ORIGINAL ARTICLE

ASSOCIATION OF LEPTIN WITH TYPE 2 DIABETES IN NON-OBESE SUBJECTS

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Background: South Asians have a high tendency to develop type 2 diabetes even at low BMI. We evaluated serum leptin levels in a group of non-obese type 2 diabetics. **Methods:** An observational study conducted on 90 subjects, 55 with Type 2 diabetes mellitus, and 35 normal controls (non-diabetics). BMI, waist circumference, serum leptin, and serum glucose were measured. The correlation between these variables was studied by multiple regression analysis. **Results:** Serum leptin levels were positively correlated with BMI in obese (r=0.976) and non-obese diabetics (r=0.956). Serum leptin was related with diabetes (r= -0.153, p=0.001). Serum leptin was highly correlated with waist circumference in obese than non-obese diabetics, (r=0.753). Mean serum leptin level was 21.4 ηg/ml in non-obese diabetics and 34.9 ηg/ml in diabetic group. It is even lower than the non-obese, non-diabetics (23.3 ηg/ml). Multivariate linear regression analysis between leptin and age, weight, BMI, waist circumference in patients shows only a strong association with BMI in diabetics (p=0.0001), while in non-diabetic it was not significant (p=0.07). Serum leptin was high in diabetics taking oral hypoglycaemic (37.8±19.1 ηg/ml), while it was low in diabetics taking insulin injections (29.3±24.2 ηg/ml). **Conclusion:** Low leptin levels are associated with type 2 diabetes mellitus independent of changes in BMI.

Keywords: Leptin, BMI, waist circumference, type-2 diabetes mellitus, serum glucose.

INTRODUCTION

Type 2 diabetes mellitus is known for its morbidity and mortality all over the globe. South Asians including Pakistanis have a high tendency to develop type 2 diabetes even at low BMI. The risk of type 2 diabetes in South Asians is about 4–5 times higher than Europeans. Diabetes tends to develop about 10 years earlier than Europeans.[1] There are defects in insulin secretion and action, and fat metabolism in type 2 diabetes mellitus.[2–4] Obesity is a well known risk factor for the development of diabetes mellitus. Leptin, a 167 amino acid adipocyte derived hormone, has been implicated in the regulation of adipose mass[5] and has been reported to alter both insulin sensitivity[6–8] and insulin secretion[9]. Although it is clear that circulating leptin is positively correlated with body fat mass[10], the relationship of diabetes to plasma leptin concentration, independent of adiposity, is less clear.

There is controversy about the level of circulating leptin whether it is reduced[11], raised[12,13] or remains unchanged[14–16] in type 2 diabetes. In studies on humans with untreated type 1 diabetes and animals with insulin deficiency, plasma leptin concentrations were found consistently low.[17,18] The variable results in type 2 diabetes are expected because subjects differed with respect to extent of obesity, age, gender and ethnic group. Most of the studies on serum leptin in type 2 diabetes have focused on white populations. In this study we determined leptin concentrations and parameters related to type 2 diabetes mellitus in Pakistanis with type 2 diabetes and normal control subjects. We also assessed the relationship between leptin, anthropometric variables and ABO blood groups.

MATERIAL AND METHODS

This was a cross-sectional study. Fifty-five Pakistani patients with type 2 diabetes mellitus and 35 normal control subjects were included in the study. The patients and controls were matched by ethnic group. Patients had been diagnosed to be diabetic for a median of 5 years (range, 0.5–20.0 years). They were taken from the outdoor clinics of Benazir Bhutto Hospital and Holy Family Hospital, Rawalpindi. One person was managed on diet, 30 patients were treated with oral anti-diabetic agents, 20 were on insulin therapy while 4 patients were using both insulin and oral anti-diabetics. Among diabetics, 33 were suffering from other diseases like hypertension and ischemic heart disease taking either ACE inhibitors or angiotensin receptor blockers, and low dose aspirin. Majority of patients were suffering from hypertension only. Controls were healthy subjects who in the last 12 months were not taking any medication and had no family history of diabetes mellitus.

The study protocol was approved by the local ethical committee and a verbal informed consent was taken by all the subjects. Anthropometric measurements were assessed in all subjects. The waist circumferences were measured using a flexible measuring tape directly applied to the subject's skin at the level of umbilicus. Random blood samples were collected for the measurement of serum leptin, glucose and lipids.

Blood samples were immediately centrifuged at 2,500 rpm for 15 minutes and the supernatants were stored at -70 °C until analysis. Serum glucose, was measured on same day by enzymatic tests using an automatic chemistry analyser (Beckmann Coulter CX 9-

Pro, USA). Serum leptin was measured by sandwich enzyme immunoassay (RD191001100 Human Leptin ELISA, Clinical Range Kit, Bio Vender Laboratorni Medicina a.s®, Czech Republic EU). The inter-assay and intra-assay coefficients of variation (CV) for leptin, were 7.6% and 4.2% respectively.

