IS LEPTIN A DETERMINANT OF BONE MINERAL DENSITY IN POST-MENOPAUSAL WOMEN WITH OSTEOPOROSIS?

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ABSTRACT

In this study, we aimed to investigate the serum leptin level whether it is a determinant of bone mineral density (BMD) in osteoporotic postmenopausal women. Forty (40) osteoporotic female patients (screened according to WHO criteria, t-score < 2.5SD, at lumbar region or femoral neck region) and forty (40) age and body mass index matched healthy female subjects(served as control group;t score >-1.0) were studied. All the women were at post-menopausal period. Serum concentrations of leptin after an overnight fast were measured by radioimmunoassay. BMD values were measured by dual energy X-ray absorptiometry (DEXA, NORLAND XR-46) at the lumbar spine, femoral neck and total body. There was no significant difference between groups regarding serum leptin levels (P=0.599).

Our study showed that there was no statistically significant difference concerning serum leptin levels in osteoporotic and non-osteoporotic postmenopausal women . However, at a local tissue level, leptin may effect bone. For this reason, leptin related studies at the bone micro-environment are promising.

Key Words : *Leptin, Bone mineral density, Post Menopausal Osteoporosis*

POSTMENOPOZAL OSTEOPOROTİK KADINLARDA, LEPTİN KEMİK MİNERAL YOĞUNLUĞUNUN BELİRLEYİCİSİ Mİ ?

ÖZET

Bu çalışmada, postmenopozal kadınlarda, serum leptin düzeyinin, kemik mineral yoğunluğunun (KMY) bir belirleyicisi olup olmadığını araştırmayı amaçladık. Kırk osteoporotik kadın hasta (Lomber veya kalça bölgesinde, t skoru <-2.5) ve yaş ve vücut kitle indeksi uyumlu 40 sağlıklı kadın (t skoru >-1) çalışmaya alındı. Tüm kadınlar post-menopozal dönemdeydi. Serum leptin konsantrasyonu bir gece aç kaldıktan sonra, radioimmunoassay ile ölçüldü. KMY değerleri, lomber bölge, kalça bölgesi (femoral neck) ve tüm vücutta, dual enerji X -ray abzorbsiyometri ile ölçüldü (DEXA NORLAND XR-46).

Serum leptin düzeyi açsından gruplar arasında anlamlı fark yoktu (p>0.05).

Çalışmamızda, postmenopazal osteoporotik ve non-osteoporotik kadınlarda, serum leptin düzeyi açısından istatiksel olarak anlamlı fark görülmemiştir. Bununla birlikte, leptin kemiği lokal olarak, doku düzeyinde etkiliyebilir. Bu nedenle, kemik mikro çevresinde yapılan, leptin ile ilgili çalışmalar, ümit vericidir.

Anahtar Kelimeler : *Leptin, Kemik Mineral Yoğunluğu, Postmenopozal Osteoporoz*

INTRODUCTION

Osteoporosis and obesity are significant health problems especially in western societies. These two diseases are inversely correlated (1). Numerous studies have shown that obesity is related with increased bone mineral density (1). Body weight is a major determinant of bone mass, moreover, fat mass and body weight are correlated with BMD in women independently of their menopausal status (2). Although the body weight is one of the important determinant of the bone mass and BMD, this relationship also remains unknown (1,2,3).

Several reports have suggested that, the effect of fat mass on BMD may be mediated by hormonal factors such as sex hormones, leptin and insulin.

Leptin, the product of the ob gene, is secreted mainly by white adipose tissue and correlated with fat mass (5). In addition to central effects,

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leptin has also effects on peripheral tissue (5). Both serum leptin and bone mass are positively correlated with body weight (6). In vitro studies have demonstrated a direct effect of leptin on osteoblasts differentiation and matrix mineralisation (7). Moreover, several clinical studies have shown that plasma leptin levels were positively correlated with BMD in women. However, there are some studies in which reported that no correlation between serum leptin levels and bone mass.

In this study, we evaluated the plasma leptin level and BMD values and also related bone metabolism markers in post menopausal osteoporotic patients by comparing with age, sex and body mass matched healthy subjects. We investigated whether serum leptin level is determinant of bone mineral density or not.

PATIENTS AND METHODS

Forty female osteoporotic patients and (t score<-2.5 at lumbar region and femoral neck region) 40 healthy female subjects (t score>-1) who had been postmenopausal for at least 12 months and visited our outpatient for the evaluation and treatment of osteoporosis were recruited into the study. All the subjects were screened according to WHO patient criteria (8).

