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ORIGINAL ARTICLE

Serum Leptin Levels in Antiretroviral Therapy Naïve HIV-1 Infected Patients in Zaria, Nigeria

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This study aimed at determining serum leptin levels in ART naïve HIV-1 infected adults in relation to body mass index (BMI), CD4 cell count and presence or absence of symptomatic HIV disease or features of AIDS. Materials & Methods: This cross sectional study was undertaken in 2008 among patients, attending Ahmadu Bello University Teaching Hospital. Serum leptin levels by ELISA were determined in 40 consecutive sex matched, HIV infected adults (20 normal weight and 20 underweight) and 26 sex matched HIV negative, healthy, normal weight controls. Symptomatic and asymptomatic HIV infected patients as well as AIDS and non-AIDS patients with similar BMI were compared. CD4 cell counts were correlated with leptin levels. Results: The median leptin levels of healthy controls and asymptomatic normal weight patients were not significantly different. Female patients tended to have lower leptin values than male ones. Median leptin was lower in underweight patients when compared to normal weight patients (13.8 vs 39ng/mL, p=0.009) and also lower in symptomatic patients when compared to asymptomatic patients (27.9 vs 43.9ng/mL, p=0.038) but not significantly different between AIDS and non-AIDS cases. Among healthy controls, leptin levels positively correlated with CD4 T counts (r=0.47, p=0.04) but in HIV/AIDS patients the correlation (r=0.28, p=0.07) was not significant. Conclusion: In wasted HIV infected patients, low leptin levels were reflective of loss of adipose mass and were worse in females. It is suggested that independent of the effect of BMI, leptin secretion is down regulated in untreated symptomatic HIV/AIDS patients with secondary infections. The results also suggest that the normal leptin induced rise with CD4 T cell counts may be blunted by untreated HIV infection.

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Introduction

Leptin is an adipocyte derived hormone involved in weight regulation, energy balance and immunity.[1,2] Studies in mice have shown that leptin promotes weight loss by acting on hypothalamic receptors to decrease appetite and increase energy expenditure,[1] as well as mediating immune response by activating proliferation of haematopoeitic stem cells.[2] Leptin also augments cellular immunity by promoting a predominant pro-inflammatory T helper cell 1 (TH1) cytokine activation.[2, 3]

Serum leptin concentrations have been shown to be directly proportional to the adipocyte mass with higher levels found in individuals with obesity compared to lean weight healthy individuals.[4] Sex differences in leptin concentrations have also been observed with women exhibiting higher leptin levels than men for any given measure of adiposity.[5,6] The

effects of oestrogen and androgens hormones in increasing and decreasing leptin mRNA expression in adipocytes may underlie the sex differences in leptin levels.[1] Some investigators have however attributed the sex differences to relative leptin resistance in women, as compared to men with similar adipose mass.[5]

In view of increased levels during acute inflammation, leptin has also been suggested as a possible mediator of the anorexia of inflammation.[2] Furthermore, considering the low levels found in patients with malnutrition and starvation[7,8] and its role in activating the immune response,[2] decreased serum leptin level has been implicated as a significant contributory factors in the pathogenesis of immunosuppression of malnutrition and starvation.[2]

Untreated HIV infection is characterized by progressive cellular immunodeficiency, opportunistic infections, anorexia, weight loss and malnutrition.[9-11] While leptin has been shown to induce anorexia resulting in weight loss in rodents,[1] studies in patients with HIV associated wasting have shown that serum leptin levels are decreased and that low levels are proportional to the degree of body fat loss.[12,13] Without regard to body fat loss however, leptin levels have also been shown to be depressed in HIV/AIDS patients with symptomatic secondary infections especially in patients with tuberculosis-HIV co-infections.[14-15] This finding has been attributed to a down regulation of leptin secretion by the persistent inflammatory response characteristics of symptomatic HIV/AIDS.[14]

In Nigeria, there are no previous studies evaluating leptin levels in HIV infected patients. This study was undertaken to determine the role of leptin in HIV disease in antiretroviral therapy (ART) naïve HIV infected Nigerians when compared to normal weight HIV negative controls, by evaluating body mass index, a marker of adiposity and nutrition, CD4 T cell count, a marker of cellular immunity as well as presence or absence of symptomatic HIV infection or features of AIDS.

