

Relationship between Leptin Levels and Suppressed CD4 Counts in HIV Patients

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Key Words

Human immunodeficiency virus · CD4 T cells · Leptin · Highly active antiretroviral therapy

Abstract

Objective: To examine the relationship between serum leptin levels and suppression of CD4 count in HIV-infected individuals with highly active antiretroviral therapy (HAART).

Subjects and Methods: Thirty seropositive HIV male patients selected from the Infectious Disease Hospital were classified into two groups according to their immunological and virological response to HAART. The first group included 15 male patients with low viral load and low CD4 counts; the second included 15 male patients with low viral load and high CD4 counts. Morning serum leptin and tumor necrosis factor- α levels of HIV patients were measured and correlated with fasting serum insulin, Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), HIV viral load and CD4 count. **Results:** Serum leptin levels were significantly higher in patients with high CD4 counts than in patients with low CD4 counts (mean serum leptin level 47.3 vs. 10.9 ng/ml, respectively; $p < 0.0001$). A positive correlation was observed between serum leptin levels and CD4 counts ($r = 0.697$; $p <$

0.0001); positive correlations were also seen between leptin levels and fasting serum insulin and HOMA-IR ($r = 0.633$, $p < 0.0001$, and $r = 0.537$, $p < 0.003$, respectively). **Conclusion:** Serum leptin level was higher in HIV patients with high CD4 count and correlated with fasting serum insulin and HOMA-IR, thereby indicating that HAART treatment could lead to decreased levels of leptin in HIV patients, which might lead to impaired immunological recovery.

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Introduction

Leptin, a hormone synthesized mainly in adipose cells, is the 16-kDa nonglycosylated protein product of the obese (ob) gene [1]. It regulates body weight in a central manner via its cognate receptor in the hypothalamus [2]. Leptin is also expressed at lower levels in other tissues, such as the placenta and stomach [3]. There is increasing evidence that leptin has systemic effects apart from those related to energy homeostasis, including regulation of neuroendocrine, reproductive, hematopoietic and immune functions [4].

Obese (ob/ob) mice and leptin-deficient (ab/ab) mice, in which the leptin receptor is truncated, display immune dysfunction and lymphoid organ atrophy, affecting thymus size and cellularity, similar to that observed in starved animals and malnourished humans [5]. Thus, these mice have reduced levels of peripheral T and B cells, suggesting that leptin may have a role in lymphopoiesis. Leptin has been shown to protect mice from starvation-induced lymphoid atrophy and increases thymus cellularity in ob/ob mice [6]. Leptin also induces the proliferation, differentiation and functional activation of hematopoietic cells, and this function may explain the role of adipose tissue present in the marrow cavity [7].

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome [8]. HIV primarily infects vital cells in the human immune system such as helper T cells (CD4+ T cells), macrophages and dendritic cells. HIV infection results in a reduction in levels of CD4+ T cells through three main mechanisms: direct viral killing of infected cells, increased rates of apoptosis of infected cells, and killing of infected CD4+ T cells by CD8+ cytotoxic T lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is impaired, and the body becomes progressively more susceptible to opportunistic infections [9].

Antiretroviral drugs keep the levels of HIV in the body at a low level, enabling the immune system to recover and work effectively. Highly active antiretroviral therapy (HAART) in HIV-1-infected individuals has a broad spectrum of clinical outcomes. In the majority of patients, the plasma viral load becomes undetectable and CD4+ T cells increase over time. However, in a number of subjects, a discrepancy between plasma viral load and CD4+ T-cell recovery has been observed. CD4+ T-cell count can rise despite persistently detectable plasma viral load (virologic nonresponders), which occurs in 7–15% of the patients [10], or conversely, CD4+ T-cell numbers do not increase despite plasma viral load suppression (immunologic nonresponders) [11]. It has been observed that 20% of patients receiving long-term HAART are 'immunologic nonresponders' [11], i.e. patients who fail to achieve a CD4+ T-cell count above 300 cells/ μ l at 6, 12, 18, 24 months of HAART [12]. Therefore, the aim of this study was to explore the role of serum leptin in the pathogenesis of suppression of CD4+ T-cell count in HIV patients despite a low viral load after HAART.

