Relation of Leptin and Adiponectin With Cardiovascular Risk Factors, Intact Parathormone, and Vitamin D Levels in Patients With Primary Hyperparathyroidism

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Background: Primary hyperparathyroidism (PHPT) is associated with high cardiovascular morbidity. The aim of the present study was to explore the relationship of leptin and adiponectin levels with cardiovascular risk factors and anthropometric parameters in patients with PHPT with and without metabolic syndrome (MS). **Methods:** A total of 62 patients with PHPT were enrolled. Weight, blood pressure, basal glucose, insulin, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, insulin, HOMA-R, intact parathormone, vitamin D, calcium, leptin, and adiponectin levels were measured in fasting condition. **Results:** Prevalence of MS with ATP III definition was 32.3% (20 patients; 15 females (75%) and 5 males (25%)) and 67.7% patients without MS ($n = 42$ patients; 35 females (83.3%) and 7 males (16.7%)). In the analysis with leptin as dependent vari-

able, the weight and HOMA-R levels remained in the model ($F = 9.2$; $P < 0.05$), with an increase of 1.31 (CI 95%: 0.24–2.31) ng/ml with each one unit of HOMA-R and an increase of 0.4 (CI 95%: 0.01–0.84) ng/ml with each 1 kg of weight. In a second model with adiponectin as dependent variable, the HOMA-R and HDL-cholesterol levels remained in the model ($F = 7.37$; $P < 0.05$), with a decrease of -0.62 (CI 95%: 0.01–1.1) ng/ml with each one point of HOMA-R and an increase of 0.18 (CI 95%: 0.04–0.38) ng/ml with each 1 mg/dl of HDL-cholesterol. In the multivariate, PTH I was not associated with other variables. **Conclusion:** There was a high prevalence of MS—32.3% of patients with PHPT presented an MS. Serum levels of leptin and adiponectin are not related with PTH I, vitamin D, and calcium levels in patients with PHPT. J. Clin. Lab. Anal. 26:398–402, 2012. © 2012 Wiley Periodicals, Inc.

Key words: adiponectin; leptin; primary hyperparathyroidism

INTRODUCTION

The current view of adipose tissue is that of an active secretory organ, sending out adipokines that modulate insulin resistance, energy expenditure, and inflammation. Adipokines are proteins produced mainly by adipose tissue (1). These molecules have been shown to be involved in the pathogenesis of the metabolic syndrome (MS) and cardiovascular disease in obese patients. For example, adiponectin is an adipocyte-derived collagen-like protein identified through an extensive search of adipose tissue. Hypoadiponectinemia increased risk of coronary artery disease together with the presence of multiple risk factors, indicating that adiponectin is a key factor of the MS (2). Leptin is a protein secreted primarily from adipocytes. Leptin suppresses food intake and increases energy expen-

diture by enhancing thermogenesis and metabolic rate. Reports suggest that leptin contributes to atherosclerosis and cardiovascular disease in obese patients (3). Most studies of serum profile of adipokines have been conducted in obese patients; with few studies have been conducted specifically in patients with primary hyperparathyroidism (4). Therefore, an area of research interest is

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the potential relationship between hyperparathyroidism, adipokine levels, and cardiovascular risk factors. The factors associated with cardiovascular disease include high blood pressure, low high-density lipoprotein (HDL), high triglyceride levels, hyperglycemia, and obesity. Many of these risk factors are the ones that make up the so-called MS.

Primary hyperparathyroidism (PHPT), caused by solitary parathyroid adenomas in 85% of cases and by diffuse hyperplasia in 15% (5), results in overproduction of parathyroid hormone (PTH) with hypercalcemia. PHPT is associated with high cardiovascular morbidity and mortality (6), structural and functional abnormalities, such as left ventricular hypertrophy and myocardial calcification (7), and hypertension (8).

Accordingly, the aim of the present study was to explore the relationship of serum leptin and adiponectin levels with cardiovascular risk factors, intact parathormone and vitamin D levels in patients with PHPT with and without MS.

