



Ghrelin level is associated with other Biochemical parameters in obese diabetic patients.

By

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Abstract

Aims; When T2DM associated with obesity they form a very harmful combination that exacerbates the consequences of each other leading to various disorders. The present study aims to assess the ghrelin hormone level in obese diabetic patients and to find out a possible relationship between ghrelin level with different parameters including lipid profile, glycated hemoglobin (HbA1c), insulin resistance (IR) parameters calculated from Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), HOMA insulin sensitivity (HOMA2-%S), and HOMA beta cells function (HOMA2-%B).

Methods; Sixty obese T2DM patients with were participated in the study. Lipid profile parameters and blood sugar were measured spectrophotometrically. Ghrelin and insulin measurements were carried out using ELISA technique. HbA1c were measured by ion-exchange chromatography. Insulin resistance parameters were calculated using HOMA2-Calculator software

Results; The results showed that obese diabetic patients have a poor glycemic control and more than half of obese diabetic patients were insulin resistant (IR) according to ($HOMA2-IR \geq 3$). Typical dyslipidemia are present in obese diabetic patients indicating higher risk factor for cardiovascular diseases. Mean ghrelin level is low in diabetic obese patients and non-IR patients have moderately higher ghrelin level than IR patients. Insulin sensitivity (HOMA2-%S) and beta-cell function (HOMA2-%B) indicators are lower in diabetic patients than control group. In obese diabetic patients, ghrelin level is negatively correlated with BMI, cholesterol, HbA1c, atherogenic ratios, and HOMA2-%S. Ghrelin level is positively correlated with HDL-C and HOMA2-IR.

Conclusions: Low ghrelin level is associated with poor diabetes control and bad prognosis parameters in obese diabetic patients.

Introduction:

Type 2 diabetes mellitus (T2DM) is often associated with obesity and results from insufficient insulin production/secretion and insulin resistance (IR) [1]. IR is a defect in insulin secretion by pancreatic β -cells and in the response of its receptors lead to hyperglycemia and the onset of diabetes [1] that a major pathophysiological role in T2DM and is tightly associated with major public health problems, including



obesity [2]. The consequence of its chronic complications including blindness, neuropathies, and cardiac and cerebral disorders [3].

Obesity is a wide prevalent disorder. Excess visceral fat generates chronic inflammation that eventually triggers insulin resistance and the associated comorbidities of metabolic syndrome including DM [4]. Coexistent T2DM and obesity-often termed “diabesity”-is represents a challenge to the treatment of both conditions and leads to increase morbidity and mortality from each condition [5]. Excess adiposity accentuates insulin resistance and complicates the treatment of T2DM [5, 6]. Ghrelin, identified as an endogenous ligand for the growth hormone secretagogue receptor, functions as a somatotrophic and orexigenic signal from the stomach [7]. Ghrelin secreted mainly from stomach and low-level ghrelin expression also occurs in several tissues outside the gut [8], including pancreatic islet cells, hypothalamus (where it stimulates the secretion of [growth hormone](#) from the [anterior pituitary gland](#), pituitary, lung, adrenal cortex, kidney, bone, testis and placenta) [8, 9].

Widmar et al (2007) [10] summarized the effects of ghrelin on the CNS, and subsequent glucose, lipid and energy metabolism. Ghrelin is the strongest GH secretagogue known to date, but the role of endogenous ghrelin in the regulation of circulating GH levels remains controversial. [11]. The preprandial increase in ghrelin levels was observed in humans that initiated meals voluntarily without any time- and food-related cues [12]. The present work aims to assess the ghrelin hormone level in obese T2DM patients and study the possible relationships between ghrelin and insulin resistance parameters estimated from Homeostasis Model Assessment-Insulin Resistance (HOMA2-IR), lipid profile, as a mean to predict the risk of cardiovascular diseases in obese diabetic patients, and glycated hemoglobin (HbA1c) percentage as a level of glycemic control.