Descriptive data were expressed as mean, standard deviation, and range of all variables. SPSS-15 was used for statistical analysis. Means of data in patients and controls were compared using the independent *t*-test. Data were also analysed by multiple linear regressions, using leptin as the dependant variable. Independent variables included age, height, weight, BMI, serum glucose random, waist circumference. Differences were considered statistically significant at $p<0.05$. Correlation between serum leptin and BMI was sought using the Pearson's correlation.

RESULTS

Ninety subjects, 55 diabetics and 35 non-diabetic controls, were included in this study. Their basic characteristics are given in Table-1. There were 36 women and 54 men (aged 19–77 Yr). Non-diabetic control group (n=35) had mean BMI<25.0 Kg/m^2 (excluding 6 obese subjects in this group) (Table-2). They had normal serum glucose and their mean serum leptin level was 23.3 ηg/ml (range 4–43 ηg/ml). In diabetic group most of the subjects had hyperglycaemia, (mean random serum glucose 274.8 mg/dl), while in non-diabetics it was within normal limits (mean 94.5 mg/dl). In the same group mean BMI was 27.5 Kg/m^2 (range: 18.3–39.5 Kg/m^2; mean serum leptin level was 34.9 ηg/ml (range: 1.0–79.0 ηg/ml); both parameters were higher in diabetic group (Table-2). Regarding use of medications, diabetics were divided into 4 groups (Table-3). Majority of the patients used oral hypoglycaemics mainly sulfonylureas (n=30), followed by insulin (n=20).

Serum leptin was high in diabetics taking oral hypoglycaemic, (37.8±19.1 ηg/ml) while it was low in diabetics taking insulin (29.3±24.2 ηg/ml). Serum leptin highly correlated with BMI in obese diabetics (r=0.976). Serum leptin also correlated with waist circumference in obese diabetics (r=0.753), while in non-obese diabetics this correlation was also significant (r= -0.153, p=0.001). Mean serum leptin level was low in non-obese diabetics (21.4 ηg/ml) compared to diabetic group (34.9 ηg/ml). It was even lower than that of the non-obese, non-diabetics (23.3 ηg/ml) (Table-4). Serum leptin was highly correlated with BMI in non-obese diabetics also(r=0.956). Mean serum leptin levels are almost similar in patients with type 2 diabetes and control subjects. When obese subjects (BMI >30 Kg/m^2) are analysed separately, serum levels of leptin are comparable in obese diabetics and obese controls (58.4±11.0 ηg/ml and 55.2±5.9 ηg/ml respectively), i.e., there is a negligible difference; BMI is almost same but mean waist circumference has significant difference, 114 Cm in obese diabetics and 102 Cm in obese controls. Women in each group have higher leptin levels than men. Other variables are also high in women than men as shown in Table-5.

Multivariate linear regression analysis between leptin and age, weight, BMI, waist circumference in patients showed a strong association only with BMI (p=0.0001) in diabetics while in non-diabetic it was not significant (p=0.07). It is observed that one unit increase in serum leptin is 4.62 Kg/m^2 increase in BMI in females. Regression model:

$$Y_{S.leptin} = -82.329 + (4.628\ BMI) - (0.31\ Age) + (0.86\ Height)$$
$$= (0.586)\ (0.070)\ (0.931)$$
$$R^2 = 94.6\%\ (95\%\ approx)$$

Table-1: Descriptive Statistics for all cases (n=90)

Variables	Range	Mean±SD	*p*
Age (Yr)	19-77	52.3±13.2	<0.001
Height (Cm)	143-187	160.9±10.2	<0.001
Weight (Kg)	45-97	69.1±12.6	<0.001
BMI (Kg/m^2)	16.3-39.5	26.1±5.1	<0.001
Waist (Cm)	39-140	94.7±16.1	<0.001
S. Glucose R (mg/dl)	61-587	205.3±131.9	<0.001
S Leptin (ηg/ml)	1-79	32.5±19.5	<0.001

Table-2: Basic characteristics

	Diabetic n=55		Non-diabetic n=35	
Variables	**Range**	**Mean ±SD**	**Range**	**Mean ±SD**
Height (Cm)	143–183	155.7±8.6	157–187	169.1±6.4
Weight (Kg)	45–94	66.4±12.7	53–97	73.3±11.3
BMI (Kg/m^2)	18.3–39.5	27.5 ±5.7	18.9–34.0	26.5 ±7.0
Waist (Cm)	66–140	99.4±16	68–110	89.1 ±10.7
S Glucose R (mg/dl)	80–587	274.7±125.5	61.0–155	249.6 ±133.8
S Leptin (ηg/ml)	1–79	34.8±21.4	4–65	29.3 ±24.2

