Correlation between Microalbuminuria and Glucose/ Leptin ratio in a sample of Iraqi patients with type 2 diabetes mellitus

العلاقة بين التركيز المتناهي للالبومين في الادرار ونسبة الكلوكوز الى الليبتين في عينة من المرضى العراقيين المصابين بالسكر من النوع 2

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Abstract

To study the association of serum leptin and glucose/leptin ratio with microalbuminuria in patients with type 2 diabetes mellitus at different durations, and to predict their effect on their renal function. A case control study was conducted in the International Diabetic Center at Al-Mustansiryia University in Baghdad-Iraq from 1st September 2008 to the 30th of July 2010. One hundred and eight male patients with type 2 diabetes mellitus (DM) were introduced in this study. Fifty three healthy subjects were used as a control. Fasting serum glucose, insulin, leptin, urea, creatinine and body mass index were estimated in both patient and control groups. Fifty three 49.07% out of 108 were diabetic for ≤2 years, while 55 patient 50.93% were diabetic >2 years. All patients were obese while healthy control subjects were not. Fasting serum glucose, insulin and leptin levels were found elevated in patients group while in control group their levels were found within normal range. Both urea and creatinine were also within the normal range for patients and control groups. Level of albumin in urine of patients with diabetes mellitus for ≤ 2years was found normal while in those with 3 to 6 or more than 6 years of DM, level of urinary albumin was found within the micro rang. A positive correlation with highly significant p value (<0.01) was found between patients (≤ 2 years and >2 years) serum leptin, glucose/leptin ratio and microalbuminuria. Conclusions: a strong association was found between serum leptin and glucose/leptin ratio with microalbuminuria in type 2 diabetic patients which can be used to predict the efficiency of their renal function at different durations and can be used as additional marker for the diagnosis of early stage of nephropathy.

المستخلص

لتَحرّي تأثيرِ التركيزِ العاليِ لهورمونِ الليبتن ونسبته مع الكلوكوز على الوظيفةِ الكلويةِ لمرضى السكري بنوعِ 2 في مددٍ مختلفةٍ ، وخصوصاً تأثيره على كمية طرحِ زلالِهم البوليِ . أجريت هذه الدراسة في مركزِ السُكّريِ الوطني في الجامعةِ المستنصرية في بغداد / العراق مِنْ الاول من /أيلولِ 2008 إلى الثلاثين مِنْ تموزِ 2010 مئة وثمانية مرضى ذكور مصابين بداء السكّري نوع 2 وثلاثة وخمسون شخصا غير مرضى اتخذوا كمجموعة قياسية . تم قياس كمية الكلوكوز الصائم ، الانسولين ، الليبتين ، اليوريا ، الكرياتينين و كتلة الجسم (Body Mass Index) للمرضى ومجموعة السيطرة . اظهرت النتائج ان ثلاثة وخمسون (49.07 %) مريضا من اصل 108مريضا كانوا مصابين بداء السكري لمدة سنتين او اقل ، بينما كان 55 مريضا

50.93 % يعانون من مرض السكري لأكثر من سنتين .على عكس المجموعة القياسية ، كان جميع المرضى مصابين بالبدانة . واظهرت الدراسة ان مستويات الكلوكوز الصائمِ ، الانسولين ومستويات الليبتن في مصلِ المرضى ، مرتفعة مقارنة مع المستوى الطبيعي في مصل الأشخاص الأصحاء وكانت معدلات اليوريا والكرياتينين ضمن المدى الطبيعيِ عند مجموعة المرضى ومجموعة السيطرةِ . وان معدل كمية الزلال في ادرارثلاثة وخمسون مريضا 49.07 % بالسكري نوع 2 ولمدة سنتين او اقل كانت ضمن المدى الطبيعي في حين ن معدل الزلال في ادرار 43 مريض بالسكري ولفترة تتراوح مابين (3-6)سنوات بالنوع 2 (78.18 %) و12 مريض (21.82 %)مصاب لفترةأكثر من 6سَنَواتِ بمرض السكري سجل ارفاعا قليلا عن معدله الطبيعي . وقد وجد ارتباط موجب قوي وبأحتماية أقل من 0.01 بين كمية الزلال في أدرار المرضى بالسكري بفترة أقل او اكثر من 2 سنة مع مصل اللبتين ونسبته مع الكلوكوز . نستنتج من ذلك ان بالأمكان استخدام مصل اللبتين ونسبته مع الكلوكوز اضافة الى كمية الزلال الدقيقة في الادرار كمؤشراضافي لاستدلال على كفاءةِ الوظيفةِ الكلويةِ لمرضى داء السكّري نوع 2 لمدد مختلفةِ كما ويساعد في التشخيص المراحل الأولى من التلف الكلوي .

