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Evaluation Of Association Of Serum Leptin With Chronic Complications Of Diabetes And Glycemic Control

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ABSTRACT

Background: The chronic complications of type-2 diabetes mellitus have a major impact over the growing mortality and morbidity worldwide as well as in India. Leptin, an adipose-derived energy balance regulating hormone has been implicated in the development of chronic complications of type-2 diabetes mellitus. This study was done to clarify the association of leptins with the complications of diabetes

Materials and methods: An analytical cross-sectional study was conducted in 160 non-obese type-2 diabetic patients, of which 80 had one or more chronic complication of diabetes and 80 were without any complications. The fasting and postprandial sugar levels, serum leptin, HbA1c and renal parameters were measured.

Results: Leptin levels in diabetic patients with complication was found to be lower than that of patients without complications in both the gender ($p < 0.001$). There was no significant difference in the leptin levels among various complications ($p = 0.620$). We found an inverse correlation between leptin and fasting blood sugar levels ($r = -0.172$; $p = 0.030$), postprandial blood sugar levels ($r = -0.194$; $p = 0.014$) and HbA1c ($r = -0.271$; $p = 0.001$).

Conclusion: Our findings suggest that leptin might have a role in regulating the glycaemic status. Also, reduction in concentration of leptin is associated with the development of complications of diabetes.

Key Words: Chronic complications of diabetes mellitus, glycaemic status and leptin.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Despite being one of the most extensively explored diseases, acceleration in its prevalence never diminishes.

Type II diabetes mellitus is the commonest of all diabetes with a complex pathophysiology which result in hyperglycemia.[2] Patients with Type II diabetes mellitus can develop acute and chronic complication due to hyperglycaemia. The two types of chronic complication which can occur in type II diabetes mellitus are microvascular and macrovascular.

Leptin is an adipose-derived energy balance regulating hormone produced from OB (Lep) gene located on chromosome 7.[3] Several studies have been done to explore the non-hypothalamic action of leptin. As a result of these studies, a significant proportion of the riddle of peripheral action of leptin has been unravelled. One such is the role of leptin is glucose homeostasis.

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Defect in this adipo-insular axis is one of the causes for the development of type 2 diabetes mellitus. Several studies have shown in leptin sensitive persons, leptin controls the secretion of insulin to adapt glucose homeostasis to the body fat store and it also increases insulin sensitivity. Obese individuals have leptin resistance which results in loss of leptin control over insulin secretion and thus results in hyperinsulinemia. Chronic hyperinsulinemia leads to pancreatic β cell failure. Due to leptin resistance, insulin sensitivity is decreased, which also leads to superadded insulin resistance. Finally, the β cell failure and reduced insulin sensitivity lead to the development of type-2 diabetes mellitus.[4]

Leptin levels are influenced by several factors like gender, BMI, adiposity, insulin levels and drugs.[5] Due to the presence of several confounding factors, the results of the studies which associate leptin levels in diabetic patients are not unified. While some studies show that leptin level is elevated in diabetics, some other studies indicate that there is no change in leptin level and there are few more studies point out that leptin levels are reduced in diabetic patients.[6-9]

This abnormal variation in leptin concentration in diabetics has been linked with the development of diabetic complications. Leptin is said to have a pleiotropic effect on complications of diabetes. studies have shown that leptin increases the nitric oxide production, which protect against the development of diabetic nephropathy.[10] On the other hand, some studies disagree with this and state that leptin increases the activity of the sympathetic nervous system and oxidative stress thereby worsening the diabetic nephropathy.[11-13] In diabetic retinopathy, few studies have shown that elevated levels of leptin worsen diabetic retinopathy due to angiogenic effect.[14-17]

Leptin has also been implicated in the development of cardiovascular events. Pietersen et al study stated that leptin might have a role in the development of atherosclerosis.[18] But Smith et al in their study pointed out that leptin has a cardio protective effect by upregulating the RISK pathway (phosphatidylinositol 3-OH kinase (PI3K)-cellular Akt/protein kinase B (Akt) and p44/42 mitogen-activated protein kinase (MAPK) extracellular signal-regulated MAPK (Erk1/2) signalling cascades) which reduces the ischaemia-reperfusion injury.[19]

Though above studies have shown that leptin is elevated in obesity related type – 2 diabetes mellitus and its relation with that of its complications, but the results on leptin levels in non – obese diabetic patients still remain contradictory, as also its association with the development of diabetic complication and this becomes our research goal.

The present study is designed to evaluate the relationship between serum leptin with non-obese type-2 diabetes mellitus and its role in the development of chronic complications and also to find the association between leptin and glycemic status.

The specific goals were to estimate leptin concentration among non-obese diabetes patients, to evaluate the difference in leptin levels between diabetic patients with and without chronic complications and to check the relation between leptin and glycemic status.

Materials and methods:

It was a hospital based analytical cross sectional study conducted by the Department of Biochemistry ,approved by the Institutional Ethics Committee.

