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Serum leptin level and microvascular complications in type 2 diabetes

ABSTRACT

Background. Type 2 diabetes (T2DM) and its complications are highly prevalent in Egypt and are considered a major health problem. Insulin resistance arising from visceral obesity is the main pathological mechanism of T2DM. Leptin is an adipokine secreted from visceral adipose tissue and its level is proved to be higher in patients with T2DM, but its association with microvascular complications is not yet well-established, for this aim the present study was conducted.

Methods. This cross-sectional study was conducted among 120 participants with T2DM recruited from the diabetes outpatient clinic of Alexandria Main University Hospital, Alexandria, Egypt. Each participant was subjected to full history taking, complete physical examination and laboratory investigations.

Results. Serum leptin level was significantly positively correlated with diabetes duration, BMI, WC, systolic and diastolic blood pressure, FPG, HbA_{1c}, serum insulin level, HOMA-IR, total cholesterol, triglycerides and LDL-C. Regarding microvascular complications, serum leptin level was highly significantly positively correlated with UACR, peripheral neuropathy and retinopathy ($P < 0.001$) and significantly negatively correlated with e-GFR ($P = 0.003$).

Conclusions. Serum leptin level is significantly correlated with microvascular complications in patients with T2DM in Alexandria, Egypt. (Clin Diabetol 2020; 9; 4: 239–244)

Key words: leptin, type 2 diabetes, microvascular complications

Introduction

Type 2 diabetes (T2DM) represents 90–95% of patients with diabetes worldwide [1]. Egypt occupies the 8th ranks regarding the prevalence of diabetes. The estimated prevalence of diabetes in Alexandria, Egypt was 16.8% in 2018 [2]. The main pathogenic mechanism of T2DM is insulin resistance that results in relative insulin deficiency [1]. Most of patients with T2DM are obese and many studies linked obesity to T2DM [3, 4]. Insulin resistance in muscle and liver results from impaired glucose uptake by adipose tissue [5]. Visceral adipose tissue is metabolically active and secretes substances called adipokines. Adipokines leads to development of insulin resistance by many mechanisms [6, 7].

Leptin is one of the adipokines secreted from adipocytes in visceral adipose tissue [8]. It is also detected in gastric mucosa, hepatic stellate cells, placenta, ovaries and mammary gland [9]. Leptin is acting as an adipose-derived endocrine signal by inducing satiety through hypothalamic receptors and enhancing lipid metabolism and energy expenditure [10]. Higher serum leptin levels were detected in patients with diabetes and leptin resistance has been implicated in the pathogenesis of diabetes and insulin resistance [11]. Leptin also has an important inflammatory role responsible for endothelial dysfunction, increased oxidative stress, vascular inflammation and proliferation of vascular smooth muscle cells (VSMC) and resultant intimal hyperplasia [12].

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Vascular inflammation is the core pathological mechanism of diabetic microvascular complications and there is evidence that adipocytokines play a probable role in vascular inflammation and endothelial dysfunction [13].

A well-established relationship between leptin and insulin resistance, diabetes, obesity, inflammation and metabolic syndrome was proved in previous studies [14–16], but its relation to microvascular complications is still unclear. Moreover, microvascular complications are major health problems in Egypt [17]. This invited us to conduct the present study.

Materials and methods

This cross-sectional study was conducted among 120 participants with T2DM recruited from patients attending diabetes outpatient clinic of Alexandria Main University Hospital, Alexandria, Egypt who accepted to participate in the research after explaining the research aim.

Inclusion criteria included patients with T2DM and BMI > 18.5 kg/m^2 while exclusion criteria included ischemic cardiovascular event in previous 3 months, severe liver impairment, recent history of major trauma or surgery, hematological disorders or malignancy, chronic inflammatory or autoimmune diseases, as well as patients with recent history of sever significant infection at study entry.

This work was done in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Helsinki Declaration in 1975 (revised in 2008). An approval was obtained from ethics committee of Faculty of Medicine, Alexandria University. All participates gave their written informed consent after explaining the nature and the aim of the study.