Introduction

Type 2 diabetes mellitus is one of the most common diseases worldwide. Through lifelong vascular complications, diabetes leads to excessive rates of myocardial infarction, stroke, renal failure, blindness and amputations [1].

It results from disorders of insulin action and insulin secretion, either of which may be the predominant feature and both of which are usually present when the disease becomes clinically manifest. The disease thus, is of insidious onset and may remain asymptomatic for many years. The true duration of disease is often not known. It has been reported that duration of more than 6 years of diabetes may have existed before diagnosis [2].

About one third of type 2 diabetics will eventually have progressive deterioration of renal function. Diabetic nephropathy is a public health concern of increasing proportions. It has become the most common single cause of end-stage renal disease all over the world [3].

Insulin resistant state characterized by hyperinsulinaemia is responsible for various disorders including endothelial dysfunction and microalbuminuria. Several factors have been linked to the development of this insulin resistance like leptin, adiponectin, free fatty acids, inflammation and some genetic factors [2].

Leptin, the product of the ob gene, is a 16 kDa cytokine-like peptide that is produced primarily by adipose tissue and has been shown to regulate food intake and energy expenditure in rodents. High levels of circulating leptin characterize most cases of obesity suggesting the development of central and/or peripheral resistance [4,5].

The exact relationship between leptin and insulin is not clear and is sometimes controversial. Although insulin secreted from the pancreatic beta cells rather than from adipocytes, the secretion of both hormones influenced by overall amount of fat stores as well as by short-term changes in energy balance. Also, insulin receptors are located in the same key hypothalamic areas as leptin receptors, whereas insulin secretion is stimulated acutely in response to meals, leptin secretion is not [6,7].

Recently, a number of studies have suggested that the fasting glucose to insulin ratio (G/I) may represent another useful method for assessing insulin resistance [8]. But the fasting G/I ratio as an index of insulin sensitivity is conceptually inappropriate, so fasting glucose/ leptin ratio was predict to be a new diagnostic marker for detection

insulin resistance (IR) in a sample of Iraqi diabetes mellitus patients in addition to G/I, Quantitative Insulin-Sensitivity Check Index (QUICKI) and Homeostasis Model Assessment for Insulin resistance (HOMA-IR) indexes, and it was found more useful than them [9].

Concerning that insulin resistance is accompanied by compensatory hyperinsulinaemia and high insulin concentrations are known to induce glomerular hyperfiltration and affect renal function. Therefore, we aimed to investigate the effect of high concentration of leptin hormone and glucose/ leptin ratio on the renal function of type 2 diabetes mellitus at different durations, especially on their urinary albumin excretion as microalbuminuria (MAU).

Methods

A case control study was conducted in the International Diabetic Center at Al-Mustansiryia University in Baghdad-Iraq from first of September 2008 to the 30th of July 2010. One hundred and eight male patients with type 2 diabetes mellitus were introduced in this study. They were classified according to there duration of disease. Fifty five (50.93%) were with duration ≤2 years while the rest fifty three (49.07%) were with duration more than 2 years (3, 4, 5, 6 and more). Fifty three healthy subjects were used as a control. A questioner was designed with different questions including diabetes type, duration of disease, family history, smoking, usage of drugs, its duration and type, body mass index, hypertension, ...etc for study groups. All patients with age ≥ 30 years, newly diagnosed with type2 diabetes mellitus [10] and failed to achieve controlled fasting blood sugars (FBS) after four weeks of recommended diabetic diet were included, while those with a history of neuropathy, hepatitis, psychiatric disorders or any other illness; taking glucocorticoids or other hormones; drugs known to interact sulphonylureas and history of allergy to the investigational drugs were excluded from this study.