Subjects and Methods:

Subjects

A-Patients: After ethics approval, eighty male patients with T2DM were participated in the study. Their age range was 52.8 ± 10.7 year. They all were obese ($BMI \geq 30$) and they were overnight fasting. The samples were collected from "Center of Diabetes and Endocrine" in Al-Sader Medical City in Najaf Governorate-Iraq during November 2010. Only 60 patients were completed all biochemical tests. They were on treatment with hypoglycemic agent glibnclamide (Daonil[®]) with or without metformin (Glucophage[®]).

Exclusion Criteria: The present study excluded the patients with the following criteria: patients with $BMI < 29$, patients with serum $TG \geq 5.32$ mmol/L to apply Fredwald's equation, and $FBS > 450$ mg/dL and fasting insulin > 57.6 mIU/l to apply the HOMA calculator software requirements. The study also excluded the patients with any obvious major diabetic complications including, heart diseases, and patients with lipid lowering medications (e.g. simvastatin or atrovastatin).

B-Controls: Thirty apparently healthy obese adult males were selected as control group. Their age and weight ranges were comparable to that of patients. None of these subjects has an obvious systemic disease.



Methods

Blood Samples

Five milliliters of venous blood samples were drawn from each patient and control. One milliliter of fresh blood were added to EDTA tube to measure the glycated hemoglobin in blood and the rest of blood was left at room temperature for 10 minutes for clotting, centrifuged 3000 rpm for 5 minutes, and then serum was separated and stored at -18°C till analysis.

Measurements

Body mass index values were calculated from the following equation $BMI = \text{Weight (Kg)} / \text{Height (m)}^2$: When BMI is larger than 30 Kg/m², the person is definitely obese.

Serum sugar and lipid profile (total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-C) were measured spectrophotometrically by enzymatic reactions using ready for use kits supplied by Biolabo[®], France. HDL-C was determined after precipitation of other lipoproteins by sodium phosphotungstate with magnesium chloride reagent and the cholesterol contents in the supernatant were measured by the cholesterol kit. Very low density lipoprotein cholesterol (VLDL-C) was calculated from $TG/2.19$ and low density lipoprotein (LDL-C) from Friedewald's formula ($LDL\text{-cholesterol} = \text{total cholesterol} - HDL\text{-C} - VLDL\text{-C}$).

Ghrelin in serum was measured by enzyme immunoassay supplied by USBio[®], USA, based on the principle of "competitive" enzyme immunoassay. Fasting serum insulin was measured using DRG[®] Insulin ELISA kit, Germany, which is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

HbA1c percentage was calculated by means of ion-exchange chromatography method employing chromatographic-spectrophotometric test using (Pre-Fil[®]) in Whole Blood supplied by Stanbio-Germany.

Insulin resistance parameters

Insulin resistance parameters were calculated from fasting glucose and insulin using HOMA calculator software downloaded freely from <http://www.dtu.ox.ac.uk/homacalculator/download.php>. It used to generate the index of insulin resistance (HOMA-IR), insulin sensitivity (HOMA-%B) and the index of β -cell function (HOMA-%B). In ideal normal-weight individual age <35 year had HOMA-IR of 1 mol. μ U/L² and HOMA-% B cell function of 100% [14]. The subject will be considered as insulin resistant when (HOMA2-IR>3) [15]

Statistical Analysis

The results were expressed as (mean $\pm$ standard deviation). Two tailed pooled t-test was used for the comparison between the patients and control groups in the measured parameters. Correlation coefficients were calculated to estimate the



correlation between parameters. All statistical analysis were performed using Excell[®] program (2007) from Microsoft Co. USA.

Results

Lipid profile components in addition to different atherogenic ratios in Table (1) showed significant increases in serum cholesterol, TG, LDL-C in obese diabetic patients as compared with control group. While there is no significant in serum HDL-C in patients in comparing with control group. The most important atherogenic ratio in the present study is TG/HDL-C that showed a significant increase in diabetic patients group in comparing with control group (Table 1). Serum ghrelin concentrations are significantly lower in obese diabetic patients than control group (Table (1)). The ghrelin levels in IR patients (55% of T2DM patients) and non-IR (45% of T2DM patients) patients were 0.620 ± 0.063 ng/ml and 0.659 ± 0.071 ng/ml, respectively. The results showed a significant increase ($p=0.031$) in ghrelin level in IR patients in comparing with non-IR patients.

