Pregnancy-associated plasma protein-a is associated with subclinical atherosclerosis in men with HIV infection

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ABSTRACT

The pathophysiology of an early and accelerated atherosclerotic process is complex and multifactorial in HIV-infected men compared with HIV-non-infected men. Several biomarkers have been well studied in the detection of the early stage of atherosclerosis, but studies are limited in HIV-infected men. The objective of this study was to investigate the association between serum pregnancy-associated plasma protein-A (PAPP-A) and carotid intima-media wall thickness (CIMT) in asymptomatic HIV-infected men. This a case–control study group comprising 118 HIV-infected men and 60 age-matched and gender-matched HIV-non-infected men. Serum PAPP-A was measured using an ELISA kit and carotid IMT was evaluated by Doppler ultrasonography in all subjects. Statistical analysis included receiver-operating characteristic (ROC) analysis, Pearson correlation and logistic regression analysis. Serum PAPP-A level was significantly higher in HIV +CIMT+ group compared with HIV +CIMT group and HIV–CIMT- group. We found a positive correlation between PAPP-A and increased CIMT (r=0.737, p<0.0001), and a negative correlation between nadir CD4 T cell counts and increased CIMT (r=−0.526, p<0.001). In multivariate logistic regression analyses, PAPP-A, nadir CD4 T cell count and age were significantly associated with subclinical atherosclerosis (p<0.001, p=0.006 and p=0.032, respectively). In ROC analysis, PAPP-A levels of >3.70 µg/mL were associated with subclinical atherosclerosis in HIV+ men with a specificity of 100% and a sensitivity of 71% (area under the curve: 0.949, 95% CI 0.875 to 1.000, p<0.001). Serum PAPP-A level was strongly correlated with increased CIMT in HIV-infected men. PAPP-A might be used as an early biomarker to identify atherosclerosis in asymptomatic HIV-infected men.

Significance of this study

What is already known about this subject?
- The prevalence of coronary artery disease in patients infected with HIV is 1.5–2 times higher than that in the normal population.
- Carotid intima-media thickness (CIMT), as measured by ultrasound, is a reliable noninvasive imaging method for detecting subclinical atherosclerosis in HIV+ and HIV– men.
- Elevated serum pregnancy-associated plasma protein-A (PAPP-A) levels are a strong biomarker for the early diagnosis of acute coronary syndrome.

What are the new findings?
- Serum PAPP-A levels were significantly higher in HIV-infected men who had increased CIMT compared with both HIV-infected men who did not have increased CIMT and the control group.
- PAPP-A levels>3.70 µg/mL were associated with subclinical atherosclerosis in HIV+ men with a specificity of 100% and a sensitivity of 71%.
- PAPP-A was very strongly positively correlated with increased CIMT in HIV-infected men.

How might these results change the focus of research or clinical practice?
- Elevated levels of serum PAPP-A might be considered a biomarker in the diagnosis of silent atherosclerosis in HIV-infected men.
- Future studies should attempt to elucidate whether serum PAPP-A level is associated with the diagnosis of silent atherosclerosis in HIV-infected men.

INTRODUCTION

The prevalence of coronary artery disease is 1.5–2 times higher in patients infected with HIV+ than in the normal population.[1] Although the reason for early atherosclerosis in HIV+ patients is still not clear, the underlying mechanisms implied include chronic inflammation, endothelial dysfunction and disturbance in the coagulation cascade.[2 3] Cardiac complications such as sudden cardiac death, heart failure, cardiomyopathy and arrhythmia are more prevalent in HIV+ patients compared with non-infected (HIV–) individuals.[4 5] Carotid intima-media thickness (CIMT), as measured

