

## **Serum Leptin in Obese Pakistani Subjects With and Without Diabetes Mellitus**

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### **ABSTRACT**

**Background:** Leptin, an adipokine, is considered to have a role in obesity and associated metabolic dysfunction. Serum leptin has generally been reported as lower in Caucasian subjects with diabetes. In non-caucasian groups, however, the data on leptin in subjects with diabetes is more controversial being reported as lower, similar or higher than controls. We report on serum leptin concentrations in obese Pakistani subjects with type 2 diabetes mellitus and their age and weight matched controls.

**Methods:** We compared fasting serum leptin concentrations in 71 (11 males & 60 females) obese Pakistani subjects with type 2 diabetes attending diabetic clinic and 73 (12 male & 61 females) age and weight matched controls, from hypertensive clinic of Shaikh Zayed Hospital, Lahore, Pakistan.

**Results:** The median ages of study cases and controls were 45.8 yrs and 40.3 yrs respectively. The median BMIs of study cases and controls were 32.4 and 33.4 kg/m<sup>2</sup> respectively. Serum leptin concentrations in females and males with diabetes [median inter quartile range, 21.5 (14.1–28.7) and 11.7 (4.7–18.5) µg/L, respectively] were lower ( $P<0.005$ ) than in their respective female and male controls [112.6 (88.6–136.2) and 43.6 (29.2–57.2) µg/L, respectively]. Serum leptin concentrations were similar in females and males with diabetes, where as in non-diabetic females leptin concentrations were found higher than non-diabetic males ( $P<0.05$ ).

**Conclusion:** We report lower serum leptin concentrations in obese Pakistani subjects with type 2 diabetes mellitus. We suggest that this may be due either to altered fat distribution or to relative insulin deficiency in type 2 diabetes or both.

**Key words:** Leptin, Diabetes Mellitus, Obesity, Ethnicity, Pakistani

## Introduction

Leptin, a protein hormone expressed and released by adipocytes, is considered to have a role in the regulation of body weight and associated energy metabolism [1]. Serum leptin concentrations are characteristically increased in obese subjects and there appears to be a linear relationship between serum leptin and body mass index (BMI) in women and to a lesser extent in men [2, 3]. Serum leptin, however, is lower in subjects with diabetes mellitus compared to weight matched controls [4]. Most of the leptin data pertains to Caucasian populations and the limited data available, suggest that serum leptin may vary by ethnicity [5, 6]. Furthermore, the data on serum leptin in non-caucasian subjects is conflicting. Compared to weight matched controls, serum leptin has been reported as being lower in Sudanese, Bangladeshi and Turkish [7-9], similar in Chinese [10] but higher in Asian Northern Indian and Saudi [11, 12] subjects with type 2 diabetes mellitus.

In Pakistan, serum leptin levels were studied in hypothyroid females [13], infertile patients [14], pre-eclampsia patients [15] and patients with liver disease [16]. No study has been carried out for leptin levels in type 2 diabetics. In this study we have compared serum leptin concentrations in obese subjects with and without diabetes mellitus at Shaikh Zayed Medical Complex Lahore, Pakistan.

## Methods

It was a cross-sectional analytic design, using one time examination and blood sampling. Obese patients with type 2 diabetes mellitus with fasting blood sugar >200 mg/dl and BMI>30kg/m<sup>2</sup>, were recruited from those attending the Diabetic Clinic at Sheikh Zayed Teaching Hospital, Lahore, Pakistan. Patients had diabetes of more than two years duration. They were receiving different treatment

regimens including sulphonylureas, metformin, acarbose and insulin, either as single agent or as combination therapy. BMI matched non-diabetic obese patients, considered as controls who were recruited from patients attending the hypertension clinic at Sheikh Zayed Teaching Hospital, Lahore, Pakistan. All participants gave informed consent to participate in this study.

Five milliliters of venous blood was collected into plain tubes for the measurement of serum leptin between 8.30 and 12.00 o'clock. Samples were centrifuged within 2 hours of collection and separated serum was stored and frozen at -20°C until analysis.

Serum leptin was measured by radioimmunoassay as prescribed [13] using reagents and methodology supplied by NETRIA, St Bartholomew's Hospital, London, UK. The assay has a detection limit of 0.8 µg/L. The intra-assay and inter-assay coefficients of variation were 6.2 % and 8.3 %, respectively.

