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SERUM LEPTIN AS A RISK FACTOR FOR DIABETES MELLITUS

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ABSTRACT

Background and objectives:

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. Leptin, a hormone released by adipocytes, is considered to have a role in regulation of body weight and energy metabolism. It reduces insulin release from human pancreatic β cells and inhibits insulin biosynthesis by decreasing preproinsulin mRNA expression in β cells. Hence serum leptin levels were measured as a risk factor for DM. A case control study was done from January 2012 to October 2012 to estimateserum leptin levels in type 2 DM patientsand compare with age and sex matched controls and to assess serum leptinlevels as an early predictor of onset of type 2 DM. The study was done on 30 type 2 DM patients, diagnosed according to the American Diabetic Association, and 30 age and sex matched controls. Patients with renal, liver and thyroid disorders were excluded. Serum leptin was measured by sandwich ELISA method. The mean and standard deviation of serum leptin levels was 12.33±3.32 ng/mL in cases and 7.11±2.91 ng/mL in controls [p value <0.001]. DM patients had significantly higher levels of leptin compared to controls. Hence increased levels of serumleptin can be used as risk factor in the development of type 2 DM in healthy individuals.

KEY WORDS

Adipocytes; Diabetes Mellitus;ELISA; Leptin; Preproinsulin

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia[1]. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [2]. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future [3].

An epidemic of Type 2 DM is underway in both developed and developing countries [4]. It has become one of the world's most important public health problems. Type 2 DM accounts for 85-90% of diabetes worldwide [5]. It is most commonly diagnosed in those over 40 years of age and the incidence rises to a peak at 60-65 years [5]. However, much younger people are now presenting with type 2 DM, following the rapid rise in childhood obesity.

Traditionally adipocytes have been viewed as energy deposits that store TAG during feeding and release FA during fasting to provide fuel for other tissues. However adipose tissue secretes numerous proteins that have important physiological function eg. Adiponectin, Leptin, Resistin, Angiotensinogen, Estrogen, Visfatin etc. These factors participate in autocrine and paracrine regulation within adipose

tissue and can affect the function of distant organs such as muscles, pancreas, liver and central nervous system.

Leptin is a protein hormone made of 167 aminoacids and released by adipocytes. It is considered to have a role in regulation of body weight and energy metabolism. It reduces insulin release from human pancreatic β cells and inhibits insulin biosynthesis by decreasing preproinsulin mRNA expression in β cells. It plays a role in chronic inflammation and autoimmunity. Serum leptin concentrations are characteristically increased in obese subjects. It is higher in subjects with type 2 DM. Highleptin levels correlate significantly with markers of insulin resistance. Leptin therapy in lipodystrophic patients was shown to improve glycemic control and insulin stimulated hepatic and peripheral glucose metabolism. It also reduces hepatic and muscle triglyceride content, suggesting that it acts as a signal that contributes to regulation of total body sensitivity to insulin [6].

MATERIALS AND METHODS

Source of data

The study was approved by the ethical committee of Bangalore Medical College and Research Institute, Bangalore (BMC & RI). It was conducted on patients with type 2 DM attending the outpatient &inpatient Department of Medicine in Victoria Hospital of BMC & RI. The patients and the controls voluntarily participated in this study.All patients with type 2 Diabetes Mellitus aged 40-70 years diagnosed according to American Diabetes Association criteria (FBS ≥126 mg/dl & 2 hour PPBS ≥ 200 mg/dl) and patients already on treatment for DM were included in the study.Patients with history of myocardial infarction, angina, liver, kidney and thyroid diseases which are known to influence serum levels of leptinwere excluded from the study. 30 age and sex matched healthy individuals were taken as controls.Informed consent was taken from patients and control subjects

Fasting blood samples were collected and the separated serum was used for estimation. Serum Leptinlevels were estimated using DRG Leptin (Sandwich) ELISA provided by DRG International, Inc., USA. This ELISA kit is a solid phase enzyme-linked immunosorbent assay based on the sandwichprinciple [1].

STATISTICAL METHODS

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.Student 't' test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Leven1s test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value: $0.01<P \leq 0.05$)

** Strongly significant (P value: $P \leq 0.01$)

RESULTS

In both cases and controls, 36.6% were males and 63.3%were females. In both the study groups, 50% were in the age group 40-50 years, 40% were in the age group 50-60 and 10% were in the age group 61-70 years. Cases and controls were appropriately age and sex matched.

