Leptin status and biochemical parameters in type 2 diabetic males from Gaza strip

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ABSTRACT

Objective: To assess leptin status and biochemical parameters in type 2 diabetic males from Gaza Strip.
Methods: The study comprised 66 type 2 diabetic males and 66 healthy non-diabetic controls from Gaza Strip. Patients and controls were age matched. Data were obtained from questionnaire interview and biochemical analysis of blood and urine samples.
Results: Diabetes was associated with family history and diet. The main self-reported complications among patients were retinopathy and cardiovascular disease. Serum leptin was significantly increased in diabetic patients compared to controls (8.1 ± 7.6 versus 5.9 ± 4.0 ng/ml, P = 0.044). Similarly, serum glucose, cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-C) were significantly higher in diabetics than controls. Conversely, serum urea, creatinine and high density lipoprotein cholesterol (HDL-C) were significantly lower in diabetics. Urinary albumin, albumin/creatinine ratio (ACR) and glomerular filtration rate (GFR) were significantly increased in diabetics whereas urinary creatinine was significantly decreased. In diabetic patients, leptin showed positive significant correlation with body mass index, BMI (r = 0.669, P < 0.001), triglycerides (r = 0.412, P = 0.001) and urinary albumin (r = 0.276, P = 0.025) and negative significant correlation with urinary creatinine (r = - 0.327, P = 0.007).
Conclusion: Hyperleptinaemia and alterations with significant values in biochemical parameters were found in type 2 diabetic patients where leptin was positively correlated with BMI, triglycerides and urinary albumin, and negatively correlated with urinary creatinine.

KEY WORDS: Leptin, Biochemical parameters, Type 2 diabetic males, Gaza Strip

Introduction

Diabetes mellitus is a chronic disease associated with hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The two main types of diabetes are named insulin dependent (type 1) and non-insulin dependent (type 2) diabetes. Lack of or severe reduction in insulin secretion due to autoimmune or viral destructions of β cells is responsible for type 1 diabetes, which accounts for 5-10% of diabetic patients. The predominant form, type 2 diabetes, accounts for more than 90% of cases [1]. Type 2 diabetes usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce it [2].

Impaired insulin secretion and increased insulin resistance in type 2 diabetes induces hepatic glucose output resulting in the development of hyperglycemia [3]. In addition, lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. Ketones are produced, and are found in large quantities in ketosis, the liver converts fat into fatty acids and ketone bodies which can be used by the body for energy [4]. Excess fatty acids in serum of diabetics are converted into phospholipids and cholesterol in liver.

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These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins [5]. Chronic hyperglycemia accompanied with dyslipidemia may largely contribute to the development of macrovascular complications (cardiovascular and cerebrovascular diseases) and microvascular

complications (diabetic nephropathy, neuropathy and retinopathy) which are the major outcome of type 2 diabetes progress [6].

Leptin is a small peptide hormone (16-kDa protein) that is mainly, but not exclusively, produced in adipose tissue. The circulating leptin concentration therefore directly reflects the amount of body fat [7]. Leptin was identified through positional cloning of the obese (ob) gene, which is mutated in the massively obese ob/ob mouse, and it has a pivotal role in regulating food intake and energy expenditure [8]. It binds to the so-called long receptor (Ob-Rb) in the hypothalamus and regulates food intake through the release of other neurotransmitters [9]. As a small peptide, leptin is cleared principally by the kidney [10]. The link of Leptin with obesity and diabetes is unclear and controversial. However, secretion of leptin is impaired in diabetes. Several authors found an increase in leptin levels in Type 2 diabetic patients [11-13], while others demonstrated a decrease in the hormone level [14,15].

The prevalence rate of diabetes mellitus in the Gaza Strip is alarming [16]. Despite that, data on the disease are lacking and restricted to annual reports emerged from the Palestinian Ministry of Health. In addition, follow up patients in Gaza hospitals and clinics are only restricted to monitoring blood glucose level when the patient visits the clinic. The present study was undertaken to assess serum leptin level as well as other biochemical parameters in blood and urine of type 2 diabetic males in Gaza Strip. Understanding the status of leptin and other biochemical parameters, and their relations could be useful in the management of the disease.

Materials and methods

Study design and study population

The present study was a case control study. The study population comprised 66 type 2 diabetic males selected randomly from Al Rimal Medical Center which is the representative clinic for diabetic patients in Gaza Strip. A total of 66 healthy non-diabetic individuals selected randomly from general population were served as controls. Patients and controls were aged matched.