by ultrasound, not only is an independent predictor of future cardiovascular events but is also used as an adjuvant to conventional cardiovascular risk factors.[6] Male subjects with >1 mm of CIMT are reported to have a twofold higher risk of coronary artery disease.[7] In addition, CIMT is a reliable non-invasive imaging method for detecting subclinical atherosclerosis in both HIV+ and HIV– men.[8] Pregnancy-associated plasma protein-A (PAPP-A) is a high-molecular-weight zinc-binding metalloproteinase. In the first and second trimesters of pregnancy, elevated serum concentrations of PAPP-A are used to detect genetic anomalies such as Down syndrome. While predominantly synthesized from placental trophoblasts, PAPP-A synthesis also occurs, in very low amounts, from vascular smooth muscle and mesenchymal cells in non-pregnant women and healthy men.[9] Studies have demonstrated that elevated serum PAPP-A levels are a strong biomarker for the early diagnosis of acute coronary syndrome in patients who present with chest pain before an elevation in cardiac troponin level in emergency settings.[10–13] Moreover, a meta-analysis reported that elevated serum PAPP-A levels strongly were associated with subclinical atherosclerosis future cardiovascular adverse events in patients with stable coronary artery disease.[9]

In the present study, we aimed to evaluate the value of PAPP-A in the early diagnosis of atherosclerosis and its association with CIMT in HIV +men.

MATERIALS AND METHODS

This was a case–control study that enrolled HIV+ male subjects (>18 years) who were followed in our HIV outpatient clinic. The control group consisted of age-matched and sex-matched HIV- subjects with similar risk factors. HIV+ patients were divided into two groups based on the presence of CIMT: HIV+ CIMT+ and HIV+CIMT–. All patients were stable under antiretroviral therapy (ART). Age, sex, body mass index, risk factors for cardiovascular disease (family history, hypertension, diabetes, hypercholesterolemia and cigarette status), duration of HIV infection and ART, electrocardiography, surface echocardiography parameters, physical exam and laboratory tests were carefully recorded for each patient. The presence of arterial hypertension, diabetes mellitus and hypercholesterolemia was defined according to the Adult Treatment Panel III criteria.[14] Chronic HIV infection diagnosis and ARV exposure were based on patient report and confirmed by medical records. Nadir CD4, CD4 and CD8 T-cell counts, and HIV viral load were assessed via review of the laboratory records. Exclusion criteria were advanced kidney or liver failure, overt heart failure, systemic inflammatory diseases and the presence of cardiovascular disease (previous coronary artery disease, stroke or intermittent claudication).

Laboratory measurements

Venous blood was drawn from an antecubital vein at the time of hospital admission. After clotting, all samples for PAPP-A measurement were centrifuged for 5 min at 4000 rpm. The serum samples were collected and stored at –20°C until analysis according to the manufacturer's instructions. After the samples were restored to room temperature, circulating PAPP-A level was analyzed with a sandwich ELISA technique using DRG PAPP-A ELISA kits (DRG International, USA) by personnel who had no information of the subjects' clinical characteristics. The results are expressed in μg/mL. The detection range of this method was 0–30 µg/mL. The intra-assay coefficient of variation (CV) was approximately 7.5% and the interassay CV was approximately 9%.

Serum fasting blood glucose, creatinine, blood urea nitrogen, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were measured from a separate fasting serum sample using standard laboratory techniques (Hitachi 7600 Automatic Biochemical Analyzer-Hitachi, Japan).

Carotid intima-media thickness assessment

Both right and left carotid ultrasound was performed by an experienced radiologist who was blinded to the clinical characteristics of the patients. The high-resolution B-mode ultrasound machine (PHILIPS EnVisor Ultrasound) with a 7.5–10 MHz linear probe was used for all participants. Measurement of CIMT was done at 1 cm proximal to the common carotid artery bifurcation, carotid bulb and the proximal segment of the internal carotid artery on both sides. All images were stored and assessed by another radiologist who was also blinded to the patient characteristics. The mean CIMT was used for analysis. If the value of CIMT was ≥0.9 mm, it was accepted as abnormal.