Participants were subjected to:

— Full medical history included following issues: personal data, detailed analysis of different cardio-metabolic risk factors, hypertension and dyslipidemia, history of macrovascular complications of diabetes (cardiovascular disease, cerebrovascular disease or peripheral arterial disease) and the use of antidiabetic, antihypertensive and antidyslipidemic drugs.

— Complete physical examination including body weight and height, body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m^2). Waist circumference (WC) was measured at the midpoint between highest point of the iliac crest and lowest point of the costal margin at the end of normal expiration according to the WHO recommendations [18].

— Vital signs including pulse and blood pressure (BP) were measured. Neurological examination was performed and diagnosis of peripheral neuropathy was done based on abnormal results of more than one diagnostic test of the following: vibration perception threshold using a 128-Hz tuning fork, temperature perception, pin-prick, ankle reflex and touch-pressure sensation (10-g Semmes-Weinstein monofilament) [19].

— Fundus examination was done in ophthalmology outpatient clinic of Alexandria Main University Hospital using slit lamp biomicroscope plus fundus lens by expert ophthalmologist. Diabetic retinopathy was diagnosed based on fundus examination findings and was divided into proliferative and non-proliferative stages:
  - non-proliferative diabetic retinopathy (NPDR): presence of microaneurysms, hemorrhages and hard exudates;
  - proliferative diabetic retinopathy (PDR): presence of neovascularization.

Laboratory investigations

Blood was drawn for metabolic, biochemical and hematological parameters after a 12 hours overnight fasting and serum was used for measurement of the following: fasting plasma glucose (FPG) and fasting serum insulin [20]. Homeostasis Model Assessment 2 (HOMA2) calculator was used to estimate insulin resistance (%S) (HOMA-IR) according to the updated computer based HOMA2 mode. Whole blood was mixed in EDTA tubes for glycated heamoglobin (HbA_{1c}) [20]. Lipid profile was measured including total serum cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and serum triglycerides [20]. Serum was collected in Eppendorf tubes and kept at –80C till assay of leptin by using ELISA according to the manufacturer [21]. Urinary albumin/creatinine ratio (UACR) was measured in a random spot urine collection [22].

Serum creatinine was measured with calculation of eGFR for staging of diabetic kidney disease using CKD-EPI equation [23]. Diabetic kidney disease was diagnosed based on the presence of albuminuria (UACR ≥ 30 mg/g) and/or reduced eGFR (< 60 ml/min/1.73 m^2).

Statistical analysis of the data was done using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percentage. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using mean and standard deviation. Significance of the obtained results was judged at the 5% level.

Table 1. Characteristics of participants (n = 120)

Measures	Mean ± SD	
Age (years)	53.03 ± 7.65	
BMI [kg/m^2]	32.11 ± 3.26	
Waist circumference [cm]	110.4 ± 10.21	
FPG [mg/dL]	139.4 ± 46.88	
Insulin level [μIU/mL]	13.37 ± 10.67	
HOMA-IR	5.26 ± 3.26	
HbA_{1c} (%)	7.75 ± 1.66	
Total cholesterol [mg/dL]	212.7 ±49.79	
Triglycerides [mg/dL]	175.0 ± 77.59	
LDL-C [mg/dL]	107.7 ± 33.65	
HDL-C [mg/dL]	56.20 ± 6.44	
eGFR [ml/min/1.73 m^2]	85.40 ± 11.26	
UACR [mg/g]	52.08 ± 35.62	
Leptin [ng/ml]	20.26 ± 7.74	
Sex	No.	%
Male	58	48.3
Female	62	51.7
Duration of diabetes		
< 5 years	48	40.0
Peripheral neuropathy	26	21.7
Diabetic retinopathy	28	23.3
Proliferative	10	8.3
Non proliferative	18	15.0
UACR ≥ 30	34	28.3

CAD — coronary artery disease; BMI — body mass index; FPG — fasting plasma glucose; HbA_{1c} — hemoglobin A_{1c}; LDL-C — low density lipoprotein-cholesterol; HDL-C — high density lipoprotein-cholesterol; eGFR — estimated glomerular filtration rate; UACR — urinary albumin to creatinin ratio

The used tests were as follows:

— Mann-Whitney test: for abnormally distributed quantitative variables, to compare between two studied groups;

— Kruskal-Wallis test: for abnormally distributed quantitative variables, to compare between more than two studied groups;

— Spearman correlation coefficient was used to identify the correlation between the level of serum leptin and the other parameters in the studied subjects.