The study population consists of 160 non-obese diabetic patients in which 80 are free of diabetic complications and the rest of them had one or more chronic diabetic complication. Type -2 diabetes mellitus was diagnosed based on the history of treatment with oral hypoglycemic drugs with American diabetic association criteria for the diagnosis of diabetes. The study population was selected based on inclusion and exclusion criteria. Both male and female patients between the age groups of 35-55 years of age with and without chronic complications of type-2 diabetes mellitus like Diabetic retinopathy, nephropathy, foot ulcers, cardiovascular complications related to diabetes were included in the study.

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The exclusion criteria were the patients with any form of renal, cardiovascular, ophthalmic, peripheral vascular disease ad subjects with BMI (Body Mass Index) ≥ 23, patients on insulin therapy, known case of hypertension.

After obtaining a written informed consent physical examination was done, BMI was calculated by measuring the height in cm and weight in kg. Fundus examination was done by an ophthalmologist to rule out the presence of retinopathy, ECG was done to find out the cardiac complication and foot examination was done to confirm the presence of foot ulcers.

Serum, plasma and urine sample was collected to estimated plasma fasting blood sugar, plasma post prandial blood glucose, HbA1c, serum urea, serum creatinine and urinary microalbumin. Serum leptin was estimated by sandwich ELISA method using Leptin- ELISA kit from DIA Source.

Statistical Analysis:

All results are expressed as mean ± standard deviation (SD). The difference in various parameters between diabetics with and without complications was done using independent student t-test. Difference in serum leptin levels between diabetic complications was seen using analysis of variance (ANOVA). The correlation between leptin with glycemic status was done using Pearson's correlation. p - value less than 0.05 is considered statistically significant. All analysis was done using Statistical Package for the Social Sciences (SPSS) version 16 for windows.

Results

Our study population consisted of 160 patients with type – 2 diabetes mellitus. Out of 160 diabetic patients, 80 patients did not have any complications and rest of them had one or more chronic complications of diabetes mellitus. In the later subgroup, the main bulk of the population was contributed by diabetic retinopathy (10%), diabetic ulcer (13.12 %) and those patients with both diabetic retinopathy and nephropathy (12.5 %).

The difference in mean age of diabetics with and without complications was found to be insignificant (Table I). Our study population was recruited in such a way that the body mass index (BMI) of the entire population was in normal range according to Asian classification for BMI. There was no significant difference in mean body mass index between subgroups (Table I). The mean fasting blood sugar, post-prandial blood sugar, HbA1c and urinary microalbumin were high in diabetic patients with complications compared to patients without complications. There was no significant difference in urea and creatinine concentration between the groups. But mean urinary microalbumin concentration was high in diabetic with complication compared to patient without complication ($p < 0.001$) (Table I).

The mean concentration of leptin levels in males was 10.46 ng/ml and in females it was 14.02 ng/ml. The difference in the leptin concentration found between the genders was statistically significant (< 0.001).

There was significant difference in mean leptin concentration between diabetic patients with and without complications in both genders. Namely, in the male subgroup the mean leptin levels in patients without complications was 12.90 ng/ml which was found to be significantly higher than that of the mean leptin concentration in patients with complications which was 8.18 ng/ml. In female subgroup the mean leptin concentration in diabetic patients without complications was 17.55 ng/ml which was found to be higher than in female patients with complications which was 10.19 ng/ml (Table II; Figure I).

We had done Analysis of variance (ANOVA) to see whether there is any difference in mean serum leptin concentration between various diabetic complications and it was found that there was no significant difference seen within and between various subgroups of diabetic complications (Table III)

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Pearson correlation was done to verify the association of leptin with that of fasting blood sugar, postprandial blood sugar and HbA1c. Our study results revealed that fasting blood sugar, post prandial blood sugar and HbA1c, correlated inversely with leptin concentration (Table IV).

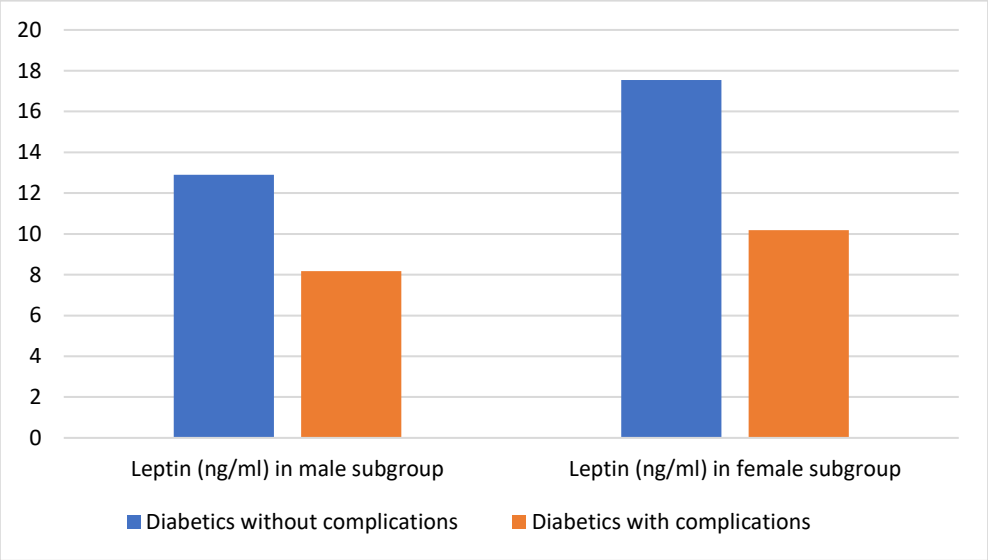

Figure I Gender specific difference in mean leptin variation among diabetic patients with and without complication