SUBJECTS AND METHODS

Subjects and Procedures

A sample of 62 consecutive patients with PHPT was enrolled. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by our Hospital. Written informed consent was obtained from all patients. The diagnosis of PHPT was established according to laboratory data characterized by high levels of total calcium, and intact parathormone (I-PTH). All patients underwent technetium Tc99m MIBI imaging and ultrasonography. None of the patients had renal insufficiency (creatinine level < 1.2 mg/dl). The exclusion criteria included: steroid treatment, pregnancy, severe comorbid conditions, and none of these subjects were receiving any medical treatment. To estimate the prevalence of MS, the definitions of the ATP III were considered (9). The cutoff points for the criteria used are three or more of the following: central obesity (waist circumference > 88 cm in females and > 102 cm in males), hypertriglyceridemia (triglycerides > 150 mg/dl or specific treatment), hypertension (systolic BP > 130 mmHg or diastolic BP > 85 mmHg or specific treatment), or fasting plasma glucose > 100 mg/dl or drug treatment for elevated blood glucose.

Weight, blood pressure, basal glucose, insulin, total cholesterol, low-density lipoprotein (LDL)-cholesterol, HDL-cholesterol, triglycerides, insulin, HOMA-R, I-PTH, vitamin D, calcium, leptin, and adiponectin levels were measured in fasting condition.

Biochemical Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA). Insulin was measured by enzyme immunoassay method (Insulin, SPI bio, Montigni, France) and the homeostasis model assessment for insulin resistance (HOMA-R) was calculated using these values (10). Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY), while HDL-cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL-cholesterol was calculated using Friedewald formula. Serum alkaline phosphatase, creatinine, and calcium were determined by automated enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Geneva, Switzerland).

Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Houston, TX) with a sensitivity of 0.05 ng/ml and a normal range of 10–100 ng/ml (11). Adiponectin was measured by ELISA (R&D systems, Inc., Minneapolis, MN) with a sensitivity of 0.246 ng/ml and a normal range of 865–21,424 ng/ml (12).

Intact PTH (I-PTH) was measured by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). 25-OH Vitamin D was measured by electrochemiluminescence immunoassay (Roche Diagnostics GmbH).

Anthropometric Measurements and Dietary Intake

Body weight was measured to an accuracy of 0.01 kg and body mass index computed as body weight/(height²). Waist circumference (narrowest diameter between xiphoid process and iliac crest) was measured. Tetrapolar body electrical bioimpedance was used to determine body composition (13). Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer, and averaged.

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instruction to record their daily dietary intake for 3 days including a weekend day. Food scales and models to enhance portion size accuracy were used. National composition food tables were used as reference (14).

Statistical Analysis

The results were expressed as mean $\pm$ standard deviation. The distribution of variables was analyzed with Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed

TABLE 1. Anthropometric and Biochemical Variables, Metabolic Syndrome Versus No Metabolic Syndrome Groups

Parameters	Metabolic syndrome (<i>n</i> = 20)	No metabolic syndrome (<i>n</i> = 42)	
Age (years)	62.8 ± 12.6	68.0 ± 9.5	ns
BMI (kg/m ²)	29.3 ± 4.1	25.1 ± 3.5	<0.05
Waist circumference (cm)	98.0 ± 11.3	87.8 ± 8.5	<0.05
Fat mass (kg)	26.7 ± 7.3	19.3 ± 6.3	<0.05
SBP (mmHg)	140.7 ± 17.6	132.1 ± 21.1	<0.05
DBP (mmHg)	79.4 ± 11.1	74.1 ± 11.4	ns
Glucose (mg/dl)	119.3 ± 22.1	95.5 ± 8.9	<0.05
Total cholesterol (mg/dl)	216.3 ± 42.9	225.4 ± 29.5	ns
LDL-cholesterol (mg/dl)	133.6 ± 39.5	142.9 ± 32.5	ns
HDL-cholesterol (mg/dl)	50.4 ± 13.3	62.7 ± 17.8	<0.05
Triglycerides (mg/dl)	159.1 ± 50.3	101.4 ± 39.1	<0.05

SBP, systolic blood pressure; DBP, diastolic blood pressure, ns, not significant.

