

Serum Leptin values in the healthy obese and non-obese subjects of Rawalpindi

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Abstract

Objective: To determine serum leptin concentrations from a sample of Rawalpindi population in relation to body mass index, age and gender.

Methods: The observational, comparative study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, and Benazir Bhutto Hospital, Rawalpindi from August 2008 to December 2008. Subjects were 100 including healthy obese, overweight and non-obese of both genders aged between 20-50 years. Sampling was done by non-probability convenience method. Body Mass Index was calculated by formula BMI= weight in kg/height in m²: non-obese subjects were defined as 18.5-23.0kg/m²; overweight 23.1-27.4kg/m²; and obese 27.5-40kg/m². Serum glucose was measured using Glucose oxidase-phenol amino phenazone (GOD-PAP) method and serum leptin by sandwich enzyme-linked immunosorbent assay method.

Results: Serum leptin concentrations were higher in obese subjects (mean 52.8±24.6 ng/mL; range 28.2-77.4ng/mL; P <0.001) than in non-obese subjects (mean 12.7±6.1ng/mL, range 6.6-18.8ng/mL). Mean Body Mass Index in obese group was 31.7±3.1kg/m² (range 28.6-34.8kg/m²) while it was 21.2±1.5kg/m² (range 19.7-22.7kg/m²) in the non-obese group. Body Mass Index was strongly positively correlated with serum leptin concentration (r=0.59, p <0.001) in the obese group. The mean serum leptin concentration was much higher in the healthy obese and non-obese women (64.4ng/mL and 8.7ng/mL respectively) than in men of both categories (40.4 ng/mL and 5.5 ng/mL respectively). Age had no significant relation with serum leptin level (p= 0.416).

Conclusions: In the study sample, serum leptin concentration was positively correlated with Body Mass Index in healthy obese and non-obese subjects of both genders. The levels were higher in women than in men. Age had no significant relation with serum leptin level in this age group.

Keywords: Serum leptin, Body Mass Index, Obese, Non-obese, Rawalpindi. (JPMA 63: 245; 2013)

Introduction

Leptin¹ is a protein hormone that plays a key role in regulating body weight and metabolism. It was discovered in 1994. Its discovery has generated much attention in the fields of obesity and metabolic research.

Leptin molecule is approximately 16 kDa and encoded by the obesity (ob) gene. It is synthesised and secreted by adipocytes, and serum concentrations reflect the amount of energy stored in adipose tissue.² Leptin binds to receptors in the hypothalamus and influences the expression of several neuro-peptides that regulate energy intake, energy expenditure, and neuro-endocrine functions, thereby decreasing bodyweight. Genetically obese mice have mutations of ob gene with resultant very low levels of leptin. Similarly, few persons with extreme genetic obesity are leptin-deficient. However, most obese persons have hyperleptinaemia proportionate to body fat and appear to be leptin-resistant.² The physiologic role of

leptin is presently not completely understood. It may be a determinant of obesity and its complications. Limited studies have been conducted to explore the relation of leptin concentrations with Body Mass Index (BMI) in local population.³⁻⁶ Leptin may have an effect on BMI. A few studies conducted in Pakistan included finding correlations of leptin with certain disease or determining serum leptin levels in cord blood.⁷ To increase our understanding about the relationship of leptin concentrations with BMI, age and gender in the population of Rawalpindi, Pakistan, we measured serum leptin concentrations in 100 obese and non-obese healthy subjects of both genders in a sample of adult population of Rawalpindi.

Subjects and Methods

The observational, comparative study was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, and the Benazir Bhutto Hospital (BBH) Rawalpindi from August 2008 to December 2008.

The study initially comprised 100 healthy obese and non-obese subjects who were picked by non-probability sampling. Among them, 50 were healthy obese, including overweight, and 50 healthy non-obese, between 20 and

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50 years of age. The subjects were labelled overweight (BMI= 23.1-27.4kg/m²); obese (27.5-40kg/m²) and non-obese (18.5-23.0kg/m²). The BMI values were used under the guidelines for Asian populations issued in 2000 by the International Association for the Study of Obesity and the International Obesity Task Force of World Health Organisation, Western Pacific Region, Australia.^{8,9} Very obese subjects having BMI >40kg/m² and pregnant female subjects were excluded from the study. The study was approved by the AFIP ethical committee, and a verbal informed consent was obtained from all the participants.

Relevant clinical and laboratory data was collected from healthy obese and non-obese attendants of patients who presented at the outpatient department in BBH and the reception of AFIP. The data was entered in a proforma. Clinical history was taken about diabetes mellitus, hypertension, depression or any acute or chronic disease to exclude such subjects from the study. Subsequently the subjects having fasting plasma glucose 126 mg/dl were excluded. The final sample size, as such, stood at 90.

For BMI, height (cms) was measured using wall-mounted stadiometer and weight (kg) was determined using a weighing balance. BMI was calculated by the following formula: BMI= Weight (kg)/ Height (m²).