Table 1: Age distribution of cases and controls

Age in years	Cases		Controls	
	No	%	No	%
40-50	15	50.00	15	50.00
51-60	12	40.00	12	40.00
61-70	3	10.00	3	10.00
Total	30	100.00	30	100.00
Mean ± SD	52.70±8.47		52.37±8.77	

Samples are age matched with p=0.881

Graph 1: Comparison of cases and controls according to age groups

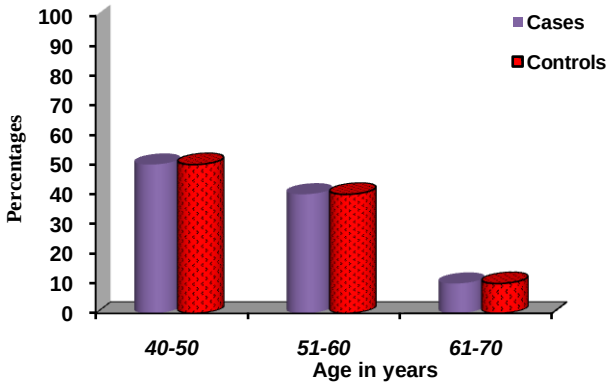
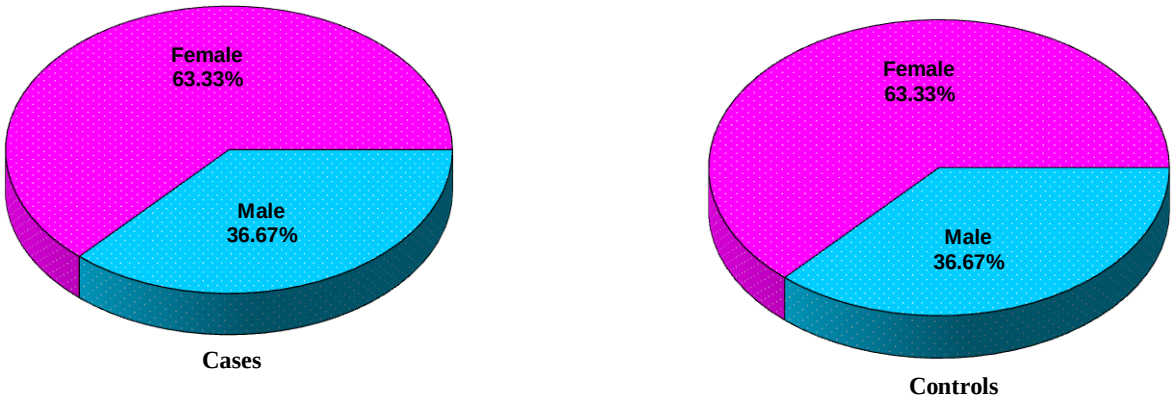


Table 2: Gender distribution among cases and controls

Gender	Cases		Controls	
	No	%	No	%
Male	11	36.67	11	36.67
Female	19	63.33	19	63.33
Total	30	100.00	30	100

Samples are gender matched with p=1.000

Graph 2: Comparison of cases and controls and according to sex



DISTRIBUTION OF GLUCOSEPARAMETERS IN CASES AND CONTROLS

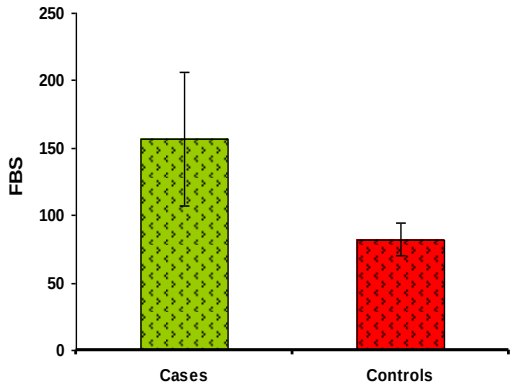
Table 3: Comparison of Glucose parameters in cases and controls

Glucose parameters	Cases	Controls	P value
FBS (mg/dL)	156.77±49.20	81.87±12.16	<0.001**
PPBS(mg/dL)	250.97±97.76	134.13±13.77	<0.001**
HbA1c(%)	8.96±1.98	5.11±0.22	<0.001**

Fasting Blood Sugar

In the 30 cases that were studied, the mean FBS level was 156 mg/dL with a SD of 49.20 mg/dL. The controls had a mean FBS level of 81.87 mg/dL with a SD of 12.16 mg/dL and p value <0.001as depicted in Table 3 and Graph no 3.