Statistical analysis

Data are reported as the mean ±SD for continuous variables and, if appropriate, compared using independent sample t-tests or Mann-Whitney U tests. Categorical variables are reported as percentages and numbers and, if appropriate, compared using the χ^2 or Fisher's exact test. The normality assumption was evaluated by the Kolmogorov–Smirnov test. Continuous variables were compared among three groups using one-way analysis of variance or the Kruskal–Wallis test. The Pearson correlation was used to find the association between the various parameters and CIMT in the study population. Logistic regression analysis was performed to indicate the association between the dependent (presence of subclinical atherosclerosis) and independent variables in HIV-infected men. The area under the receiver operating characteristic (ROC) curve (area under the curve (AUC)) along with its 95% CI was calculated to detect the different cut-off points for the circulating PAPP-A levels in the diagnosis of subclinical atherosclerosis. The AUC (and its 95% CI), sensitivity and specificity were estimated by selecting a probability threshold that resulted in comparable sensitivity and specificity values. For all statistical tests, a p value<0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows, V.21.

RESULTS

In total, 116 patients and 60 controls met the inclusion criteria. We enrolled 34 patients (mean age, 40.0±11.22) in the HIV+ CIMT+ group, 82 patients (mean age, 39.1±8.87) in the HIV+ CIMT group and 60 patients (mean age, 39.6±7.87) in the control group. The demographic characteristics, clinical features, laboratory findings and cardiac risk profiles of all subjects are shown in table 1.

Table 1 Clinical, demographic and laboratory parameters in patients with and without HIV

	HIV (+) CIMT (+) (n:34)	HIV (+) CIMT (–) (n:82)	HIV (–) CIMT (–) (n:60)	P values
Age (years)	40.0±11.22	39.1±8.87	39.6±7.87	0.728
BMI (kg/m^2)	24.96±2.64	24.78±2.64	25.28±2.20	0.633
Current smoker, n (%)	9 (60.0)	28 (68.3)	26 (55.3)	0.458
Systolic blood pressure (mm Hg)	113.7±11.6	110.5±12.1	110.0±8.3	0.356
Diastolic blood pressure (mm Hg)	68.33±6.98	66.46±6.73	66.98±6.57	0.416
Fasting glucose (mg/dL)	87.71±11.29	85.93±7.54	86.65±7.60	0.769
Creatinine (mg/dL)	0.88±0.13	0.95±0.12	0.90±0.09	0.067
Total cholesterol (mg/dL)	171.73±32.69	178.47±41.43	176±32.79	0.825
LDL-C (mg/dL)	107.73±19.55	113.48±38.68	104.55±27.86	0.440
Triglycerides (mg/dL)	142.33±78.94	129.13±50.83	132.67±74.91	0.466
10-year risk of CHD disease (Heartscore),%	2.1±0.5	1.9±0.5	2.0±0.4	0.814
Resting heart rate (bpm)	78.0±11.55	78.76±11.29	72.97±11.17	0.175
E/A ratio	1.34±0.29	1.34±0.32	1.33±0.30	0.450
LVEF (%)	66.5±5.81	65.9±0.5.20	66.1±5.33	0.856
PAPP-A (µg/mL)	3.81±2.20	0.14±0.05	0.11±0.03	<0.001
Duration of HIV (months)	15.9±14.8	16.9±15.15	–	0.318
Viral load				
≥1000 copies/mL, n (%)	5 (14.7)	11 (13.4)	–	0.495
<1000 copies/mL, n (%)	29 (85.3)	71 (86.6)	–	0.518
Nadir CD4 T cell count (cells/mm^3)	186.0±78.64	465.55±135.95	–	<0.001
CD4 T cell count	598.2±304.4	616.2±310.5	–	0.752
Duration of ART use (months)	15.2±12.1	16.5±11.5	–	0.419

Categorical variables are expressed as frequencies (%). Continuous variables are expressed as the mean (SD).
*HIV+CIMT+ vs HIV+CIMT, p<0001.
†HIV+CIMT+ vs HIV– CIMT–, p<0001.
ART, antiretroviral therapy; BMI, body mass index; CHD, coronary heart disease; CIMT, carotid intima-media wall thickness; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; PAPP-A, pregnancy-associated plasma protein-A.

