DOI: 10.7860/NJLM/2021/46249:2467

Original Article

Biochemistry Section

Adipocytokines in Diabetes Mellitus: A Study from a Rural Setting in Haryana, India

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ABSTRACT

Introduction: Diabetes Mellitus (DM) and Obesity are the biggest public health challenges of 21st century. Both these disorders are associated with several co-morbidities like hypertension, hyperlipidemia, Cardiovascular Diseases (CVD) etc., that may be linked to the underlying insulin resistance, hyperglycaemia, dyslipidemia, hyperinsulinemia and altered levels of adipocyte-derived hormones. Furthermore, clinical studies in humans have suggested the possible correlation of plasma concentration of several adipocytokines and measures of adiposity, insulin resistance and endothelial function in humans.

Aim: To estimate and compare the serum levels of leptin and adiponectin in patients with Type 2 Diabetes Mellitus (T2DM) and in non-diabetic subjects with and without obesity.

Materials and Methods: In the study, 200 T2DM patients (with and without obesity) and 200 non-diabetic subjects (with and without obesity) aged between 30-70 years of either sex were included. In all the subjects included in the study, serum leptin and adiponectin levels were estimated using Enzyme Linked Immunosorbent Assay (ELISA) method.

Results: It was observed that the serum adiponectin levels decreased while leptin levels increased significantly ($p<0.001$) in obese than non-obese diabetics. Similarly, obese non-diabetics showed higher serum leptin and lower adiponectin levels than their non-obese counterparts ($p<0.001$).

Conclusion: Serum levels of leptin and adiponectin are altered in subjects with T2DM and obesity which may indicate the potential role of adipocytokines as an important link between increased fat mass, insulin resistance, deranged glucose metabolism and endothelial dysfunction especially in diabetic patients.

Keywords: Cardiovascular disease, Insulin resistance, Obesity

INTRODUCTION

Adipose tissue, the largest endocrine organ of the body, was traditionally considered as a storage depot of fatty acids having only passive functions. Several studies, over the past few years, have revealed the endocrine function of adipose tissue which is now known to secrete a large number of hormones and cytokines also known as adipocytokines, e.g., Tumour Necrosis Factor (TNF-α), Interleukin-6, Adiponectin, Leptin, Angiotensinogen and Plasminogen Activator Inhibitor (PAI-1) etc., involved in glucose and lipid metabolism, inflammation, coagulation, blood pressure and feeding behaviour [1]. Thus, adipocytokines play a significant role in the metabolism and functions of many organs and tissues including muscle, liver, vasculature and brain. Besides chronic low grade inflammation and an increased predisposition for the development of insulin resistance, DM and/or vascular diseases, obesity has been found to be associated with adipose tissue dysfunction characterised by hypersecretion of pro-atherogenic, pro-inflammatory and pro-diabetogenic adipocytokines and decreased production of adiponectin [2].

Obesity, mainly abdominal obesity is also associated with decreased levels of the vascular protective adipokine, adiponectin. It is the gene product of the adipose tissue most abundant gene transcript – 1 (apM 1) gene; bearing structural homology to collagen VIII, X and complement C1q as well as TNF-α. It is a 244-amino acid polypeptide with molecular weight of 30 kDa that is exclusively secreted by adipocytes of white adipose tissue and acts as a hormone with anti-inflammatory, anti-atherogenic and insulin sensitising properties [3]. Thus, adiponectin, by various mechanisms aimed at suppressing hepatic gluconeogenesis, enhancing fatty acid oxidation in liver as well as skeletal muscle and promoting glucose uptake in skeletal muscle as well as insulin secretion, may reduce the risk of T2DM [4]. The levels of adiponectin, in contrast to other adipocytokines is found to be decreased in obesity which could be due to the reason that the accumulation of visceral fat might produce inhibiting factors for adiponectin synthesis or secretion such as TNF-α [5]. Studies have suggested the possible role of adiponectin as a link between the markers of inflammation, endothelial dysfunction; obesity and risk factors of T2DM [6,7]. Low adiponectin levels, through the interplay of genetic factors and environmental factors, results in obesity, which in turn leads to insulin resistance, metabolic syndrome and thus augmenting the risk of T2DM. In addition, hypoadiponectinemia may be an unconventional and significant risk factor for Coronary Artery Disease (CAD) and hypertension [8].