Data are expressed as median with inter-quartile. Since the data was not normally distributed, non-parametric one way analysis of variation with Kruskal-Wallis post-test analysis was used to compare between group variables and Spearman correlation was used to assess the association, of within group variables, using GraphPad, InStat computer software (GraphPad Software; San Diego, USA).

The selection of non-diabetics for both genders was mainly by matching the weight and gender.

## Results

The results are shown in the Table 1. Females and males with diabetes were older ( $P<0.005$ ) than those without diabetes, but otherwise BMI were similar in all groups. Serum leptin concentrations were higher in females without diabetes than those with diabetes ( $P<0.001$ ), males with diabetes ( $P<0.001$ ) and males without diabetes ( $P<0.05$ ).

**Table 1- Clinical and demographic characteristics of study subjects**

Groups	Obese Diabetic Patients		Obese non-diabetic subjects	
	Female	Male	Female	Male
Number	60	11	61	12
Age (years)	45 <sup>£</sup> (45.8 – 50.6)	50 (40.7 – 64.7)	40* (38.5 -43.1)	42 (36.7 – 51.1)
BMI (kg/m <sup>2</sup> )	32.5 (32.2 – 34.0)	32 (30.8 – 35.0)	33.2 (33.0-34.9)	34.5 (33.2 – 35.0)
Leptin (µg/L)	21.5 <sup>£</sup> (16.9 – 26.9)	11.7 <sup>§,£</sup> (7.1 -16.2)	112.6 <sup>*,§</sup> (96.8- 128.5)	43.6 <sup>§,£</sup> (32.8- 54.3)

Results are expressed as medians with 95% confidence intervals in parentheses

Where \* is  $P<0.05$  compared to female diabetic subjects

§ is  $P<0.05$  compared to male diabetic subjects

£ is  $P<0.05$  compared to female non-diabetic subjects

Serum leptin was higher in men without diabetes than in those with diabetes ( $P < 0.005$ ). In female patients with diabetes, serum leptin concentrations correlated inversely with BMI ( $r = -0.354$ ;  $P = 0.006$ ). There were no other significant correlations.

## Discussion

We report lower circulating leptin levels in the obese diabetics and non-diabetics subjects from an urban population of Lahore, Pakistan. These finding are consistent with those studies reporting lower serum leptin in subjects with diabetes in Caucasian and non-caucasians populations [4, 7-9], but different from those reporting similar or higher serum leptin concentrations in subjects with diabetes [10-12]. It is difficult to reconcile these differences, but they may be related to selection of patient groups, different analytical techniques and type I or II statistical errors.

A possible explanation of lower leptin levels in diabetic subjects is altered body fat distribution in diabetes. Subjects with diabetes have increased visceral fat and less subcutaneous fat [17]. Visceral fat produces less leptin than subcutaneous fat [17, 18]. Subjects with diabetes, therefore, would be expected to have lower circulating leptin than weight-matched controls as reported in this and other studies [4, 5-9].

Relative insulin deficiency in type 2 diabetes may offer an alternative explanation for the lower leptin levels in diabetic subjects, since insulin is important stimulator of leptin production [19-21]. This also suggests that leptin may be a marker of insulin secretion rather than insulin sensitivity.

In this study, females had higher serum leptin concentrations than males, but this did not reach statistical significance in diabetic subjects. The higher leptin levels in females than in males have previously been reported in population

studies [22] and are probably due to gender differences in body fat distribution. Subcutaneous fat produces more leptin than visceral fat [17, 18]. Women, carry most of their higher body fat content subcutaneously, whereas men carry most of their lower body fat content viscerally [3, 17, 23, 24]. The higher subcutaneous fat content in women would, therefore, explain their higher serum leptin concentrations. It however, has also been suggested that the gender differences in serum leptin may be related to the differences in sex hormones [23]. Serum leptin levels of obese diabetic and non-diabetic subjects have also been reported higher as compared with lean diabetic patients and non-diabetic subjects ( $P < 0.05$ ) [25].

In summary, we reported that obese Pakistani women and men with type 2 diabetes mellitus have lower serum leptin than weight-matched obese controls. It remains to be established whether the lower leptin levels in Pakistani subjects with diabetes are explained by altered fat distribution or relative insulin deficiency or both. Further studies are needed to prospectively evaluate the relationship between reported factors and leptin levels among various groups of Pakistani population.

The study had some limitations as did not include anthropometric data like waist circumferences. It also did not homologize the diabetics in view of glycemic control and various treatment modalities. Regarding a non probability sample, it may be difficult to generalize the results to the general community of patients and healthy individuals.

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