Statistical significance was set at P value ≤ 0.05.

Results

The present study was conducted among 120 participants with T2DM recruited from diabetes outpatient clinic of Alexandria Main University Hospital, Alexandria, Egypt. Participants' characteristics are mentioned in Table 1.

Table 2. Correlation between the level of serum leptin and the other parameters in the studied subjects

	Patients	
	r_s	P
Age (years)	0.167	0.068
Duration of DM (years)	0.280	0.002*
BMI [kg/m^2]	0.389	< 0.001*
Waist circumference [cm]	0.413	<0.001*
Systolic blood pressure [mm Hg]	0.383	< 0.001*
Diastolic blood pressure [mm Hg]	0.181	0.048*
FPG [mg/dL]	0.428	< 0.001*
Insulin [μIU/mL]	0.247	0.006*
HOMA-IR	0.323	< 0.001*
HbA_{1c} (%)	0.282	0.002*
Total cholesterol [mg/dL]	0.363	< 0.001*
Triglycerides [mg/dL]	0.338	< 0.001*
LDL-C [mg/dL]	0.331	< 0.001*
HDL-C [mg/dL]	0.086	0.350
eGFR [ml/min/1.73 m^2]	–0.273	0.003*
UACR [mg/g]	0.469	< 0.001*

r_s — Spearman coefficient; * — statistically significant at P ≤ 0.05
BMI — body mass index; FPG — fasting plasma glucose; HbA_{1c} — hemoglobin A_{1c}; LDL-C — low density lipoprotein-cholesterol; HDL-C — high density lipoprotein-cholesterol; eGFR — estimated glomerular filtration rate; UACR — urinary albumin to creatinin ratio

Serum leptin level was significantly positively correlated with diabetes duration, BMI, WC, systolic and diastolic blood pressure, FPG, HbA_{1c}, serum insulin level, HOMA-IR, total cholesterol, triglycerides and LDL-C (Table 2).

Correlation between serum leptin level and microvascular complications of diabetes

There was highly statistically significant positive correlation between serum leptin and UACR (Table 2, Figure 1), peripheral neuropathy and retinopathy (Table 3) (P value < 0.001) and statistically significant negative correlation between serum leptin and eGFR (Table 2, Figure 2) (P value < 0.001).

Discussion

Type 2 diabetes is a major health problem due to its high prevalence and the burden of its chronic complications [1]. Leptin is one of the adipokines incriminated in the pathogenesis of insulin resistance and T2DM. The present work was conducted to study the relation between serum leptin level and diabetic microvascular complications in a cohort of 120 participants with T2DM in Alexandria, Egypt. In the current study, serum leptin level was significantly positively correlated with