Ethical consideration

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Local Ethics Research Committee. All subjects provided written informed consent prior to the study.

Questionnaire interview

A meeting interview was used for filling in the questionnaire. All interviews were conducted face to face by one investigator who had a Bachelor degree of Medical Technology. The questionnaire was based on diabetic clinic questions of Al Rimal Medical Center with some modifications [17]. Most questions were the yes/no type, which offer a dichotomous choice [18]. A questionnaire was piloted with 8 patients not included in the population sample, and modified as necessary. The questionnaire included questions related to age, smoking, family history of diabetes, diet, duration of diabetes and diabetic complications.

Body mass index

The body weight and height of each individual dressed in light clothing without shoes were measured using a carefully calibrated balance (Detecto, CAP-180 Kg, USA) for weight and vertical measuring rod for height. The BMI was calculated as Kg body weight/height in meter squared. People with BMI=18.5–24.9 were considered to have normal weight, people with BMI=25.0–29.9 were classified overweight and people with BMI $\geq$ 30.0 were considered obese [19].

Blood and urine sampling and processing

Fasting overnight venous blood samples (about 8 ml each) were collected into vacutainer tubes from 66 type 2 diabetic patients and 66 controls by a well-trained medical technologist. The blood samples were left for a while without anticoagulant to allow blood to clot. Then, serum samples were obtained by centrifugation at room temperature using Rotina 46 Hettich Centrifuge, Japan at 4000 rpm/10 minutes to be used for biochemical analysis. Random urine samples were also collected from the same patients and controls. The samples were then centrifuged by the same way as serum to precipitate all the debris and then used for urine analysis. For urinary creatinine determination, urine samples were diluted 1/20 (25 urine/475 distilled water).

Biochemical analysis

Serum leptin level was determined by competitive enzyme immunoassay technique (Diagnostic System Laboratories, USA [20]. Serum glucose was measured by glucose oxidase (GOD)/glucose peroxidase (POD) method using Labkit Kits, Spain [21]. Serum urea and creatinine were determined by urease-glutamate dehydrogenase (GDH)/UV method and by Alkaline Picrate method, respectively using BioSystems kit, Spain [22,23]. Serum cholesterol and triglycerides were estimated by cholesterol oxidase (COD)/POD method and by glycerol phosphate oxidase/peroxidase method, respectively using BioSystems kit, Spain [24,25]. High density lipoproteins (HDL-C) was determined by precipitating method using Labkit kit, Spain [26]. Low density lipoproteins (LDL-C) was calculated using the empirical relationship of Friedewald [27].

Urine analysis

Urinary albumin was determined by Immunoturbidometry-Latex method using BioSystems kit, Spain [28]. Urinary creatinine was measured by kinetic test without deproteinization using DiaSys reagent kits [29]. Albumin/creatinine ratio (mg/g) = microalbumin in urine (mg/L) x1000 /creatinine in urine (mg/dl) x10. The urine creatinine value was multiplied by 10 in order to convert mg/dL into mg/L, then divide the urine albumin value by the urine creatinine value to arrive at the ratio, then the ratio was multiplied by 1000 to express the value as (mg albumin/g creatinine). Glomerular filtration rate was calculated by Schwartz equation: GFR (ml/min/1.73m^2) = 0.55x length / serum creatinine.

Statistical Analysis

Data entry and statistical analyses were performed using Statistical Package for Social Sciences Inc, Chicago, Illinois (SPSS) computer program version 21 for windows. Simple distribution of the study variables and the cross tabulation were applied. Chi-square (χ^2) was used to identify the significance of the relations, associations, and interactions among various variables. The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between diabetics and controls leptin hormone. Pearson's correlation test was applied. The results in all the above mentioned procedures were accepted as statistical significant when the P-value was less than 5% ($P < 0.05$). Range as minimum and maximum values was used. The percentage difference was calculated according to the formula: Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100. Percent difference = (| (V1 - V2) | / ((V1 + V2)/2)) * 100.

Results

General characteristics of the study population

As indicated in Table 1, the mean age of controls was not significantly different from that of diabetics (50.0 ± 7.4 versus 49.7 ± 6.4 years, t = 1.146, P = 0.254). Similarly, there was no significant difference in the mean BMI between controls and diabetics (27.8 ± 3.9 versus 29.2 ± 4.5 kg/m^2, t = 1.866, P = 0.064). Nine (13.6%) controls and 15 (22.7%) diabetics were smokers whereas 57 (86.4) controls and 51 (77.3) diabetics were not (χ^2 = 1.833, P = 0.176). Eighteen (27.3%) controls and 36 (54.5%) diabetics reported family history of diabetes compared to 48 (72.7%) controls and 30 (45.5%) diabetics who did not (χ^2 = 10.154, P = 0.001), indicating that family history is associated with diabetes.

