Original Article

Leptin and Bone Mineral Density in Haemodialysis Patients

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Abstract

Introduction: There are very few data about relations between leptin and bone mineral density (BMD) in regular haemodialysed patients. We aimed to examine the relationship of serum leptin levels with BMD values in dialysed patients. We also assessed whether leptin is a significant predictor of BMD in haemodialysed patients. Materials and Methods: Leptin levels were studied using commercially available kits and BMD values were calculated using dual energy X-ray absorptiometry (DEXA) at femoral neck and distal radius in 74 (30 men and 44 women) haemodialysis patients. Results: BMD values at the femoral neck and distal radius did not differ significantly between the 2 genders. BMD at the distal radius correlated positively with bone alkaline phosphatase (BAP) (r = 0.503, *P* = 0.005) in male patients and correlated positively with phosphorus (r = 0.343, *P* = 0.02) in female patients. The time on dialysis treatment was longer in men (59 ± 48 vs 44 ± 41) but the difference was not statistically significant. Leptin levels were negatively correlated with BMD at the distal radius (r = -0.250 and *P* = 0.03) in all patients. Serum leptin levels were also correlated with body mass index (BMI) in all the patients (r = 0.749 and *P* = 0.001) and in both genders (r = 0.653 and *P* = 0.001 in women, r = 0.704 and *P* = 0.001 in men). In multivariate regression analysis, it was found that leptin level was not an independent determinant of BMD at all skeleton sites measured. Conclusions: There was significant difference between the 2 genders with reference to leptin levels, BMI, phosphorus and creatinine. Serum leptin levels are not significant predictors of BMD in the current study.

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Key words: Bone mass, Dialysis patients, Gender, Serum leptin

Introduction

Leptin, a protein hormone product of the obesity (ob) gene, is synthesised and secreted mainly by white adipose tissue. This hormone has been initially considered mainly as an anti-obesity hormone as by its action or the hypothalamic centre, through its OBRb receptor, it suppresses appetite and increases basal metabolism. Besides being an energy homeostasic hormone, leptin also has been shown to be involved in gonadal maturation and in somatotropic and adrenocorticotropic functions regulating the immune system and body development.[1]

Leptin might be a mediator between body fat and bone because fat mass correlate positively with serum leptin in healthy subjects.[2] In humans, the relationship of leptin concentration and bone density is still uncertain.[3] There was an inconsistent positive relationship of leptin with bone mineral density (BMD) at regional sites in premenopausal women in 1 study,[4] but bone density was associated inversely with leptin concentration in a study on men.[5]

In vitro studies have demonstrated direct effect of leptin on osteoblast differentiation and matrix mineralisation.[6] Furthermore, in vivo experiments of systemic leptin administration have been shown to stimulate bone growth.[7] In contrast, its intracerebroventricular administration in wild or ob/ob leptin deficient mice resulted in bone loss.[8]

It has been suggested that leptin levels in chronic renal failure is elevated[9] while BMD decreased.[10] However, there are very few data about correlations between leptin and BMD in haemodialysis patients.

In this study, we aimed to examine the relationship of serum leptin with BMD in 74 patients maintained on haemodialysis. Therefore, we assessed BMD, serum leptin levels, body mass index (BMI), bone alkaline phosphatase (BAP), intact PTH, phosphorus, albumin, urea, creatinine, total protein and calcium.

Materials and Methods

Patients

Thirty men and forty-four women (age range, 18 to 78

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years; mean age, 47.08 ± 14.8 years) on haemodialysis at the Cumhuriyet University Medical School Haemodialysis Unit, Private Sivas Dialysis Center Haemodialysis Unit, Sultan Izzettin Keykavus Hospital Haemodialysis Unit and the SSK Sivas Hospital Haemodialysis Unit between January 2005 and February 2006 were included in this study. Twelve out of the 44 women were in postmenopausal state. In these 4 haemodialysis units, the same dialysis membrane (hemaphein dialyser membranes) was used. Patients were excluded if they were on dialysis for less than 1 year, anuric, or were on dialysis with jugular or subclavian catheter and long-term permanent port catheter. Similarly, patients with diabetes mellitus, chronic pulmonary disorders, and hepatic cirrhosis or hepatitis B, hepatitis C carriers as well as those on active tuberculosis therapy were excluded. All participants agreed to have their BMD measured in addition to serum biochemistry evaluation. They were on dialysis for 50 ± 44 months and they were haemodialysed for 4 to 5 hours a day, 3 times a week. All participants gave written consent. The study was approved by the Local Ethics Committee.