Materials & Methods:

This cross sectional study was carried out in 2008 at the Ahmadu Bello University Teaching Hospital (ABUTH), after obtaining institutional ethical approval and informed consent from all study participants. Forty ART naïve HIV-1 infected adult patients, 20 normal weight and 20 underweight patients, age and sex matched, were recruited consecutively from the medical outpatient clinic and the medical wards of ABUTH. Patients with diabetes, hypertension and kidney disease were excluded. Twenty six (13 males, 13 females) HIV negative normal weight healthy adults, who were neither hypertensive nor diabetic were recruited as controls.

Data collection and clinical examination:

Demographic data and clinical history of all study subjects were recorded. Weight, in kilograms, and height, in metres, were measured and body mass index was calculated as weight (kg)/ height (m^2). According to the WHO criteria[16] all subjects with BMI=18.5-24.9 kg/m^2 were classified as normal weight, and, those with BMI <18.5kg/m^2, as underweight.

All patients were clinically examined and evaluated for opportunistic infections. HIV disease was staged using the WHO criteria.[17] Patients with CD4 T cell count < 200cells/ul and or Stage 4 disease were grouped as AIDS cases while those with CD4 T cell count ≥200 and any with stage 1 to 3 HIV disease were grouped as non-AIDS cases. Asymptomatic patients (those without symptoms or secondary infection at recruitment) and symptomatic patients (those with active symptoms or secondary infection at recruitment) were identified and grouped accordingly.

Laboratory methodology: HIV antibody screening was undertaken using serial rapid test as recommended by the WHO.[18] All positive results were confirmed by Western blot (Immunetics Inc, Qualicode HIV 1/2 kit, Boston, USA) according to manufacturer's specifications. CD4 T cell count was determined by flow

cytometry (Partec, GmbH, Munster,Germany) accordingtomanufacturer's specification.

Aprotinin(Trasylol)200KIu/mLwas added to serum samples obtained from all study subjects after which samples were stored at -20ºC until collectively assayed for immunoreactive leptin using an ELISA based assay (Diagnostic Automation Inc, USA, Cat no 1742-6), according to the manufacturer's brochure. Leptin concentrations of serum samples were extrapolated from a standard curve by using the corresponding absorbance of each serum sample. The lowest detectable level of leptin of the assay was 1ng/mL.

Statisical analysis: Statistical package of social science (SPSS 13) was used for data analysis. Results were expressed as median and interquartile ranges (IQR). Parametric tests (Students t-test and Pearson's correlation) were used to analyze normally distributed quantitative variables (healthy controls); quantitative variables in patients lacked normal distribution and were hence analyzed by non-parametric tests (Mann-Whitney and Spearman's correlation). P<0.05was considered significant.

Results

The demographic data and laboratory results of healthy controls, normal weight patients and underweight patients are shown in Table 1.

The median ages (and age ranges) of healthy controls, normal weight patients, underweight patients were 32yrs (19-60yrs), 34yrs (22-54yrs) and 36yrs (24-52yrs) respectively (Table1).Of 20 underweight patients, 4(25%), who had severe wasting (BMI<16kg/m^2), were all females.

