Serum adipokines and HIV viral replication in patients undergoing antiretroviral therapy

Victoria Aramă,[1] Cătălin Tilișcan,[2] Daniela Adriana Ion,[3] Raluca Mihăilescu,[4] Daniela Munteanu,[5] Anca Streinu-Cercel,[6] Ana Maria Tudor,[7] Adriana Hristea,[8] Viorica Leoveanu (†),[9] Ioana Olaru,[10] Ștefan Sorin Aramă[11]

Abstract

Introduction **Several studies have reported that cytokines secreted by adipose tissue (adipokines) may be linked to HIV replication. The aim of the study was to evaluate the relationship between HIV replication and serum levels of adipokines, in a Caucasian HIV-infected population of men and women undergoing complex antiretroviral therapy.**

Methods **A cross-sectional study was conducted in an unselected sample of 77 HIV-1-positive patients. Serum adipokines levels were measured including circulating adiponectin, leptin, resistin, tumor necrosis factor alpha (TNF-alpha) and interleukin-6 (IL-6). Patients were divided into two groups: Group 1 - with undetectable viral load and Group 2 - with persistent HIV viral replication. Differences between groups were tested using independent-sample t-test for Gaussian variables and Mann–Whitney–Wilcoxon test for non-parametric variables. Pearson's chi-squared test was used for correlation analysis.**

Results **A total of 77 patients (age range: 17-65, mean: 32.5 years) including 44 men (57.1% men, age range: 17–63 years, mean: 34.1 years) and 33 women (42.9% women age range: 19–65 years, mean: 30.3 years) were included in the study. TNF-alpha had significantly higher serum levels in patients with detectable viral load (16.89 vs. 9.35 pg/mL), (p=0.043), but correlation analysis lacked statistical significance. Adiponectin had median serum levels of 9.22 µg/mL in Group 1 vs. 16.50 µg/mL in Group 2 but the results lacked statistical significance (p=0.059). Higher leptin, IL-6 and resistin serum levels were noted in patients with undetectable HIV viral load, without statistical significance.**

Conclusions **The present study reported higher TNF-alpha serum levels in patients with persistent HIV viral load. We found no statistically significant correlations between adiponectin, leptin, resistin and IL-6 and HIV viral load in our Caucasian HIV-positive study population, undergoing antiretroviral therapy.**

Keywords **Adipokines, adiponectin, leptin, resistin, TNF-alpha, HIV**

[1]MD, PhD, Associate Professor, Department of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [2]Assistant Lecturer, Department of Physiopathology and Immunology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; [3]Professor, Department of Physiopathology II, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; [4]Researcher, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [5]Researcher, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [6]MD, PhD, Lecturer, Department of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [7]MD, PhD, Lecturer, Department of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; MD, PhD, [8]Lecturer, Department of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [9]Biochemist, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [10]MD, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', Romania; [11]MD, PhD, Professor, Department of Physiopathology and Immunology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

*Corresponding author: Cătălin Tilișcan, MD, National Institute for Infectious Diseases 'Prof. Dr. Matei Balș', 1 Dr. Calistrat Grozovici street, Bucharest, Romania, Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu street, Bucharest, 020022, Romania, catalin_tiliscan@yahoo.com

Introduction

Adipokines are adipocyte-synthesized cytokines that have various immunomodulating roles. Adipocytes release adiponectin, leptin, resistin, tumor necrosis factor-alpha (TNF-alpha) and various interleukins (IL).[1]

The introduction of complex combined antiretroviral therapy (cART) has determined a significant increase in the life expectancy of HIV-positive patients but has also led to fat redistribution (lipodystrophy) and complex metabolic changes.[2-4] Lipodystrophy associates subcutaneous lipid wasting with central adiposity.[3,5] Lipodystrophy may be associated with chronic inflammation and high levels of TNF-alpha.[6] TNF-alpha may promote HIV replication via activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB).[7] Leptin regulates caloric intake via hypothalamic feedback and contributes to energy homeostasis and neuroendocrine function. Leptin also exerts immunomodulatory activity and may be associated with HIV viral replication.[8]

The aim of the study was to evaluate the relationship between HIV replication and serum levels of adipokines, in a Caucasian HIV-infected non-diabetic population of men and women, undergoing cART.

