Impaired adipokine profile in HIV positive patients without metabolic syndrome: the role of antiretroviral therapy

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Key words: Adiponectin; leptin; osteoprotegerin; HAART

ABSTRACT

Adipose tissue secretes a range of biologically active proteins called adipokines. Dysregulation of adipokines is implicated in the etiology of insulin resistance and metabolic syndrome, but the relation between adiponectin, leptin and antiretroviral therapy has been poorly studied and subject to controversy. This study investigated the relation between plasma adipokine levels and different antiretroviral drug exposure in HIV positive subjects with similar metabolic and anthropometric parameters and without metabolic syndrome. Our data show that antiretroviral drugs impair the adipokine and osteoprotegerin profiles in HIV positive patients without metabolic syndrome and in the absence of impaired glycemic and lipidic parameters.

Introduction

Since 1996 treatment with a combination of antiretroviral agents has become the standard care for HIV positive patients. These highly active antiretroviral therapies (HAART) have dramatically reduced HIV-related morbidity and mortality. The management of HIV therapy, however, has been complicated by the long-term toxicity of antiretroviral medications. HIV-infected individuals receiving HAART have a high rate of metabolic disorders associated with an increased risk of cardiovascular disease. These disorders have become an important cause of morbidity and mortality in HIV-infected patients. Insulin resistance syndrome plays an important role in the pathogenesis of cardiovascular diseases and metabolic syndrome (Samaras et al., 2007). Multiple mechanisms are thought to contribute to pathogenesis of metabolic syndrome: among them, adipose tissue excess is known to play a major role, although the functions of molecular mediators remain unclear (Friedman, 2002; Kershaw and Flier, 2004; Maeda et al., 2002). Adipose tissue is the site of expression and secretion of a range of biologically active proteins called adipokines, e.g. leptin and adiponectin (Staiger et al., 2003). Adipokine dysregulation is known to be involved in the etiology of insulin resistance (Mynarcik et al., 2002). Adiponectin has anti diabetic and antiatherogenic properties (Shimabukuro et al., 2003; Yamauchi et al., 2003). Adiponectin plasma levels are significantly reduced in obese subjects, insulin-resistant subjects and type 2 diabetic patients, and increased after weight reduction (Spranger et al., 2003; Weyer et al., 2001). Adiponectin influences insulin sensitivity and lipid metabolism, but it is not clear whether these effects are correlated with fat mass or distribution (Goldfine & Kahn, 2003; Stefan et al., 2002; Tscherter et al., 2003). Leptin is inversely correlated with adiponectin; its plasma levels affect energy homeostasis by inhibiting food intake and stimulating energy expenditure. Both leptin synthesis and circulating levels are increased in obese patients and are correlated with the fat mass; most obese patients are leptin resistant (Friedman & Halaas, 1998). In addition leptin has been shown to be expressed in osteoblasts and to promote bone mineralization, whereas adiponectin expression is enhanced during osteoblast differentiation.

The relation between adiponectin, leptin and osteoprotegerin and antiretroviral therapy has been poorly studied and is subject to controversy. Moreover data on adipokines and their biological effects are based primarily on research in non–HIV-infected populations.

The purpose of this study was to investigate at the relation between plasma adiponectin, leptin and osteoprotegerin levels, metabolic parameters, anthropometric factors and antiretroviral drug exposure.

Methods

Patients

The study population consisted of 59 subjects: 49 HIV-positive patients were recruited from the Day Hospital of the Department of Tropical and Infectious Diseases, “Sapienza” University of Rome, Italy. All HIV-positive patients were on a stable and effective antiretroviral therapy: 20 patients were on a first line antiretroviral therapy with lopinavir/ritonavir (LPV/r), 14 patients were on a first line antiretroviral therapy with efavirenz (EFV), 15 patients were on a simplified therapy with abacav-
Patients with metabolic syndrome served as controls. After signing their written and informed consent to participate in the study, subjects provided a fasting blood sample and completed an interview about their smoking status, sedentarity and current medications. Demographic data were collected for each patient: age, weight, height, duration of HIV infection, duration and types of all antiretroviral therapy, CD4+ cell count and HIV-RNA copies. The patients had no signs of ongoing infections and had fasting glucose below 110 mg/dl.