Leptin is a 16 kDa peptide hormone produced by adipocytes which has got a key role in the regulation of energy intake and expenditure by controlling appetite and glucose metabolism [9]. Leptin levels are directly proportional to the adiposity and may play a role in the pathogenesis of obesity and its related disorders [10]. Serum leptin levels are higher in females than males. Leptin resistance in beta cells might result in hyperinsulinemia due to lack of its inhibitory effect on insulin secretion thus resulting in T2DM [11]. Several studies have observed increased or unchanged leptin levels in diabetic patients [12,13].

Keeping in view the above facts, the present study was planned to estimate and compare the serum levels of adipocytokines- leptin and adiponectin in T2DM patients and non-diabetic subjects with and without obesity.

MATERIALS AND METHODS

The present case-control, observational study was carried out in the Department of Biochemistry in collaboration with Department of Medicine, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India, from April 2014 to March 2015 amongst 400 subjects in the age range of 30-70 years of either sex. The study was undertaken after approval by Institutional Ethics Committee (IEC) vide no. IEC/MMIMSR/14/46 dated 29/03/2014. Informed consent, both in English as well as vernacular language was taken from all the participants enrolled in the study. The study subjects were selected by simple random sampling technique.

Sample size calculation: The sample size was calculated by applying the formula:

$$N = \frac{z^2 \times p\,(1-p)}{C^2}$$

with confidence level of 95%, 5% margin of error and population proportion of 50% which came out to be 384. So, sample of 400 subjects was included in the study.

Inclusion criteria: Two hundred persons aged between 30-70 years of either sex suffering from T2DM and under treatment with and without obesity and 200 normal subjects in the same age range having no diabetes with and without obesity were included.

Exclusion criteria: The patients suffering T1DM, rheumatoid arthritis, CAD, hyperthyroidism/ hypothyroidism, cushing syndrome or any other endocrinopathy besides DM, pregnant females and patients on antioxidant drugs, corticosteroids and insulin were excluded from the study.

The patients were diagnosed as diabetic as per diagnostic criteria for DM issued by American Diabetes Association (ADA) [14] [Table/Fig-1].

Condition	Fasting glucose (mg/dL)	2 hours after glucose (mg/dL)	HbA1c (%)
Normal	<100	<140	<5.7
Impaired fasting glycaemia	100-125	-	5.7-6.4
Impaired glucose tolerance	-	140-199	5.7-6.4
Diabetes mellitus	≥126	≥200	≥6.5

[Table/Fig-1]: American Diabetes Association (ADA) diagnostic criteria for diabetes mellitus.

DM is diagnosed by demonstrating any one of the following:

- HbA1c level ≥6.5%
- Fasting plasma glucose level ≥126 mg/dL
- Plasma glucose ≥ 200 mg/dL two hours after a 75g oral glucose load as in glucose tolerance test.
- Symptoms of hyperglycaemia or hyperglycaemic crisis and random plasma glucose ≥200 mg/dL.

The calculation of Body Mass Index (BMI) was done by dividing body weight (in kilograms) by squared height (in square metres) according to World Health Organisation (WHO) [15] and the subject was classified as:

Normal weight: BMI between 18.5 to 24.9 kg/m^2

Overweight: BMI between 25 to 29.9 kg/m^2

Obesity: BMI of 30 kg/m^2

Grouping of subjects: All the 400 subjects included in the study were divided into four groups in the following manner:

Group I: 100 subjects having T2DM and obesity.

Group II: 100 subjects having T2DM and no obesity.

Group III: 100 subjects having only obesity and no diabetes.

Group IV: 100 subjects having no obesity and no diabetes.

Collection and processing of blood samples: On a pre-informed date and after an overnight fasting of 10-12 hours, the venous blood sample (about 5 mL) was collected from the ante-cubital vein of the subjects under the aseptic conditions. After allowing the blood to stand in acid-washed vial for about 30 minutes and after the formation of clot, the supernatant was centrifuged to perform the estimation of serum leptin and adiponectin levels.

Serum leptin and adiponectin levels were measured using ELISA technique based kits [16,17].