Ten milliliters of blood samples were aspirated from each patient and control. They asked to be fast at least (8-12) hours and not more than 12hours. Blood samples were left to clot for 10 minutes and then centrifuged for 15 minutes at 1500 xg (2500 rpm) to separate the serum.

Serum was used to evaluate fasting glucose, insulin, leptin, urea and creatinine. Serum glucose was determined using the enzymatic colorimetric method (Biomérieux kit) at 510 nm (normal reference range was 65-110 mg/dl).

Leptin and insulin were measured using High Performance Liquid Chromatography (HPLC) method at wavelength of 233 for leptin and 214 nm for insulin respectively [11]. The normal reference ranges for both leptin and insulin were (7.36±3.73) ng/ml, 2-25 µIU/ml respectively according to the American Medical Association.

Glucose/insulin ratio (G/I) ratio is easily calculated, with lower values depicting higher degrees of insulin resistance [12].

RANDOX Kits were used to determine serum urea and creatinine levels. Urinary albumin value was determined on fresh morning mid-stream urine by the RANDOX immunoturbidimetric method at 340 nm. If albumin level found between < 20 mg/day then it is normal, between (20-300) mg/day (microalbuminuria), and >300 mg/day is macroalbuminuria [13].

Statistical analysis

SPSS version 17 was used to analyze data in term of descriptive statistics such as mean and Standard Deviation (SD), student t-test and Pearson's correlation. P value <0.05 was considered to be significant.

Results

One hundred and eight male patients with type 2 diabetes mellitus (DM) were classified according to their duration of disease. Fifty three (49.07%) were ≤2 years, while 55 patient (50.93%) have DM more than 2 years. Patients and control age were comparable as shown in Table (1). Fasting serum glucose, insulin and leptin levels with their ratios in patients group were higher than those found in control serum. Levels of patient's urea and creatinine were higher than that found in control's group, but they were both within the normal range. A significant difference was found between patient's and controls BMI mean values. Patients were obviously obese while control subjects were not. A normal comparable mean standard deviation of urinary albumin values were found between patients with ≤ 2 years type 2 DM and the control subjects, while it was found to be higher in patients with > 2 years type 2 DM which exhibit the presence of microalbuminuria as shown in Table (1).

Table (1): Mean standard deviations of control (n=53) and patients group (n=108) classified according to their disease duration.

Characteristics	Type2 duration time		Control
	≤ 2 years (n=53)	>2 years (n=55)	(n=53)
Age (years)	38.3±6.3	38.2±7.2	38.2±6.2
FSG (mg/dl)	158.4±7.6	160.2±7.9	76.0±0.2
FI (µIU/ml)	28.1±2.9	28.2±1.5	14.4±1.4
FL (ng/ml)	25.3±1.0**	26.3±1.1**	7.9±1.7
F(G/I) ratio	5.5±0.8	5.6±0.7	4.1±0.1
F(G/L) ratio	6.0±1.9*	6.1±0.2*	3.4±0.8
BMI(Kg/m^2)	30.3±3.5*	30.1±1.1*	21.1±1.2
SU (mg/dl)	37.5±0.3	41.3±0.2	18.0±1.1
SCr (mg/dl)	0.8±0.2	1.0±0.1	0.6±0.1
UA (mg/day)	8.3±0.2NS	23.9±0.8*	8.0±0.0

FSG= Fasting serum glucose, FI= fasting insulin, FL= fasting leptin, BMI= body mass index, SU= serum urea, SCr= serum creatinine, and UA= urinary albumin. * *t-test* between patients group (general) and control group. NS=not significance. (*)= significance when p<0.05.

