39, 50)	0.727
Sex male (%)	59 (83.09)	56 (78.87)	0.335
Framingham risk Score	7 (1, 14)	8 (8.61)	0.368
Current Smokers (%)	38 (53.52)	26 (36.61)	0.035
Hypertension (%)	29 (40.84)	25 (35.21)	0.302
Dyslipidemia (%)	39 (54.92)	36 (50.7)	0.490
Type 2 Diabetes (%)	5 (7.04)	7 (9.85)	0.194
Total Cholesterol, mg/dl	189 (153, 218)	194 (171, 234)	0.010
LDL cholesterol, mg/dl	110 (82, 134)	122 (103, 148)	0.033
Tryglicerides, mg/dl	139 (83, 212)	110 (79, 169)	0.946
HDL cholesterol, mg/dl	42.5 (37.7, 53)	46.5 (39.7, 57)	0.312
Body mass index, kg/m2	24.26 (21, 27.5)	26.15 (23.8, 28)	0.612
Waist circumference, cm	87 (80, 95)	91.5 (81.4, 98)	0.364
sPLA-2 (pg/ml)	8.35 (5.36, 9.6)	1.22 (0.59, 2.62)	<0.001
VCAM (ng/ml)	945.63 (655.9, 1404.05)	678.35 (530.39, 831.04)	<0.001
hsCRP (mg/L)	3.3 (1.27, 5.28)	3.05 (2.68, 3.27)	0.05
SOD (U/mL)	0.29 (0.25, 0.35)	0.41 (0.31, 0.5)	0.008
TBARS (uM/L)	1.69 (1.39, 1.97)	7.47 (5.03, 10.4)	<0.001
Adiponectin (ug/mL)	8.53 (5.9, 11.29)	8.47 (6.4, 10.3)	0.919
Resistin (ng/ml)	4.93 (4.02, 5.86)	5.89 (3.07, 6.3)	0.282

Results are expressed as median (IQR) or number (%). VCAM, vascular cell adhesion molecule; sPLA2, secretory phospholipase A2; hsCRP, high sensitivity C-reactive protein; SOD, Superoxide dismutases; TBARS, thiobarbituric acid reactive

**Table 1:** Clinical characteristics, fasting metabolic parameters and biomarkers of HIV infected patients and healthy control group.



predictors of HIV status. sPLA2 was the best predictor of HIV status (OR 3.42, IC95% 1.88-6.22;  $p < 0.001$ ).

To identify predictors of detectable viral load only in HIV infected group, a binary logistic regression analysis was carried out. Considering detectable viral load as dependent variable, VCAM was the best predictor (OR 1.03, IC 95% 1.01-1.02;  $p = 0.034$ ).

There was a positively correlation between VCAM and sPLA2 (spearman's correlation coefficient (scc) 0.328;  $p < 0.001$ ), resistin (scc 0.268;  $p = 0.003$ ), IMT (scc 0.242;  $p = 0.044$ ) and plasma HIV viral load (scc 0.391;  $p = 0.001$ ) and negatively with TBARS (scc -0.241;  $p < 0.004$ ) and Total cholesterol (scc -0.27;  $p = 0.001$ ). There was a positively correlation between sPLA2 and hsCRP (scc 0.218;  $p = 0.022$ ) and negatively with SOD (scc -0.283;  $p = 0.001$ ), TBARS (scc -0.56;  $p < 0.001$ ) and total cholesterol (scc -0.219;  $p = 0.01$ ). There was a positively correlation between hsCRP and resistin (scc 0.35;  $p < 0.001$ ). There was a positively correlation between SOD and TBARS (scc 0.282;  $p = 0.001$ ). There was a positively correlation between TBARS and total cholesterol (scc 0.184;  $p = 0.03$ ) and inversely with time of ART exposure (scc -0.256;  $p = 0.031$ ). There was a positively correlation between adiponectin and HDL cholesterol (scc 0.282;  $p = 0.002$ ) and negatively with Framingham risk score (scc -0.203;  $p = 0.03$ ) and IMT (scc -0.260;  $p = 0.032$ ). There was a positively correlation between resistin and IMT (scc 0.369;  $p = 0.002$ ).

## Discussion

In this study, we have found that endothelial activation (VCAM) and inflammatory markers (sPLA-2 and hsCRP) were higher in HIV-infected patients compared to a control group of healthy subjects matched for age, gender and cardiovascular risk factors. Furthermore, HIV infected patients had low levels of TBARS and SOD than healthy subjects. Patients with undetectable viral load had higher biomarker of endothelial activation and inflammation than healthy subjects. However, VCAM was the only biomarker that was higher when no

suppressed patients were compared with suppressed HIV infected patients, suggesting that control of viral replication can reduce inflammation or modify oxidative stress but not improve endothelial dysfunction completely.

sPLA2 enzymes are expressed at very high levels in acute and chronic inflammatory disorders and are involved in multiple steps in atherosclerosis that include lipoprotein remodeling, generation of proinflammatory bioactive lipids, and activation of inflammatory pathways [10]. sPLA2 have important roles in the initiation, progression, and/or rupture of lipid-rich atherosclerotic plaques. To date, there are very limited data in the literature documenting the effect of HIV or ART on levels of sPLA2. Protein Nef and Tat of VIH can induce release of proinflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6 [11] that induce synthesis of sPLA2 in arterial smooth muscle cells and hepatocytes [10]. In our study, sPLA2 was positively correlated with hsCRP, a marker of inflammation and an important predictor of cardiovascular disease (CVD) in the general population [12] that is higher in HIV-infected subjects [13]. We also found a strong correlation between sPLA-2 levels and VCAM, corroborating its role in the development of endothelial activation and CVD.

Reactive oxygen species and oxidatively modified LDL have been implicated in the initiation, progression, and final rupture of the atherosclerotic lesion [14]. We have observed lower concentrations of TBARS in HIV-infected group. Although the pathways is unclear, the inversely relationship between TBARS and time of exposure to ART, suggest that ART could have an antioxidant effect. This is consistent with previous studies that found NNRTI were associated with low peroxide concentrations [14]. This effect was independent of SOD since this antioxidant enzyme was lower in HIV infected patients.

Resistin may promote endothelial cell activation and upregulate several adhesion molecules and cytokines [15]. Adiponectin is a metabolically active adipokine, which is inversely associated with obesity, insulin resistance, and atherosclerosis [15]. Prospective studies have shown that higher plasma adiponectin concentrations are associated with decreased risk of type 2 diabetes mellitus and cardiovascular disease [15]. In our study, no differences were observed between HIV infected patients and healthy subjects due to both groups had very similar body mass index and waist circumference. Although adiponectin was no correlated with others biomarkers, there was an inversely correlation with IMT. Furthermore, resistin was correlated positively with IMT, VCAM and hsCRP indicating that both resistin directly and adiponectin inversely could be cardiovascular risk marker in those patients.

Some study limitations should be acknowledged. First, its transversal nature means that the associations found can not be classified as causal. Second, the number of patients included was not high, that limiting the power and accuracy of the analysis. Nevertheless, the number of patients included allow us to detect differences between subjects with and without HIV and between suppressed and no suppressed patients.

In conclusion, HIV infection is associated with chronic inflammation and endothelial activation that is not completely suppressed by the treatment, regardless of beneficial effect on oxidative stress of ART. The association of new serum biomarkers (sPLA2, TBARS, SOD and resistin) with traditional and no traditional cardiovascular risk factors and IMT suggest that can be useful to predict cardiovascular risk in HIV infected patients.

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