Exclusion criteria for HIV positive patients were a diagnosis of metabolic syndrome defined by National Cholesterol Education Program Adult Treatment Panel III report as a constellation of the following metabolic risk factors: waist circumference ≥ 102 cm (male) and ≥ 88 cm (female), triglycerides = 150 mg/dl, HDL cholesterol < 40 mg/dl (male) and < 50 mg/dl (female), blood pressure = 130/85 mmHg, fasting glucose ≥ 110 mg/dl. A diagnosis of metabolic syndrome was made if a subject had three or more of the above features. (Samaras et al., 2007)

Cardiovascular risk of HIV positive patients was evaluated by the Framingham study and "Progetto Cuore" model.

Methods

Blood samples were obtained after an overnight fast. Serum chemistry was performed on the day of blood collection. Serum was then separated and stored at −80 °C for the other measurements. Serum glucose, cholesterol, triglycerides, HDL cholesterol, creatinine and SGPT were measured using an automated Kodak dry chemistry system. LDL cholesterol, creatinine and SGPT were measured using commercially available kits: leptin (DSL, Texas, USA, intra-assay coefficient of variation 4.9%); adiponectin (LINCO Research, Inc, St Charles, MO, USA intra-assay coefficient of variation < 9.5%); osteoprotegerin measurements as well as adipokine assays were performed in duplicate. Insulin measurements were performed using commercial chemiluminescent assays (Immulite, DPC, LA).

Bone density was measured using the following criteria: osteopenia/osteoporosis, high creatinine levels or known renal diseases, known liver diseases and use of corticosteroids. Patients on lipid lowering and/or on antihypertensive medication were not included in the analysis.

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Table 1: Antiretroviral therapy of HIV positive patients

<table>
<thead>
<tr>
<th>N° PATIENTS</th>
<th>BACKBONE</th>
<th>% PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Patients: lopinavir/r (1st Line therapy)</td>
<td>didanosine + lamivudine</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>tenofovir + emtricitabine</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>zidovudine + lamivudine</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>abacavir + lamivudine</td>
<td>15</td>
</tr>
<tr>
<td>14 Patients: efavirenz (1st Line therapy)</td>
<td>didanosine + lamivudine</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>tenofovir + emtricitabine</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>zidovudine + lamivudine</td>
<td>21.4</td>
</tr>
<tr>
<td>15 Patients: trizivir + tenofovir (Simplified therapy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Patients: controls</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients were classified according to components of the metabolic syndrome defined by the National Cholesterol Education Program Adult Treatment Panel III report as a constellation of the following metabolic risk factors: waist circumference ≥ 102 cm (male) and ≥ 88 cm (female), triglycerides = 150 mg/dl, HDL cholesterol < 40 mg/dl (male) and < 50 mg/dl (female), blood pressure = 130/85 mmHg, fasting glucose ≥ 110 mg/dl. A diagnosis of metabolic syndrome was made if a subject had three or more of the above features. (Samaras et al., 2007)

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a quantitative reverse polymerase chain reaction (Amplicor HIV Monitor; Roche Diagnostic Systems, Branchburg, NJ). The limit of detection was 50 copies/ml.

**Statistical analysis**

Descriptive statistics were used to describe the sample. The chi square test was used to compare categorical variables, and the Student’s test for continuous variables. Univariate analysis was performed to examine the relation with adherence level by computing analysis of variance. To obtain adjusted estimation, we used a multiple logistic regression model. A P-value < 0.05 was considered statistically significant for all analyses.

All statistical analyses were performed using the SPSS software programs (SPSS Inc., Chicago, Illinois, USA).

