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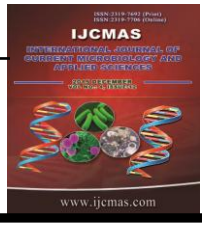

Original Research Article

Serum Leptin and Body Mass Index in Type 2 Diabetes Mellitus Patients of Dehradun, Uttarakhand, India

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A B S T R A C T

The aim of this study was to evaluate the variation in serum leptin levels in obese and non-obese type 2 diabetes mellitus (type 2 DM) patients and compared them with normal healthy controls. The study group consisted of forty patients of type 2 DM and twenty normal healthy controls. Blood glucose (FBS and PPBS), HbA1c, Serum lipids and lipoproteins, Serum leptin, and Body mass index (BMI) were estimated in obese and non-obese type 2 DM patients and normal healthy controls. The Mean±SD of serum leptin levels were significantly higher in obese (9.83± 1.72 ng/ml) and non-obese (6.09±1.83 ng/ml) type 2DM patients than normal healthy controls (5.38±2.20 ng/ml). BMI was found to be significantly higher in obese and non-obese patients than controls (28.1 ± 1.1, 23.5 ± 3.4 and 22.8 ± 2.3 respectively). Serum lipid and lipoprotein levels were observed to be significantly raised in patients of type 2 DM.The study concluded increased leptin levels were significantly correlated with high BMI (obesity) and type 2 DM. Hence increased levels of serum leptin can be used as risk factor in the development of type 2 DM.

Keywords

BMI,
Type 2 DM,
Leptin,
Obese,
Non-Obese,
HbA1c

Introduction

Diabetes mellitus (DM) is one of the world's major public health problems. In 2010, 285 million people were affected worldwide and by the year 2030, this number will reach to about 435 million (Sarath and Rajkumar, 2011). With this increasing incidence worldwide, it will be a leading cause of morbidity and mortality for the anticipated future (Alvin, 2012).

Approximately 80% of the subjects with type 2 DM are obese. Traditionally adipocytes were considered to store triglycerides during feeding and release free fatty acids during fasting. However adipose tissue secretes more than 50 bioactive peptides (adipokines) like adiponectin, leptin, resistin, visfatin etc. These factors participate in autocrine and paracrine

regulation and can affect the function of distant organs such as muscles, pancreas, liver and central nervous system (Rajala and Scherer, 2003).

Human leptin is 16-kDa, protein product of the obesity (*ob*) gene. There are six isoforms of the leptin receptor Ob-R (a-f) are known, which vary in length, location, and functionality (Lee et al, 1996). Most of the leptin receptors (Ob-R) are present in the arcuate nucleus of the hypothalamus. These isoforms are closely related to the class I cytokine receptor family. Ob-Ra and Ob-Rb are dominant isoforms of the heart, whereas the others are expressed at low levels and are not well conserved among species. Ob-Re is the secretary form that binds circulating leptin and regulates the concentration of free leptin (Ge H et al, 2002). When leptin binds with receptor, it undergoes homo-oligomerization and transduces different signal to distant organs. The major signaling pathways are Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) and mitogen-activated protein kinase (MAPK) (Nakashima et al, 1997).

Leptin stimulates the synthesis of pro-opiomelanocortin, which is processed to produce α-melanocyte–stimulating hormone and activate downstream melanocortin-3 and 4 receptors to reduce appetite (Cowley et al, 2001). It controls feeding and energy expenditure through JAK2/STAT3 signaling pathways therefore it reduces hyperphagia and obesity. But obesity is associated with high levels of leptin, chronic over-expression of these high levels reduces leptin receptors, diminished signaling, and impaired responsiveness to exogenous leptin, which suggests that obese humans are resistant to this adipocytes hormone (Scarpace and Zhang, 2007) . The other mechanisms of leptin resistance are genetic mutation, leptin self-regulation, restricted tissue access and cellular or circulating molecular regulation (Haynes, 2005).

