

## Association of serum leptin with various indices of bone turn over in renal transplant patients.

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### Abstract

Leptin is a small peptide hormone that is mainly produced in adipose tissues. Leptin plays an important role in regulating appetite and energy expenditure and may be involved in modulating bone mineralization. The relationship between leptin and bone mineral density and bone biochemical parameters is not completely understood. This study was designed to examine the relationship between some biochemical parameters such as alkaline phosphatase (ALP), phosphorus (P), intact parathormone (iPTH) and calcium (Ca) with serum leptin level in renal transplant recipients. We studied 72 renal transplant patients (47 men and 25 women) with mean ages of 44 ( $\pm$  12) years and mean body mass index (BMI) of  $24.2 \pm 3.9$  Kg/m<sup>2</sup>. Serum Ca, P, ALP, iPTH and serum leptin were measured. In this study a significant difference of serum leptin between males and females with higher values in females was seen. In patients a significant positive correlation of serum leptin with serum iPTH was found. There was not relationship between serum leptin with ALP, P, Ca, BMI, age and creatinine clearance. A negative relationship between serum leptin and the duration of kidney transplantation was found. Positive relationship of serum leptin with serum parathormone suggests that leptin may affect bone metabolism in renal transplant patients.

**Key words:** Leptin, Parathormone, Kidney, Transplantation, Renal function test

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### Introduction

Renal transplantation (RT) is a treatment of choice for patients with end-stage kidney diseases [1-5]. Kidney transplantation corrects most of the metabolic disorders [4, 5]. Post-transplant bone disease is a heterogeneous disease with various effects of age, gender, persistent hyperparathyroidism, corticosteroids and therapy with calcineurin inhibitors [4-10]. Leptin is released from fat tissues and circulating levels of leptin have been shown to be positively associated with fat mass in both lean and obese individuals [10,11]. Previous studies have shown elevated serum leptin levels in renal failure patients [12]. Leptin level is increased before transplantation, and decreased in the first year of RT [13]. Increase in serum leptin level may be seen thereafter and may be due to increase in fat mass, insulin resistance or steroid use in kid-

ney transplant patients [14]. Leptin first emerged as a component of a regulatory fat mass and energy expenditure but recent evidence showed that leptin might be a potential mediator of the protective effects of fat mass on bone tissues [14,15]. Both a peripheral and a central action of leptin on bone have been proposed. Peripherally, leptin is found to exert positive effects on bone formation, whereas it is thought to reduce bone formation through a central control mechanism when binds to its specific receptors located on the hypothalamic nuclei [16-19]. However, the exact role of leptin on bone metabolism has not yet been clarified. It seems that leptin has the ability to affect directly the bone remodeling by modulating both osteoblast and osteoclast activities [17-19]. This study was designed to evaluate the association between various indices of bone turn-over with serum leptin level in kidney transplant patients.

## Material and Methods

### Patients

This cross-sectional study was conducted on a group of stable kidney transplant patients, referred to the clinic of nephrology to continue their treatment. All patients signed the consent form for participation in this study. The etiologies of renal failure were diabetic nephropathy, hypertension, various glomerular diseases, autosomal dominant poly cystic kidney disease (ADPKD) or urinary tract infections [20-27]. Exclusion criteria included presence of acute rejection, any active or chronic infection, taking antibiotics during the past two months, and taking medications such as non steroidal anti-inflammatory drugs. After admission, all patients were examined for blood pressure (BP) and body mass index. Patients' histories concerning the length of the time that they were undergone kidney transplantation and their treatments were also obtained. Body mass index (BMI) was calculated using the standard formula (weight in kilograms/height in square meters) [28].

The basic immunosuppressive regimen of the recipients consisted of a combination of Cyclosporine at a mean dosage of  $190 \pm 60$  mg/day (median=200 mg/day), Prednisolon 7.5 mg/day for all of the patients and Mycophenolate mofetile in 46% of the patients at a dosage of  $1500 \pm 500$  mg/day (median=1500 mg/day)/or Azathioprine at a dosage of 50 to 100 mg/day in the rest of the patients. Also patients were under treatment of Calcium carbonate and active vitamin D3 (Calcitriol; Rocaltrol) at various doses.