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM SPSS, Chicago, USA). Mean±Standard Deviation (SD) were calculated for different characteristics of the subjects. For continuous variables, Student's t-test and one-way Analysis of Variance (ANOVA) were used to compare the statistical differences between these variables.

RESULTS

The physical parameters of both type 2 diabetic and non-diabetic subjects with and without obesity, i.e., age and BMI were reported previously [18]. Briefly, the mean age (in years) of obese diabetics was lower than non-obese diabetics (50.68±9.41 vs. 51.69±9.41) while the BMI in diabetic, obese subjects (32.4±2.84 kg/m^2) was higher than in non-obese subjects (23.55±1.77 kg/m^2) with difference amongst two groups being statistically highly significant (p<0.001) [18]. Similarly, the mean age (in years) of obese, non-diabetics (48.17±11.71) was comparable to that of non-obese, non-diabetics (48.49±11.26). Further, in both obese as well as non-obese, non-diabetics, BMI (kg/m^2) showed highly significant statistical difference (p<0.001) with obese subjects having higher BMI (33.39±5.12) than non-obese counterparts (23.6±0.96). [Table/Fig-2] shows levels of serum leptin and adiponectin in obese and non-obese type 2 diabetic subjects.

Parameter	Diabetic, obese subjects (n=100) Mean±SD	Diabetic, non-obese subjects (n=100) Mean±SD	p-value
Leptin (ng/mL)	32.44±34.98	14.13±17.24	p<0.001
Adiponectin (µg/mL)	9.73±5.71	14.75±7.02	p<0.001

[Table/Fig-2]: Serum leptin and adiponectin in diabetic subjects.
*Data are presented as Mean±SD; Highly significant difference (p<0.001) between diabetic obese and diabetic, non-obese subjects in the levels of leptin and adiponectin was observed

Serum adiponectin levels decreased significantly (p<0.001) in obese diabetics than non-obese subjects with diabetes. In contrast, serum leptin levels were found to be significantly increased (p<0.001) in obese diabetics than non-obese diabetics.

Furthermore, the obese non-diabetics also showed significantly higher levels of leptin than non-obese subjects without diabetes (p<0.001) [Table/Fig-3]. In contrast, serum adiponectin concentration was found to be lower in obese as compared to non-obese non-diabetic subjects and; the difference between the two groups being statistically highly significant (p<0.001).

Parameter	Non-diabetic, obese subjects (n=100) Mean±SD	Non-diabetic, non-obese subjects (n=100) Mean±SD	p-value
Leptin (ng/mL)	27.09±27.81	13.53±11.83	p<0.001
Adiponectin (µg/mL)	10.89±3.74	13.96±4.87	p<0.001

[Table/Fig-3]: Serum leptin and adiponectin in non-diabetic subjects.
*Data are presented as Mean±SD; Highly significant difference (p<0.001) between diabetic obese and diabetic, non-obese subjects in the levels of leptin and adiponectin was observed

ANOVA revealed statistically highly significant difference amongst mean leptin and adiponectin levels of subjects included in four groups under study with F=14.69 (p<0.001) and 19.28 (p<0.001) for leptin and adiponectin, respectively.

DISCUSSION

The DM and obesity have close relationship with each other as far as their onset and pathophysiology is concerned. Insulin resistance seems to be a common aetiologic mechanism underlying both T2DM and obesity [19]. The insulin resistance has been associated with altered levels of adipocyte-derived hormones besides hyperglycaemia and hyperinsulinemia; all of these factors together promote the development of CVD [20,21]. The adipocytokines secreted by adipose tissue may simply lead to emergence of diseases linked with obesity [22].

One of the adipocytokine, leptin has been observed to play an important role in directing the intake of food, storage of energy besides managing the metabolism of carbohydrates and lipid in the body. Although obesity and insulin resistance are associated with the defective regulation of food intake, the role of leptin in the development of these diseases is not well understood. Few studies have predicted leptin as an independent risk factor for CVD which may suggest its role as an important link in the development of CVD and obesity. In a study conducted by Wannamethee SG et al., obese individuals had markedly increased plasma leptin levels [23]. Several studies carried out on diabetics reported high serum leptin levels in diabetics than non-diabetics [24,25].