Table (1) showed that the obese diabetic patients have a hyperinsulinemia and Insulin/Glucose ratio are higher in diabetic patients than control group. There is also a significant decrease in insulin sensitivity (HOMA2-%S) and β cell function (HOMA2-%B) in diabetic patients as compared with control group.

Table (2) showed the results of fasting insulin, Insulin/Glucose, HOMA2-IR, HOMA2-%S, and HOMA2-%B in IR diabetic patients and non-IR diabetic patients subgroups and control group. The results indicated severe hyperinsulinemia in obese diabetic patients and low beta cell function in non-IR patients.

The results in Table (3) showed negative correlation between ghrelin level and each BMI, HbA1c, cholesterol, cholesterol/HDL-C, and HOMA2-%S while there is a positive correlation between ghrelin and HOMA2-IR.

Table (4) showed same correlations between ghrelin and insulin resistance parameters in IR and Non-IR Patients. However, non-IR diabetic patients showed a higher correlation coefficient than IR diabetic patients.

Discussion:

Correlation between Ghrelin & Other Parameters:

According to the American Diabetes Association (ADA) guidelines 2007, the value of HbA1c should be kept below 7% in all diabetic patients [16]. HbA1c of the patients group = 8.51% which is also found in previous work and found to be correlated with different complications in T2DM patients [17]. However, in comparison with a recent study, Maki et al (2009) [18] found that HbA1c in a diabetic patients group was $6.7 \pm 0.1\%$ indicating that the obese patients in the current study need more monitoring and modifying of treatment regimn to control the disease consequences.

The dyslipidemia noticed in the patients group are common in diabetic patients and has different explanations [19]. However, HDL-C is not significantly changed due to the fact that HDL-C level increases by exercise and decrease in the physically inert people like the obese subjects under study. Subsequently, these patients are at high risk for heart diseases due to the atherosclerotic events of hypercholesterolemia and triglyceremia.



The increase in TG level and the decrease in HDL-C in the obese diabetic patients affect the TG/HDL ratio and produce more profound changes than other ratios [20]. These results may connect the ghrelin level and cardiovascular diseases through its correlation with lipid profile parameters.

The decline in ghrelin level in obese diabetic patients can be explained via the fact that circulating ghrelin levels are increased by fasting and energy restriction but decreased in T2DM [21]. Furthermore, among patients with T2DM, fasting ghrelin levels are lower in obese than lean patients [22, 23]. However, the decrease in ghrelin level by increasing glucose and insulin revealed a controversy in T2DM disease. If T2DM results from insulin deficiency (as in fasting state) then the ghrelin level should be increased. But if the T2DM pathophysiology due to insulin resistant (as in the obesity state), in this case the ghrelin level should be increased. The results in the present study indicated high ghrelin level in T2DM patients that in accordance with IR state as a main cause for T2DM in the patients group [24]. The body becomes more resistant to insulin with increasing duration of diabetes, so that insulin level is high or normal in the body but the available insulin is insufficient [25]. However, the controversy still present due to obesity of the patients that supposed to decrease the ghrelin level [24]. The best possible explanation for the high ghrelin level in diabetic patients group supposes a competition between the factors that increase ghrelin level (insulin deficiency) and factors that decrease ghrelin level (obesity, glucose, and hyperinsulinemia). The high percentage of IR diabetic patients may give the reliability to this explanation because the overall decrease in ghrelin level in diabetic patients group is due mainly to both high glucose level and obesity in addition to the increase in insulin level in IR patients.

When the results of insulin for each patient studied individually, the hyperinsulinemic patients have a very large value of insulin that affects the overall mean of insulin level of the diabetic patients group. Therefore, the subdivision of patients group into IR and non-IR is very important to obtain accurate results about the insulin level and resistant state. Hence, the patients were subdivided into IR and non-IR groups and the results of each subgroup were compared with control group and with each other as presented in Table (2).