Using student t-test, no significance was found between patient's urinary albumin with ≤ 2 years duration of DM and the urinary albumin of control. But a significant p value < 0.05 was found between urinary albumin of patients with > 2 years duration of DM and the control.

In Table (2), urinary albumin, serum leptin, Glucose/leptin ratio and body mass index were classified according to patient's duration of diabetes mellitus. It was found in general that all patients with different duration of disease were obese with BMI ≥ 30 Kg/m^2. The urinary albumin (UA) in patient's ≤ 2 years duration of disease was found normal unlike those with DM more than 2 years as shown in Table-2. In addition to that, patients with normal UA have serum leptin less than patients with higher urinary

albumin than normal level (microalbuminuria). Regarding to the G/leptin ratio, it was higher in patients with > 2 years of DM than those with ≤ 2 years of disease.

Table (2): Urinary albumin, serum leptin, Glucose/leptin ratio and body mass index mean standard deviations in patients group classified according to their duration of disease.

	Patients (n=108)	*Duration (Years)*	*UA (mg/day)*	*Serum leptin (ng/ml)*	G/leptin ratio	BMI(Kg/m^2)
n=55	n=53	≤ 2	8.3±0.2	25.3±1.0	6.0±1.9	30.3±3.5
	n=43 (78.18%)	3-6	23.8±1.2	26.2±0.0	6.1±0.2	30.1±0.4
	n=12 (21.82%)	+ 6	24.0±0.3	26.4±2.0	6.1±0.3	30.2±1.8

UA=urinary albumin, n=number of patients, BMI= body mass index and G=glucose.

Using the same classification according to patient's duration of disease, correlations were studied between their urinary albumin, serum leptin, Glucose/leptin ratio and body mass index as shown in Table (3). A significant positive correlation was found between fasting leptin level and the urinary albumin in patients with >2 years DM (p=0.04). But it was found highly with p = 0.001 in those patients with ≤*2* years duration of DM. Also, a positive highly significant correlation was found between Glucose/leptin ratio and urinary albumin in patients with >2 years DM (p=0.002). While it was found significant with p = 0.04 in those patients with ≤*2* years duration of DM. Serum leptin and its ratio were found positively correlated with BMI of the diabetic patients in their different duration Table (3).

Table (3): Correlations and P values between fasting serum leptin levels and its ratio in type 2 diabetic patients classified according to their duration of disease.

	Serum leptin concentrations (r-value) , P value		Serum G/leptin ratio (r-value) , P value	
Duration	***≤2(Years)***	***>2(Years)***	***≤2(Years)***	***>2(Years)***
BMI(Kg/m^2)	**0.719,0.02***	**0.706,0.029****	**0.823,0.001****	**0.796,0.001****
UA(mg/day)	**0.788,0.001****	**0.678,0.04***	**0.668,0.04***	**0.793,0.002****

UA=urinary albumin, G=glucose and BMI= body mass index. (*)= significance when p<0.05. (**)= high significance when p<0.01.

Discussion

The term microalbuminuria is defined by a urinary albumin excretion rate higher than normal but lowers than 300mg/day, which is considered as the lowest detection limit of proteinuria as measured by standard laboratory methods in the absence of urinary tract infection and acute illness including myocardial infarction. Albumin excretion in healthy individuals ranges from (2–20)mg/day. The presence of microalbuminuria precedes the development of overt diabetic nephropathy by (10–14) years. It is at this stage that one can hope to reverse diabetic nephropathy or prevent its progression [14].

In this case-control study, 55 patients with type 2 DM more than 2 years were found microalbuminuric. Forty three out of them (78.18%) have diabetes from (3 – 6) years, while 12 out of 55 (21.82%) have diabetes more than 6 years. This result was found higher than those of [14, 15] study. Which were 37% and 25% respectively for the

same duration of disease. This increase may be due to the fact that most of the type 2 DM patients were on irregular treatment and/or poor glycemic control. Method of estimation of urinary albumin and/or microalbuminuria as well as ethnical differences would have also played a role in giving higher percentage in the present study.