Similarly, the present study also observed higher serum leptin levels in obese, diabetics in comparison to non-obese type 2 diabetics. The possible explanation for the increased serum leptin levels, hyperleptinemia seen in these patients may be due to leptin resistance that develops because of defective transport of leptin across Blood Brain Barrier (BBB); impaired leptin receptor signalling and blockages in neuronal circuits present downstream [26]. Even insulin resistance has been associated indirectly with increased serum leptin levels [27]. This hyperleptinemia further results in increased *ob* gene expression (the gene product of which is leptin only) leading to enhanced leptin secretion [28].

Adiponectin levels in plasma in contrast to all the other known adipocytokines, have been reported to be decreased in various conditions associated with hyperinsulinemia and insulin resistance such as obesity, T2DM, CVD etc. The exact mechanism underlying this association is not well understood but hyperinsulinemia tends to down regulate apM1 gene expression in adipose tissue which may lead to lower levels of adiponectin [29]. The present study also found significantly lower levels of adiponectin in obese diabetics than non-obese diabetic subjects in agreement with the studies conducted by Bu J et al., and Annuzzi G et al., which observed significantly decreased adiponectin levels in obese diabetics [30,31].

The results of the present study also confirmed that the presence of significantly higher serum leptin levels in obese than non-obese non-diabetic subjects. The levels of adiponectin were significantly lower in obese than non-obese non-diabetics. Studies conducted by Jaleel F et al., and Weyer C et al., have revealed that overweight and obese subjects had elevated leptin and decreased adiponectin levels [22,29]. The increase in leptin levels was proportionate to the degree of adiposity, thus indicating the possible role of hyperleptinemia in the development of various complications related to obesity [32]. Further, large number of leptin receptors are present in brain and peripheral tissues and any mutation in the gene encoding the receptor leads to defective transport of leptin across BBB or deranged signal transduction. This, in turn results in interference in the regulation of neuropeptide Y by leptin which would result in increased food intake and decreased expenditure of energy that ultimately leads to obesity. Hence, leptin is unable to exert its effects in spite of excessive production. This phenomenon, known as leptin resistance, leads to increased concentration of leptin in obese subjects [33].

Surprisingly, although adipose tissue is the main source of adiponectin, research findings suggest that the blood level of adiponectin is reduced in obese or type 2 diabetics who have large reserves of fat tissue. This may in part be related to hyperinsulinemia and chronic insulin resistance seen in T2DM due to overproduction of TNF-α by adipose tissue and interference in its signalling in endothelial cells by adiponectin [34]. Reduction in metabolic function of adipocyte with ageing leading to decreased adiponectin mRNA expression in adipose tissue has also been postulated for low serum adiponectin levels in obesity. Previous studies performed by Al-Kayatt TH et al., Mohammadzadeh G et al., and Abdelgadir M et al., also confirmed hypoadiponectinemia in obesity and T2DM [35-37].

Limitation(s)

The major limitation of the present study was a small sample size which makes it difficult to generalise its findings to the larger population. Therefore, prospective studies with large sample size should be conducted for better inferences.

CONCLUSION(S)

The present study findings revealed that the blood levels of adipocyte-derived hormones namely leptin and adiponectin are altered in subjects with T2DM and obesity, clearly suggesting the presence of pro-inflammatory, atherogenic and pro-diabetogenic adipocytokine profile which may be related to obesity, hypertension and CVD. In conclusion, estimation of serum leptin and adiponectin levels in obese subjects may help in assessing the risk of developing insulin resistance and thereafter, diabetes and CVD in such individuals. This would enable suitable preventive measures to be implemented in such high-risk subjects in order to protect them against DM and its complications. This also offers a new field for the development of novel adipocytokine-related treatment strategy for the alleviation of obesity and obesity-related metabolic disorders like T2DM, CVD etc.

Acknowledgement

The authors express deepest gratitude to Late Dr. Rajesh Pandey unending support throughout the conduct of the study.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 17, 2020
- Manual Googling: Oct 29, 2020
- iThenticate Software: Jan 19, 2021 (25%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Aug 11, 2020**
Date of Peer Review: **Sep 24, 2020**
Date of Acceptance: **Nov 23, 2020**
Date of Publishing: **Apr 01, 2021**