

Evaluation of Plasma Leptin in Type 2 Diabetes Mellitus

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Abstract:

Background: Leptin is an adipokine that has been shown to be involved in pathways influencing the risk of cardiovascular disease and diabetes mellitus.

Aims: To estimate and compare plasma leptin levels in type 2 diabetes mellitus (T2DM) patients with normal healthy individuals.

Materials and methods: 60 T2DM patients and 50 age matched healthy individuals were taken as cases and controls respectively, in a case-control study conducted from October 2013 to September 2015 in the Department of Biochemistry in collaboration with the Department of Medicine, RIMS, Imphal. Fasting plasma leptin levels was estimated by DRG Leptin (Sandwich) ELISA kit manufactured by DRG Instruments GmbH, Germany Frauenberstrabe 18, D-35039 Maburg. Serum lipid profile estimation was done by enzymatic colorimetric test with lipid clearing factor (LCF) by kits marketed by Human Gesellschaft fur Biochemica and Diagnostica GmbH Germany.

Results: The mean duration of diabetes in cases was 5.56±3.30 years. The mean value of leptin (ng/ml) was 9.91±5.62 in cases and 5.11±2.66 in controls. Out of 60 patients, 20 had high plasma leptin levels whereas, 40 had leptin levels within the normal range. Leptin was positively correlated with waist-hip ratio ($r=0.626$, $p=0.000$), total cholesterol ($r=0.39$, $p=0.002$) and LDL-Cholesterol ($r=0.315$, $p=0.014$) and it was negatively correlated with HDL-Cholesterol ($r=-0.293$, $p=0.023$).

Conclusion: Increased levels of plasma leptin is an important risk factor for developing diabetes.

Keywords: Leptin, Type 2 diabetes mellitus, adipokine, ELISA- Enzyme linked immunosorbent assay.

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I. Introduction

Diabetes mellitus is a metabolic disorder affecting millions of people. According to the 2016 data from the World Health Organization (WHO), an estimated 422 million adults are living with DM.¹ The International Diabetes Federation (IDF) estimates the number of diabetics in India to be 72.9 million in 2017 and this number is expected to increase to 134.3 million by the year 2045.²

Diabetes can be classified into: (a) Type 1 diabetes (b) Type 2 diabetes (c) Gestational diabetes mellitus (d) Specific types of diabetes due to other causes (monogenic diabetes syndromes, diseases of the exocrine pancreas, and drug- or chemical-induced diabetes).³

T2DM is the most common type of diabetes. It can be due to insulin resistance with relative insulin deficiency or an insulin secretory defect with insulin resistance. It is mainly contributed by factors such as sedentary lifestyle and poor dietary habit leading to overweight and obesity.⁴

Leptin, a 16 kDa protein product of Ob gene is an adipokine that was discovered in 1994.⁵ It is predominantly produced by adipose cells and is also produced by syncytiotrophoblasts, ovaries, skeletal muscle, lower part of the fundic glands in stomach, mammary epithelial cells, bone marrow, pituitary and liver.^{6,7} Leptin acts through their receptors (LEPRs) on specific populations of neurons in the brain.⁸ It helps to regulate energy balance by inhibiting hunger and is also known as the "satiety hormone". The net action of leptin is to inhibit appetite, stimulate thermogenesis, decrease glucose and reduce body weight and fat.⁹ In obesity, a decreased sensitivity to leptin occurs resulting in an inability to detect satiety despite high energy stores.¹⁰ Leptin also plays a role in other physiological processes. It interacts with other hormones and energy regulators indirectly mediating the effects of insulin, insulin-like growth factor, growth hormone, glucocorticoids, cytokines and metabolites.¹¹

There are suggestions that the association between plasma leptin and diabetes may be a manifestation of an underlying leptin resistance mediated by obesity. Several pre-clinical studies indicate that leptin improves insulin resistance, and glucose and lipid imbalances in mouse models of T2DM.^{12,13} However, some recent clinical trials showed that leptin therapy is ineffective or only marginally effective in improving diabetes and insulin resistance in obese people affected by T2DM.¹⁴

This study was taken up with the aim to i) estimate and compare the leptin levels in T2DM with normal healthy individuals.

II. Materials And Methods

Study setting and study population: The case-control study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, from October 2013 to September 2015. The study population consisted of 60 type 2 diabetic patients above 18 years old attending medicine OPD in RIMS, Imphal taken as cases and 50 healthy individuals aged matched taken as controls.