After an overnight fast, blood samples were obtained in

the morning between 0800-0900 hours. Blood samples were poured into plain tubes and allowed to clot at room temperature. The serum for leptin was separated 20 minutes after collection by centrifugation at a speed of 2000-3000 G for 10 minutes. Serum samples were aliquoted and frozen at -20°C for analysis later on.

The quantitative determination of serum leptin was conducted by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique, using commercially available reagent kit, DRG® Leptin (Sandwich) ELISA (EIA-2395) by RUO, Germany.

All statistical analyses were performed using SPSS 15. For each variable, mean, standard deviation, and ranges were calculated. P values for comparison of serum leptin level were determined in healthy obese and non-obese subjects; p <0.05 was considered significant. For correlation between serum leptin and BMI in healthy obese and non-obese subjects, r-values were determined by Pearson's correlation test.

Results

Comparison of baseline characteristics in terms of age, height, weight, BMI, fasting plasma glucose, and serum leptin levels of the subjects was noted (Table-1).

The mean age in the obese (n=40) and the non-obese

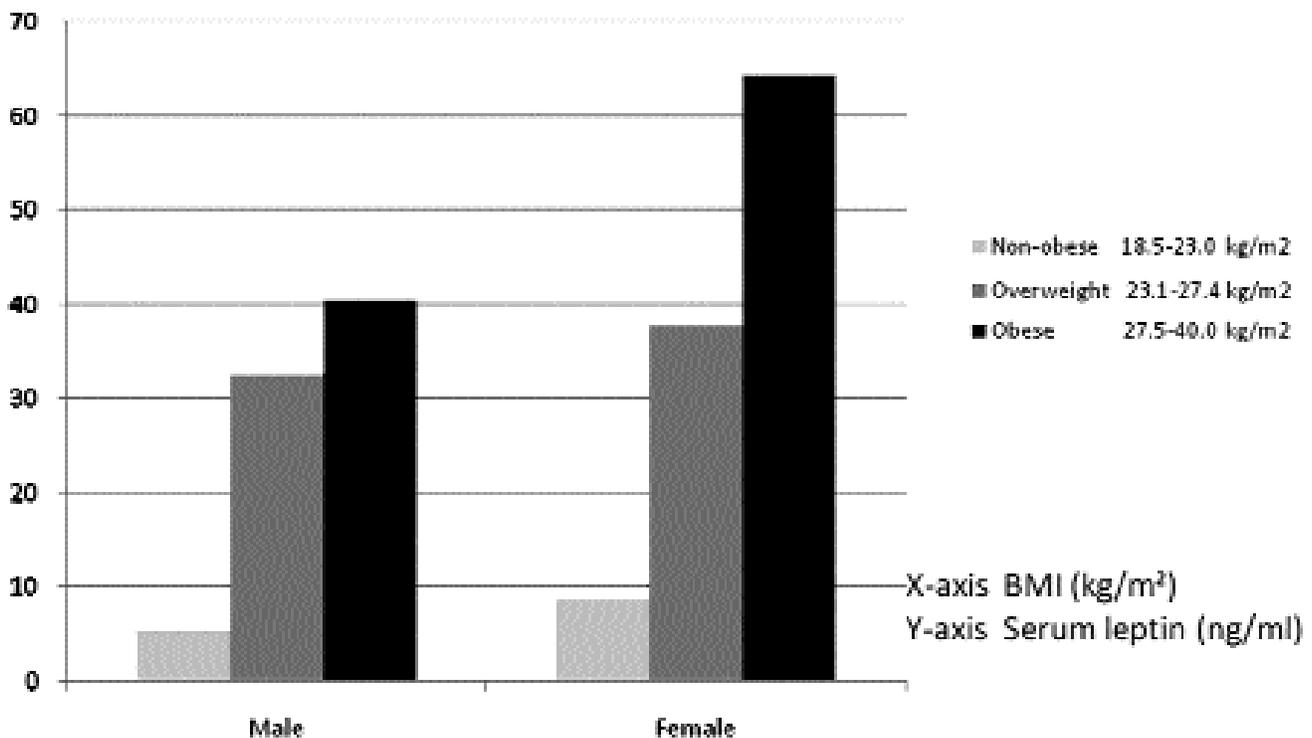


Figure-1: Mean serum leptin levels (ng/mL) in males and female subjects with reference to their body mass index categories.

Table: Baseline characteristics.

Parameters	Obese (n=40) Mean±SD	Non Obese/Controls (n=50) Mean±SD	P-values
Age (years)	34.8±4.6	32.7±6.1	0.416
Height (cms)	163±6.7	167±6.7	0.004
Weight (kg)	80±9.7	59±6.6	0.001
BMI (kg/m ²)	31.6±3.1	21.2±1.5	0.001
FP Glucose (mg/dl)	90±19.8	88.2±16.2	0.001
S leptin (ng/ml)	52.8±24.6	6.3±3.1	0.001