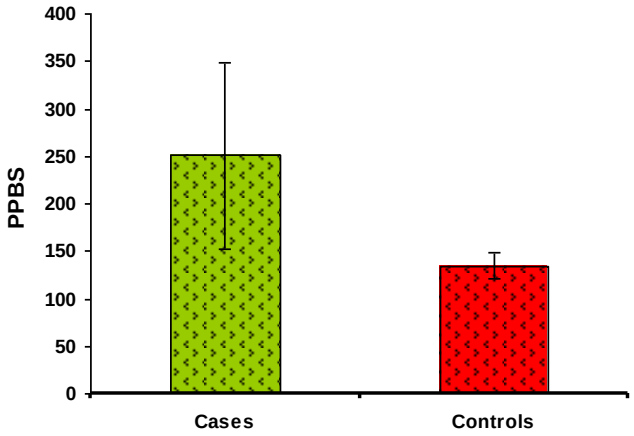
Graph 3: FBSIn Cases and Controls



Post Prandial Blood Sugar

In the 30 cases that were studied, the mean PPBS level was 250.97 mg/dL with a SD of 97.76 mg/dL. The controls had a mean PPBS level of 134.13 mg/dL with a SD of 13.77 mg/dL and p value <0.001 as depicted in Table 3 and Graph no 4.

Graph 4: PPBSIn Cases and Controls



HbA1c

From Table 3 and Graph no 5, the mean HbA1c in the 30 cases was 8.96% with a SD of 1.98%. Mean HbA1c in controls was 5.11% with a SD of 0.22% and p value <0.001.

Graph 5: HbA1c In Cases And Controls

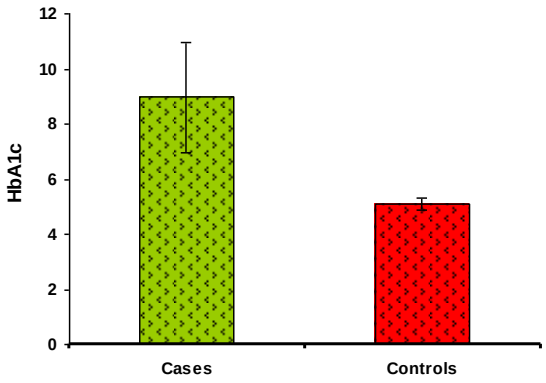


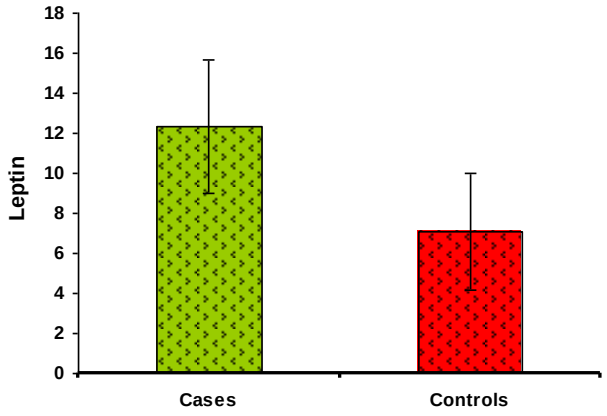
Table 4:Comparison of Biochemical parameters in the two groups studied

Bio-chemical parameters	Cases	Controls	P value
Urea (mg/dL)	23.33±11.19	22.10±4.99	0.615
Creatinine(mg/dL)	0.82±0.28	0.82±0.14	0.991
Total Bilirubin (mg/dL)	0.52±0.25	0.55±0.26	0.664
Direct Bilirubin (mg/dL)	0.08±0.075	0.070±0.06	0.583
Total protein (mg/dL)	7.12±0.67	7.44±0.36	0.026*
Albumin (mg/dL)	3.69±0.55	4.04±0.30	0.004**
Globulin (mg/dL)	3.41±0.53	3.40±0.43	0.936
ALP (U/L)	99.47±35.77	25.87±8.31	<0.001**
ALT (U/L)	27.60±26.32	20.60±9.70	0.177
AST (U/L)	31.77±26.08	89.30±25.54	<0.001**
TSH (µIU/mL)	2.26±1.27	1.87±1.15	0.226

Table 5: Comparison of serum Leptinlevels in the two groups studied

	Cases	Controls	P value
Leptin(ng/mL)	12.33±3.32	7.11±2.91	<0.001**

Graph 6: Serum Leptinlevels in cases & controls



Normal serum leptin levels is 4 – 8 ng/mL.