Student's *t*-test. Nonparametric variables were analyzed with the Mann–Whitney *U*-test. Correlation analysis was performed with Pearson and Spearman tests. A multiple regression model (step by step) was used to study the dependent variables (leptin, adiponectin, and I-PTH). A *P*-value under 0.05 was considered statistically significant.

Results

Sixty-two patients gave informed consent and were enrolled in the study. The mean age was 64.5 ± 11.8 years, the mean BMI was 26.4 ± 4.2.

Prevalence of MS with ATP III definition was 32.3% (20 patients; 15 females (75%) and 5 males (25%)) and 67.7% patients without MS (*n* = 42 patients; 35 females (83.3%) and 7 males (16.7%)).

Table 1 shows the subjects differences in anthropometric and biochemical parameters with and without MS. Patients with MS had higher weight, BMI, waist circumference, systolic blood pressure, glucose and triglycerides levels than patients without MS. Patients with MS had lower HDL-cholesterol levels than patients without MS.

Table 2 shows the subject's levels of adipokines, I-PTH, vitamin D, calcium, and alkaline phosphatase in MS and no MS. Patients with MS had higher HOMA-R, insulin, and leptin levels than patients without MS.

Serial assessment of nutritional intake with 3 days written food records showed a calorie intake of 1,854 ± 710 kcal/day, a carbohydrate intake of 196.7 ± 81.3 g/day, a fat intake of 90.6 ± 33.2 g/day, and a protein intake of 94 ± 23.7 g/day. Dietary intakes were similar in patients without and with MS; calories (No MS: 1,866.7 ± 541.8 kcal/day vs. MS: 1,688.8 ± 541.1 kcal/day; ns), carbohydrates (No MS: 202.2 ± 71.6 g/day vs. MS: 176.9 ± 49.1 g/day; ns), pro-

TABLE 2. Insulin, Adipokines, and Calcium Metabolism Parameters, Metabolic Syndrome Versus No Metabolic Syndrome Groups

Parameters	Metabolic syndrome (<i>n</i> = 20)	No metabolic syndrome (<i>n</i> = 42)	
Insulin (mUI/l)	24.1 ± 12.9	17.3 ± 13.1	<0.05
HOMA	7.1 ± 4.1	4.2 ± 3.5	<0.05
Leptin (ng/dl)	24.8 ± 13.1	15.5 ± 13.1	<0.05
Adiponectin (ng/dl)	9.1 ± 4.5	18.3 ± 20.1	ns
Phosphatase alkaline (U/L)	70.3 ± 16.1	73.8 ± 23.5	ns
Calcium (mg/dl)	10.2 ± 0.7	9.7 ± 0.7	ns
PTH I (pg/ml)	124.3 ± 52.6	123.1 ± 62.2	ns
Creatinine (mg/dl)	0.83 ± 0.21	0.75 ± 0.18	ns
Vitamin D (nmol/l)	23.4 ± 13.1	26.3 ± 16.5	ns

PTH I, parathormone intact; ns, not significant.

TABLE 3. General Correlation Analysis in the Total Group

Characteristics	Leptin	Adiponectin	I-PTH
BMI	<i>r</i> = 0.4	ns	ns
Weight (kg)	ns	ns	ns
Waist circumference (cm)	<i>r</i> = 0.49	ns	ns
Fat mass (kg)	<i>r</i> = 0.41	ns	ns
Glucose (mg/dl)	<i>r</i> = 0.34	ns	ns
HOMA	<i>r</i> = 0.5	ns	ns
LDL-cholesterol (mg/dl)	<i>r</i> = 0.35	ns	ns
HDL-cholesterol (mg/dl)	ns	<i>r</i> = 0.65	ns
Vitamin D (nmol/l)	ns	ns	<i>r</i> = −0.3
Triglycerides (mg/dl)	ns	ns	<i>r</i> = 0.48
Calcium (mg/dl)	ns	ns	<i>r</i> = 0.49