There were no significant differences among the groups with respect to age, body mass index (BMI), systolic and diastolic blood pressure or smoking status (p>0.05 for all; table 1). Creatinine, LDL-C, TC, TG and fasting glucose of each group were similar (p>0.05, for all; table 1). Serum PAPP-A levels were significantly higher in the HIV+ CIMT+ group compared with both the HIV+ CIMT group and HIV– CIMT– group (3.81±2.20, 0.14±0.05, 0.11±0.03, p<0.001, respectively, table 1). However, serum PAPP-A levels were similar between the HIV+ CIMT group and the HIV– CIMT– group (p>0.05). Considering echocardiographic parameters, there was no difference in left ventricular ejection fraction or left ventricular diastolic function between the groups (p>0.05, for all; table 1). We used the ROC analysis to find a cut-off value for predicting subclinical atherosclerosis. We found that PAPP-A levels of >3.70 µg/mL were associated with subclinical atherosclerosis in HIV+ men with a specificity of 100% and a sensitivity of 71% (AUC: 0.949, 95% CI 0.875 to 1.000, p<0.001;figure 1). Pearson correlation coefficients were calculated in order to analyze associations between increased CIMT and PAPP-A, nadir CD4 T cell count, HbA1c, duration of HIV infection, ART duration and LDL-C. PAPP-A was significantly and very strongly positively correlated with increased CIMT (r=0.737, p<0.0001, table 2). Nadir CD4 T cell count was moderately inversely correlated with increased CIMT (r=−0.526, p<0.001, table 2). In addition, PAPP-A level was inversely correlated with nadir C4 T cell count (r=−0.676, p<0.001). However, HbA1c, duration of HIV infection, ART duration and LDL-C did not show any correlation with increased CIMT (r=0.342, p=0.08; r=−0.110, p=0.421; r=−0.158, p=0.246; r=0.099, p=0.481, respectively, table 2). Multivariate logistic regression analysis including serum PAPP-A level, nadir CD4 T cell count, age, current smoker, glucose, creatinine and LDL-C showed that only serum PAPP-A level, nadir CD4 T cell count and age were significantly associated with subclinical atherosclerosis in HIV-infected men (OR 5.718, 95% CI 3.526 to 8.801, p<0.001; OR 0.984, 95% CI 0.972 to 0.995, p=0.006; OR 1.211, 95% CI 1.016 to 1.444, p=0.032, respectively, table 3).

DISCUSSION

Our study is the first to investigate the association between serum PAPP-A levels and increased CIMT, which is considered an early marker of atherosclerosis in HIV+ men. Our findings showed that HIV+ men with increased CIMT had higher serum PAPP-A levels than that in their counterparts with normal CIMT. The HIV+ and HIV– men with normal CIMT did not significantly differ in terms of serum PAPP-A levels. For the detection of early atherosclerosis in HIV+ men, the strongest positive correlation was between elevated CIMT and PAPP-A; there was a moderately strong negative correlation between elevated CIMT and low nadir CD4. Mutivariate logistic regression analysis indicated that subclinical atherosclerosis was associated with serum PAPP-A level, nadir CD4 T cell count and age.

