

ASSESSMENT OF SERUM LEPTIN LEVELS IN DIFFERENT CATEGORIES OF BODY MASS INDEX

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ABSTRACT

Objective: To assess leptin concentrations in different categories of Body Mass Index (BMI) in a sample of local population of Rawalpindi, Pakistan.

Study design: Observational study.

Place & Duration of Study: Armed Forces Institute of Pathology and Benazir Bhutto Shaheed Hospital, Rawalpindi, 2009-2010 (8 months).

Materials and Methods: Fasting serum leptin concentrations were measured in 100 healthy obese, overweight and non-obese individuals aged 20-50 years, including both sexes. ELISA test was done for serum leptin assay. For each statistical analysis SPSS version 15 was used. The mean, standard deviation, ranges p-values and r-values.

were calculated for statistical inference

Results: Serum leptin concentrations were strongly associated with sex and the body mass index. Serum leptin level was found comparatively higher in women, in same category of BMI, (mean-64.4 ng/ml, obese group) than in men (mean-40.4 ng/ml, obese group). The mean serum leptin concentration in non-obese women was (12.7 ng/mL) and in men (6.4 ng/mL). Body mass index was strongly positively correlated with serum leptin concentration ($r=0.874$, $P<0.001$) in obese group. Serum leptin concentrations were higher in obese individuals (mean 52.8 \pm 24.6 ng/mL, P value <0.001) than in non-obese individuals (mean 12.7 \pm 6.1 ng/mL). As the grade of obesity increased so does serum leptin levels. It was higher in overweight males (32.6) females (37.8), grade I males (40.4), females (64.4) obesity.

Conclusion: In this sample of the local population, serum leptin concentrations correlated positively in different categories of BMI in both sexes.

Key words: Leptin, Population, Obese, Overweight, Non-obese, Grades of body mass index.

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INTRODUCTION

In Pakistan and many other countries, overweight and obesity in affluent class of society are problems reaching epidemic proportion which increase the risk of developing complications [1,2]. Therefore, accurate and easily measured indicators of obesity are needed to perform comparisons across populations. They are also needed to monitor trends and risks of developing obesity. They have a definite place in clinical medicine. BMI is a commonly used surrogate for obesity that is inexpensive and easily measured, but its correlation with serum leptin levels in local population is imperfect. The Asians have

greater tendencies of developing insulin resistance and other complications of obesity at a lower BMI level [3,4]. The protein product of the obesity (ob) gene, leptin may be a determinant of obesity and its complications [5,6]. Leptin has attracted much attention in the fields of obesity and metabolic research since its discovery in 1994 [7]. Adipocytes synthesize and secrete leptin. Serum levels of leptin reflect the amount of energy stored in adipose tissue. Leptin binds to receptors in the hypothalamus and influences the expression of several neuropeptides that regulate energy intake, energy expenditure, and neuroendocrine function [8]. Although few persons with extreme obesity are leptin deficient, most obese persons have hyperleptinemia proportionate to body fat and appear to be leptin resistant [9].

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concentrations to different grades of BMI, as a surrogate for obesity, we measured it in 100 obese, overweight, and non-obese subjects of both sexes in the local population.

MATERIALS AND METHODS

This was an observational study, conducted at Armed Forces Institute of Pathology (AFIP) Rawalpindi and Benazir Bhutto Shaheed Hospital (BBH) Rawalpindi. Study was approved by Ethical committee of Armed Forces Institute of Pathology. A verbal consent was obtained by all participants.

One hundred (100) healthy obese, overweight and non-obese subjects were selected by non-probability sampling technique. Among them, 40 were obese, 10 overweight and 50 non-obese. Subjects were between the ages of 20–50 years. They were labeled as overweight having BMI 23.1–27.4 kg/m², obese 27.5–40 kg/m² and non-obese 18.5–23.0 kg/m². These BMI values were used under the guidelines for Asian populations issued in year 2000 by World Health Organization, Western Pacific Region, Australia (10). Grading of obesity was done as under:

Grade 1	27.5–32.4.0 kg/m ²
Grade 2	32.5–37.4 kg/m ²
Grade 3	37.5–40.0 kg/m ²
Severe obesity	>40kg/m ²

Subjects who were suffering from certain metabolic disorders like diabetes mellitus etc, and very obese subjects having BMI >40 kg/m² were excluded from the study. Also persons who reported without fasting (n = 12), whose serum specimens were unavailable (n = 3) or of insufficient quantity to perform the leptin assay (n = 4), or who had missing BMI data (n = 3) were excluded. Their number was not included in total no of participants.