The results in subgroups revealed also an increase in insulin level in IR and non-IR patients than control group. The result also are cited in other previous work [18] that found $HOMA2-IR=2.48\pm0.26$ and 1.00 ± 0.11 for diabetic patients and control group, respectively. The $HOMA2-IR$ level in the present work is higher than Maki et al results due mainly to the obesity in the patients under study that may exacerbate the insulin resistance [26]. This finding indicate a state of insulin resistant that due mainly to receptor defects.

Correlation between Ghrelin & Other Parameters:

The results in Table (3) showed an inverse relationship between BMI and ghrelin level even the correlation coefficient (r) value is -0.48 and has not reach the cut off value of the correlations (i.e., 0.5). These results were noticed in many other works revealed that plasma ghrelin concentration is low in obese people and high in lean people [22]. It is assumed that low total ghrelin concentrations and some genetic polymorphisms of ghrelin have been associated with obesity-associated diseases (reviewed by Olavi Ukkola 2011) [27]. In CNS Ghrelin affects the hypothalamic



appetite-regulating pathways, while in the periphery ghrelin increases adipose tissue accumulation and has a diabetogenic effect on the liver and pancreas [28]. Ghrelin levels are in general reduced in obese individuals and in subjects with IR [28].

The results in Table (3) indicating that FBS is not the major contributing factor for ghrelin level and other factors such as insulin, obesity, and lipids may affect the ghrelin level and overcome its role. These results are corresponding to the finding of Caixas et al (2002) [29] who found that, unlike the suppression of ghrelin after a meal-induced rise in insulin, parenteral administration of glucose and insulin had little effect on ghrelin levels [29]. However, both oral and intravenous glucose loads are shown to regulate ghrelin secretion in humans [30] and carbohydrate feeding resulted in lower ghrelin than fat feeding [31].

There is a negative correlation between the serum ghrelin in diabetic patients and HbA1c percentage even the r -value did not reach the cut off value but it is close to 0.05. Therefore the increase in HbA1c level means poor control and leads to decrease in ghrelin level in most but not all diabetic patients under study. The patients group in the current study was fasting and obese, hence there are many factors affecting the ghrelin level in those patients including insulin secretion [23], poor control of the diabetic patients, and IR state [26] that appears in many patients (Table 2).

The correlation between mean ghrelin level and lipid profile indicated that ghrelin level is associated with high HDL-C that is good indicator for heart health. There are many observational studies reported a positive association between ghrelin and HDL-C [33].

In adults, low total ghrelin concentration has been associated with some features of metabolic syndrome, including elevated BP, hypertriglyceridemia, high FBS, and low HDL-C [34]. Ghrelin has diverse cardiovascular effects, which are most probably ghrelin receptor mediated rather than GH mediated, since expression of ghrelin receptor has been reported in the cardiovascular system [35]. Ghrelin enhances lipogenesis and gluconeogenesis, increases triglyceride levels, and reduces fatty acid oxidation-stimulating activity; its effect to promote triglyceride deposition is greater in liver than skeletal muscle [36]. The lipid abnormalities are prevalent in diabetes mellitus because IR or deficiency affects key enzymes and pathways in lipid metabolism. In particular, the following processes are affected: apoprotein production, regulation of lipoprotein lipase, action of cholesteryl ester, transfer proteins and hepatic and peripheral actions of insulin [37].

The overall results showed negative correlations between ghrelin level and atherogenic ratios in general with a favorable to Chol/HDL-C ratio as a best ratio correlated with ghrelin level ($r=-0.549$). These results indicated an increased risk of cardiovascular diseases in diabetic patients as ghrelin level decreased. From previous biochemical results in diabetic patients, low ghrelin level are associated with most of the parameters related to harmful prognosis e.g., with insulin resistance, cholesterol, LDL-C, and TG. Risk factors for atherosclerotic events and cardiovascular disease include male sex, increased age, elevated plasma total cholesterol and LDL-C, decreased HDL-C, high blood pressure, smoking and diabetes mellitus [38]. Most of these risk factors are available in the patients under study. In the present study, low ghrelin level may be another factor associated with the risk factors for coronary heart diseases.



There is no correlation between ghrelin level and fasting insulin and Insulin/FBS ratio (Table 3) due to the fact that the diabetic patients are not a homogenous group in the secretion of insulin, some of them are IR and the others are Non-IR. Some patients were hyperinsulinemic while others are hypoinsulinemic depending on the IR state and the correlation is not linear. Hence, the insulin level only is not a good indicator for ghrelin state in obese diabetic patients.