Table I: Demographic characteristics and laboratory findings of study population

Variables	Diabetics without complications n = 80	Diabetics with complications n = 80	p value
Age in years	45.75 ± 5.73	46.80 ± 5.60	0.243
BMI (kg/m^2)	21.42 ± 1.00	21.09 ± 1.36	0.085
FBS (mg/dl)	149.35 ± 61.94	176.26 ± 73.07	0.013*
PPBS (mg/dl)	237.21 ± 83.77	281.98 ± 106.95	0.004*
HbA1c (%)	7.67 ± 0.67	8.18 ± 0.95	< 0.001*
Serum urea (mg/dl)	25.48 ± 6.74	25.15 ± 6.20	0.741
Serum creatinine (mg/dl)	0.73 ± 0.15	0.77 ± 0.14	0.092
Urinary microalbumin (mg/L)	18.45 ± 4.92	47.83 ± 45.41	< 0.001*

* $p < 0.05$ is considered as statistically significant.

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Table II: Gender specific difference in leptin levels between diabetics with and without complications

Parameter	Diabetics without complications (Mean ± SD)	Diabetics with complications (Mean ± SD)	p value
Leptin (ng/ml) (Male Subgroup)	12.90 ± 1.89	8.12 ± 1.84	< 0.001*
Leptin (ng/ml) (Female Subgroup)	17.55 ± 1.79	10.19 ± 2.76	< 0.001*

** p < 0.05 is considered as statistically significant.*

Table III: Alteration in Leptin concentration in various diabetic complications

Subgroups	N	Serum Leptin Mean ± SD	p value
Diabetic retinopathy	16	8.70 ± 2.09	0.675
Diabetic nephropathy	11	8.04 ± 1.46	
Diabetic ulcer	21	9.39 ± 2.80	
Cardiovascular complications	6	8.92 ± 0.82	
Diabetic retinopathy and nephropathy	20	9.45 ± 3.29	
Cardiovascular complications and nephropathy	5	10.18 ± 1.50	
Diabetic ulcer and diabetic nephropathy	1	8.50	

* p < 0.05 is considered as statistically significant.

Table IV: Association between leptin levels with glycemic status.

Parameter	Leptin	
	Pearson Correlation r value	**p value**
Fasting blood sugar (mg/dl)	-0.172	0.030*
Post prandial blood sugar (mg/dl)	-0.194	0.014*
HbA1c (%)	-0.271	0.001*

* p < 0.05 is considered as statistically significant.

DISCUSSION:

The association between serum leptin levels and obese diabetic patients with diabetic complications has been studied extensively, but the studies which explains the relationship between leptin with that of non-obese diabetic patients and its complications are meagre and their results aren't unified. So, to clarify whether leptin alteration in diabetics is independent of BMI and also to explore its association with its complications we had conducted this study.

In this study, serum leptin concentration in the female sub group was greater than that of the leptin concentration in male study group population. The possible reason behind this variation in leptin concentration could be explained by differences in the sex hormone levels between male and female. Studies have shown that testosterone and oestrogen can alter the concentration of leptin. [20,21,22]

To rule out the gender influence over the result, we had divided the study population into two groups based on gender. The difference in leptin levels between diabetic patients with and without complications was done in each group separately. In both male and female subgroup, the leptin concentration in patients without complications is higher than that of leptin level in patients with diabetes complication. There was no difference in the leptin levels among the

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various complication of diabetes (Table III). So, our study postulate that leptin levels will be elevated in early stages and reduced in late stages of diabetes mellitus. This results of ours may be due to reduction in the β cell mass with severe insulin resistance in late stages of diabetes mellitus which result in reduced stimulation of insulin over leptin production.

Several studies have stated that leptin is reduced in late stages namely in patients with diabetic complications. And some of them had pointed out leptin might have a preventive role over the development of diabetic nephropathy, atherosclerosis, retinopathy. [19,23,26] In our study we found that leptin concentration is inversely correlating with glycemic status (FBS, PPBS and HbA1c). This finding in our study could be explained by the role of leptin over glucose homeostasis. Leptin increases insulin sensitivity, thereby reducing blood glucose levels. Leptin also reduces blood sugar levels by reducing hepatic glucose production. Even though leptin improves glycemic status, the magnitude of the increased glucose uptake elicited by leptin is generally much lower than that achieved by insulin.[27] This may explain the weak but significant association between leptin and glycemic status, which is seen in our study.

CONCLUSIONS:

From this study, we conclude that leptin might have a role in the control of glycemic status among diabetics and the development of diabetic complications is associated with reduction in leptin levels.

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