### Laboratory methods

Serum leptin was measured as follows; blood samples were drawn after an overnight fasting, and centrifuged within 15 minutes of drawing. The levels were measured

by an enzyme-linked immunosorbent assay (ELISA) method using DRG kit (DRG diagnostic, Berline, Germany). Serum leptin normal range for males was considered to be  $3.84 (\pm 1.79)$  and for females  $7.36 (\pm 3.73)$  ng/mL. Intact parathormone (iPTH) was measured by radioimmunoassay method (normal range, 10-65 pg/mL). After an overnight fast peripheral venous blood samples were collected for biochemical analysis including serum creatinine (Cr), blood urea nitrogen (BUN), albumin (Alb), serum magnesium (Mg), phosphorus (P), calcium (Ca), alkaline phosphatase (ALP) and serum albumin (Alb) levels, using standard kits. Creatinine clearance was evaluated from serum creatinine, considering age and body weight [29].

### Statistical Analysis

The results were expressed as the mean  $\pm$  SD and median values. A statistical correlation was assessed using a partial correlation test. Comparison between groups were done using student's *t* test. For normalization of the iPTH and leptin data, their second square were used. All statistical analyses were performed with the SPSS statistical package (version 11.500 for Windows; SPSS, Chicago, USA). Statistical significance was determined at a *P*-value  $< 0.05$ .

## Results

A total of 72 patients were enrolled in this study, including 47 men, 25 women, in whom, 11 patients had diabetes mellitus. The mean patients' age was  $44 (\pm 12)$  years. The mean length of the time that they had received a transplant kidney was  $67.5 (\pm 42)$  months (median: 62 years). The patients data are shown in table 1. In this study there was a significant difference of serum leptin between females and males ( $r < 0.001$ ; Figure 1). There was no significant

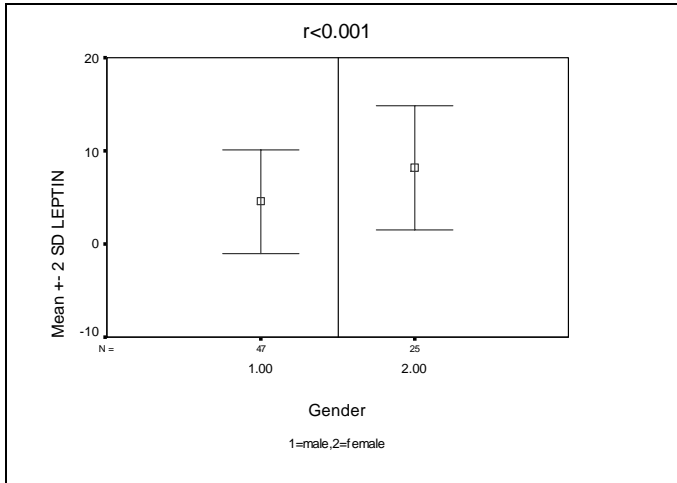
**Table 1.** Data of the patients

N=72	Minimum	Maximum	Mean + SD	Median
Age (year)	13	61	$44 \pm 12$	44
DKT (month)*	4	162	$67.5 \pm 42$	62
BMI (kg/m <sup>2</sup> )	15	33	$24 \pm 4$	17
Ca (mg/dL)	6.7	10.7	$9.5 \pm 0.7$	9.6
P (mg/dL)	2.5	5	$3.7 \pm 0.6$	3.7
Leptin (ng/mL)	0.25	16	$5.8 \pm 3.4$	4.8
iPTH* *(Pg/mL)	4	40	$18.4 \pm 8.2$	16.5
Alb (g/dL)	2.5	6.2	$4.2 \pm 0.5$	4.2
Mg(mg/dL)	1.5	2.5	$1.9 \pm 0.2$	1.9
ALP(IU/L)	35	963	$169 \pm 133$	131