Methods

A cross-sectional study was performed in a Caucasian cohort of HIV-1-positive patients attending the National Institute of Infectious Diseases 'Prof.Dr. Matei Balș' in Bucharest, Romania, between June 2009 and June 2010. The study was part of a prospective Romanian research grant (SLD-ART no. 62077/2008) on HIV infected patients. The research was approved by the local research ethics committee (protocol no. 190/15.01.2009).

The study enrolled 77 consecutive patients who gave their written informed consent to participate in the study. All enrolled patients had undergone cART for at least 6 months at the time the blood samples were collected. A standard questionnaire was filled out by the patients for demographic parameters and for personal and family medical histories.

We calculated the body mass index (BMI), defined according to WHO criteria.[9] Patients were classified as being overweight and obese with BMI of 25-29.9 kg/m^2 and ≥ 30 kg/m^2, respectively.

Blood samples were collected and tested for: HIV viral load (Roche LightCycler® 480 Real-Time PCR System); CD4 T cell count (BD FACSCanto II flow cytometer); leptin (BioSource EASIA (Enzyme Amplified Sensitivity Immunoassay), KAP2281, Belgium); adiponectin (BioSource ELISA, KAPME09, Belgium); resistin (BioSource ELISA, KAPME 50, Belgium); TNF-alpha (BioSource EASIA (Enzyme Amplified Sensitivity Immunoassay), KAP 1751, Belgium); interleukin-6 (IL-6) (BioSource EASIA (Enzyme Amplified Sensitivity Immunoassay), KAP 1261, Belgium); fasting insulin (ELx50 Bioelisa Washer BioTek, Spain) and fasting plasma glucose, fasting triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol (dry slide technology, Vitros 950, Johnson & Johnson, USA).

Insulin resistance was measured using the Homeostatic Model Assessment of Insulin Resistance, known as the HOMA-IR, described by Matthews et al.[10] Using the fasting insulin and fasting glucose levels, HOMA-IR was calculated in the following formula:

[Fasting insulin (μU/mL) x Fasting plasma glucose (mmol/L)]/22.5.

Values > 3 were considered to indicate insulin resistance.

Statistical analysis

The Shapiro–Wilk test was used to determine whether the samples came from normally distributed populations. Gaussian distribution variables were expressed as means (±

Article downloaded from www.germs.ro
Published on 1 March 2012
© GERMS 2012
ISSN 2248 – 2997
ISSN – L = 2248 – 2997

standard deviation [SD]). For non-normally distributed variables we used medians.

Differences between groups were tested using independent-sample t-test for normally distributed variables and Mann–Whitney–Wilcoxon test for non-parametric variables. Pearson's chi-squared test was used for correlation analysis. The level of statistical significance was set at 5%. SPSS® Statistics software v.17.0, IBM®, New York, USA was used to perform all analyses.

Results

A total of 77 patients (mean age 32.5 years, range: 17-65) were enrolled, including 44 men (57.1%, mean age 34.1 years, range: 17-63), and 33 women (42.9%, mean age 30.3 years, range: 19-65).

More than half (57.9%) of the patients had at least 2 antiretroviral combinations in the past. 76.84% of the patients had at least one PI in their current cART regimen. The mean BMI of study patients was 23.7 kg/m^2. Overall, 23.4% of the patients were overweight and 6.5% were obese. The median overall CD4 T cell count of enrolled patients was 515.00 cells/cmm. Clinical and biological characteristics of study patients are presented in tables I and II and compared in table III.

Out of the enrolled patients, 75.32% had serum undetectable HIV viral load at the time of blood sampling and 24.68% had HIV viral loads ranging from 344 to 364 000 copies/ml. Study subjects were divided into two groups: Group 1 (with undetectable viral load): 58 subjects; and Group 2 (with persistent HIV viral replication): 19 subjects.

Patients with undetectable HIV viral load had lower median concentrations of adiponectin (9.22 vs. 16.50 μg/mL) than Group 2 but the results lacked statistical significance (p=0.059). The significance level of Pearson's chi-squared test was 0.058.