Table 1. General characteristics of the study population.

Characteristic	Controls (n=66)	Diabetics (n=66)		test	P-value
Age ± SD (years) *(Min - max)*	50.0 ± 7.4 *(40 - 60)*	49.7 ± 6.4 *(40 - 60)*	t	1.146	0.254
BMI ± SD (kg/m^2) *(Min - max)*	27.8 ± 3.9 *(20.8 - 34.2)*	29.2 ± 4.5 *(21.6 - 41.0)*	t	1.866	0.064
Smoking					
Yes	9 (13.6)	15 (22.7)	χ^2	1.833	0.176
No	57 (86.4)	51 (77.3)			
Family history					
Yes			χ^2	10.154	0.001
No	18 (27.3)	36 (54.5)			
	48 (72.7)	30 (45.5)			
Diet					
Yes	12 (18.2)	24 (36.4)	χ^2	5.500	0.019
No	54 (81.8)	42 (63.6)			

BMI: Body Mass Index. People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight and people with BMI ≥ 30.0 were considered obese [19]. Values are n (%) except age and BMI where values are expressed as means ± SD.

Concerning diet, 12 (18.2%) controls and 24 (36.4%) diabetics followed diet with respect to 54 (81.8%) controls and 42 (63.6%) diabetics who did not ($\square 2 = 5.500$, P = 0.019).

Duration of diabetes and diabetic complications

Table 2 shows that diabetic patients since ≤ 5 years were 44 (66.7%), whereas those with diabetic duration of 6 - 10 years were 13 (19.7%). The rest of patients 9 (13.6%) had diabetes for more than 10 years. In addition, self-reported complications among diabetic patients were retinopathy 18 (27.3%), cardiovascular diseases 12 (18.2%), recurrent infection 6 (9.9%), neuropathy 3 (4.5%) and skin lesions 3 (4.5%).

Table 2. Distribution of diabetic patients (n=66) by the duration of the disease.

Duration of diabetes (Year)	Number (%)
≤ 5	44 (66.7)
6 - 10	13 (19.7)
> 10	9 (13.6)

Leptin and other biochemical parameters of the study population

Table 3 summarized the mean levels of serum leptin and other biochemical parameters in controls and patients.

Table 3. Leptin and other biochemical parameters of the study population.

Variable	Control (n=66)	Diabetics (n=66)	% difference	t-test	p-value
Leptin (ng/ml) *(Min - max)*	5.9 ± 4.0 *(0.58 - 16.8)*	8.1 ± 7.6 *(1.7 - 39.6)*	31.4	2.032	0.044
Glucose (mg/dl) *(Min – max)*	86.6 ± 14.4 *(67 - 133)*	177.3 ± 92.7 *(78 - 445)*	68.7	7.979	< 0.001
Urea (mg/dl) *(Min – max)*	30.0 ± 5.9 *(40 - 60)*	20.2 ± 6.5 *(10 - 31)*	-39.0	-9.842	< 0.001
Creatinine (mg/dl) *(Min – max)*	0.71 ± 0.16 *(0.2 - 1.0)*	0.55±0.18 *(0.3 - 0.9)*	-25.4	5.623	< 0.001
Cholesterol (mg/dl) *(Min – max)*	161.6 ± 47.9 *(75 - 285*)	183.6 ± 38.3 *(88 - 242)*	12.7	2.956	0.004
Triglycerides (mg/dl) *(Min – max)*	155.0 ± 130.7 *(91 - 718)*	215.4 ± 72.7 *(79 - 314)*	32.6	3.336	0.001
HDL-C (mg/dl) *(Min – max)*	51.6 ± 8.5 *(32 - 66)*	43.3 ± 3.9 *(37 - 51)*	-17.5	-7.313	< 0.001
LDL-C (mg/dl) *(Min – max)*	79.1 ± 36.5 *(12 - 132)*	97.2±37.3 *(28 - 163)*	20.5	2.872	0.005

HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol. All values are expressed as mean ± SD. P < 0.05: Significant.