Table 1: Leptin levels and CD4 Tcell count in controls and patients

	Controls (n=26)	Normal weight Patients (n=20)	Underweight Patients (n=20)	P values	
				C vs N	U vs N
Age(yrs)	32 (29-41)	34 (30-38)	36 (31-44)	NS	NS
BMI (kg/m2)	22.8 (20.5-24.8)	21.3 (20-22.9)	17.5 (16.8-18.1)	NS	P<0.0001 Z= -5.41
CD4 count (cells/uL)	698 (587-885)	289 (116-599)	344 (132-386)	P<0.0001 Z=-3.65	NS
Leptin (ng/mL)	53.8 (37.5-71.3)	39 (25.6-48.2)	13.8 (3.8-35.9)	P<0.025 Z=-2.26	P<0.001 Z= -2.56

NB: Data for all subgroups are expressed as median and inter-quartile range. n=number of patients, C= controls, U =underweight patients, N= normal weight patients.

The median CD4 cell counts (and ranges) of healthy controls, normal weight patients and underweight patients were 698 cells/μL (420-1315), 298 cells/μL (84-1010), 344 cells/μL (8-810) respectively. Controls had significantly higher median CD4cellcounts when compared with normal weight patients (p<0.0001) and underweight patients (p<0.0001). The difference in the median CD4 cell counts between underweight and normal weight patients was not statistically significant (Table 1)

Body mass index and leptin levels: Serum leptin levels in controls, normal weight patients and underweight patients are shown in table 1. The median serum leptin level was significantly lower in normal weight patients when compared to controls (p=0.024, Z=-2.26, Table 1). Median serum leptin was also significantly lower in underweight patients when compared to normal weight patients (p=0.009, Z=-2.56 table 1).

Grouped data of underweight and normal weight patients, showed a marginal positive correlation between leptin levels and BMI (r=0.3, p=0.06).

HIV clinical categories and leptin levels: Of the 40 patients, 16 (4 males, 12 females) were asymptomatic at the time of recruitment while 24 (16 males, 8 females) were symptomatic. The clinical diagnosis of symptomatic patients at recruitment included HIV related diarrhoea (n=7), tuberculosis of lungs and lymph nodes diagnosed by lymph node histopathology and chest x-ray findings (n=5), pulmonary tuberculosis diagnosed by chest x-ray findings (n=3), Plasmodium falciparum malaria (n=2) typhoid sepsis (n=1), Escherichia coli related urinary tract infections (n=1), Herpes genital ulcers (n=1), sputum culture negative bronchopneumonia (n=2), upper respiratory tract infection (1 patient) and oral thrush (n=1).

The differences in median ages and BMI of asymptomatic patients (35yrs and 19.3 kg/m^2) and symptomatic patients (35yrs, 18.3kg/m^2) were not statistically significant (p>0.05). The median CD4 cell count of symptomatic patients (163cells/µL, Interquartile range IQR= 107-345) was however significantly lower than that of asymptomatic patients (646 cells/µL, IQR= 140-765, p=0.008, Z=-2.62), as was the median leptin level of symptomatic patients (27.9ng/mL, IQR=4.9-36.4) compared to that of asymptomatic patients (43.7ng/mL IQR= 11.8-48.2, p=0.038, Z=-2.07).

To determine if the presence of symptoms contributed to the lower leptin levels found in normal weight patients, leptin levels of normal controls were compared with those of 10 of the 20 normal weight patients, who were asymptomatic. The results revealed that the median serum leptin level in the 10 asymptomatic normal weight patients (45.7ng/mL, IQR=38.5-49.8) was not significantly different from that of normal controls (53.8ng/mL, IQR=37.5-71.3,p>0.05).

According to WHO HIV clinical staging 6, 3, 18 and 13 patients had stage 1, 2, 3 and 4 diseases respectively. Based on CD4 counts and WHO HIV staging, 19 patients (7 males, 12 females) and 21 patients (13 males, 8 males) were grouped as AIDS cases and non-AIDS HIV cases respectively.

The differences in the median ages and BMI of AIDS cases (34 yrs and 18.5kg/m2) and non-AIDS cases (36 yrs and 18.2kg/m2) were not statistically significant (P>0.05). Although AIDS cases had a lower median leptin level (22ng/mL, IQR=3.8-40) than non-AIDS cases (35ng/mL, IQR=25.9-45.6), this difference was not statistically significant.