We excluded the subjects who had diabetes mellitus, thyroid disorder, renal and hepatic disorders or metabolic bone diseases. None were taking drugs or hormones influencing bone metabolism. Women had no any vertebral compression fractures on lateral spine radiographs and no history of trauma, smoking, alcohol abuse and hysterectomy.

All the subjects gave informed consent for the monitoring biochemical parameters and BMD values.

Weight and height were measured by standard technique. Body mass index (BMI) was calculated as body weight (Kg) divided by height squared (m^2).

Biochemical measurements

Sample Preparation:

Whole venous blood samples were taken from patients. Blood samples were allowed to clot for 30 min at room temperature and centrifuged for 10 min at 5000 rpm. Serum samples were removed and stored at -20°C until used for assay.

The levels of osteocalcin were analyzed with quantitative determination N-MID osteocalcin in serum. The concentrations of -crossLaps (CTX) were quantitatively determined by analyzing degradation products type I collagen in serum. The electrochemiluminescence immunoassay (ECLIA) was used for determination of these tests. The levels of PTH was also analyzed with electrohemiluminescence immunoassay method (Roche Electsys 2010 immunoassay analyzer, Mannheim, Germany). The activity of total alkaline phosphates was measured according to recommended reference method of the International Federation of Clinical Chemistry (IFCC). The levels of calcium were analyzed with method according to Schwarzenbach with o-cresolphtalein complex and the concentrations of inorganic phosphate were analyzed with direct phosphomolybdate method according to Daly and Ertingshausen (Cobas Integra 700 analyzer, Roche Diagnostics, Mannheim, Germany).

Leptin assay:

The levels of leptin were analyzed by a competitive enzyme immunoassay (EIA) measuring the natural and recombinant forms of the cytokine leptin in osteoporotic patients and controls with this assay method, goat anti-rabbit antibodies were used to capture a specific leptin complex in each sample consisting of leptin antibody and biotinylated leptin. The biotinylated leptin conjugates of samples are competed for leptin specific antibody binding sites. The assay is visualized using a streptavidin alkaline phosphatase

conjugate and an ensuing chromogenic substrate reaction. The amount of leptin detected in each sample was compared to a leptin standard curve, which demonstrated an inverse relationship between absorbance and its concentration. Assay procedure was done according to suggestions of manufacturer (Accucyte Human Leptin, Lot. No. AL010-DA, Cytimmune Science Inc., MD, US).

BMD values were measured by dual-energy-X-ray absorptiometry (DEXA) using NORLAND - XR46 (Norland Co, Fort Atkinson, WI, USA) at the lumbar spine (L2-L4), femoral neck region and whole body. BMD was automatically calculated from the bone area and bone mineral content and expressed absolutely in g/m^2.

Lateral radiographs of the thoracic and lumbar spine were taken for the evaluation of any compression fractures.

Statistical analysis

BMD values, demographic and biochemical parameters were expressed as mean SD for patients. Statistical analysis was performed using the SPSS 9.0. The two groups were compared with student t test. Spearman correlation coefficients were calculated for some correlations in patients and controls. Simple linear regression analysis was performed for the effect of leptin on BMD in patients and controls. P values less than 0.05 were considered significant.

RESULTS

The characteristics of the patients and controls are summarized in Table I,II,III.

There was no statistically significant difference between groups concerning serum leptin levels (P=0.599).

Tablo I. Clinical characteristics of the patients and controls

	Patients(n=40)	Controls (n=40)	*P* value
Age (years)	54.3±6.3	55.7±7.0	0.425
BMI(Kg/m^2)	27.8±4.3	28.2±4.3	0.701
Age at menarche	13.6±1.5	13.5±1.2	0.808
Years since menopause	10.6±7.5	9.2±4.1	0.736

Data are shown as mean ± s.d.

Tablo II. BMD at lumbar region, femoral neck region, total BMD and BMC of the patients and controls.