**Results**

**Patient characteristics**

The study population comprised 35 males (71.4%) and 14 females (28.6%) with a mean age of 44.7 years. Of 49 patients enrolled in the study the primary mode of HIV transmission was heterosexual exposure (26 patients, 53%), followed by homosexual exposure (12 patients, 24.5%), bisexual exposure (8 patients, 16.4%), and finally injection drug abuse (3 patients, 6.1%). No patient was an active drug abuser. The sex ratio (female/male) was 0.4 for the total population, 0.4 for the HIV-positive group and 0.42 for the control group. No differences in sex or age were observed in the two groups of patients. Self-reported adherence data were available for all patients. At enrolment an optimal adherence was reported in all participants and all patients had a sedentary lifestyle. HIV/HBV co-infection was present only in one subject; HIV/HCV co-infection was found in two patients. All cases of co-infection were in the group on therapy with trizivir plus tenofovir.

**Immuno-virological findings**

Nadir level of CD4+ cells was 162.6±118.4 cells/mmc (mean±SD). HIV-RNA zenith was 188800±165100 copies/ml (mean±SD). CD4+ cell count at the time of enrolment was 511.6±250.5 cells/mmc (mean±SD) and all patients presented an undetectable HIV viral load.

**Antiretroviral regimen**

The duration of HAART (mean±SD) was 22.1 ± 10.4 months for patients on first line therapy and 39.5 ± 11.3 months for patients on simplified therapy. No patient had a history of virologic failure.

**Anthropometric and metabolic findings**

The study of anthropometric parameters in the HIV positive group showed: BMI 24.08±2.08 (mean±DS), ponderal index 12.61±0.37 (mean±DS), Khosla's obesity index 5.04±0.41 (mean±DS), BSA 1.84±0.12 m² (mean±DS) and WHR 0.89±0.093 (mean±DS). Clinical and laboratory features of the 49 HIV-positive subjects included in the study are shown in Table 2. There was no significant difference in mean age, blood pressure, fasting glucose, fasting insulin, HOMA-IR, total cholesterol, HDL cholesterol, triglycerides, BMI, ponderal index, Khosla's obesity Index, BSA or WHR.

**Cardiovascular risk evaluation**

Cardiovascular risk of HIV positive patients, evaluated by the Framingham study and "Progetto Cuore" model, was not higher than the risk of the general population. No statistical differences were found between groups on different antiretroviral therapy (Table 3).

**Adipokines and osteoprotegerin dosages**

Plasma adiponectin, leptin and osteoprotegerin levels are shown in Table 4. Plasma adiponectin levels were similar in the group of HIV positive

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<table>
<thead>
<tr>
<th>Lopinavir/r</th>
<th>Efavirenz</th>
<th>Trizivir + Tenofovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean ± DS</td>
<td>mean ± DS</td>
<td>mean ± DS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.3</td>
<td>9.08</td>
</tr>
<tr>
<td>CD4+ nadir (cells/mmc)</td>
<td>145.7</td>
<td>105.6</td>
</tr>
<tr>
<td>CD4+ actual (cells/mmc)</td>
<td>409.5</td>
<td>154.4</td>
</tr>
<tr>
<td>HIV-RNA zenith (copies/ml)</td>
<td>172000</td>
<td>150700</td>
</tr>
<tr>
<td>HIV-RNA actual (copies/ml)</td>
<td>&lt; 50</td>
<td>0</td>
</tr>
<tr>
<td>BMI</td>
<td>23.61</td>
<td>1.524</td>
</tr>
<tr>
<td>Ponderal index</td>
<td>12.64</td>
<td>0.2286</td>
</tr>
<tr>
<td>Khosla's obesity index</td>
<td>4.976</td>
<td>0.2653</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.805</td>
<td>0.1086</td>
</tr>
<tr>
<td>Waist circumference (cm) - male</td>
<td>84.17</td>
<td>5.006</td>
</tr>
<tr>
<td>Waist circumference (cm) - female</td>
<td>68.75</td>
<td>4.234</td>
</tr>
<tr>
<td>WHR</td>
<td>0.8585</td>
<td>0.1018</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.968</td>
<td>0.6093</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>161.8</td>
<td>16.42</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>34.7</td>
<td>4.911</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>141</td>
<td>17.8</td>
</tr>
</tbody>
</table>
patients and in HIV-seronegative controls with the metabolic syndrome (P = 0.3). The analysis of groups of HIV positive patients on therapy showed plasma adiponectin levels significantly higher in the LPV/r group than the EFV group and trizivir + tenofovir group (P <0.005).

