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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Association of Serum Leptin with Various Biochemical Parameters of Bone Turnover in Maintenance Hemodialysis Patients

Hamid Nasri M.D.
Department of internal medicine, Hajar Medical, Educational and Therapeutic Center,
Shahrekord University of Medical Sciences, Shahrekord, Iran

Abstract: Leptin is a small peptide hormone that is mainly but not exclusively, produced in adipose tissue. leptin is cleared principally by the kidney. Serum leptin concentrations and bone mass are directly related. This cross-sectional study was conducted on patients with end-stage renal disease (ESRD), who were undergoing maintenance hemodialysis. Serum calcium, phosphorus, predialysis serum creatinine, blood urea nitrogen and also alkaline phosphatase (ALP) and also intact serum PTH (iPTH) and serum Leptin were measured too. In this study a significant difference of serum leptin between males and females of diabetic patients with more values in females was seen. In all patients a significant positive correlation of logarithm of serum leptin with logarithm of serum iPTH and a significant positive correlation of serum leptin and BMI in were found. In male hemodialysis patients a near significant and inverse correlation of serum ALP with serum leptin was seen, moreover in female hemodialysis patients a near significant inverse correlation of serum leptin with serum phosphorus and also a significant inverse correlation of serum leptin with CaXP products were found too. In hemodialysis patients serum leptin affects bone activity and need further investigation this aspect of hemodialysis patients.

Key words: Serum leptin, alkaline phosphatase, hemodialysis, bone turnover, PTH

Introduction

The adipose tissue cytokine leptin is a small peptide hormone that is mainly but not exclusively, produced in adipose tissue (Zoccali *et al*., 2004). Leptin exerts several important metabolic effects on peripheral tissue, including modification of insulin action, induction of angiogenesis, and modulation of the immune system (Wolf *et al*., 2002). Leptin reaches the brain by a saturable transport mechanism and via direct effects on the hypothalamus, decreases appetite and increases metabolism (Stenvinkel, 1999). Serum leptin concentrations in normal humans have been reported to correlate with the body mass index (BMI) as well as with the body fat mass (Nakazono *et al*., 1998). The hormone leptin is considered to have a role in the prevention of osteoporosis and probably acts on bone tissue through inhibition of osteoclasis (Ibanez *et al*., 2000). Several recent studies have demonstrated that, leptin is cleared principally by the kidney. Thus serum leptin concentrations are increased in patients with chronic renal failure and those undergoing maintenance dialysis (Wolf *et al*., 2002), and it has been speculated that hyperleptinemia may contribute to uremic anorexia and malnutrition (Stenvinkel, 1999). The recent discovery that leptin is a bone mass determinant (Pasco *et al*., 2001 and Matkovic *et al*., 1997) is an important new facet of the physiological repertoire of this protein. In fact human studies have shown that serum leptin concentrations and bone mass are directly related (Pasco *et al*., 2001 and Matkovic *et al*., 1997). Evidence for a stimulatory role of leptin on bone mineralization in humans includes direct relationships between serum leptin concentrations and bone mineral mass and BMD in lean women (Pasco *et al*., 2001) and lean girls (Matkovic *et al*., 1997).In this regard, it appears particularly intriguing that altered plasma leptin concentration has been reported in diseases which are typically associated with osteopenia as in liver cyrrhosis (Ducy *et al*., 2000) and in type 2 diabetes (Gordeladze and Reseland, 2003). Studies concerning the relationship of serum leptin and biochemical markers of bone turn over in dialysis patients are scarse and effects of serum leptin levels on bone activity in patients with chronic renal failure and hemodialysis have not been widely investigated. Preliminary results revealed that high plasma leptin in male patients on chronic dialysis therapy is associated with biochemical evidence of reduced bone turnover (Zoccali *et al*., 2004 and Ghazali *et al*., 2003). Contradictory data also have been reported. This cross-sectional study aims to examine relationships between serum leptin levels and some biochemical parameters of bone turnover in maintenance hemodialysis patients.