Relevant clinical and laboratory data were collected from healthy obese and non-obese attendants of patients presenting at outpatient department in BBH and reception of AFIP, and entered in a Performa.

Clinical history was taken about diabetes mellitus, hypertension, depression or any acute or chronic disease to exclude such subjects and to select healthy obese subjects. Similarly healthy non-obese subjects were selected. Also, no subject was included in this study having fasting plasma glucose > 7.0 mmol/L.

Height (cms) was measured using wall-mounted stadio meter and weight (kg) was determined using a weighing balance to calculate BMI (as an expression of obesity). BMI was calculated by the following formula:

$$\text{BMI} = \text{Weight (kg)} / \text{Height (m}^2\text{)}$$

Where, kg = kilogram, m = meter

Blood samples were obtained in the morning between 0800–1100 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, frozen at -20°C, for analysis later on. The quantitative determination of serum leptin was conducted by 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 the statistical package for the social sciences (SPSS, Version15). For each variable, descriptive statistics were calculated. *p*-Values for comparison of serum leptin level were determined in healthy obese and non-obese individuals by chi-square test, *p*-Value <0.05 was considered significant. To determine correlation between serum leptin and body mass index in obese and non-obese individuals, *r*-values were determined.

RESULTS

One hundred individuals, 40 obese, 10 overweight and 50 non-obese were included in this study. Comparison of baseline characteristics i.e.

age, weight, height, BMI, fasting plasma glucose, fasting plasma insulin and serum leptin levels of these individuals, is shown in the table-1.

There were 33 (66%) females in obese group and 32 (64%) females in non-obese group (P=0.834). Both the groups were comparable with respect to age and a highly significant difference was observed in BMI ($p < 0.001$).

Male to female ratio for mean BMI and serum leptin levels in non-obese and obese groups is shown in figure-1. Significant differences were observed between male and female subjects with respect to

mean serum leptin levels and body mass index (BMI) categories as shown in table 2. The mean serum leptin concentration was much higher in healthy obese women and also in non-obese women (64.4 ng/mL & 8.7 ng/mL respectively) than in men in these groups (40.4 ng/mL & 5.5 ng/mL respectively). It was also observed that there is a significant correlation between different categories of BMI and serum leptin levels ($p < 0.001$). As the grade of BMI increases so does serum leptin level, as shown in figure-2.

Table-1: Baseline characteristics of obese and non-obese subjects.

Parameters	Obese (n=40)		Non-obese/ Controls (n=5)		p-values
	Mean	SD	Mean	SD	
Age (years)	34.8	4.6	32.7	6.1	0.948
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 (mmol/l)	5.0	1.1	4.9	0.9	0.001
FP Insulin (IU/ml)	11.0	5.1	0.9	0.4	0.001
S leptin (ng/ml)	52.8	24.6	6.3	3.1	0.001

Table-2: Mean serum leptin levels in different categories of BMI in male and female subjects.

BMI Categories	Mean Serum Leptin Levels (ng/ml)	
	Males	Females
Non-Obese 18.5-23.0 kg/m ²	5.5±0.4	8.7±0.5
Overweight 23.1-27.4 kg/m ²	32.6±3.5	37.8±3.7
Obese 27.5-40.0 kg/m ²	40.4±4.7	64.4±7.8

Table-3: Comparison of present study with the study of Paul RF *et al.*

BMI Categories	Present study Kazmi <i>et al.</i>		Other Local study Paul RF <i>et al.</i>	
	Mean Serum Leptin Levels (ng/ml)			
	Males	Females	Males	Females
Non-obese 18.5-23.0 kg/m ²	5.5±0.4	8.7±0.5	2.6±1.5	17.3±10.3
Overweight 23.1-27.4 kg/m ²	32.6±3.5	37.8±3.7	9.9±6.8	34.8±13.6
Obese 27.5-40.0 kg/m ²	40.4±4.7	64.4±7.8	21.3±14.2	48.2±21.1

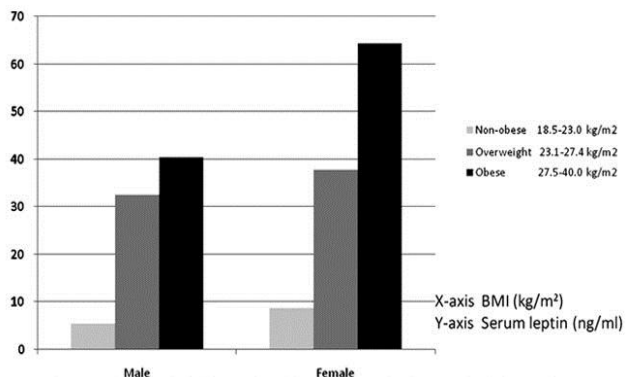


Figure-1: Mean serum leptin levels (ng/ml) in males and female subjects with reference to their body mass index categories.