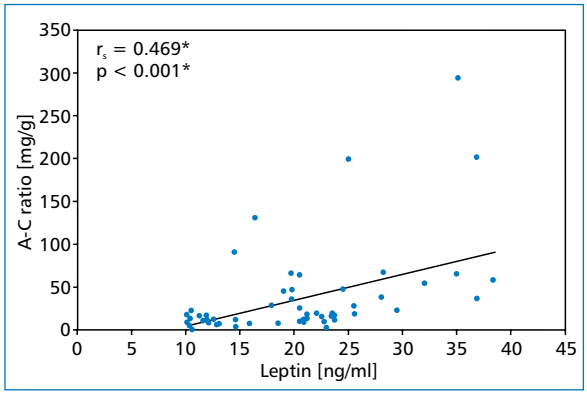

Figure 1. Correlation between leptin and UACR in studied subjects

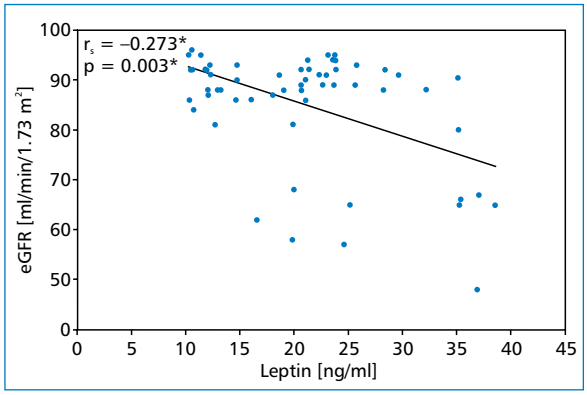

Figure 2. Correlation between leptin and eGFR in studied subjects

Table 3. Correlations between serum leptin level and peripheral neuropathy and retinopathy in the studied group

	N	Leptin Mean ± SD	Test of sig.	P
Peripheral neuropathy				
No	94	18.10 ± 6.14	U = 466.0*	< 0.001*
Yes	26	28.05 ± 8.01		
Fundus examination				
Normal	92	18.14 ± 6.20	H = 21.322*	< 0.001*
Proliferafive	10	29.26 ± 7.13		
Non proliferafive	18	26.09 ± 8.86		

U — Mann-Whitney test; H — H for Kruskal-Wallis test; p — p value for association between leptin and different parameters; * — statistically significant at P ≤ 0.05

diabetes duration, BMI, WC, systolic and diastolic blood pressure, FPG, HbA_{1c}, serum insulin level, HOMA-IR, total cholesterol, triglycerides and LDL-C. In agreement with the results of the present study, Cha et al. [24] found that plasma leptin level is positively correlated with BMI, fasting plasma glucose HbA_{1c} and total cholesterol values in patients with T2DM. Yassin et al. [25], who studied serum leptin level in patients with T2DM also found significant positive correlations between serum leptin and diabetes duration, cholesterol, triglycerides and LDL-C. Zulfania et al. [26] in concordance with the results of the present study found that serum leptin concentration was significantly correlated with BMI, FPG and HbA_{1c} in patients with T2DM.

Regarding the relation between serum leptin level and microvascular complications, the present study showed a significant positive correlation between serum leptin level and UACR, peripheral neuropathy and retinopathy and a significant negative correlation between serum leptin level and e-GFR. In concordance with the present study a meta-analysis by Rodríguez et al. [27] concluded that higher leptin levels were associated with microalbuminuria, macroalbuminuria and neuropathy, but in disagreement with the results of the present study, no association was found between serum leptin level and retinopathy. This difference may arise from the different patients' characteristics in studied cohorts. Cha et al. [24] showed similar results as they found a significant positive correlation between serum leptin level and urinary albumin excretion, and significant negative correlation with creatinine clearance. Yassin et al. [25] also showed a significant positive relation between serum leptin level and urinary albumin excretion and they concluded that serum leptin may be used as a marker of progression of diabetic kidney disease. On the other hand, Sari et al. [28], who studied the relation between serum leptin level and diabetic complications in patients with T2DM, found no significant difference between patients with and without diabetic nephropathy, retinopathy or neuropathy. Jung et al. [29], in concordance with the results of the present study, showed that serum leptin was significantly higher in patients with neuropathy than in patients without neuropathy. Uckaya et al. [30] studied the relation between serum leptin level and diabetic retinopathy and their results were similar to the

results of the present study as they found a significantly higher leptin level in patients with proliferative and non-proliferative diabetic retinopathy than in patients without retinopathy.

Conclusions

From the results of the present study we concluded that serum leptin level is associated with microvascular complications in T2DM. This may be due to the pro-inflammatory potential of leptin and its involvement in subclinical inflammation and endothelial injury which are incriminated in the pathogenesis of microvascular complications. Further studies may declare the link between serum leptin level and progression of microvascular complications and the use of serum leptin level as a prognostic factor in patients with microvascular complications.

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Conflict of interest

The authors declare to have no conflict of interest.

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