Materials and Methods

This cross-sectional study was carried out on patients with end-stage renal disease (ESRD), who were undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membrane. According to the severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism (SHPTH) was given

Table 1: Mean ±SD, Minimum and Maximum of age, duration and dosage hemodialysis and also laboratory results of total hemodialyzed patients

Total patients N=36	Minimum	Maximum	Mean±SD	Median
Leptin ng/ml	0.10	51.9	7±9.2	4.2
BMI kg/m^2	16	33	21.33±3.98	20.5
AGE years	16	80	45.7±16.5	43
DH* months	2	156	30±36	17.5
Dosages sessions	18	1584	285±396	144
PTH pg/ml	16	1980	435±454	309
Ca mg/dl	5	10	7.7±0.93	8
P mg/dl	3.4	10	6.4±1.8	6.2
ALP IU/L	150	5487	633±891	444
CAXP mg^2/dl^2	25	80	51±15	50
URR %	39	76	59±9.2	57.5
Creat mg/dl	3	18	9.4±3.6	9.5
BUN mg/dl	30	180	82±34	78

*Duration of hemodialysis

oral active vitamin D3 (Rocaltrol), calcium carbonate tablets and Rena-Gel capsules at various doses. After 12-hour fasting, levels of serum calcium (Ca), phosphorus (P), predialysis serum creatinine also pre and post dialysis blood urea nitrogen(BUN) and also alkaline phosphatase (ALP) were measured using standard kits. Intact serum PTH (iPTH) was measured by the radioimmunoassay (RIA) method using DSL-8000 of USA (normal range of values is 10-65 pg/ml). Serum Leptin (normal range of values for males is 3.84±1.79 and for females is 7.36±3.73ng/ml were measured by enzyme-linked immunosorbent assay (ELISA) method using DRG kits of Germany. For the efficacy of hemodialysis the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data. The body mass index (BMI) was calculated using postdialysis weight and height (kg/m^2). Duration and dosages during hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 h. For statistical analysis, the data are expressed as the mean±SD. Comparison between the groups was done using Student's t-test. Statistical correlations were assessed using partial correlation test. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at a *p*-value <0.05.

Results

Of the total patients (n=36), 26 were non-diabetic HD patients (F=10, M=16) and 10 (F=4, M=6) were diabetic HD patients. Table 1, 2 and 3 show the patients' mean ± SD age, the length of time they were on hemodialysis, the dialysis dosage, and the results of laboratory tests of total, non diabetic and diabetic dialysis patients. The mean patients' age was 46±18 years. The mean length of the time patients had received hemodialysis was 30±36 months (median: 17.5).

The mean serum leptin was 7±9.2 ng/ml (median:4.2 ng/ml). The mean serum leptin values within the diabetic and non-diabetic groups were 7.63±4.63 (median: 7.8) and 6.85±10.47 (median: 3.45) ng/ml, respectively.

The mean serum iPTH was 435±454 (median: 309) pg/ml. The mean iPTH values within the diabetic and non-diabetic groups were 218±287 (median: 43) and 519±482 (median: 335) pg/ml, respectively. In this study, no significant differences between age, BMI, duration of hemodialysis treatment, dialysis dosage, Cax P products, URR, serum iPTH, serum leptin, Ca, P, ALP, Creat or BUN between males and females of total patients were found (*p* N.S.). However, there was a near significant difference of serum iPTH levels between diabetic and non-diabetic HD patients (p = 0.075). In this study no significant differences of serum leptin between males and females of non diabetic HD patients was seen, however a significant difference of serum leptin between males and females of diabetic HD population was seen (r=0.035). In all patients a significant positive correlation of BMI with serum leptin (r= 0.45, p = 0.008) (adjusted for age) was existed. In all patients a significant positive correlation of logarithm of serum leptin with logarithm of serum iPTH (r =0.42 p = 0.045) (adjusted for age, duration and dosage of dialysis, serum ca, p, URR, gender, presence of DM and also BMI) was found. In male hemodialysis patients a significant inverse correlation of dialysis efficacy (as determined by URR) with serum leptin (r = -0.46, p = 0.036), and also a near significant inverse correlation of serum ALP and serum leptin (r = -0.39, p = 0.079) were seen (adjustment for duration of hemodialysis treatment for two above correlations) too. Moreover in this group a significant positive correlation of age and serum leptin (r = 0.44, p = 0.046) (adjusted for hemodialysis dosage) and a significant positive correlation of hemodialysis dosage and serum leptin (r = 0.44, p = 0.046) (adjustment for age) were seen too. In this group also a near significant positive correlation between duration of