From Table no 5 & Graph no 6, the mean serum leptin levels in cases is 12.33 ng/mL with a SD of 3.32 ng/mL and p value <0.001. The mean serum leptin levels in controls was 7.11 ng/mL with SD of 2.91ng/mL and p value <0.001.

DISCUSSION

Several distinct types of DM are caused by a complex interaction of genetic and environmental factors. Depending on the etiology of DM, factors contributing to hyperglycemia includereduced insulin secretion, decreased glucose utilization and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual and the health care system. The function of adipose tissue as an endocrine organ has important implications for understanding the pathophysiologic relationship between excess body fat and pathologic state such as insulin resistance and type 2 DM.

Leptinis coded by the *obese* gene and is released by adipocytes. The discovery of leptin revolutionized the understanding of nutritional physiology. It is postulated to act as a satiety factor bybinding to receptors in the hypothalamus and is considered to have a role in regulation of body weight and energy metabolism. It appears to serve a critical link between energy stores and neuronal networks in the brain involved in the regulation of appetite and energy expenditure.

Leptin has a restraining effect on normal insulin secretion by the pancreas; it has been proposed that in obesity leptin resistancemight occur in β-cells, thus adding to hyperinsulinaemia observed inobese subjects. Moreover, leptin'santi-apoptotic effects in β-cellscould be diminished in the leptin-resistant state. Anti-apoptoticeffects of leptin may include inhibition of nitric oxide (NO) production via reduction of triglyceride content[7]. In addition to leptin, renal sodium reabsorption is enhanced under insulin-resistant conditions and associated hyperinsulinaemia[8].Elevated plasma levels of leptin are associated with adipocyte dysfunction in the presence of risk factors [increased BMI, CRP, LDL-c (low density lipoprotein cholesterol), and TG] [9, 10]. Fat massand gender are the main independent predictors of leptin concentration in type 2 DM patients, and that insulin secretion and the degree of insulin resistance contribute significantly to leptin levels[11]. Leptin receptors that are found in pancreatic β-cells raise the possibility that leptin may modulate insulin secretion. Exogenous leptin lowers plasma insulin levels and in vitro, leptin suppresses insulin release in human islet cells[12]. There is evidence to suggest that leptin may play a role in the pathophysiology of diabetes, possibly by suppressing insulin secretion. Since elevated baseline insulin is associated with both diabetesrisk and elevated leptin levels, it could confound an association between leptin levels and diabetes[12].

S. Fischer et al observed in their study that a functional relationship exists between insulin resistance and leptin concentrations in insulin-

resistant type 2 diabetic men, independently of body composition [12].

Marguerite J. Mcneely et al in their follow-up study found that higher baseline leptin levels predicted diabetes risk independent of baseline total fat, insulin, insulin resistance, glucose, or age in separate multiple logistic regression models [13].

S.Goyawannamethee et al in their prospective study observed that elevated IL 6, leptin and low adiponectin were associated with increased risk of type 2 diabetes [14].

Our results suggest that serum leptin levels could be used as an indicator for risk of diabetes mellitus, in addition to the established risk parameters such as obesity and physical activity. Limitations of the study were small sample size and cross sectional study.

CONCLUSION

From our study we can conclude that elevated serum leptin levels can be used as a risk factor for development of type 2 diabetes mellitus. It can be a promising new biomarker. Leptin levels represent an integrated marker of adiposity, insulin resistance and vascular dysfunction that could prove useful in future approaches to cardiovascular risk stratification in clinical practice.

It is interesting to note that leptin levels can be modified throughpharmaceuticalandlifestyle interventions, which can be used as preventive measures in reducing the incidence of type 2 DM and its dreaded complications in our country which is the "Diabetes capital of the world." It can also be used as a potential drug target.

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