Inclusion criteria:

Cases: Aged >18 years, T2DM patients. The diagnosis of T2DM was based according to American Diabetes Association Criteria which include fasting plasma glucose ≥ 126 mg/dl or post prandial plasma glucose ≥ 200 mg/dl or HbA1c $\geq 6.5\%$.¹⁵

Controls: Aged >18 years healthy individuals free from any systemic diseases.

Exclusion criteria : <18 years of age, Type 1 DM, pregnancy, <10 years residence in Manipur, patients on steroids, statins and chemotherapy, patients not willing to give consent.

Ethical approval was obtained from Research Ethics Board RIMS, Imphal. Written informed consent was obtained from all study participants.

Data collection: All the cases were interviewed after getting their consent. Detailed information about the disease duration, their medications, other illness, personal habits and family history was collected. Blood pressure was recorded 2 times with a standard mercury sphygmomanometer taken at intervals longer than 2 minutes after the participants had been sitting for 30 minutes. Anthropometric measurements (weight & height-measured without shoes, waist circumference- measured at level midway between the lower rib margin and iliac crest, hip circumference- measured at the maximal circumference over the buttocks) were taken and their BMI was calculated.

Collection of sample: 5ml of venous blood was collected by venipuncture from antecubital vein after an overnight fast. 2ml was collected in EDTA vial for estimation of plasma leptin. The sample was immediately centrifuged at 3000 rpm x 10 minutes. The plasma collected was immediately stored in aliquots at -20°C. 2 ml of blood was collected in plain vial and 1 ml in EDTA vial for estimation of lipid profile and HbA1c respectively, on the same day.

Biochemical and statistical analysis:

Leptin was estimated by DRG Leptin (Sandwich) ELISA kit manufactured by DRG Instruments GmbH, Germany Frauenberstrabe 18, D-35039 Maburg. This assay has an analytical sensitivity of 0.7 ng/ml. The normal values of plasma leptin for apparently healthy adults by this assay is 3.84 ± 1.79 (males) and 7.36 ± 3.73 (females) in ng/ml. Serum lipid profile were estimated by enzymatic colorimetric test with lipid clearing factor. Serum total cholesterol estimation was done by CHOD PAP method, serum triglyceride by GPO-PAP method, high density lipoprotein cholesterol (HDL-C) by precipitation technique¹⁶ and low density lipoprotein cholesterol (LDL-C) by Friedewald formula.¹⁷ HbA1c was estimated by fast ion exchange resin separation method.

Statistical analysis were performed using SPSS Statistics Version 20 and Minitab Release 11.12, 32 Bit. Quantitative data is presented as mean \pm SD and qualitative variables as number of cases and percentages. Chi-square test, independent sample t-test were applied whenever necessary. Pearson correlation coefficient 'r' was used for correlation. All comparisons were two-sided and p-values of <0.05 was considered as statistically significant.

III. Results And Observations

Table 1: Baseline characteristics of cases and controls

Parameters	Groups	
	Cases (n=60)	Controls (n=50)
Age (yrs)	59.35±9.61	54.94±7.02
Male(%)	37(61.7)	26(48.0)
Alcohol (%)	34(56.67)	28(56.0)
Smoking (%)	32(53.33)	24(48)
Duration of diabetes (yrs)	5.56±3.30	-
Waist-circumference (cm)	88.90±7.36	102.58±23.24
Hip-circumference (cm)	93.96±9.40	116.20±27.14
Waist-hip ratio	0.92±0.12	0.84±0.05
BMI(kg/m ²)	25.64±0.56	22.39±2.24
SBP(mm/Hg)	130.85±19.05	120.00±8.08
DBP(mm/Hg)	83.66±11.30	77.00±6.14
HbA1c (%)	6.59±0.72	5.4±0.22

Table 1 shows the baseline characteristics of all the cases and controls. Majority (61.7%) of the cases with T2DM were males whereas females were more in the control group (52%). The mean duration of diabetes in cases was 5.56±3.3 years.

Table 2: Mean±SD plasma leptin levels in cases and controls

Parameters	Cases	Controls	p-value
Leptin (ng/ml)	9.91±5.62	5.11±2.66	<0.0001

The plasma leptin levels was significantly higher in cases than in the control group.