Leptin decrease pre-proinsulin mRNA expression in β cells thus decrease the synthesis of insulin. It also reduces the release of insulin from human pancreatic β cells, which leads to the development of type 2 DM. Evidence suggested that chronic leptin treatment improves insulin-stimulated hepatic and peripheral glucose metabolism in severely insulin-resistant lipodystrophic patients. This improvement was associated with a marked reduction in hepatic and muscle triglyceride content, so it can be used as a therapeutic agent (Peterson et al, 2002).

Study done in 2001, reported unchanged levels of leptin in diabetic patients (Ozata et al, 2001). The medical literature studied so far did not show any clear consensus regarding the relationship between serum leptin and type 2 DM. So, the present study was planned to evaluate the variation of fasting blood sugar, post-parandial blood sugar, HbA1c, lipid and lipoprotein levels, BMI (obesity) and serum leptin in obese and non-obese patients of type 2 DM and their comparison with normal healthy controls.

Materials and Methods

The present study was conducted in the Department of Biochemistry, Himalayan Institute of Medical Sciences, Dehradun, over a period of 12 months (March 2012 to March 2013). Sixty patients diagnosed as type 2 DM attending medicine OPD of Himalayan hospital were enrolled in the study. The patients suffered from type 1 DM, Gestational diabetes and patients treated with insulin therapy were excluded from the study. The control group comprised of twenty, age and sex matched normal non-

obese healthy subjects. All the participants (patients and controls) were given an oral and written explanation about the study, including its procedures and were asked to read and sign an informed consent document. The study protocol and ethical aspects were approved by the ethics committee of the Himalayan institute.

Demographic data, any concurrent illness history, and information of medication were collected by interviews. The diagnosis of type 2 diabetes was based upon the classification of American Diabetes Association criteria (fasting plasma glucose ≥126 mg/dl, post parandial blood glucose ≥ 200mg/dl and HBA1c ≥6.5%) (Alberti and Zimmet, 1998). Anthropometric assessments included measurement of weight and height. Body weight was measured to the nearest 0.1 kg using the Seca 713 scales. Height of the participants without shoes was determined using measuring tape, and subsequently body mass index (BMI) was calculated by dividing weight (kg) by squared height (m^2). In this study subjects with BMI > 25 kg/m^2 were considered as obese. The patients who had total cholesterol [TC] level of >200 mg/dl, triglyceride [TG] >150 mg/ dl, high density lipoprotein-cholesterol [HDL-C] levels <40 mg/dl in males and <50 mg/dl in females, low density lipoprotein-cholesterol [LDL-C] levels >100 mg/dl were considered to be dyslipidemic.

All subjects (patients and controls) were instructed to observe an overnight fast for 8-10 hours and blood samples were drawn under aseptic conditions. Blood was being drawn in a grey topped vaccutainer for the estimation of plasma glucose (FBS and PPBS). Red top gel separated vaccutainer was used for the estimation of serum leptin, serum lipids included total cholesterol (TC) and triglycerides (TG) and serum lipoproteins included high density lipoprotein-cholesterol (HDL-C) and low density lipoproteins-cholesterol (LDL-C). For the estimation of Glycated hemoglobin (HbA_{1c}) purple topped EDTA vaccutainer was used.

Plasma glucose levels were done by hexokinase method (end point method) (Sonowane et al, 1976). Serum Leptin levels were estimated by using DRG Leptin ELISA (sandwich) provided by DRG International, USA (Imagawa et al, 1998). HbA1c was done on Mini -Vidas which was based upon the principle of HPLC (high performance liquid chromatography) (Little and Robert, 2009). TC, TG and HDL-C were estimated by commercially available enzymatic reagents on auto-analyzer [Beckman culter, DxC900]. LDL-C was calculated using Friedwald's formula (LDL-C (mg/dl) = TC - (TG/5)-HDL-C) for samples with TG value less than 350 mg/dl (Friedwald et al, 1972).