Plasma leptin levels were significantly lower in the EFV group than the LPV/r group and trizivir + tenofovir group. Likewise, leptin levels of patients on EFV were significantly lower than leptin levels of HIV-seronegative controls with the metabolic syndrome. On the other hand, plasma leptin levels were similar in HIV-seronegative controls and in the group of patients on therapy with LPV/r (P = 0.07) and the trizivir + tenofovir group (P = 0.3) (Figure 1). These differences remained significant also after data adjustment for age and sex.

Plasma osteoprotegerin levels were similar in HIV-seronegative controls and in the group of patients on LPV/r and the trizivir + tenofovir group. Plasma osteoprotegerin levels of patients on EFV were significantly higher than osteoprotegerin levels of HIV-seronegative controls with the metabolic syndrome (P = 0.006). Analysis of the relation between serum osteoprotegerin levels and cardiovascular risk factors showed that in this cohort osteoprotegerin was not correlated with BMI, waist, systolic or diastolic blood pressure. Osteoprotegerin was not found to be positively correlated with adiponectin levels; no significant correlation was reported with serum leptin levels.

Discussion
Since the introduction of highly active antiretroviral therapy, multiple metabolic and morphologic changes have been described among HIV infected patients and are thought to be the result of direct and indirect effects of antiretroviral medications. The development of a metabolic syndrome has a pivotal role in the pathogenesis of cardiovascular diseases and morphologic changes. Multiple mechanisms are thought to contribute to the pathogenesis of metabolic syndrome although the functions of molecular mediators remain unclear (Friedman, 2002; Kershaw and Flier, 2004; Maeda et al., 2002; Samaras et al., 2007).

Adipokine dysregulation is known to be involved in the etiology of insulin resistance and metabolic syndrome, but the relationship between adiponectin, leptin and antiretroviral therapy has been poorly studied and is subject to controversy (Haque et al., 2002; Kosmiski et al., 2003; Lindegaard et al., 2004). Previous papers indicated that plasma adiponectin and plasma leptin concentrations are correlated with several clinical and metabolic variables of the subjects studied. Plasma adiponectin is negatively correlated with BMI, waist circumference, waist-to-hip ratio, systolic and diastolic blood pressures, fasting plasma glucose and insulin, HOMA-IR, total cholesterol/HDL-cholesterol ratio and triglycerides, whereas it is positively correlated with age and HDL-cholesterol (Addy et al., 2003; Arita et al., 1999; Das et al., 2006; Sweeten et al., 2007; Yang et al., 2001). In addition, plasma leptin levels are positively correlated with BMI, waist circumference, WHR, fasting plasma glucose and insulin, HOMA-IR, and HDL-cholesterol and negatively correlated with age and total cholesterol/HDL-cholesterol (Grinspoon et al., 1996; Ryan et al., 2003).

The aim of this study was to investigate the relation between plasma adipokine levels and different antiretroviral drug exposure in HIV positive subjects with similar metabolic and anthropometric parameters and without metabolic syndrome. Our
data showed that plasma adiponectin levels were similar in groups of HIV positive patients and in HIV-seronegative controls with the metabolic syndrome. Plasma adiponectin levels were significantly higher in the LPV/r group than the EFV group and the trizivir + tenofovir group. On the other hand, leptin levels of patients on EFV were significantly lower than leptin levels of HIV-seronegative controls with the metabolic syndrome. Moreover plasma leptin levels were similar in HIV-seronegative controls and in groups of patients on LPV/r and the trizivir + tenofovir group.