Serum leptin was significantly increased in diabetic patients compared to controls (8.1 ± 7.6 versus 5.9 ± 4.0 ng/ml, % difference = 31.4 and P = 0.044). Similarly, serum glucose, cholesterol, triglycerides and LDL-C of diabetics (177.3 ± 92.7, 183.6 ± 38.3, 215.4 ± 72.7 and 97.2 ± 37.3 mg/dl, respectively) were significantly higher than that of controls (86.6 ± 14.4, 161.6 ± 47.9, 155.0 ± 130.7 and 79.1 ± 36.5 mg, respectively) with % differences 68.7, 12.7, 32.6 and 20.5, and P-values of < 0.001, 0.004, 0.001 and 0.005, respectively). In contrast, serum urea, creatinine and HDL-C were significantly lower in diabetics with respect to controls (20.2 ± 6.5, 0.55 ± 0.18 and 43.3 ± 3.9 mg/dl versus 30.0 ± 5.9, 0.71 ± 0.16 and 51.6 ± 8.5 mg/dl, respectively) with % differences 39.0, 25.4 and 17.5, and P < 0.001, respectively.

Urinary Albumin, creatinine, ACR and GFR of the study population

As illustrated in Table 4, urinary albumin concentration was significantly increased in diabetics compared to controls (17.3 ± 8.5 versus 9.9 ± 6.9 mg/dl, % difference = 54.4, P < 0.001). Conversely, urinary creatinine concentration was significantly decreased in diabetics than in controls (114.6 ± 39.9 versus 156.6 ±67.2 mg/dl, % difference = 31.0, P < 0.001). ACR and GFR were significantly higher in diabetics with respect to controls (13.9 ± 5.2 mg/g and 194.0 ± 64.0 ml/min/1.73m² versus 10.8 ± 9.2 mg/g and 127.2 ± 21.9 ml/min/1.73m², % differences = 25.1 and 41.6, P = 0.018 and P < 0.001, respectively).

Table 4. Urinary Albumin, creatinine, ACR and GFR of the study population.

Variable	Control (n=66)	Diabetics (n=66)	% difference	t-test	p-value
Urinary Albumin (mg/dl) *(Min - max)*	9.9 ± 6.9 *(1.3 - 25.0)*	17.3 ± 8.5 *(3.0 - 36.0)*	54.4	5.447	< 0.001
Urinary creatinine (mg/dl) *(Min - max)*	156.6 ± 67.2 *(39 - 327)*	114.6 ± 39.9 *(42 - 203)*	-31.0	4.428	< 0.001
ACR (mg/g) *(Min - max)*	10.8 ± 9.2 *(1.3 - 29.0)*	13.9 ± 5.2 *(2.5 - 22.6)*	25.1	2.399	0.018
GFR (ml/min/1.73m²) *(Min - max)*	127.2 ± 21.9 *(67 - 153)*	194.0 ± 64.0 *(102 - 330)*	41.6	8.214	< 0.001

ACR: Albumin/creatinine ratio, GFR: Glomerular filtration rate. All values are expressed as mean ± SD. P < 0.05: Significant.

Serum leptin in relation to studied parameters of controls and diabetics

Correlations between serum leptin level and other studied parameters in both controls and diabetics are pointed out in Table 5.

Table 5. Serum leptin in relation to studied parameters of controls and diabetics.

Parameter	Serum leptin level (ng/ml)			
	Control (n=66)		Diabetics (n=66)	
	r	P-value	r	P-value
Age (years)	0.065	0.607	0.059	0.635
BMI (kg/m^2)	0.844	< 0.001	0.669	< 0.001
Glucose (mg/dl)	0.011	0.930	-0.105	0.402
Urea (mg/dl)	0.300	0.015	-0.124	0.320
Creatinine (mg/dl)	0.064	0.611	0.189	0.128
Cholesterol (mg/dl)	0.034	0.784	0.167	0.180
Triglycerides (mg/dl)	0.369	0.002	0.412	0.001
HDL-C (mg/dl)	-0.380	0.002	-0.178	0.154
LDL-C (mg/dl)	-0.131	0.295	0.029	0.814
Urinary Albumin (mg/dl)	0.321	0.009	0.276	0.025
Urinary creatinine (mg/dl)	0.144	0.248	-0.327	0.007
ACR (mg/g)	0.239	0.054	0.105	0.400
GFR (ml/min/1.73m²)	-0.042	0.736	-0.180	0.149

BMI: Body Mass Index, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, ACR: Albumin/creatinine ratio, GFR: Glomerular filtration rate. The correlation was analyzed using Pearson correlation coefficient (normally distributed data). P < 0.05: Significant, P > 0.05: Not significant.