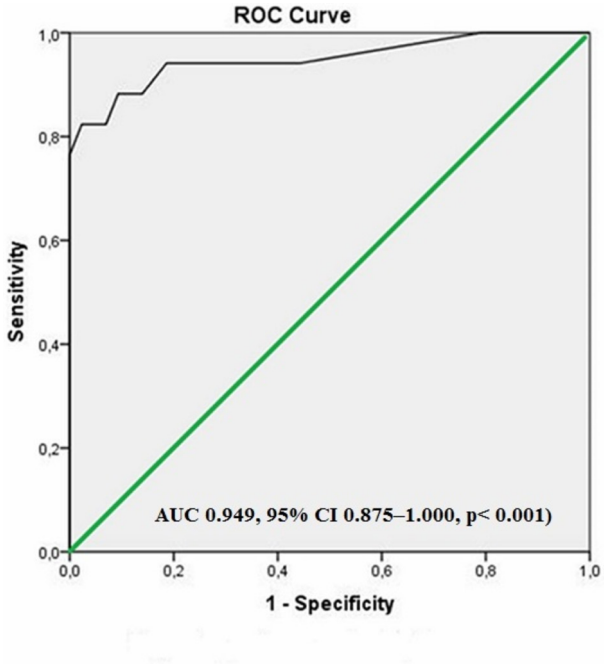

Figure 1 Receiver operating characteristic curve (ROC) shows that pregnancy-associated plasma protein-A (PAPP-A) level >3.70 µg/mL has a sensitivity of 71% and a specificity of 100% is associated with subclinical atherosclerosis in HIV-infected men (area under the curve (AUC) 0.949, 95% CI 0.875 to 1.000, p<0.001).

In addition, this study further revealed that serum PAPP-A levels>3.70 µg/mL were associated with subclinical atherosclerosis in HIV + male patients with a specificity of 100% and a sensitivity of 71%.

Atherosclerosis plays a major role in the development of cardiovascular diseases.[15] Increase in CIMT occurs prior to the formation of plaque in the artery or clinical signs. As increased CIMT is an independent and strong predictor of future cardiovascular and cerebrovascular events, it is often used for early diagnosis of atherosclerosis.[16 17] Several studies have reported the coexistence of increased CIMT in HIV and other chronic infectious diseases. Although the pathophysiology of cardiovascular diseases in HIV-infected patients is thought to be multifactorial, it still remains uncertain. Nevertheless, adirect effect of the virus, opportunistic infections and ART are among the most implied factors in the pathophysiology of atherosclerosis in HIV+ patients. In addition, low nadir CD4 T cell lymphocyte counts were reported to increase activation of T cells, which play a key role in the disease pathophysiology in HIV-infected patients.[8 18 19] In our study, we tried to minimize conventional cardiovascular risk factors in the HIV-infected male participants to avoid bias. Our subjects did not have hypertension, diabetes, dyslipidemia or risk factors regarding family history. However, over half (approximately 60%) were smokers. All patients had a low 10-year cardiovascular risk, as calculated by the Heartscore method (2% on average). The prevalence of CIMT was 23.6% in patients with low cardiovascular risk (n=34).

PAPP-A, which is a member of the insulin-like growth factor (IGF) family that has proteolytic activity, is a metalloproteinase causing an elevation in the local concentration of IGF-1. An elevated IGF-1 level was suggested to lead to both the activation of cytokines in atherosclerotic plaque and migration of vascular smooth muscle cells, which are important for monocyte chemotaxis. Therefore, increased IGF-1 levels are thought to play a critical role for coronary atherosclerosis and progression of restenosis.[20 21] Since metalloproteinases cause degradation of extracellular matrix and instability of lipid-rich atherosclerotic plaques in coronary arteries, initial clinical trials with PAPP-A were performed in patients with acute coronary syndrome.[10–13] In an autopsy study after sudden cardiac death, PAPP-A-directed monoclonal human antibody was used in coronary arteries with rupture and eroded plaques, and PAPP-A was especially identified in the shoulder region of the plaques. Serum PAPP-A levels were also measured in patients with unstable angina (UA), myocardial infarction (MI) and stable angina in the same study, where a very severe PAPP-A elevation was reported in patients with UA and MI compared with a minimal increment in patients with stable angina.[12] Bonaca *et al*[22] found that increasing PAPP-A was an independent predictor of recurrent cardiovascular events in patients with non–ST segment elevation acute coronary syndrome. Lund *et al*[23] reported that PAPP-A levels>2.9 mIU/L were the best cut-off value for the combined primary end point at 6 months follow-up in patients with acute coronary syndrome and not yet elevated cardiac troponin-I. The present study prospectively demonstrated a significant relationship between increased serum PAPP-A level and subclinical atherosclerosis in HIV-infected men. Moreover, we found that a serum PAPP-A level of >3.70 µg/mL might be