Table-3: Medications used by Diabetics

Medication	Diabetics
No medication	36
Oral hypoglycaemics	30
Insulin	20
Both insulin and oral hypoglycaemics	4
Total	90

Table-4: Comparison of mean BMI, waist circumference, random serum glucose, and serum leptin in different groups

Parameters	Diabetics (n=55)	Non-obese Diabetic (n=35)	Non-obese Non-diabetic (n=29)
BMI (Kg/m^2)	28.9	25.6	24.3
Waist (Cm)	99.4	87.4	84.4
S Glucose R (mg/dl)	214.8	96.3	94.5
S Leptin (ηg/ml)	34.9	21.4	23.3

Table-5: Comparison of parameters in obese female and male

	Obese Female (n=19)		Obese Male (n=7)	
Variables	**Range**	**Mean ± SD**	**Range**	**Mean±SD**
BMI (Kg/m^2)	30.1–39.5	34.0±2.5	30.0–34.0	32.2±1.4
S Leptin (ηg/ml)	40.0–79.0	58.3±11.2	49.0–65.0	56.0±5.9
S Glucose R (mg/dl)	80.0–369.0	229.8±98.8	82.0–326.0	132.6±87.0
Waist (Cm)	96.0–140.0	114.7±9.9	89.0–116.0	104.0±8.5

DISCUSSION

South Asians, including Indian, Pakistanis, Sri Lankans and Bangladeshis, have a high tendency to develop type

2 diabetes despite low prevalence of obesity. We determined serum leptin levels in a group of type 2 diabetics residing in district Rawalpindi, Pakistan. The main findings of study are: type 2 diabetes is associated with marked reduction in serum leptin level in both men and women; serum leptin level is strongly associated with BMI in obese person- diabetics or non diabetics; in multiple regression analysis only BMI predicted serum leptin level; raised serum leptin has got no relationship with any particular blood group.

We found low serum leptin level in non obese diabetics; the decrease was even lower than non diabetic obese, i.e., normal controls (Table-4). This may be due to associated metabolic derangements as these patients showed hyperglycaemia. Hyperglycaemia is usually associated with high insulin resistance. The role of leptin in diabetes is controversial; some workers have reported increased[6], decreased[4,5,7] or unchanged[7,14] leptin levels. Adiposity and gender are the main determinants of leptin level in normal and diabetic patients.[19] Many researchers described leptin alterations only in obese and overweight. Some have studied only men[6], or women.[12] Our results are similar to two other reports, one in non obese Indian diabetics[20], other in non obese Bangladeshi women.[12] Our findings of marked reduction of serum leptin level in diabetic man and women are important particularly in the light of alarming rise in diabetes among Pakistanis and paucity of data on leptin.

The decreased serum leptin in our diabetics may be due to following reasons: Diabetic patient selected for comparison are non obese and differ from the patients used in the above reports[4,6,7,] Insulin treatment of type 2 diabetes also increases plasma leptin levels[21] as also seen in the UKPDS study.[22] Other reports indicate insulin deficiency as a cause for lower leptin levels in diabetics.[11]

Serum leptin is found high in diabetics taking oral hypoglycemic, mean 37.8±19.1 ng/ml while it is low in diabetics taking insulin injections 29.3±24.2 ng/ml. This may be due to decreased insulin secretion in patients taking exogenous insulin. In some studies treatment of diabetes with sulfonylureas has been reported to increase serum leptin levels[4,23] but not in other studies.[5,21,24] In these studies, the effect of sulfonylureas was mediated through changes in body weight[22] or improved insulin secretion.[4] We observed a clear difference between BMI of both the groups. It is high in patients (n=30) receiving oral hypoglycaemics (mostly sulfonylureas) and low in patients (n=20) receiving insulin which is in accordance with above mentioned studies.

In the multiple regression analysis using leptin as the dependent variable, only BMI remained as significant predictor of leptin. It is also suggested that in diabetes, leptin levels are predicted by increased waist circumference, the effect of which may be mediated through insulin levels. This is an interesting finding which is also observed in an Indian study of urban population.[19]

Zimmet *et al*[25] have reported relationship of serum leptin to waist circumference in normal subjects from Western Samoa. We observed similar finding; changes in waist circumference of our study subjects contribute to leptin, due to accumulation of s/c fat, the major contributor of serum leptin. In our study serum leptin was highly correlated to waist circumference in diabetics r=0.753, and in non diabetics non obese r=464. This is in line with previous study on immigrant Indians wherein higher body fat (waist circumference)[13] and insulin resistance[9], were seen even when they are young and non obese compared to Caucasian men of similar BMI. We have not measured body fat percentage directly this is a minor short coming of this study.

Future studies with larger sample size having both sexes along with quantification of body fat content are needed to understand the role of leptin in detail in local population.

CONCLUSIONS

Low leptin levels are associated with type 2 diabetes mellitus independent of changes in BMI.

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