The analysis of groups on different therapy showed that plasma leptin levels were significantly lower in the EFV group than the LPV/r group and trizivir + tenofovir group. Despite the increasing number of recent reports on the role of adipokines in metabolism impairment in HIV negative patients, their role in HIV positive patients on antiretroviral therapy also remains unclear. Recent studies have shown that adiponectin levels are lower in HIV-infected patients than in healthy controls and that the levels decrease further with non-NRTI-based antiretroviral therapy (Lee et al., 2004). Other studies have shown that serum adiponectin levels changed after substitution of nevirapine for protease inhibitors (Petit et al., 2004).

A recent paper reported that plasma adiponectin levels increased in patients on therapy with TPV/r: in this case the increases in adiponectin levels may be a compensatory response to the dyslipidemic effects of the regimen rather than to insulin resistance. This possibility is supported by a study that showed adiponectin administered to mice receiving ritonavir significantly reduced lipid levels (Xu et al., 2004). Moreover in HIV lipodystrophy syndrome, adiponectin levels have been found to be relatively low despite the lower fat mass (Kosmiski et al., 2003), suggesting that adiponectin does not retain its normal inverse relationship with total adiposity. Different antiretroviral drugs may impair the adipokine system in different ways, but the biological mechanism and clinical implications of these changes are uncertain and require a better understanding. Our data show that antiretroviral drugs also impair the adipokine profiles in HIV positive patients without clinical signs of metabolic syndrome. Moreover these changes in plasma concentration of adipokines were observed in our cohort in the absence of impaired glycemic or lipidic parameters. Another important finding of this study is that adiponectin and leptin levels were altered in patients with a cardiovascular risk (evaluated by Framingham and "Progetto Cuore" programs) similar to the risk of the general population of the same sex and age.

The relation between osteoprotegerin and adipokines has been poorly studied and is subject to controversy. Osteoprotegerin is mainly secreted by bone but is also secreted by a variety of different tissues including cells of the cardiovascular system. For example, some studies have identified endothelial and smooth muscle cells as cellular sources of osteoprotegerin (Hofbauer et al., 2001; Collin-Osdoby et al., 2001). The contribution of skeletal versus non-skeletal osteoprotegerin to circulating levels remains unclear. In our study osteoprotegerin was not correlated with leptin and adiponectin levels, and plasma osteoprotegerin levels were similar in HIV-seronegative controls and in the group of patients on therapy with LPV/r and the trizivir + tenofovir group. On the other hand, we found that plasma osteoprotegerin levels of patients on EFV therapy were significantly higher than osteoprotegerin levels of HIV-seronegative controls with the metabolic syndrome. Recent reports of Gannage-Yeared showed that circulating osteoprotegerin levels are favourably associated with some components of the metabolic syndrome in HIV negative subjects, and osteoprotegerin was a risk factor for progressive atherosclerosis and cardiovascular disease (Gannage-Yeared et al., 2006; Gannage-Yeared et al., 2008). The present study disclosed that plasma osteoprotegerin levels were also modified in HIV positive patients without metabolic syndrome but the mechanisms of this alteration remain unclear.

Osteoprotegerin, leptin and adiponectin plasma concentrations were found to correlate with many anthropometric parameters in the HIV population but their relationship with antiretroviral drugs requires a better understanding. The transformation of HIV infection from an ultimately fatal disease to a chronic disease has been a remarkable medical achievement but it has come at a cost. The challenge now is to understand the molecular mechanisms related to the side effects of antiretroviral drugs to help clinicians develop treatment strategies and drug regimens that minimize the risks of these complications and optimize their management.

REFERENCES