Table 2 Pearson correlation coefficients for PAPP-A and other variables in relation to CIMT in HIV-infected men

	r Values	P values
PAPP-A	0.737	<0.001
Nadir CD4 T cell counts	−0.526	<0.001
HbA1c	0.342	0.08
Duration of HIV infection	−0.110	0.421
ART duration	−0.158	0.246
LDL-C	0.099	0.481

ART, antiretroviral therapy; CIMT, carotid intima-media wall thickness; LDL-C, low-density lipoprotein cholesterol; PAPP-A, pregnancy-associated plasma protein-A.

Table 3 Association between subclinical atherosclerosis and other variables using multivariate logistic regression analysis in HIV-infected men

	OR (95% CI)	P values
PAPP-A>3.70 µg/mL	5.71 (3.52 to 8.80)	<0.001
Nadir C4 T cell count	0.98 (0.97 to 0.99)	0.006
Glucose	1.00 (0.88 to 1.14)	0.915
LDL-C	0.98 (0.94 to 1.02)	0.475
Current smoker	2.18 (0.68 to 8.33)	0.097
Age	1.21 (1.01 to 1.44)	0.032
Creatinine	1.31 (0.79 to 2.01)	0.269

LDL-C, low-density lipoprotein cholesterol; PAPP-A, pregnancy-associated plasma protein-A.

useful in the diagnosis of silent atherosclerosis in HIV+ men. While the mechanism for elevated PAPP-A levels is defined for unstable plaques, the reason for elevation in patients with stable plaques is not clear for. In coronary arteries, unstable plaques originate from gradual enlargement of the lipid core and thinning of the fibrous cap in stable plaques. This is important as it implies that PAPP-A seems to be a cause of the acute coronary syndrome, not a consequence as is the case for cardiac troponins. Macrophages, vascular smooth muscle cells, fibroblasts and endothelial cells have major roles in the atherosclerotic process.[24] T lymphocytes and macrophages are known to be predominantly active in the vascular bed in HIV+ patients with low nadir CD4 levels. Therefore, reduced nadir CD4 levels and elevated PAPP-A levels may be the two main factors for the initiation of the atherosclerotic process in HIV-infected male patients. We speculate that PAPP-A, which is synthesized by activated fibroblasts, vascular smooth muscle cells and macrophages, plays a significant role in the initiation and progression of the asymptomatic atherosclerotic process in HIV-infected male patients with reduced nadir CD4 levels. Therefore, we suggest that measurement of serum PAPP-A levels in HIV-infected men may help to detect subclinical atherosclerosis and may prevent further cardiovascular events. Several limitations of the study should be considered in commenting on these results. First, this is a single-institution study involving only HIV-infected males. Therefore, it is not appropriate to use our findings in HIV-infected women. Second, the size of the HIV+ CIMT+ subgroup in this study was relatively small. Third, an HIV CIMT+ subgroup was not taken into consideration in this study. Fourth, the PAPP-A level of each subject was analyzed only once during follow-up in the outpatient clinic. Our study reveals that circulating PAPP-A level is strongly associated with subclinical atherosclerosis in HIV-infected men. An elevated level of serum PAPP-A might be considered as a biomarker in the diagnosis of silent atherosclerosis in HIV-infected men.

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Competing interests None declared.

Patient consent Not required.

Ethics approval The study was performed according to the recommendations of the Declaration of Helsinki on Biomedical Research Involving Human Subjects and the Good Clinical Practice Guidelines. The study protocol was approved by the ethics committee at our institute.

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