Assays

In all subjects, weight and height were measured by the standard technique. BMI was calculated as body weight (kg) divided by height squared (m^2).

Venous blood samples were collected in the morning after an overnight fast of 12 hours before the beginning of haemodialysis. A total of 10 cc venous blood sample was drawn between 8:00 and 9:00 am, from all the patients to establish serum leptin levels and other parameters.

The levels of serum leptin were analysed by radioimmunoassay through employing a DPC Gambyt-CR (Japan) gamma counter at the Department of Nuclear Medicine. The DSL-23100 RCA (Diagnostic systems Lab, USA) commercial kits were used for serum leptin levels.

The following parameters were assessed: total protein, albumin, urea, creatinine, calcium, phosphorus and alkaline phosphatase by means of standard laboratory methods. Serum levels of high sensitive C-reactive protein (hs-CRP), tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6 were determined by enzyme-linked immunosorbent assay. Serum BAP was measured using the ELISA kit from Metra Biosystem, USA. The levels of intact-parathyroid hormone (I-PTH) were measured by radioimmunoassay method using commercially available kits from Cis, France.

Dual X-ray Absorptiometry

BMD at the femoral neck area (mixed bone) and distal radius (mainly cancellous bone) were measured by dual-energy X-ray absorptiometry (DEXA) using an QDR-2000 apparatus (Holocig Inc. Waltham, MA, USA). All measurements were performed by an experienced radiologic technician and were made using the same instrument. The manufacturer's recommendations for calibration of the instrument were closely followed. BMD was automatically calculated from the bone area and expressed absolutely in $g/cm^{2.}$ BMD were also evaluated by DEXA. The results are given by *t* scores (number of standard deviations from the mean BMD for young sex-matched normal controls) and *z* scores (number of standard deviations from the mean BMD for age and sex-matched normal controls).

Statistical Analysis

Statistical analysis was performed using SPSS 12.0 software. Results were expressed as mean ± SD. Data were compared using Student's *t*-test and Mann-Whitney U test as appropriate. Pearson correlation coefficients were used in comparing parameters. Multiple regression analysis was also employed. *P* values <0.05 were considered as statistically significant.

Results

Anthropometric and biochemical data measured for the all patients with a subdivision according to gender are summarised in Table 1. Between the 2 genders, there was no significant difference with respect to age, serum calcium, BAP, total protein, albumin, urea, I-PTH, hs-CRP, TNF-α and IL-6. No statistically significant differences in BMD with reference to the menopausal status of the female patients were found. The time on dialysis treatment was longer in women (59 ± 48 vs 44 ± 41) but the difference was not statistically significant. There was significant difference in the 2 genders with regard to leptin levels, BMI, phosphorus and creatinine. Leptin levels, creatinine and phosphorus were significantly higher in women than in men (5.10 ± 1.56 vs 4.34 ± 1.33, 9.71 ± 3.74 vs 8.15 ± 1.75 and 68.0 ± 42.2 vs 16.9 ± 23.2, respectively). The BMI values were significantly higher in men than in women (27.5 ± 7.0 vs 22.8 ± 3.1, respectively). BMD values at the femoral neck and distal radius did not differ significantly between the 2 genders. In women, t-score at the femoral neck ranged from 2.94 to -4.45 while the z-score ranged from 3.24 to -4.0, t-score at distal radius ranged from 5.21 to -16.00, z-score ranged from 5.96 to -3.48.