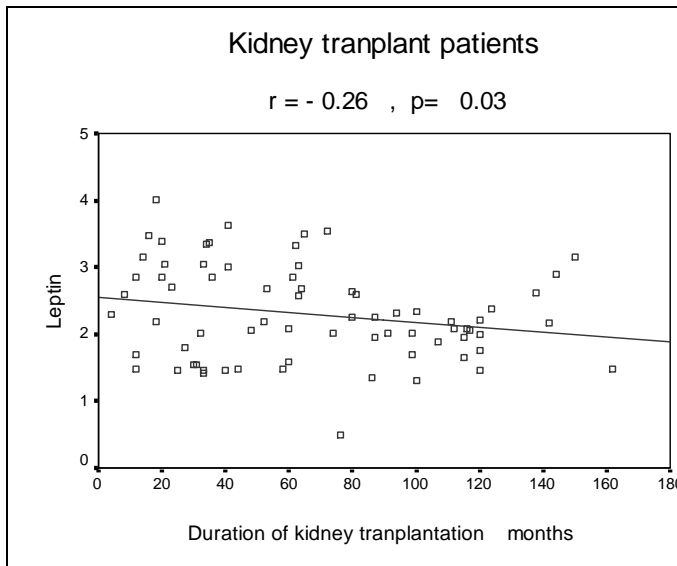
\*Duration of kidney transplantation, \*\*Intact PTH (iPTH)

difference of serum leptin between diabetic and non diabetic patients. In this study, there was a negative relationship between serum iPTH and creatinine clearance ( $r = -$

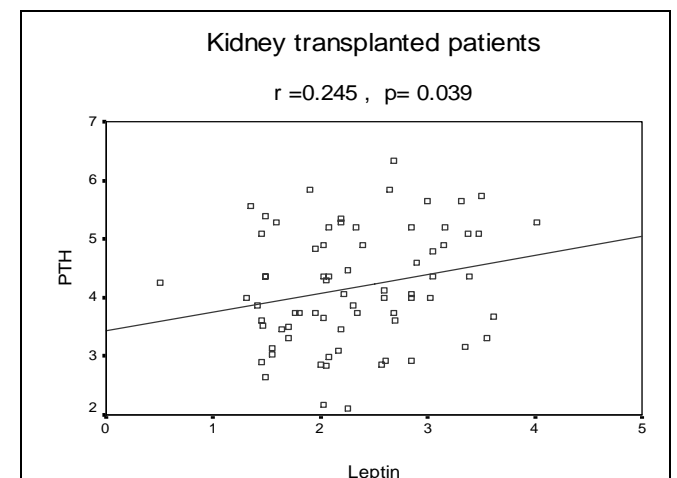
$0.44$   $P < 0.001$ ). There was not any correlation between the duration of renal transplantation and serum iPTH ( $P > 0.05$ ).



**Figure 1.** Significant difference of serum leptin between females and males



**Figure 2.** Inverse association between serum leptin and duration of renal transplantation



**Figure 3.** Positive association between serum leptin and serum iPTH

There were inverse correlations of serum ALP with age ( $r=-0.35$ ,  $P=0.02$ ) and duration of kidney transplantation ( $r=-0.29$ ,  $P=0.01$ ). In this study, there was an inverse association between serum leptin and duration of renal transplantation ( $r=-0.26$ ,  $P=0.03$ ; Figure 2). There was not any significant association between serum leptin and creatinine clearance, BMI or ages of the patients ( $P>0.05$ ). In this study, we also found a positive association between serum leptin and serum iPTH ( $r=0.24$ ,  $P=0.03$ ; Figure 3). The association of serum leptin with serum Ca, P and ALP was not significant ( $P>0.05$ ).