Statistical Analysis

The data analysis was carried out by using Statistical Package for the Social Sciences [SPSS] Version 17.0. Results were expressed as Mean (± SD). The statistical significance of difference between the various groups was determined by using the student's test; $p>0.05$ not significant, $p<0.01$ was significant, $p<0.001$was highly significant and $p<0.0001$ was extremely significant.

Results and Discussion

The mean age of the type 2DM patients and normal controls were 30-50 years. On the basis of BMI, the patients were divided into two groups, obese and non-obese type 2 DM patients. Out of sixty patients, thirty seven were obese and twenty three were non-obese. The calculated BMI was found to be

22.8 ± 2.3 in normal controls, 23.5 ± 3.4 in non-obese type 2 DM patients and 28.1 ± 1.1 in obese type 2 DM patients. BMI was found to be highly significantly raised in non-obese type 2 DM patients as compared to controls. Statistical analysis showed extremely significant raised BMI when comparison was made between normal healthy controls and obese patients (Table I).

The Fasting blood sugar levels were found to be 154.4 ± 2.2 mg/dl and 173.9± 5.9 in non-obese and obese patients of type 2 DM as compared to 82.3 ± 1.7mg/dl in normal healthy controls. The post-parandial blood sugar levels were extremely significantly higher in obese (274 ± 6.7mg/dl, $p<0.0001$) and non-obese patients (210 ± 6.7mg/dl, $p<0.001$) than normal controls (126 ± 3.2mg/dl). The levels of glycated hemoglobin were observed to be statistically significantly higher in type 2 DM patients (8.1% in non-obese) and (9.2 % in obese patients) as compared to normal healthy individuals (4.2%) (Table II).

Serum cholesterol levels were observed to be 153.4±18.17mg/dl in normal healthy controls as compared to 189.53±23.81mg/dl in non-obese and 289.53±31.97mg/dl in obese type 2 diabetes patients. The serum triglycerides were found to be significantly high in obese (272.83±51.65 mg/dl) and non-obese patients (172.83±31.10 mg/dl) as compared to healthy controls (130.15±22.18 mg/dl). The levels of serum HDL-cholesterol were observed to be less in obese and non-obese groups of type 2 DM patients (38.05±9.34 and 43.05±5.43mg/dl respectively) than normal healthy controls (64.45±8.41mg/dl). LDL-Cholesterol levels were found to be highest in of obese group patients (272.51±47.43mg/dl), high in non-obese group patients (198.51±46.13mg/dl) than normal individuals (115.15±15.94 mg/dl). The levels of VLDL-Cholesterol were found to be significantly more in diabetic subjects (54.56±9.41mg/dl in obese and 34.56±5.46mg/dl in non-obese) than normal healthy individuals (26.15±3.60mg/dl). The levels of serum lipids and serum lipoproteins were summarized in Table III.

Serum leptin levels were found to be high in non-obese diabetic patients (7.09±1.83) as compared to normal healthy controls (5.38±2.20) and relationship was significant. Mean ± SD of serum leptin was observed to be significantly higher in obese diabetes patients (9.83± 1.72) than normal controls (5.38±2.20) (Table IV).

Leptin is an important adipose tissue derived hormone that has been shown to be involved in patho-physiological mechanisms related to diabetes. However, few studies have examined an association between leptin and diabetes mellitus in humans. The present study showed that serum leptin levels were statistically significantly raised in obese, high in non-obese type 2 DM patients and normal or low in healthy controls. Our study supported by findings of previous study, who observed that elevated leptin levels could confound an association with diabetes. They concluded that leptin may play a role in the pathophysiology of diabetes, possibly by suppressing insulin secretion (Fischer et al, 2002). S.Goyawannamethee et al conducted a prospective study in 2007. The study found that increased levels of serum leptin and low adiponectin were associated with increased risk of type 2 diabetes (Goya et al, 2007).