Higher levels of median CD4 T cell count (549.50 vs. 334.00 cells/cmm) were observed in Group 1 compared to Group 2 (p=0.02). Higher leptin, IL-6 and resistin serum levels were noted in patients with undetectable HIV viral load, however this was not statistically significant. TNF-alpha had significantly higher serum levels in patients with detectable viral load (16.89 vs. 9.35), (p=0.043), however Pearson's chi-squared test had a significance level of 0.14 (table IV).

Characteristics	Total number of patients* N=77
Age (years)	32.53 (±13.73)
BMI (kg/m^2)	23.73 (±3.89)
Total cholesterol (mg/dl)	199.00 (±43.08)
HDL cholesterol (mg/dl)	44.19 (±13.26)
LDL cholesterol (mg/dl)	114.76 (±38.98)
Triglycerides (mg/dl)	204.12 (±127.54)

*Results presented as Mean (±SD)

Table I. Clinical and biological characteristics of study patients (parametric variables)

Characteristics	Total number of patients* N=77
CD4 T cells (cells/cmm)	515.00 (±320.63)
Adiponectin (μg/mL)	10.84 (±14.86)
Leptin (ng/mL)	1.87 (±4.34)
Resistin (ng/mL)	5.40 (±3.11)
TNF-alpha (pg/mL)	11.00 (±13.73)
IL-6 (pg/mL)	25.21 (±83.87)
HOMA	3.4 (±5.86)

*Results presented as Median (±SD)

Table II. Clinical and biological characteristics of study patients (non-parametric variables)

Characteristics	Group 1 (undetectable viral load)* (±SD)	Group 2 (persistent HIV viral replication)* (±SD)	Significance (2-tailed)
Age (years)	32.60 (±13.31)	32.32 (±15.32)	0.93
BMI (kg/m^2)	24.04 (±3.96)	22.84 (±3.65)	0.25
Total cholesterol (mg/dl)	200.19 (±42.40)	195.37 (±46.11)	0.67
HDL cholesterol (mg/dl)	45.10 (±14.03)	41.42 (±10.40)	0.29
LDL cholesterol (mg/dl)	115.50 (±39.11)	112.49 (±39.53)	0.77
Triglycerides (mg/dl)	201.76 (±130.67)	211.32 (±120.55)	0.77

*Results presented as Mean (±SD)

Table III. Differences in means between Group 1 and Group 2

Characteristics	Group 1 (undetectable viral load)* (±SD)	Group 2 (persistent HIV viral replication)* (±SD)	Asymptotic significance (2-tailed)
Adiponectin (µg/mL)	9.22 (±11.73)	16.50 (±21.20)	0.059
Leptin (ng/mL)	2.05 (±3.64)	1.28 (±6.09)	0.34
Resistin (ng/mL)	5.54 (±3.22)	5.10 (±2.68)	0.16
TNF-alpha (pg/mL)	9.35 (±13.44)	16.89 (±14.10)	0.043**
IL-6 (pg/mL)	26.55 (±95.82)	24.79 (±15.87)	0.43
HOMA	3.63 (±4.85)	2.67 (±8.36)	0.52
CD4 T cell (cells/cmm)	549.50 (±313.64)	334.00 (±321.15)	0.02**

*Results presented as Median (±SD); **p<0.05 Group 1 vs. Group 2

Table IV. Differences in medians between Group 1 and Group 2

Discussion

Adiponectin is exclusively and significantly expressed in human adipose cells. Studies have reported that adiponectin may act as an endogenous insulin sensitizer.[11] In non-HIV patients, lower levels of adiponectin were associated with the development of components of the metabolic syndrome such as obesity, insulin resistance and type II diabetes.[12-14]

Li L. et al. have compared the levels of adiponectin in patients with untreated HIV infection vs. healthy subjects. The HIV-positive naive patients had lower serum concentrations of adiponectin compared to the included healthy subjects.[15]

In treated HIV-positive patients, the levels of adiponectin may be lower than in naive HIV-patients. Lagathu C. et al. have reported in 2007 that both protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) altered adipokine secretion from human subcutaneous adipocytes.[16]

Sankale et al. reported that human subcutaneous adipocytes exposed to HIV-1 dramatically increased the secretion of adiponectin.[17] Our study has found no significant statistical evidence to confirm that adiponectin levels are associated with persistent viral replication, although the patients in Group 2 had higher median concentrations of adiponectin than those in Group 1.