Table 2: Mean±SD, Minimum and Maximum of age, duration and dosage hemodialysis and also laboratory results of non diabetic hemodialysis patients

Non diabetic patients N=26	Minimum	Maximum	Mean±SD	Median
Leptin ng/ml	0.10	51.9	6.85±10.5	3.45
BMI kg/m^2	16	80	43.6±16.5	19
AGE years	16	33	21±4.5	41.5
DH* months	2	156	36.8±40.9	20.5
Dosages sessions	18	1584	348.8±451.3	154
PTH pg/ml	22	1980	519±483	335
Ca mg/dl	6	9	7.8±0.7	8
P mg/dl	3.4	10	6.9±1.8	6.5
ALP IU/L	150	5487	749±10	479
CAXP mg^2/dl^2	25	80	52.31±16	53
URR %	50	76	61±7.9	60
Creat mg/dl	3	15	9.54±3	10
BUN mg/dl	30	180	79±33	74

*Duration of hemodialysis

hemodialysis treatment and serum leptin (r = 0.42, p = 0.052) (adjustment for age) was seen too. In female hemodialysis patients, a near significant and inverse correlation between serum leptin and serum phosphorus (r = -0.54, p = 0.057) was existed, in this group also a significant inverse correlation of serum leptin and CaXP products was found too (r = -0.62, p = 0.025) (adjustment for age of the patients for two above correlations).

Discussion

The principal findings of this study were, a near significant differences of serum iPTH levels between diabetic and non-diabetic HD patients. A significant difference of serum leptin between males and females of diabetic HD patients with more values in females. In all patients a significant positive correlation of logarithm of serum leptin with logarithm of serum iPTH and a significant positive correlation of BMI and serum leptin were seen. In male hemodialysis patients a significant inverse correlation of dialysis efficacy and serum leptin and also a near significant inverse correlation of serum ALP and serum leptin were seen too, in this group also a significant positive correlation of age and serum leptin and a significant positive correlation of hemodialysis dosage with serum leptin were seen too. More over in this group a near significant positive correlation between duration of hemodialysis treatment and serum leptin was seen too. In female hemodialysis patients a near significant inverse correlation of serum leptin and serum phosphorus and a significant inverse correlation of serum leptin and CaXP products were found too. Leptin is a hormone produced by adipose tissue and its serum levels correlates with total fat mass (Considine *et al.*, 1996).Several direct effects on bone recently have been attributed to leptin. It was shown in vitro that leptin acts on marrow stromal cells by inhibiting after differentiation into adipocytes, whereas favoring osteoblastic different iontion (Coen *et al.*, 2003). In experimental animals leptin inhibited bone resorption (Burguera *et al.*, 2001), also it have been shown that leptin deficient mice have increased bone mass associated with increased rate of bone formation (Ducy *et al.*, 2000). In fact apart from age, aluminum intoxication, diabetes, calcium and magnesium overload, advanced glaciation end product accumulation and beta-blocker use, factors reducing serum PTH and/or bone turnover in patients with end-stage renal diseases (ESRD) remain incompletely understood. As mentioned plasma leptin is raised in dialysis patients (Merabet *et al.*, 1997). Studies concerning the relationship between plasma leptin concentration and bone biochemical parameters showed interesting results, in the study conducted by Ghazali on 17 female and 16 male chronic dialysis patients, showed Leptin levels were twice as high in female patients. Ghazali concluded that higher leptin levels in post-menopausal female haemodialysis patients than in male patients may account for their slower bone loss with ageing, also the study suggested that serum leptin in haemodialysis patients may have a bone-sparing effect only when the serum levels of leptin were higher than the presumed threshold of blood–brain transport saturation and higher leptin levels in post-menopausal female haemodialysis patients than in male patients may account for their slower bone loss with ageing (Zoccali *et al.*, 2004). In a study conducted by Coen *et al.* (2003) on 46 hemodialysis patients (32 men, 14 women; age, 57.2 ± 11.4 years), firstly found a positive relation between serum leptin level and BMI and greater serum levels in women compared with men, and in total patients an inverse significant correlation with serum PTH was seen however this correlation was more significant in male group. Coen's study are infavor of an inverse association between serum leptin levels and some histomorphometric and histodynamic indexes of bone turnover which is in accordance with