Leptin levels are strongly linked to body fatness so it may be considered as a good biomarker of obesity. Various studies done, were positively correlated the circulating leptin concentrations and obesity, despite of the anti-obesity actions of leptin (Bullo et al, 2002; Skurk et al, 2007).

Table.1 Anthropometric parameters in controls and Obese and Non obese type 2 DM patients

Anthropometric parameters	Controls	Non-obese Type 2 DM patients (Mean ± SD)	Obese Type 2 DM patients (Mean ± SD)	P_1-value (Mean ± SD)	P_2-value (Mean ± SD)
Age	42 ± 1.2	44 ± 6.9	44 ± 6.9	NS	NS
Weight (Kg)	58.4 ± 7.4	61.1±7.3	72.9±8.4	0.001*	0.001*
Height (m)	1.60 ± 2.9	1.61 ± 8.3	1.61 ± 8.3	NS	NS
BMI (Kg/m²)	22.8 ± 2.3	23.5 ± 3.4	28.1 ± 1.1	0.001*	0.0001**

NS- non significant * highly significant ** extremely significant

P_1- The comparison between normal controls and non-obese type 2 DM patients.

P_2- The comparison between normal controls and obese type 2 DM patients

Table.II Changes in fasting, post-parandial glucose and HbA1C levels in obese and non obese Type-2 Diabetic Patients and normal healthy subjects

Parameters	Controls	Non-obese Type 2 DM Patients	Obese Type 2 DM Patients	P_1-value	P_2-value
FBS (mg/dl)	82.3 ± 1.7	154.4 ± 2.2	173.9 ± 5.9	0.001*	0.0001**
PPBS (mg/dl)	106 ± 3.2	210.0 ± 6.7	274.8 ± 6.7	0.001*	0.0001**
HBA1c(%)	4.2 ± 1.8	8.1±2.5	9.2 ± 1.5	0.001*	0.0001**

* highly significant ** extremely significant

P_1- The comparison between normal controls and non-obese type 2 DM patients.

P_2- The comparison between normal controls and obese type 2 DM patients.

Table.III Comparison of lipids and lipoprotein levels in patients of type 2 DM to that of normal healthy controls

Biochemical parameters	Controls (Mean ± SD)	Non-obese Type-2 DM Patients (Mean ± SD)	Obese Type-2 DM Patients (Mean ± SD)	P_1-value	P_2-value
Total Cholesterol (mg/dl)	153.4±18.17	189.53±23.81	289.53±31.97	0.0001	0.0001
Triglycerides (mg/dl)	130.15±22.18	172.83±31.10	272.83±51.65	0.0001	0.0001
HDL- Cholesterol (mg/dl)	64.45±8.41	43.05±5.43	38.05±9.34	0.0001	0.0001
LDL- Cholesterol (mg/dl)	115.15±15.94	198.51±46.13	272.51±47.43	0.0001	0.0001
VLDL- Cholesterol (mg/dl)	26.15±3.60	34.56±5.46	54.56±9.41	0.0001	0.0001

P_1- The comparison between normal controls and non-obese type 2 DM patients.

P_2- The comparison between normal controls and obese type 2 DM patients.

Table.IV Variation in levels of Serum leptin in normal healthy controls and obese and non-obese patients of type 2 DM

Parameter	Controls (Mean ± SD)	Non-obese Type-2 DM Patients (Mean ± SD)	Obese Type-2 DM Patients (Mean ± SD)	P_1-value	P_2-value
Serum leptin (ng/ml)	5.38±2.20	7.09±1.83	9.83± 1.72	0.01*	0.0001**

* significant ** extremely significant

P_1- The comparison between normal controls and non-obese type 2 DM patients.