Subjects and Methods

This study was conducted between December 2009 and March 2011 at the Infectious Disease Hospital, Kuwait, a tertiary care hospital that has been providing primary care to HIV-positive patients since 1990. The patients in this study were diagnosed as seropositive HIV 2 years prior to the study and were started on HAART, a combination of three or more anti-HIV medications from at least two different classes of nonnucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs), or protease inhibitors. All subjects received the HAART regimen according to individual needs but there was no variation between the two groups as both groups were treated with similar classes of drug with regard to the HAART regimen. Of 148 patients who were on HAART for more than 2 years, 118 were excluded due to advanced age, female gender, higher or lower body mass index (BMI; lower BMI: <18.5; normal BMI: 18.5–24.9; higher BMI: 25–29.9), diabetic state, any opportunistic infections, coinfection with viral hepatitis, advanced stage of the disease or history of previous treatment interruption. This was intended to exclude factors that affect serum leptin levels. Thirty patients were selected and classified into two groups. The first group (group I) included 15 male patients with low viral load and low CD4 count; the second (group II) 15 male patients with low viral load and high CD4 count. All 30 patients were subjected to full history taking, thorough clinical examination, BMI (kg/m²), liver function test, kidney profile, complete blood count, fasting blood sugar, estimation of fasting serum insulin, estimation of the levels of the proinflammatory cytokine tumor necrosis factor- α (TNF- α ; Beckman Coulter, France) and the anti-inflammatory cytokine interleukin-10 (IL-10, Beckman Coulter, France), and estimation of morning serum leptin (Diagnostic Systems Laboratories, USA) using enzyme-linked immunosorbent assay. Calculation of Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was performed using the following equation: fasting plasma insulin (μ U/ml) \times fasting plasma glucose (mmol/l)/22.5, which is highly correlated with insulin resistance [13].

Statistical Analysis

Data were first tested by One-Sample Kolmogorov-Smirnov Normality Test and subsequently analyzed with respective parametric or nonparametric tests. Comparisons between the groups were made using independent Student's t test for parametric variables and by Mann-Whitney U test for nonparametric variables. Pearson's correlation and Spearman rank correlation were used to measure association between quantitative data whether parametric or nonparametric; p value was considered significant if it was less than 0.05.

Results

The age and BMI of the patients in both the low viral/CD4 count and low viral/high CD4 count groups were compared, no statistically significant differences in the means in regard to age ($p < 0.66$) and BMI ($p < 0.29$) between the groups were observed (table 1). For all patients in both groups, there were no morphological changes in

the body due to HIV lipodystrophy, nor was there any disruption of normal eating patterns or any changes in BMI from the time the HAART regimen was initiated until the endpoint of the study. CD4 counts in group I (238 cells/ μ l) were significantly lower compared to those in group II (691 cells/ μ l, $p < 0.0001$). Group I also had a significantly lower viral load than group II. Leptin levels were significantly higher in group II (47.3 ng/ml) than in group I (10.9 ng/ml, $p < 0.0001$). While fasting serum insulin levels were significantly different, there was no significant difference in HOMA-IR (table 1). A positive correlation was observed between leptin levels and CD4 counts ($r = 0.697$, $p < 0.0001$; table 2). Similarly, there was a positive correlation between leptin levels and insulin ($r = 0.633$, $p < 0.0001$) as well as between leptin levels and HOMA-IR ($r = 0.537$, $p < 0.003$).

With regard to serum cytokine levels, there were significantly higher levels of the proinflammatory cytokine TNF- α in group I ($p < 0.045$); the TNF- α /IL-10 ratio was higher in group I than in group II ($p < 0.042$), suggestive of a stronger proinflammatory bias in group I. Furthermore, there was a significant negative correlation between the TNF- α and the CD4 counts ($r = -0.410$, p value 0.027; table 2). No significant correlations were observed between leptin levels and age, BMI, viral loads and serum levels of TNF- α or IL-10.

Discussion

Our results showed no significant difference in HOMA-IR between group I and group II; fasting glucose levels were normal in all subjects, presumably because all diabetic patients were excluded from the study. Nevertheless, it is relevant to note that patients with insulin resistance tend to remain euglycemic as long as there is an adequate compensatory increase in insulin output from the pancreas. Impaired glucose tolerance and diabetes ensue when the level of insulin resistance exceeds the compensatory increase in pancreatic insulin output. However, insulin resistance has been widely reported to occur among HIV-infected patients [14, 15]. It has been suggested that several factors may contribute to the risk of developing IR in these subjects, including type and duration of antiretroviral agents, severity and duration of HIV infection, undetectable viral load, age, weight, BMI, HCV infection, and lipid abnormalities [16], but the small sample size of our study could have limited the power to detect factors related to impairment of glucose homeostasis.

Table 1. Comparison of age, BMI, serum leptin, serum insulin, HOMA-IR, TNF- α , CD4 counts and viral load in groups I and II

	Group I (target group) (n = 15)	Group II (control group) (n = 15)	Significant difference p value
Age	40.4 \pm 1.4	39.4 \pm 1.74	0.66
BMI	23.5 \pm 0.39	24.02 \pm 0.35	0.29
FBS	6.09 \pm 0.18	5.66 \pm 0.26	0.184
Serum insulin	7.8 \pm 1.01	10.9 \pm 0.68	<0.016
HOMA-IR	2.1 \pm 0.26	2.69 \pm 0.17	0.061
CD4+	238.3 \pm 26.17	691 \pm 43.93	<0.0001
Viral load	309.1 \pm 67.1	697.3 \pm 128.06	0.014
Serum leptin	10.9 \pm 2.99	47.3 \pm 7.95	<0.0001
TNF- α	11.14 \pm 1.76	5.58 \pm 0.45	0.045
IL-10	10.23 \pm 2.37	12.11 \pm 3.08	0.89
TNF- α /IL-10	1.34 \pm 0.23	0.79 \pm 0.09	0.042

FBS = Fetal bovine serum; CD4+ = cluster of differentiation 4, a glycoprotein expressed on the surface of T helper cells; viral load = a term used to describe the amount of HIV in blood; it is a measure of the severity of a viral infection. Data are given as mean values \pm SD.