**Discussion**

In the current study we found a significant difference of serum leptin between females and males, while there was no significant difference of serum leptin between diabetic and non-diabetic patients. There was a negative relationship between serum iPTH and creatinine clearance, however, there was not any correlation between the duration of renal transplantation and serum iPTH. There was inverse correlation between the serum ALP and age of the patients or duration of kidney transplantation. There was an inverse association between serum leptin and duration of renal transplantation. There were not any significant association between serum leptin and creatinine clearance or age of the patients. This study showed also a positive association between serum leptin and serum iPTH and finally the association of serum leptin with serum Ca, P and ALP was not significant.

This study used the association between parathyroid hormone and serum leptin to define bone turn over in kidney transplant patients. Evenepoel et al. studied 1332 kidney allograft recipients, transplant between 1989 and 2000 [30]. They found that 4.1% of their patients had persistent hyperparathyroidism and needed parathyroidectomy after a first successful kidney transplantation. They concluded that persistent hyperparathyroidism requiring parathyroidectomy after successful kidney transplantation is a common clinical problem [30]. While in the present study our results are different, the patients' mean iPTH being  $18.4 (\pm 8.2)$  pg/mL (median: 16.5 pg/mL) means there was not any persistent hyperparathyroidism. Studies have shown that parallel changes in body weight and bone mass can be partially mediated via circulating leptin. Among the hormones involved in bone and mineral metabolism, such as oestrogens, testosterone and parathormone, leptin has recently become a subject of considerable interest [31-35]. It is thought that in kidney transplant recipients, serum leptin levels are often elevated and bone mineral density (BMD) decreased [31-35]. Souza et al. carried out a study on thirty-two RT patients and found no correlation between leptin and renal function in their study. They showed also that during the first post transplant year, serum leptin levels decreased significantly in relation to pre-transplant period [13]. Published data ex-

plaining leptin effects on bone metabolism remain conflicting, with positive and negative effects [14, 15, 31-35]. It has been shown that increased fat is accompanied by greater serum leptin concentrations, bone mineral mass and a lower risk of osteoporosis [36-42]. Malnutrition reduces serum leptin concentrations and bone mineralization in men [43-50]. It was also found that both serum leptin concentrations and bone mineral are increased at the initiation of puberty [45-54]. These findings show that leptin may modulate bone mineral. Steppan et al. showed in young *ob/ob* mice that treatment with leptin elevates bone size, bone mineral content, and density, indicating that leptin is needed for bone growth and modeling [55].

Previously we conducted a study on a group of hemodialysis patients. We found a significant positive correlation of serum leptin with iPTH. We concluded that in hemodialysis patients serum leptin may affect bone activity [56]. Agras et al. found that elevated leptin level is associated with increased bone mass at lumbar sites in renal transplant recipients. This finding showed that increased leptin might have a bone-sparing effect, especially in the lumbar region, in RT patients [57]. In a study on 27 kidney allograft recipients, Malyszko et al. studied markers of bone turnover and leptin [58]. They found that leptin was correlated with the percentage of body fat, serum creatinine, and urea. They concluded that leptin levels were associated with graft function and body fat in kidney allograft recipients and leptin was not related to nutritional status or bone metabolism in kidney allograft recipients, but it was associated with the current dosage of immunosuppressant and the serum calcium [58]. Recently, in a study on a group of healthy women, Maetani et al. observed strong positive association between leptin and PTH levels [59]. In the present study, we tried to consider the interrelationship between serum leptin and bone turn over or parathyroid activity in a group of kidney transplant patients. Further studies are needed to clarify significance of our findings.

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*Ethical Issues.* This study was approved by the ethical committee of Shahrekord University of Medical Sciences.

*Conflict of Interest.* The authors declare that they have no conflict of interest.

*Authors' Contributions.* HN and MRK defined the aims of research and the design of experiment and carried out the experiments. AB and SK participated in the design of the study and performed the statistical analysis. HS and MRT reviewed the paper. All authors read and approved the final manuscript.