P_2- The comparison between normal controls and obese type 2 DM patients

A study conducted on 143 participants, who developed diabetes across 5 years. They concluded that visceral fat and BMI were independent predictors of diabetes (Kanaya et al, 2006). Study done in 2006, enrolled 35 obese Omanis and 20 non-obese subjects.

Serum leptin levels were observed to be higher in obese group and positively correlated with body fatness and obesity (Maskari and Alnaqdy, 2006). Our study also concluded strong positive correlation of obesity and BMI with increased leptin levels in obese and non-obese diabetic patients (with higher BMI) than normal healthy subjects.

The current study observed higher leptin levels in women than in men in both groups (cases of type 2 DM and non-diabetic controls). Our findings were supported by other studies which gave various causes of elevated serum leptin levels in women than in men, these causes were (i) high adiposity and subcutaneous fat (ii) the existence of negative correlation between leptin and testosterone levels (Liuzzi et al, 1999; Vettor et al, 1997). (iii) the stimulation of leptin mRNA production by 17β-estradiol, which is one of the women's sexual hormones (Sweeney,2002). Latest study done in 2014 included 145 men and 177 women. Leptin levels were found to be 3.1 times more in females than males. Study concluded that structural difference in hypothalamus could be one of the causes for this gender difference (Mirrakhimov et al, 2014).

The levels of serum TGs, serum cholesterol were observed to be significantly higher in obese and non-obese type 2 DM patients than normal healthy controls. Previous study done in 1998, explained this relationship as triglycerides stored in adipose tissue as the main form of energy so they were correlated with leptin (Comizio et al, 1998). Other studies showed a significant positive correlation between leptin and HDL-C (Rainwater et al, 1997) and TG (Leyva et al, 1998). 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] (Brennan et al, 2007).

In conclusion, this study indicated an association between increased serum leptin levels and type 2 DM patients. Higher leptin levels may consider as an additional risk factor in patients of type 2 DM with high BMI (obesity) and dyslipidemia.

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Reference

Alberti KG and Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Medicine. 1998;15(7) :539-53.

Alvin C. Powers. Diabetes Mellitus. In: Longo, Fauci, Kasper, Hauser, Jameson, Loscalzo. Harrison's Principles of Internal Medicine. 18th edition. New York: McGraw-Hill Medical publishing division. 2012; 2968-74.

Brennan AM, Li TY, Kelesidis I, Gavrila A, Hu FB, Mantzoros CS. Circulating leptin levels are not associated with cardiovascular morbidity and mortality in women with diabetes: a prospective cohort study. Diabetologia.2007; 50:1178–1185.

Bullo M, Garcia-Lorda P, Salas-Salvado J. Plasma soluble tumor necrosis factor receptors and leptin levels in normal-weight and obese women: effect of adiposity and diabetes. Eur J Endocrinol. 2002; 146 : 325–331.

Comizio R, Pietrobelli A, Tan YX, Wang Z, Withers RT, Heymsfield SB, Boozer CN: Total body lipid and triglyceride response to energy deficit: relevance to body composition models.Am J Physiol 1998, 274:860–866.

Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*. 2001;411: 480–484.

Fischer. S, M. Hanefeld, S. M. Haffner, C. Fusch, U. Schwanebeck, C. Köhler et al. Insulin- resistant patients with type 2 diabetes mellitus have higher

serum leptin levels independently of body fat mass. ActaDiabetologica. September 2002; 39(3):105-110.

Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preoperative ultracentrifuge. Clin Chem.1972; 18(1): 499-502.

Ge H, Huang L, Pourbahrami T, Li C. Generation of soluble leptin receptor by ectodomain shedding of membrane-spanning receptors in vitro and in vivo. J Biol Chem.2002; 277:45898–45903.

Goya W.S, Gordon D.O .Lowe, Rumley A, Cherry L, Whincup H.P, Sattar.N . Adipokines and Risk of Type 2 Diabetes in Older Men. Diabetes Care.2007; 30:1200–1205.