DISCUSSION

World population is facing epidemic increase in overweight and obesity. It's a matter of concern for health authorities. It's obvious that increase in complications of obesity result in financial burden. BMI is a commonly used surrogate for obesity that is inexpensive and easily measured. Women have a higher proportion of body fat than do men with the same BMI. The Asians have greater tendencies of developing insulin resistance and other complications of obesity at a lower BMI levels [3,4]. For example, the age-standardized prevalence of diabetes is higher in people of South Asian origin compared with the white European population [13]. Holman *et al*, for example, estimated that the prevalence of diabetes (both diagnosed and undiagnosed) in South Asian people in England in 2010 was 14.0%, compared with 6.9% in the general population (those defined as not black or South Asian [14]. WHO suggested separate BMI cutoff values for Asians [10] and we have used the same values in present study. It was, therefore, pertinent to study whether serum leptin levels correlate with higher grades of obesity in local population also? This may help us to explore means to reduce serum leptin levels and thereby reduce obesity and its complications for our population.

Our study found a strong relationship between BMI and serum leptin level. Serum leptin level was significantly higher in obese and overweight

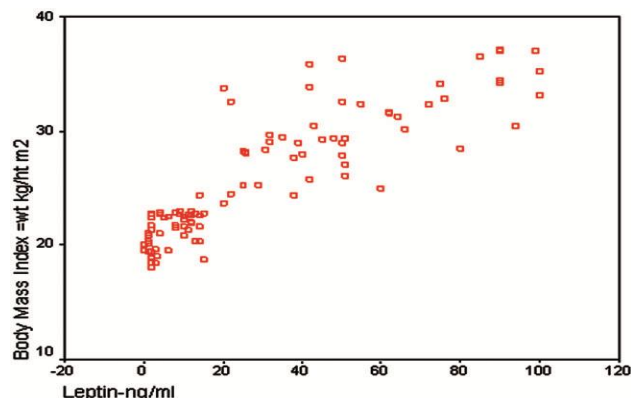


Figure-2: Correlation between serum leptin levels and body mass index.

individuals than non-obese individuals. This was in agreement with some previous studies [11,15]. Similar is the observation in a recent local study by Paul RF *et al* [16]. A comparison of present study with Paul RF *et al*'s study is hereby shown in table [3]. They have used standard values for categorization of obesity while we used WHO's suggested values for Asian populations. If we look at table-3, there is huge difference in serum leptin levels between males and female in Paul RF *et al* study while this is not the case with our study. It shows that their male participants may have very low percentage of body fat. The difference between ours and Paul RF *et.al* may be due to ethnicity. Our study population is Punjabis while theirs Pathans or Hazara of Khyber Pakhtunkhwa.

In another closely related study, conducted by Considine *et al*, [17], Serum leptin concentrations were found higher at higher grades of BMI. In this study, 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 in 100 obese and non-obese subjects (BMI > 27.4 in both sexes) using sandwich ELISA technique. 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 ± 24.1 ng/mL in the obese subjects and 9.3 ± 7.5 ng/mL in the normal weight subjects ($p < 0.001$). In the present study, these were 52.8 ± 24.6 ng/mL in the obese subjects and 6.3 ± 3.1 ng/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.* This may be due to different methods of analysis and/or study population.

CONCLUSION

In this sample of the local population, serum leptin concentrations correlated positively with different categories of BMI in both sexes. This shows the important role of leptin in human obesity

AUTHORS CONTRIBUTION

Dr. Ahsan Kazmi: Entire research work, sample collection, analysis, write-up.

Brig (Rtd) Prof Rizwan Hashim: Literature review, help in sample collection.

Brig (Rtd) Prof Abdus Sattar: Planning of research, arrangement of reagent kits, sample analysis.

Maj Gen (Rtd) Prof Farooq Ahmad Khan: Concept and overall supervision.

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