Leptin and CD4 T cell counts

Among healthy controls, leptin levels positively correlated with CD4 T cell counts (r=0.467, p=0.044, Pearson's correlation, Figure1). There was however only a marginal correlation between leptin and CD4 T cell count in patients (r=0.284, p=0.076).

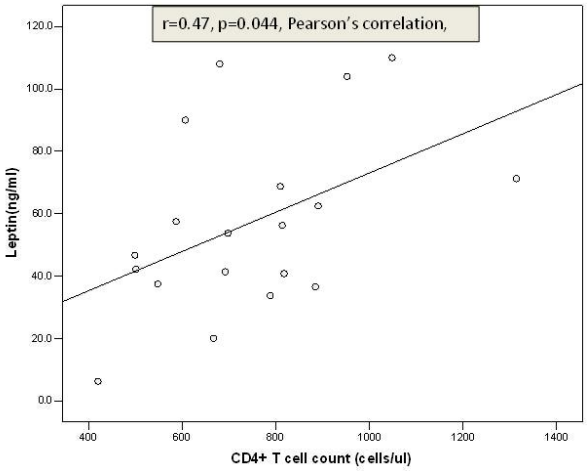

Fig 1. Serum Leptin concentrations in relation to CD4+ T cell counts in HIV negative controls. There is a signific an T positive correlation between leptin levels and CD4 T cell count (r=0.467, P=0.044, Person's correlation)

Gender differences in leptin levels

Gender differences in leptin levels and other laboratory results of controls and

patients are shown in table 2. No sex differences in the median ages and BMI of controls were observed (p>0.05). Median serum leptin was higher in female (56.3 ng/mL) than male controls (41.4 ng/mL), difference not statistically significant (Table 1).

In HIV/AIDS patients, no sex differences in the median ages of ages, BMI and CD4 cell counts were observed. Median leptin levels tended to be lower in females patients than male patients (p=0.05, table 2).

Table 2: Leptin levels and CD4 T cell counts in normal weight patients and controls.

	Controls		HIV patients		
	M (n=13)	**F (n=13)**	**M (n=20)**	**F (n=20)**	**M vs F**
Age (yrs)	34 (30-40)	30 (23-42)	34 (31-41)	35 (28-38)	NS
BMI (kg/m^2)	23.1 (21.3-24.5)	21.5 (20-24.9)	18.5 (17.6-22.1)	18.4 (16.9-20.8)	NS
CD4 count (cells/μL)	607 (548-692)	816 (707-938)	344 (149-386)	159 (89-665)	NS
Leptin (ng/mL)	41.4 (31.9-58.1)	56.3 (41-87.6)	35.6 (27.7-43.2)	7.42 (3.2-44.3)	P=0.05

NB: Data for all subgroups are expressed as median and inter-quartile range. n= number of subjects, M=male, F=female.

Discussion

The results of this study show that serum leptin levels are significantly lower in wasted HIV infected patients when compared to normal weight HIV infected patients from Zaria, Nigeria. A marginal positive correlation between BMI and leptin levels of patients was also found, These findings are supportive of the documented parallel relationship between serum leptin levels and degree of adiposity[1,4] assessed by BMI in this study. In rodents, circulating high leptin levels and high levels of exogenously injected leptin have been shown to cause anorexia leading to weight loss.[1] Paradoxically, the finding of low leptin levels in wasted HIV patients in this study does not seem to suggest that high leptin levels induced or maintained HIV related wasting. Ballinger et al[12] and Shikuma et al[13] have similarly reported low leptin levels in patients who had HIV associated wasting and attributed this to the loss of adipose tissue mass, reflected in the low BMI.