Correlation between serum leptin levels and variables in all patients and in both genders are summarised in Table 2. Serum leptin levels were strongly correlated with BMI in all the patients (r = 0.749 and *P* = 0.001) and in both genders (In women, r = 0.653 and *P* = 0.001. In men, r = 0.704 and *P* = 0.001). Leptin levels were negatively correlated with BMD at the distal radius (r = -0.250 and *P* = 0.03) in all patients and this significance persisted after adjustment to BMI (r = -0.247, *P* = 0.03). The correlation of serum leptin with BMD at the femoral neck showed a significantly positive correlation in the male patients (r = 0.441 and *P* = 0.01), but this significance was lost after adjustment to

Table 1. Clinical and Anthropometric Characteristics (Mean ± SD) in Patients

Characteristics	Women	Men	*P* value*
Number of patients	44	30	-
Age (y)	47 ± 15	48 ± 15	0.731
Time on dialysis (months)	59 ± 48	44 ± 41	0.159
Total protein (mg/dL)	6.6 ± 0.6	6.7 ± 0.7	0.469
Calcium (mg/dL)	8.7 ± 1.0	8.3 ± 1.3	0.115
Albumin (mg/dL)	3.56 ± 0.66	3.72 ± 0.67	0.335
BUN (mg/dL)	59.0 ± 18.95	57.5 ± 16.63	0.729
Creatinine (mg/dL)	9.71 ± 3.74	8.15 ± 1.75	0.020
Phosphorus (mg/dL)	5.1 ± 1.6	4.3 ± 1.3	0.036
PTH (pg/mL)	145.9 ± 105.6	236.5 ± 252.8	0.072
hs-CRP (ng/L)	10.5 ± 12.0	12.2 ± 23.0	0.689
IL-6 (pg/mL)	42.8 ± 32.0	43.9 ± 34.1	0.901
TNF-α (pg/mL)	64.2 ± 65.0	64.2 ± 70.4	0.713
Bone alkaline phosphatase (U/L)	24.4 ± 20.1	32.8 ± 30.0	0.188
Leptin (ng/mL)	68.0 ± 42.2	16.9 ± 23.2	0.000
Body Mass Index (kg/m^2)	22.8 ± 3.1	27.5 ± 7.0	0.002
BMD-femur neck (g/cm^2)	0.72 ± 0.15	0.68 ± 0.10	0.228
z-score	-0.83 ± 1.46	-0.72 ± 1.27	0.722
t-score	-1.76 ± 1.58	-1.82 ± 1.03	0.855
BMD- distal radius (g/cm^2)	0.52 ± 0.18	0.44 ± 0.15	0.071
z-score	-0.07 ± 2.16	-0.04 ± 1.77	0.797
t-score	-0.51 ± 2.13	-1.33 ± 3.26	0.192

* (Student's *t*-test) women vs men.

BMI (r = 0.288 and *P* = 0.13). BMD at the distal radius correlated positively with BAP (r = 0.503 and *P* = 0.005) in male patients and correlated positively with phosphorus (r = 0.343 and *P* = 0.02) in female patients. Serum leptin levels, after multivariate regression analysis, showed no association with BMD.

Discussion

Our findings indicate that BMD is not associated with leptin levels but with age and BMI. These findings suggest that leptin levels do not have a direct effect on bone mass in regular haemodialysed patients.

The role of serum leptin levels on BMD is still unclear. Some studies of selected samples of women[4] or men[5] and a population study of men and women[6] have, likewise, found no strong associations of leptin concentration with bone density. Martini et al[11] concluded that serum leptin have no direct effect on bone mass. Iwamoto et al[4] also reported that leptin is not a key regulator of bone metabolism, although it may have some effects on bone metabolic markers and BMD regionally. Rauch et al[12] also failed to find a relationship between bone mass and serum leptin levels by examining total and trabecular bone density at the distal radius in adult women. On the contrary, Pasco et al[13] reported a persistent association (after adjustment for age, body weight and body fat mass) between serum leptin and whole body, femoral and lateral spine BMD in non-obese women. In another study, Yoneda et al[14] reported a positive correlation between serum leptin and ultradistal radius BMD (but this latter was not adjusted to BMI) in 25 Japanese post-menopausal haemodialysis patients. In another study, Blain et al[15] reported that leptin is a significant and independent predictor of BMD in post-menopausal women. Coen et al[16] also reported that the serum leptin level is connected to bone resorption and bone formation; both are inversely related to serum leptin levels.