Table 3: Correlation between leptin and each parameter in cases

Parameters	Leptin	
	r	p-value
Duration of diabetes (yrs)	0.216	0.098
Hip circumference (cm)	-0.433*	0.001
Waist circumference (cm)	0.417*	0.001
Waist-hip ratio	0.626**	0.000
BMI (kg/m ³)	0.029	0.827
SBP	0.147	0.263
DBP	0.206	0.114

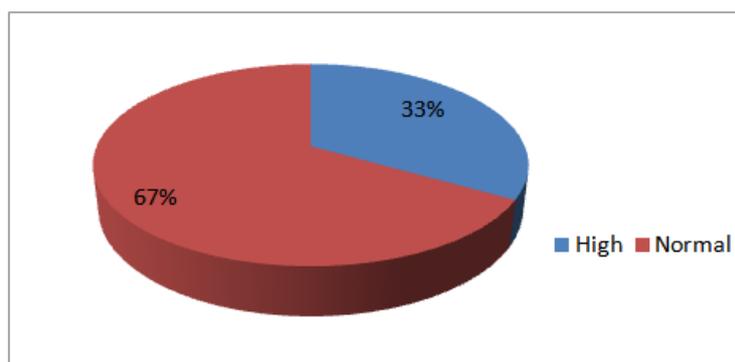
A significant positive correlation was seen between waist circumference and waist-hip ratio with the levels of plasma leptin.

Table 4: Mean±SD of lipid profile and their correlation with leptin in cases

Parameters	mean±SD	Leptin	
		r	p-value
Total Cholesterol (mg/dl)	213.71±52.10	0.394*	0.002
HDL(mg/dl)	34.61±10.51	-0.293	0.023
LDL (mg/dl)	111.56±36.77	0.315*	0.014
Triglycerides (mg/dl)	99.80±24.80	0.175	0.180

Table 4 shows that plasma leptin is positively correlated with TC and LDL, and has a negative correlation with HDL.

Table 5: Distribution of cases according to plasma leptin levels



Out of 60 cases, only 33% had high plasma leptin levels whereas all the healthy controls had plasma leptin within the normal range.

IV. Discussion

This is the first study that is conducted in the state of Manipur among type 2 diabetic patients to estimate plasma leptin levels and compare it with normal healthy individuals. In this study, diabetes mellitus was defined according to ADA criteria.¹⁴ According to studies done by Maahs DM et al¹⁸ and Haffner SM et al¹⁹, there is no association between plasma leptin levels and diabetes, whereas Soderberg S et al²⁰ reported positive association between plasma leptin levels and diabetes only in men. Although 66.7% of the cases in this study had normal plasma leptin levels, the difference was found to be significant when compared with that of controls (p-value= <0.0001). All the 50 healthy controls had plasma leptin levels within normal range.

Another finding in this study is a positive association between waist circumference and waist-hip ratio in diabetic cases with plasma leptin. This is similar to the findings of Li et al.²¹ Obesity is a well known contributing factor to development of type 2 diabetes mellitus. Leptin, an adipose tissue derived hormone acts directly on leptin receptors in the cell membrane of different types of cells in the human body. It reduces appetite as a circulating signal. However, obese individuals generally have a higher circulating concentration of leptin than normal weight individuals due to their higher body fat percentage.²² Most obese individuals show resistance to leptin which is similar to resistance of insulin in type 2 diabetes. Despite the elevated levels of circulating leptin, it fails to control hunger and ultimately lead to weight gain.

Mohiti et al²³ suggested that insulin concentration may contribute to the pathogenesis of leptin and its effects on glucose metabolism and hyperglycemia. Leptin decreases the synthesis of insulin by decreasing the pre- proinsulin mRNA expression in β cells.²⁴ Type 2 DM is mostly associated with increasing weight leading to insulin resistance. A decrease in sensitivity to leptin occurs which results in an inability to detect satiety despite high energy stores, eventually leading to leptin resistance and their increased level in the body with people suffering from T2DM who are above their normal body weight.⁹ Increase leptin fail to resist the development of obesity. Several mechanisms have been proposed to explain leptin resistance, including i) defective leptin transport across the blood-brain barrier,²⁵ ii) attenuation of leptin signaling,²⁶ iii) the deficiency and variations of LEP and LEPR genes,²⁷ iv) ER stress,²⁸ v) inflammation²⁹ and vi) excessive bioavailability of heavy metals such as Zn.³⁰

In this study, total cholesterol and low density lipoprotein cholesterol also showed a significant positive correlation with leptin. Kumar et al³¹ suggested that higher leptin levels may be an additional risk factor in patients of type 2 DM with obesity and dyslipidemia. A study done by Matsuda et al³² found that the mean levels of leptin were significantly higher in diabetic patients with neuropathy than in those without. Hence, the level of leptin may be much more in those diabetic patients with complications compared to those without.

Limitations

The study has a small sample size, therefore, results cannot be generalized.

V. Conclusion

Obesity, mainly central obesity is one of the most important contributing factor leading to insulin resistance and thereby T2DM. In our study, there is a positive association between plasma leptin and type 2 diabetes mellitus.

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