Matarese et al. showed that serum leptin concentration positively correlated with CD4+ lymphocyte number in a cohort of naive HIV-positive patients.[18] Azzoni et al. reported in 2010 that leptin levels are inversely associated with HIV replication, independent of disease progression, in a cohort composed only of ART-naïve women with a narrow CD4 range. The authors hypothesized that the inverse relationship between leptin levels and viral replication may be independent of the amount of adipose tissue. Chronic inflammation in HIV-patients with viral replication may affect adipocytes and may cause lipoatrophy and lower leptin levels.[19]

Contrary to the above, other studies prior to Azzoni et al. report that testing whether viral replication was associated with serum adipokines did not evidence a significant association between HIV viral load and leptin levels.[20] Our study similarly found no statistical evidence regarding the association between leptin and HIV viral load, in our cohort of cART multiexperienced patients.

In humans, prior work has demonstrated that patients with chronic HIV infection typically show elevations in serum or plasma TNF-alpha levels.[21-23] In a study published in 2011, Keating et al. compared cytokines profiles in three women clinical groups, including cART responders, untreated HIV patients and HIV-uninfected subjects. Untreated, progressive HIV infection was associated with increased levels of inflammatory mediators such as TNF-alpha. cART responders had cytokine profiles that more closely resembled those of HIV-uninfected women.[24] TNF-alpha is involved in a paracrine loop in which HIV-1 binding to CD4 induces TNF-alpha production, and TNF-alpha stimulates HIV-1 gene expression by a nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB).[6] Roberts et al. have reported that during primary HIV infection TNF-alpha levels correlated positively with quantitative viral load.[25] In our study, serum TNF-alpha levels were significantly higher in patients with persistent HIV replications – a finding that concurs with the

previously mentioned studies – but the correlation analysis lacked statistical significance.

The most important limitation of this study was that the number of patients with persistent HIV viral replication (Group 2) was too small and as a result the statistical power was low. Also, the results should be interpreted in light of the cross-sectional design of our study, which does not permit cause-effect relationships interpretations in the studied variables.

The present study reported higher TNF-alpha serum levels in patients with persistent HIV viral load undergoing antiretroviral therapy. We found no statistically significant correlations between adiponectin, leptin, resistin and IL-6 and HIV viral load in our study population. Further studies with larger number of patients and prospective designs may be required to draw statistically significant and relevant conclusions regarding the association between adipokines and HIV replication.

Funding The study was funded by the National Authority for Scientific Research (Grant No. 62077/2008, 2008-2011). Coordinating center: National Institute of Infectious Diseases 'Prof.Dr. Matei Balș', Bucharest, Romania. Project Manager: Professor Adrian Streinu-Cercel, MD, PhD.

Conflict of interest All authors – none to declare.

Authors' contributions All authors had equal contributions.

Acknowledgement The authors thank all study participants for their contribution to the research, as well as all medical staff involved in this study, especially Dr. Cristina Loredana Benea for the involvement in recruiting and in the follow-up of the patients.