The results of our descriptive study of chronic haemodialysed patients do not provide evidence for a direct effect of serum leptin concentration on BMD. In our study, serum leptin levels and BMD at the femoral neck showed a significantly positive correlation in the male patients (r = 0.441 and *P* = 0.01) but this significance was lost after adjustment to BMI (r = 0.288, *P* = 0.13). In addition, leptin concentration does not appear to mediate the positive relationship of BMD with BMI.[12]

The lack of a direct correlation of leptin concentration with bone density in our study and other studies may be caused by, at least in part, decreased leptin function despite high levels that occur with leptin resistance.[17]

We confirmed the results of Isidori et al[18] regarding BMI as an independent contributor to serum leptin levels. In this study, we found a significant relationship between leptin levels and BMI values (r = 0.749 and *P* = 0.001).

Our findings confirmed the gender dimorphism on serum leptin levels in haemodialysis patients as they were more than twice as high in women as in men. Considine et al[2] pointed out that women and men with equivalent percentages of body fat had comparable levels of serum leptin. The mean value of serum leptin in our patients was 68.0 ± 42.2 ng/mL in women, and 16.9 ± 23.2 ng/mL in men.

In the present study, the association of leptin levels and other factors known to influence BMD in haemodialysis patients (such as age, serum calcium, BAP, total protein, albumin, urea and I-PTH levels) were comparable. Similar to the study by Ghazali et al,[19] the time of dialysis was found to be longer (the difference was not statistically significant) in women (59 ± 3 vs 48 ± 2) and BAP was measured and no correlation with leptin was found. We also found no correlation with serum I-PTH.

Kokot et al[20] suggested that there was no association between PTH and serum leptin concentration in haemodialysis patients. Our observations are consistent with this suggestion.

Table 2. Correlation Between Serum Leptin Levels and Variables in Patients

Variable	All patients		Women		Men	
	R	*P* value	R	*P* value	R	*P* value
Time on dialysis	0.038	0.75	0.120	0.43	-0.232	0.21
PTH (pg/mL)	0.099	0.40	-0.059	0.70	-0.071	0.71
BAP (U/L)	0.033	0.77	-0.185	0.22	-0.039	0.83
BMD-femur neck (g/cm^2)	0.039	0.74	-0.051	0.74	0.441	0.01
After adjustment to BMI	-0.055	0.64	-0.105	0.50	0.288*	0.13*
BMD-distal radius (g/cm^2)	-0.250	0.03	-0.158	0.30	-0.173	0.36
After adjustment to BMI	-0.247	0.03	-0.030	0.84	-0.339	0.07
BMI (kg/m^2)	0.749	0.00	0.653	0.00	0.704	0.00
Total protein (mg/dL)	0.083	0.48	0.183	0.23	-0.065	0.73
Calcium (mg/dL)	-0.002	0.99	0.122	0.43	0.162	0.39
Albumin (mg/dL)	0.100	0.39	0.066	0.67	0.018	0.92
BUN (mg/dL)	-0.038	0.74	-0.018	0.90	-0.017	0.92
Creatinine (mg/dL)	-0.111	0.35	0.088	0.57	0.020	0.91
Phosphorus (mg/dL)	-0.070	0.55	0.059	0.70	0.166	0.38
hs-CRP (ng/L)	-0.034	0.77	0.136	0.38	-0.173	0.36
IL-6 (pg/mL)	0.036	0.76	0.152	0.32	-0.068	0.72
TNF-α (pg/mL)	0.182	0.12	0.262	0.09	0.308	0.09

BAP: bone alkaline phosphatase; BMD: bone mineral density; BMI: body mass index; BUN:blood urea nitrogen; CRP: C-reactive protein; IL: interleukin-6; PTH: parathormon; TNF-α: tumour necrosis factor-alpha

Conclusions

In conclusion, local production of leptin, which may enhance osteogenic activity and inhibit adipogenic activity[21] may play a partial role in bone metabolism. However, the long-term effect of leptin on BMD should be investigated.

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