	Patients(n=40)	Controls(n=40)	*P* value
Total BMD(g/cm^2)	0.876±6.9	1.01±5.1	∗0.001
Total BMC (g)	2130.7±22	2556.3±15	∗0.001
Lumbar(g/cm^2)	0.741±6.0	1.061±0.1	∗0.001
Femoral –neck(g/cm^2)	0.712±9.8	0.879±1.0	∗0.001

Data are shown as mean ± s.d.
∗Significant

Tablo III. Serum leptin levels and bone related markers of both groups

	Patients(n=40)	Controls(n=40)	*P* value
ALP(U/L)	206.2±50	199.8±60	0.595
P(mg/dl)	3.8±0.9	3.5±0.5	0.167
Ca (mg/dl)	9.4±0.9	9.1±1.3	0.293
Osteocalcin(ng/dl)	27.0±10.0	31.4±13.9	0.126
β-crossLab(ng/dl)	0.331±0.1	0.381±6.2	0.296
Leptin(ng/ml)	11.1±7.1	10.0±8.3	0.599

Data are shown as mean ± s.d.

There was statistically significant difference between groups regarding BMD at lumbar region, femoral neck region measured, total bone mineral content and total bone mineral density (p=0.000). Serum leptin levels were not correlated with lumbar BMD, femoral neck region, total BMD and total BMC in the patients and controls (r=0.174;P=0.310, r=-0.080; P=0.632, r=0.115;p=0.477 r=-0.117;P=0.489, r =0.143;P=0.332, r=0.112;P=0.425, r=0.071; P=0.613, r=0.140;p=0.320, respectively).

In patients and controls, serum leptin levels were correlated with BMI (r=0.429;p=0.009, r=0.361;p=0.04).

In patients and controls, there was no correlation between serum leptin levels and total ALP, osteocalcin, beta-crosslap (r=-0.012; P =0.951, r=-0.117; P =0.530, r=-0.038; P =0.838;).

DISCUSSION

Our finding indicates that, circulating leptin levels are not associated with BMD or leptin is not a determinant of BMD in postmenopausal osteoporotic women.

The role of circulating leptin levels on bone mass is unclear. However, serum leptin levels are increased in obesity. They are strongly and directly related to fat mass (9).Yamauchi et al. reported strong positive correlation between %fat and plasma leptin concentrations (10). Isidori et al reported that BMI was an independent contributor of serum leptin levels (11). In the study, we found significant relationship between leptin level and BMI in all subjects. However, we could not find any relationship between serum leptin level and BMD in these patients.

Goulding et al. also found no significant correlation between circulating leptin levels and biochemical markers of bone and thus speculated that leptin played no significant role in the regulation of bone cell activity (12). Although our results related with bone markers are consistent with the study of Goulding et al, biochemical bone markers are known to reflect current bone metabolism (12).

As mentioned earlier, the exact role of leptin on bone mass remains controversial. Ducy et al reported that leptin was a potent inhibitor of bone formation acting through the central nervous system (13). They found that leptin deficient (ob/ob) and leptin receptor -deficient (db/db) mice in fact have increased bone mass associated with increased rates of bone formation and leptin caused bone loss in leptin deficient mice (13). Martini et al. concluded that serum insulin GF -1 and serum leptin have no direct effect on bone mass (14). Iwamoto et al. 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 (15). Goulding et al. revealed that their results do not support the hypothesis that leptin mediates the bone sparing effects of obesity (12). Rauch et al. also failed to find a relationship between bone mass (at distal radious) and serum leptin levels (16). In the study, we failed to observe any significant difference between osteoporotic patients and healthy subjects regarding serum leptin levels and also found no any correlation between serum leptin levels and BMD in both groups. Therefore we may suggest that leptin is not a major regulator of BMD in these measured regions. The presence or absence of leptin receptors on osteoblast and osteoclast is also not known (15). We speculate that if leptin receptors exist on bones, their effect may be different in postmenopausal period or their effect of mechanism is different or changed at post menopausal period. In addition, the central effect of leptin following binding to its specific receptors located on hypothalamic nuclei may result in decreased bone formation especially in postmenopausal period as a result of imbalance between central and peripheral

effects (17). It has been also suggested that, leptin's influence may be less significant for the mature bone at the postmenopausal period (16). Therefore we suggest that the long term effect of leptin on bone mass should be investigated at postmenopausal period.

In conclusion, our study revealed that leptin might not play a major role in regulating bone mass, but, at a local level, bone marrow adipocytes may produce leptin which may enhance osteogenic activity and inhibit adipogenic activity (18). Thus it may be possible that local production of leptin may partly play a role in bone metabolism and whether it has effects on women at the postmenopausal period should be investigated in future.

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