References

1. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med. 2008; 14(11-12):741-51.
2. Guaraldi G. Evolving approaches and resources for clinical practice in the management of HIV infection in the HAART era. GERMS; 2011; 1(1):6-8
3. Falutz J. Management of fat accumulation in patients with HIV infection. Curr HIV/AIDS Rep. 2011; 8(3):200-8.
4. Giralt M, Domingo P, Villarroya F. Adipose tissue biology and HIV-infection. Best Pract Res Clin Endocrinol Metab. 2011; 25(3):487-99.
5. Tilișcan C, Aramă V, Mihăilescu R, Streinu-Cercel A, Ion D, Aramă ȘS. Methods for monitoring mitochondrial toxicity of nucleoside-analogue reverse-transcriptase inhibitors in HIVpatients during antiretroviral therapy. Therapeutics, Pharmacology and Clinical Toxicology 2011; XV(4):289-292
6. Bulló M, García-Lorda P, Peinado-Onsurbe J, et al. TNFalpha expression of subcutaneous adipose tissue in obese and morbid obese females: relationship to adipocyte LPL activity and leptin synthesis. Int J Obes Relat Metab Disord. 2002; 26(5):652-8.
7. Herbein G, Varin A, Larbi A, et al. Nef and TNFalpha are coplayers that favor HIV-1 replication in monocytic cells and primary macrophages. Curr HIV Res. 2008; 6(2):117-29.
8. Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. Ann Intern Med. 1999; 130(8):671-80
9. World Health Organization (2000) Obesity: Preventing and Managing the Global Epidemic. Technical Report Series No. 894. WHO, Geneva, 2000.
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412-9.
11. Ziemke F, Mantzoros CS. Adiponectin in insulin resistance: lessons from translational research. Am J Clin Nutr. 2010; 91(1):258S-261S.
12. Kubota N, Terauchi Y, Yamauchi T, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem. 2002; 277(29):25863-6
13. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001; 86(5):1930-5
14. Yamamoto Y, Hirose H, Saito I, et al. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin Sci (Lond) 2002; 103(2):137-42
15. Li L, Chen J, Sun FY, et al. Markers of endothelial injury and plasma adipocytokine in antiretroviral-naive HIV patients. Zhonghua Nei Ke Za Zhi. 2011; 50(2):136-9.
16. Lagathu C, Eustace B, Prot M, et al. Some HIV antiretrovirals increase oxidative stress and alter chemokine, cytokine or adiponectin production in human adipocytes and macrophages. Antivir Ther. 2007; 12(4):489-500.
17. Sankalé JL, Tong Q, Hadigan CM, Tan G, Grinspoon SK, Kanki PJ, Hotamisligil GS. Regulation of adiponectin in adipocytes upon exposure to HIV-1. HIV Med. 2006; 7(4):268-74.

18. Matarese G, Castelli-Gattinara G, Cancrini C, et al. Serum leptin and CD4+ T lymphocytes in HIV+ children during highly active antiretroviral therapy.Clin Endocrinol (Oxf). 2002; 57(5):643-6.
19. Azzoni L, Crowther NJ, Firnhaber C et al. Association between HIV replication and serum leptin levels: an observational study of a cohort of HIV-1-infected South African women. J Int AIDS Soc. 2010; 13:33.
20. Scherzer R, Shen W, Bacchetti P, et al. Simple anthropometric measures correlate with metabolic risk indicators as strongly as magnetic resonance imaging-measured adipose tissue depots in both HIV-infected and control subjects. Am J Clin Nutr. 2008; 87(6):1809-17.
21. Lähdevirta J, Maury CP, Teppo AM, Repo H. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am J Med 1988; 85(3):289-91.
22. von Sydow M, Sonnerborg A, Gaines H, Strannegård O. Interferon-alpha and tumor necrosis factor-alpha in serum of patients in various stages of HIV-1 infection. AIDS Res Hum Retroviruses 1991; 7(4):375-80.
23. Zangerle R, Gallati H, Sarcletti M, Wachter H, Fuchs D. Tumor necrosis factor alpha and soluble tumor necrosis factor receptors in individuals with human immunodeficiency virus infection. Immunol Lett 1994; 41(2-3):229-234.
24. Keating SM, Golub ET, Nowicki M, et al. Women's Interagency HIV Study. The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of women. AIDS 2011; 25(15):1823-32.
25. Roberts L, Passmore JA, Williamson C et al. Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. AIDS 2010; 24(6):819-31.

Please cite this article as:
Aramă V, Tilișcan C, Ion DA, Mihăilescu R, Munteanu D, Streinu-Cercel A, Tudor AM, Hristea A, Leoveanu V, Olaru I, Aramă SS. Serum adipokines and HIV viral replication in patients undergoing antiretroviral therapy. GERMS. 2012;2(1):12-17