Table 2. Correlation between serum leptin levels and TNF- α and other parameters in study groups

	Serum leptin		TNF- α	
	r	p value	r	p value
Serum insulin	0.633	0.0001	-0.229	0.241
HOMA-IR	0.537	0.003	-0.217	0.267
Serum leptin	1.000	0	-0.233	0.233
CD4+	0.697	0.0001	-0.410	0.027
Viral load	0.428	0.082	-0.288	0.137

CD4+ = Cluster of differentiation 4, a glycoprotein expressed on the surface of T helper cells; viral load = a term used to describe the amount of HIV in blood; it is a measure of the severity of a viral infection.

Insulin resistance appeared to be a consistent feature in the HIV patients of this study, especially in group II. There are three possible explanations: first, protease inhibitor drugs were used as a constituent of HAART and some of these drugs directly bound to and blocked the insulin-sensitive glucose transporter (GLUT 4), thus inhibiting glucose transport [17, 18]. This effect, which has been demonstrated in both adipocytes and myocytes, induces peripheral insulin resistance in skeletal muscle and adipose tissue and impairs the ability of beta cells to compensate [19].

Second, protease inhibitors vary in their ability to induce insulin resistance. Our subjects were not randomly assigned to receive different types of therapy or protease inhibitors. Therefore, we could not demonstrate an association between the use of protease inhibitors and the development of insulin resistance. Third, HIV itself has been linked to insulin resistance [20]. While there is ongoing debate on the role of HIV in adipose tissue inflammation, the ability of the virus to modify adipocyte phenotype has been demonstrated in a study by Sankalé et al. [21]. Moreover, infected macrophages in lipodystrophic adipose tissue show modified characteristics. When activated, these cells secrete proinflammatory cytokines such as TNF- α and IL-6 that control adipocyte metabolism, decrease adiponectin production and induce insulin resistance and lipolysis [22]. This concept, called lipotoxicity, may explain why chronic HIV disease is associated with abnormal metabolic changes including insulin resistance and dyslipidemia in the absence of antiretroviral drugs [23].

An interesting feature of this study is the finding of a significant difference in the levels of the proinflammatory cytokine TNF- α between the two groups (table 1), which is in agreement with Resino et al. [24], who found higher levels of TNF- α at lower HIV viral loads. Another report by Hestdal et al. [25] describes higher levels of serum TNF- α in HIV patients with lower CD4+ counts. This is in accord with our observation of elevated TNF- α levels in subjects with decreased CD4 counts. A plausible explanation for high TNF- α levels in subjects with low CD4 counts is that the increased TNF- α may have been produced by other cells such as CD8+ T cells, the levels of which may have been elevated in these subjects. The ratio of TNF- α to IL-10 is higher in group I, i.e. patients with a lower CD4 and lower viral load. That is, the Th1/Th2 cytokine ratio is higher in this group, again suggestive of a CD8+ T cell predominance in these subjects.

TNF- α levels did not correlate with HOMA-IR and fasting insulin levels in both groups (table 2), suggesting but not unequivocally confirming that TNF- α does not have a significant role in the pathogenesis of insulin re-

sistance. Yet another mechanistic explanation of insulin resistance in HIV patients is that NRTIs may contribute to insulin resistance, usually indirectly through metabolic changes and fat redistribution [26]. Oxidative stress and/or mitochondrial insult from NRTI exposure may represent a common pathway for increased lactate levels and decreased adiponectin from adipocytes, which ultimately result in insulin resistance in patients with HIV infection who are receiving antiretroviral therapy [27].

In this study, the serum leptin concentration was positively correlated with HOMA-IR, which reflects the degree of insulin resistance and concentration of serum insulin. Our results are similar to other studies [28], which describe an important role for insulin in the state of hyperleptinemia. Compensatory hyperinsulinemia due to insulin resistance stimulates adipocytes to produce leptin [29]. We hypothesized that the hyperleptinemia in group II was due to hyperinsulinemia. Although it has been reported that proinflammatory cytokines such as TNF- α induce production of leptin from adipocytes [30], a significant correlation between serum leptin levels and TNF- α in both groups was not found, suggesting that TNF- α did not play a role in hyperleptinemia in group II.

Despite the fact that virological suppression was better in group I than in group II, the CD4 counts in group II were higher than in group I (table 1). In addition, a clear positive correlation between serum leptin levels and CD4 counts (table 2) was found, supporting the assumption that the hyperleptinemia in group II had an important role in improving CD4 counts in HIV patients.

Conclusion

Serum leptin level was higher in HIV patients with high CD4 count and correlated with fasting serum insulin and HOMA-IR, thereby indicating that HAART treatment could lead to decreased levels of leptin in HIV patients, which might lead to impaired immunological recovery.

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