In diabetics, Pearson correlation coefficient test showed positive significant correlation of serum leptin with BMI (r = 0.669, P < 0.001), triglycerides (r = 0.412, P = 0.001) and urinary albumin (r = 0.276, P = 0.025), and negative significant correlation with urinary creatinine (r = -0.327, P = 0.007). In controls, leptin showed positive significant correlation with BMI (r = 0.844, P < 0.001), urea (r = 0.300, P = 0.015), triglycerides (r = 0.369, P=0.002) and urinary albumin (r = 0.321, P=0.009), and negative significant correlation with HDL-C (r = -0.380, P = 0.002). However, there were no significant correlations of serum leptin level with age, glucose, creatinine, cholesterol, LDL-C, ACR and GFR in both controls and diabetics.

Discussion

Although diabetes mellitus is prevalent in Gaza strip, there is under-diagnosis and under-reporting of the disease, even no real national figure exists on its prevalence. The present study provides a wide view on biochemical features not only in blood but also in urine of type 2 diabetic patients from Gaza Strip. Serum leptin determination and its correlation with other biochemical parameters were targeted for the first time in those patients. This broad assessment may give a clearer picture on the patient condition and may help in the disease management.

The mean age of type 2 diabetic patients was 49.7 ± 6.4 years coincides with the fact that type 2 diabetes mellitus usually develops after age 40 years [30]. The family history as a risk factor for diabetes is in agreement with other studies [31,32]. The poor compliance with diet reported among diabetic patients necessitates launching of educational programs to show the importance of diet in controlling the disease. A low glycaemic load diet that adequately adheres to the principles of the traditional Mediterranean diet may reduce the incidence of type 2 diabetes [33]. The finding that almost two-thirds of the patients had diabetes for 5 years or less does confirm the idea that type 2 diabetes has a long asymptomatic pre-clinical phase which frequently goes undetected. At the time of diagnosis, the patient could have one or more diabetes complications [34]. The more frequent self-reported complications among our diabetic patients were retinopathy and cardiovascular diseases.

The present results revealed that serum leptin level was significantly increased in diabetic patients compared to controls. Similar results were previously documented [12,13,35]. However, lower levels were observed in diabe-

tes [14]. This implies that the role of leptin in type 2 diabetes is controversial and still needs further investigation. Nevertheless, it is accepted that leptin is cleared from plasma mainly by the kidney [36]. Therefore, serum hyperleptinaemia recorded in the present study may be attributed, in part, to deterioration of kidney function as indicated by disturbance in serum urea and creatinine concentrations as well as in urinary albumin, creatinine, ACR and GFR. The observed decrease in serum urea and creatinine in diabetic patients compared to controls may be explained on the basis of glomerular hyperfiltration. Marked increase of GFR in diabetic patients does support this view. Significant elevation in urinary albumin levels in our diabetic patients was documented by other authors and explained mostly as a result of impairment of kidney filtration efficiency [37]. Such elevation of urinary albumin as well as of ACR is known as a case of proteinuria and reflects a condition of diabetic nephropathy. However, it is difficult to determine the onset and stages of such changes and this may lead to controversial results.

With respect to serum lipid profile, cholesterol, triglycerides and LDL-C levels were significantly increased in diabetic patients when compared to controls whereas HDL-C level was significantly decreased in diabetics. Such findings are in concurrent with that declared in the literature [16,38]. The abnormal levels of serum lipids in diabetics is due mainly to increase in the mobilization of free fatty acids from fat depots, since insulin inhibits the hormone sensitive lipase. Excess serum fatty acids are converted into triglycerides, phospholipids and cholesterol in liver which may be discharged into blood [39].

As depicted from Pearson's correlation test, serum leptin in diabetic patients showed significant positive correlation with BMI, triglycerides and urinary albumin, and negative significant correlation with urinary creatinine. Positive association of leptin with BMI, triglycerides and urinary albumin in type 2 diabetic patients was reported [40-42]. Such associations mean alterations of serum leptin level in type 2 diabetes. The accompanying raise of leptin level with urinary albumin indicates impairment of kidney function and probably progression towards diabetic nephropathy. Leptin level was shown to be increased with the progression of diabetic nephropathy in patients with type 2 diabetes [43]. However, the exact stage of diabetic nephropathy is beyond the scope of this study. On the light of the previous results, one can say that serum leptin was elevated with significant alterations in biochemical parameters in type 2 diabetic patients where leptin was positively correlated with BMI, triglycerides and urinary albumin, and negatively correlated with urinary creatinine.

Conflict of Interest

We declare that we have no conflict of interest.

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