Haynes WG. Role of leptin in obesity-related hypertension. Exp Physiol. 2005; 90 (5):683–688.

Imagawa K, Matsumoto Y, Numata Y, Morita A, Kikuoka S, Tamaki M et al. Development of a sensitive ELISA for human leptin, using monoclonal antibodies. Clin Chem.1998; 44(10): 2165- 2171.

Kanaya AM, Wassel FC, Vittinghoff E, Harris TB, Park SW, Goodpaster BH et al. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. Arch Intern Med. 2006; 166 (3): 350 – 356.

Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI et al. Abnormal splicing of the leptin receptor in diabetic mice. Nature. 1996;379(6566): 632–5.

Leyva F, Godsland IF, Ghatei M, Proudler AJ, Aldis S, Walton C et al. Hyperleptinemia as a component of a metabolic syndrome of cardiovascular risk. Arterioscler Thromb Vasc Biol.1998; 18:928–933.

Little RR and Robert LW. A Review of Variant Hemoglobins Interfering with Hemoglobin A1c Measurement. J Diabetes Sci Technol. 2009; 3 (3): 446-451.

Liuzzi A, Savia G, Tagliaferri M, Lucantoni R, Berselli ME, Petroni ML, et al. Serum leptin concentration in moderate and severe obesity: relationship with clinical, anthropometric and metabolic factors. Int J Obes Relat Metab Disord. 1999;23(10):1066-73.

Maskari Al YM and Alnaqdy AA. Correlation between Serum Leptin Levels, Body Mass Index and Obesity in Omanis. Sultan Qaboos Univ Med J. 2006 ; 6(2): 27–31.

Nakashima K, Narazaki M, Taga T. Leptin receptor (OB-R) Oligomerizes with itself but not with its closely related cytokine signal transducer gp130. FEBS Lett. 1997; 403(1):79–82.

Ozata M, Gungor D, Turan M, Ozisik G, Bingol N, Ozgurtas T, et al. Improved glycemic control increases fasting plasma acylation-stimulating protein and decreases leptin concentrations in type II diabetic subjects. J Clin Endocrinol Metab. 2001; 86(8):3659-64.

Peterson KF, Oral EA, Dufour S, Befroy D, Arivan C, Yu C et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest. 2002;109 (10):1345–1350**.**

Rainwater DL, Comuzzie AG, VandeBerg JL, Mahaney MC, Blangero J.Serum leptin levels are independently correlated with two measures of HDL. Atherosclerosis 1997; 32:237–243.

Rajala MW and Scherer PE. Minireview: the adipocyte–at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology. 2003;144: 3765–3773.

Sarath R and Rajkumar B. Role of Leptin in Diabetes Mellitus. Indian Journal of Fundamental and Applied Life Sciences. 2011; 1 (2): 209-214.

Scarpace PJ and Zhang Y. Elevated leptin: consequence or cause of obesity? Frontiers in Bioscience. 2007;12:3531–3544.

Skurk T, Alberti-Huber C, Herder C, Hauner H: Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 2007, 92:1023–1033.

Sonowane M, Savory J, Cross E, Heintges H and Chester B. Kinetic measurement of glucose with a centrifugal analyzer, Hexokinase and glucose oxidase procedures compared.Clic Chem. 1976; 22 (7):1100-01.

Sweeney G. Leptin signalling. Cell Signal. 2002;14(8):655-63.

Vettor R, De Pergola G, Pagano C, Englaro P, Laudadio E, Giorgino F et al. Gender differences in serum leptin in obese people: relationships with testosterone, body fat distribution and insulin sensitivity. Eur J Clin Invest. 1997;27(12):1016-24.

Mirrakhimov EM, Kerimkulova A, Lunegova O, Mirrakhimov A, Malik P, Nabiev PM et al.The association of leptin with dyslipidemia, arterial hypertension and obesity in Kyrgyz(Central Asian nation) population. BMC Research notes.2014; 7: 411:415.