[16] reported about the importance of leptin hormone secretion and its association with insulin level. They said that leptin is important in weight regulation and acts to control food intake and energy expenditure. They found that leptin concentrations increase with obesity and tend to decrease with weight loss and correlate with insulin levels in patients with hyperinsulinaemia [16] . Results showed in Table (1) were agreed with what they found because our patients were hyperinsulinemic, hyperleptinemic, and obese (BMI >30 Kg/m^2) whether the duration of disease was ≤2 years or >2 years. While BMI, insulin and leptin levels of healthy subjects were within their normal ranges Table (1).

The expression of leptin in adipocytes and its plasma concentration are both positively correlated with total adiposity. Therefore, it is generally believed that leptin represents a lipostatic factor contributing to the regulation of body weight via a negative feedback loop. In addition, plasma leptin concentrations can be acutely modulated by a variety of physiological conditions (starvation-refeeding and cold exposure) and hormonal factors (insulin, catecholamines, glucocorticoids, thyroid hormones, gonadal steroids, etc.). The observations that starvation decreases both plasma insulin and leptin levels and that obesity is strongly associated with hyperinsulinaemia and hyperleptinemia have led many researchers to investigate the effects of insulin on leptin secretion. Although several studies found that insulin stimulates leptin expression and secretion in adipocytes in vitro, others found little or no effect of insulin. The in vivo effects of insulin on leptinemia are also contradictory; some groups reported that insulin increases plasma leptin levels in rodents or humans, whereas others found that insulin does not appear to acutely regulate leptin expression or secretion. Hyperglycemia, insulin resistance and hyperleptinemia are some of the consequences of obesity [17].

It has been shown that leptin levels display ethnic differences. For example, a large population study from the United States showed that leptin levels were higher in non Hispanic blacks than in white subjects of Mexican American origin having leptin levels in between [18]. Inhabitants of the Pacific island of Kitava had lower leptin levels than whites, [19] whereas subjects of south Asian origin seem to have a higher leptin level than whites or Chinese [20, 21] In subjects of African origin, the information is not conclusive since studies showing higher leptin than in other ethnic groups [22] or no difference [23] have been published. To further explore whether differences exist in leptin levels in Arabs with and without diabetes, a study done by [24] concluded that Kuwaiti patients with type 2 diabetes showed evidence of an unfavorable metabolic profile despite having leptin levels similar to controls, and obesity influences serum leptin levels more significantly in type 2 diabetes, in which leptin levels tends to be low [24].

In this study, Iraqis as another Arab ethnic population specially those who lives in the Middle East area shows a high leptin level and high insulin level with a significant

correlation in addition to their obesity. This finding differs from [24], and it may be due to gender, because this study includes only male patients while their study included both male and female and exhibit their result as a general result [24].

Serum leptin concentrations were higher in Type 2 diabetic patients with nephropathy than normoal buminuric diabetic patients and controls.

In a previous study [9], G/L ratio was examined as a new diagnostic marker for patients with DM. It was suggested that this ratio can be used in addition to other diabetic markers to assess insulin resistance in patients with hyperglycemia. For further study and to predict whether this ratio can be used to avoid any further renal damage in patients with type 2 DM, correlation with MAU and BMI were analyzed as shown in Table (3).

From the highly significant positive correlations found between leptin, urinary albumin and G/L ratio as well as BMI, it can be conclude that increased leptin level in type 2 diabetes patients may reflect the cause of increase in their fat in addition to impairment in their renal leptin degradation which highlighted the early stage of renal disease. So, leptin and G/L ratio in addition to urinary albumin in a sample of Iraqi patients' different durations, especially ≤ 6 years can be used to predict the efficiency of their renal function and help in diagnose of the earlier stage of nephropathy. The later finding needs extensive future studies.

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