Table 3: Mean±SD, Minimum and Maximum of age, duration and dosage hemodialysis and also laboratory results of diabetic hemodialysis patients

Diabetic patients N=10	Minimum	Maximum	Mean±SD	Median
LEPTIN ng/ml	0.20	15.2	7.6±4.63	7.8
BMI kg/m^2	27	75	51±15.9	22.5
AGE years	20	24	22±1.6	55
DH * months	6	24	14±6.3	12
Dosages sessions	54	216	119±55.5	99
PTH pg/ml	16	860	218±287	43
Ca mg/dl	4	10	6±2	7.5
P mg/dl	175	584	330±155	6
ALP IU/L	32	70	48±12.8	289
CAXP mg^2/dl^2	39	75	53.6±10.36	49
URR %	5	10	7.5±1.3	54
Creat mg/dl	3	18	9.5±4.8	9
BUN mg/dl	30	140	90±37	98

Duration of hemodialysis

experimental data showing decrease osteoblastic activity related to the administration of leptin *in vivo* (Coen *et al*., 2003, Burguera *et al*., 2001) or the addiction of leptin to preosteoclastic *in viro* (Coen *et al*., 2003, Burguera *et al*., 2001, Holloway *et al*., 2002). Previously in a study conducted by Kokot *et al*. (1998) (the inverse relationship between serum leptin and PTH1-84 was also noted (Kokot *et al*., 1998). Zoccali *et al*. (2004) in a study on 161 hemodialysis (HD) patients to find the association between plasma leptin and biochemical bone turnover indicators, showed plasma leptin was sex-dependent, being significantly higher in female dialysis patients than in male dialysis patients and it related directly to body mass index (Zoccali *et al*., 2004). In an agreement with the studies of Coen *et al*., (2003) and Zoccali *et al*. (2004), we also found a significant positive relation between serum leptin and BMI. In the aspect of sex-dependency we have a higher values of serum leptin only in females of our diabetic HD population. The other findings of study which was conducted by Zoccali *et al*. (2004) were; in male patients, plasma leptin after adjusted for BMI related inversely to serum intact PTH, serum PTH1-84, C-PTH fragment and serum PTH1-84/C-PTH fragment ratio, while no such relationships were found in female patients in males. Zoccali *et al*. (2004) found that the link between plasma leptin and bone turnover markers was independent of other factors. Also plasma leptin related inversely to skeletal alkaline phosphatase in male patients but not in female patients. In an agreement with mentioned studies we showed a significant positive correlation of logarithm of serum leptin with logarithm of serum iPTH, we also have a very near significant and inverse correlation of serum leptin and serum alkaline phosphatase in male dialysis population. In this group we also had some other findings, a significant positive correlation between age and serum leptin and a significant inverse correlation between dialysis efficacy and serum leptin a significant positive correlation between hemodialysis dosage and serum leptin were seen too, moreover in this group a very near significant positive correlation between duration of hemodialysis treatment and serum leptin was seen as well. Interestingly in female hemodialysis patients a near significant and inverse correlation between serum leptin and serum phosphorus and a significant inverse correlation between serum leptin and CaXP products were found. We concluded that this inverse correlation between leptin and phosphorus or CaxP products could indirectly show the negative association of bone activity with serum leptin in this group too, while the high serum phosphorus or CaxP products exist in the cases of uncontrolled secondary hyperparathyroidism, could support the negative association of bone activity and serum leptin in dialysis patients. Our distinct data needs more attention to investigate with larger hemodialysis population to clarify this aspect of hemodialysis patients.

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