BMI, body mass index; HOMA, homeostasis model assessment; ns, not significant. *P*-value of *r* coefficient is below 0.05 (significant).

teins (No MS: 80.2 ± 21.6 g/day vs. MS: 76.6 ± 21.6 g/day; ns), and lipids (No MS: 81.6 ± 28.1 g/day vs. MS: 70.9 ± 35.8 g/day; ns). Calcium intake did not reach 1 g per day in both groups (No MS: 788.9 ± 320 mg/day vs. MS: 818.1 ± 361 mg/day; ns). Dietary intake of vitamin D was similar in both groups (No MS: 2.9 ± 4.8 μg/day vs. MS: 3.2 ± 3.3 μg/day; ns).

Table 3 shows the correlation analysis among adipocytokines and other parameters in the global group. A positive correlation was observed among leptin levels, BMI, waist circumference, fat mass, glucose, HOMA-R, and LDL-cholesterol. A positive correlation was detected between adiponectin levels and HDL-cholesterol. A negative correlation was observed between PTH I levels and vitamin D, and positive correlation among PTH I, triglycerides, and calcium levels.

Tables 4 and 5 show the correlation analysis in MS and no MS patients, respectively. In patients with MS (Table 4), a positive correlation was observed among leptin levels, waist circumference, HOMA-R, and LDL-cholesterol. A positive correlation was detected between

TABLE 4. General Correlation Analysis in the Metabolic Syndrome Group

Characteristics	Leptin	Adiponectin	I-PTH
BMI	ns	ns	ns
Weight (kg)	ns	$r = -0.31$	ns
Waist circumference (cm)	$r = 0.54$	ns	ns
Fat mass (kg)	ns	ns	ns
Glucose (mg/dl)	ns	ns	ns
HOMA	$r = 0.57$	ns	ns
LDL-cholesterol (mg/dl)	$r = 0.53$	ns	ns
HDL-cholesterol (mg/dl)	ns	$r = 0.63$	ns
Vitamin D (nmol/l)	ns	ns	ns
Triglycerides (mg/dl)	ns	ns	ns
Calcium (mg/dl)	ns	ns	$r = 0.41$

BMI, body mass index; HOMA, homeostasis model assessment; ns, not significant. *P*-value of *r* coefficient is below 0.05 (significant).

TABLE 5. General Correlation Analysis in the No Metabolic Syndrome Group

Characteristics	Leptin	Adiponectin	I-PTH
BMI	$r = 0.46$	ns	ns
Weight (kg)	ns	ns	ns
Waist circumference (cm)	$r = 0.38$	ns	$r = 0.68$
Fat mass (kg)	$r = 0.47$	ns	ns
Glucose (mg/dl)	$r = 0.34$	ns	ns
HOMA	$r = 0.5$	ns	ns
LDL-cholesterol (mg/dl)	$r = 0.35$	ns	ns
HDL-cholesterol (mg/dl)	ns	$r = 0.65$	ns
Vitamin D (nmol/l)	ns	ns	$r = -0.7$
Triglycerides (mg/dl)	ns	ns	$r = 0.88$
Calcium (mg/dl)	ns	ns	$r = 0.76$

BMI, body mass index; HOMA, homeostasis model assessment; ns, not significant. *P*-value of *r* coefficient is below 0.05 (significant).

adiponectin levels and HDL-cholesterol and an inverse correlation between adiponectin levels and weight was observed. A positive correlation was detected between PTH I and calcium levels.

In patients without MS (Table 5), a positive correlation was observed among leptin levels, BMI, waist circumference, fat mass, glucose, HOMA-R, and LDL-cholesterol. A positive correlation was detected between adiponectin levels and HDL-cholesterol. A negative correlation was observed between PTH I levels and vitamin D, and positive correlation among PTH I triglycerides, and calcium levels.