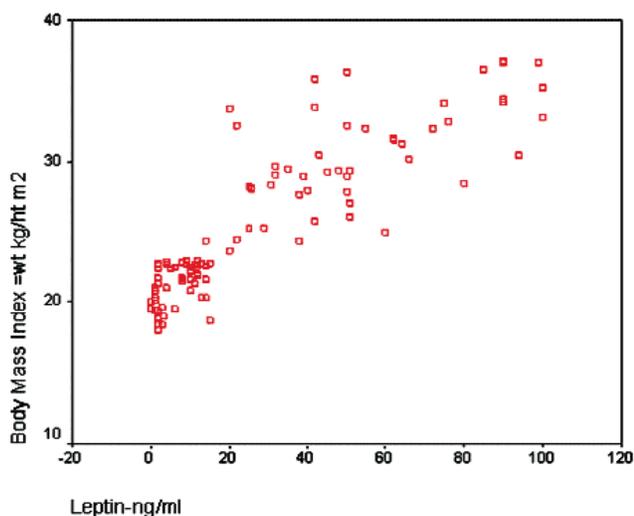


Figure-2: Correlation between Serum leptin level and Body Mass Index.

(n=50) groups was 34.8±4.6 years (range 30.2-39.4 years) and 32.7±6.1 years (range 26.6-38.8 years) respectively. There were 33 (66%) females in the obese and overweight group, and 32 (64%) females in the non-obese group (p=0.834) (Figure-1). The mean height in obese and non-obese groups was 163±6.7cms (range 156.3-169.7cms) and 167±6.7cms (range 156.3-173.7cms) respectively. Mean weight in obese and non-obese groups was 80±9.7 kg (range 70.3-89.7kg) and 59±6.6kg (range 52.4-65.6kg) respectively. Both the groups were comparable with respect to age, gender and weight/BMI. Age of the subject had no significant effect on fasting serum leptin levels in both the groups (p=0.416).

Mean BMI and serum leptin levels were compared in male and female obese and non-obese subjects, which showed significant differences. The mean serum leptin concentration was much higher in healthy obese and non-obese women (64.4 ng/mL & 8.7 ng/mL respectively) compared with men in these groups (40.4 ng/mL & 5.5 ng/mL respectively).

Highly significant difference was observed between

mean BMI of obese and non-obese groups. Mean BMI in obese group was 31.7±3.1kg/m² (range 28.6-34.8 kg/m²), while it was 21.2±1.5kg/m² (range 19.7-22.7kg/m²) in the non-obese group. Similar was the difference between mean serum leptin level (52.8±24.6ng/dl, range 28.2-77.4ng/mL) in the obese group and (6.3±3.1ng/dl, range 6.6-18.8ng/mL) in the non-obese group. Highly significant correlation between serum leptin level and BMI was found (r= 0.59, p= 0.001) (Figure-2). The mean serum leptin level in overweight subjects was in between that of obese and non-obese groups and it also correlated well with BMI.

Discussion

The study found a strong relationship between BMI and serum leptin in the obese group. Serum leptin level was significantly higher in obese than non-obese subjects. This was in agreement with some previous studies.^{5,10} Maffei et al measured serum leptin levels in obese and weight-reduced subjects. In the present study, the same was done in apparently healthy obese and non-obese subjects (normal healthy adults).¹⁰ In another study conducted by Considine et al,² serum leptin concentration were measured by using radioimmunoassay in 136 normal-weight subjects and 139 obese subjects (BMI >27.3 for men and >27.8 for women). In the present study, serum leptin was measured using Sandwich ELISA technique in 100 obese and non-obese subjects. The advantage of present study is that it was more specific, as the chance of cross-reactivity to other biological products of human origin was nil. Cross-reactivity with mice leptin is 0.2% (manufacturer's data), which is negligible. In the above-mentioned study, the mean serum leptin concentrations were 31.3±24.1ng/mL in the obese subjects and 9.3±7.5ng/mL in the normal-weight subjects (p <0.001). In the present study, these were 52.8±24.6ng/mL in the obese subjects and 6.3±3.1ng/mL in the normal-weight subjects (p <0.001). The leptin concentration was relatively much higher in obese subjects in the present study than that of Considine et al.² This may be due to different methods of analysis and/or study population. Our results showed significantly low levels of serum leptin levels, signifying more accuracy at lower values of the assays. Similar is the case with high levels of leptin. This means that our method had better linearity and detection limit; it could detect leptin both at low and high levels (range from 0-100 ng/mL in serum).

Serum leptin concentrations were not related with age in this group (20-50 years, P= 0.416). However, serum leptin concentrations were found higher in females than males in the same age group and BMI level. Panarotto et al,

observed that for a given bodyweight, the levels were higher in women than in men, but the reason for this difference was not clear.¹¹ This may be due to greater amount of percent body fat mass in females or the inducing effects of oestrogen-progesterone combined with suppressive effects of androgens on leptin.¹² Our observation is also in agreement with some other international studies^{5,13-15} in which serum leptin levels were found to be higher in females.

A small sample size and convenience sampling were used in this study which was a limitation of the exercise. More studies with larger sample size and different sampling technique may be planned to find out the relationship between serum leptin and BMI.

Conclusions

In this sample of Rawalpindi population, BMI strongly correlated with serum leptin concentrations in healthy obese and non-obese subjects of either gender. Moreover, serum leptin concentrations were found to be higher among the females. There was no significant relationship between age and serum leptin level in this age group.

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