The study data has also shown that leptin levels are depressed during symptomatic HIV/AIDS, independent of the effect of BMI. Similarly, Grunfeld and co-workers[14] also reported a BMI independent suppression of serum leptin levels by secondary infection in AIDS patients. These findings are in contrast with the known increase in circulating leptin levels that occurs during inflammatory events such as sepsis and in response to inflammatory agents such as bacterial endotoxin and tumour necrosis factor α.[19,20] Van Crevel and colleagues[21] suggested exhaustion of leptin production by chronic inflammation of tuberculosis when they reported low serum leptin concentrations in HIV negative tuberculosis patients, independent of the effect of BMI. Down regulation of leptin may also underlie the low levels of leptin found in our symptomatic HIV/AIDS patients, as 8 (33%) of the 24 symptomatic patients were cases with tuberculosis while others had various types of opportunistic infections. This hypothesis requires confirmatory prospective studies in Nigerians.

It is notable that leptin levels in asymptomatic normal weight HIV infected patients were not significantly different from those of normal controls, findings suggesting that without weight loss and or symptomatic

HIV/AIDS infection, leptin levels of HIV infected patients are comparable with those of normal weight HIV negative individuals. Grunfeld et al[14] also reported an insignificant difference in the leptin levels between asymptomatic AIDS patients and normal HIV negative individuals of similar BMI.

Studies in mice and humans have shown that leptin stimulates proliferation of T cells in vitro and promotes pro-inflammatory TH_1 cytokines cellular immune responses;[7,22,23] in agreement with this positive relationship between leptin levels and T cells, we found a significant positive correlation between CD4 T cells and leptin levels in healthy controls. In HIV infected patients however, the positive correlation was only marginal. HIV preferentially infects and depletes CD4 T cells and it is plausible that untreated HIV infection leads to a blunting of the normal positive correlation between serum leptin and CD4 T cell count. Other factors affecting leptin levels such as weight loss and symptomatic HIV infection may also be contributory. These findings suggest a role for leptin supplementation in countries that can afford it, compared to indirect leptin enhancement via use of vitamin E and zinc in poorer countries, especially in Africa.[21,23,24]

Leptin levels have been shown to be higher in females than males for any given measure of adiposity.[5] Leptin levels have also been found to rise more rapidly in women than men with progressive increases in body fat.[5,6] Similarly, we found a trend towards higher leptin levels in female controls when compared to male controls. However, this trend did not reach statistical significance possibly because only normal weight individuals were evaluated. In contrast, female HIV infected patients tended to have lower leptin levels when compared to male patients. Although the limited sample size precluded appropriate sex comparisons of leptin between all groups of HIV infected patients, the reversal of sex differences in leptin levels may be attributed to a more severe lowering of BMI in female patients. Indeed, all patients with severe wasting ($BMI<16kg/m^2$) were females.

There are limitations to our study. First, although BMI correlates with total body fat, it is not as sensitive as techniques directly measuring total body fat.[1] BMI is however one of simplest, convenient and reasonably sensitive markers of adiposity appropriate to our setting. Waist circumference measurement is equally a simple and reliable marker of abdominal fat that may be correlated with serum leptin levels in future studies in Nigerians. Second, the assessment of cellular immunity was restricted to CD4 cell counts which was determined in a cross sectional design. A prospectively designed study evaluating serial changes in CD4 cell counts in relation to leptin levels and also determining the relationship between leptin and other markers of cellular immunity such as CD8 (cytotoxic) T cells, CD3 (total T cells) cells and TH1 cytokines would have shed more light on the effects of leptin on the cellular immune system in untreated HIV infected Nigerians.

In conclusion, the results of this study have shown that serum leptin levels are low in wasted ART naïve HIV infected Nigerians. Symptomatic HIV/AIDS suppressed serum leptin levels, independent of the effect of BMI and in the absence of weight loss and or symptomatic HIV infection; the leptin levels in HIV infected patients are not different from those of normal weight healthy HIV negative individuals. The study also revealed that in HIV infected patients, there was a blunting of the normal leptin induced rise in CD4 T cell count, found in controls. The use of leptin supplementation as an adjunct in the restoration and activation of the depressed cellular immune responses, characteristic of HIV infection, may be of benefit to resource rich countries.

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