Multivariate Analysis

After univariate analysis, we performed a multivariate analysis with leptin, adiponectin, and PTH I as dependent variables. In this analysis adjusted by weight, MS presence/absence, dietary intake, age, and sex with leptin as dependent variable, the weight and HOMA-R levels re-

mained in the model ($F = 9.2$; $P < 0.05$), with an increase of 1.31 (CI95%: 0.24–2.31) leptin ng/ml with each one unit of HOMA-R and an increase of 0.4 (CI95%: 0.01–0.84) leptin ng/ml with each 1 kg of weight. In a second model adjusted by weight, MS presence/absence, dietary intake, age, and sex with adiponectin as dependent variable, the HOMA-R and HDL-cholesterol levels remained in the model ($F = 7.37$; $P < 0.05$), with a decrease of -0.62 (CI95%: 0.01–1.1) adiponectin ng/ml with each one point of HOMA-R and an increase of 0.18 (CI95%: 0.04–0.38) adiponectin ng/ml with each 1 mg/dl of HDL-cholesterol. In the third multivariate analysis adjusted by weight, MS presence/absence, age, and sex with I-PTH as a dependent variable, all variables were excluded of the model.

DISCUSSION

The major finding of this study was that serum levels of leptin and adiponectin are not related with I-PTH, vitamin D, and calcium levels in patients with PHPT. A second significant finding is that 32.3% of patients with PHPT presented an MS as defined by the Adult Treatment Panel III criteria.

In the literature, the most important variable that determines leptin concentration is body fat mass (15). Our study shows a correlation between leptin and weight (total fat mass and central fat mass as waist circumference) in patients with PHPT. Adiponectin decreases lipid synthesis and glucose production in the liver and causes decreases in glucose and free fatty acid levels in the blood. Some studies (16) have described a positive significant correlation of adiponectin levels with HDL-cholesterol concentrations, as our data showed in patients with PHPT.

To our knowledge, this is the second study of adipokines levels in patients affected by PHPT (4), but the design and control of confounder factors was different. Our study did not show association of these adipokine levels with I-PTH, vitamin D, and calcium levels. In the study of Delfini et al. (4), an increase in circulating leptin in PHPT patients (with and without MS) and a decrease in circulating adiponectin in PHPT patients with MS were detected. It is possible that these differences were due to fat mass; this previous study (4) controlled only weight without an evaluation of total fat mass with bioimpedance, a potential bias could be implied. Other study has found that leptin levels were related to bone resorption (17) and bone formation in hemodialysis patients and that leptin levels were associated inversely to serum PTH in male dialysis patients (18). However, this relationship could be only extrapolated to patients with secondary hyperparathyroidism. Other limitation of the previous study (4) is that it did not study insulin resistance, which occurs in MS. As our data show, in multivariate analysis, leptin and

adiponectin levels were associated with insulin resistance (HOMA-R) without a significant correlation with I-PTH or vitamin D levels. The lack of dietary control could be other bias; this variable was controlled in our design.

Our finding, the lack of association of adipokine levels with PHPT, has been confirmed in other study with an intervention design (19). Bhadada et al. (19) have shown that insulin resistance, and adiponectin and leptin levels did not change 3 months after curative parathyroidectomy.

There were some limitations in our study, too. First, the cross-sectional design of the study precludes comment on causality in the relationships. Second, the sample size was small, limiting its statistical power for detecting associations. Nevertheless, this study represents a novel investigation of relationship between serum adipokine (leptin and adiponectin) levels and cardiovascular risk factors in patients with PHPT.

In conclusion, 32.3% of patients with PHPT presented an MS. Serum levels of leptin and adiponectin are not related with I-PTH, vitamin D, and calcium levels in patients with PHPT. Further studies are needed to analyze this unclear topic area with clinical and potential therapeutic implications.

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