The correlation between ghrelin and insulin hormones is arising from many facts associated with energy expenditure and appetite. Ghrelin and insulin are two hormones which play a relevant role in body weight regulation [7]. In one research it is found that after meal the rise in insulin suppress ghrelin level [39]. In contrast with these studies, Schaller et al (2003) [40] found the decrease in ghrelin after meal is not directly regulated by glucose or [29; 40]. Ghrelin secreted also from pancreas [41] and acts by paracrine effect to restrict glucose-induced insulin release at least partly via attenuation of calcium ion signaling, and that this insulinostatic action may be implicated in the upward control of blood glucose [42]. Therefore it can be concluded that ghrelin secretion may be affected by adiposity through insulin and/or glucose metabolism and plays a role in meal initiation and satiety in an inverse pattern to that of insulin.

In IR patients, the decrease in insulin sensitivity (HOMA-%S) compensatory hyperinsulinaemia develops initially (increased HOMA-%B), but the first phase of insulin secretion is lost early in the disorder. As the β -cells fail to compensate for the prevailing IR, impaired glucose tolerance and DM develops. As glucose levels rise, β -cell function deteriorates (decrease HOMA-%B) further, with diminishing sensitivity to glucose and worsening hyperglycaemia. The pancreatic islet cell mass is reported to be reduced in size in diabetic patients; humoral and endocrine factors may be important in maintaining islet cell mass [43].

The negative correlation between ghrelin level and HOMA-%S are seen also in another work that revealed that low ghrelin leads to augment insulin sensitivity [44]. Although longitudinal changes in HOMA-%B in subjects on insulin secretagogues can be useful in determining β -cell function over time, it must be remembered that any initial increase in HOMA-%B following initiation of treatment simply reflects the mechanism of action of the drug. β -Cell function cannot be interpreted in the absence of a measure of insulin sensitivity, and therefore HOMA-%S should always be reported alongside HOMA-%B. The use of HOMA to assess insulin sensitivity in subjects treated with insulin has many potential problems and needs further validation [45].

In order to obtain a better conclusion about the correlation between ghrelin level and insulin resistant parameters, diabetic patients divided into insulin resistant ($\text{HOMA-IR} \geq 3$) and insulin non resistant ($\text{HOMA} < 3$) and each group were correlated with the corresponding ghrelin level and r-values were calculated (Table 4).

It's obvious that HOMA2-IR are positively correlated with ghrelin level and HOMA2-%S was negatively correlated with ghrelin level and the r-values are greater in non-IR diabetic than in IR patients. However, HOMA2-%B is not significantly correlated with ghrelin in both groups even the r-value is greater in Non-IR patients than IR diabetic patients group. These results can be explained, in part by the failure of beta cells to respond to the changes in glucose level and the receptors are not work



properly to respond to insulin in addition to the paracrine effect of ghrelin level on insulin secretion [10].

The correlation between mean ghrelin level and every HOMA-IR, HOMA-%S, and HOMA-%B showed a significant positive correlation ($r=0.65$) between ghrelin level and insulin resistance indicator (HOMA-IR) and slight positive correlation ($r=0.30$) with beta cell function indicator (HOMA-%B). While there is a significant negative correlation ($r=-0.63$) between ghrelin level and insulin sensitivity indicator (HOMA-%S). All these findings refers to a mutual correlation between ghrelin and insulin resistance parameters but not with insulin alone or insulin/FBS ratio due to different other parameters embedded in the HOMA software that take into account the steady state between blood glucose and insulin secretion and beta cell activity in respond to the glucose change. HOMA-%B is a measure of β -cell activity, not of β -cell health or pathology [45].

Recent reports have linked the adiposity signaling hormone ghrelin to IR and T2DM. A decreased ghrelin level will improve ghrelin-related suppression of insulin secretion from β -cells [46]. Compensatory insulin secretion to IR and insulin sensitivity is the major contributors associated with the regulation of ghrelin [47]. Additionally phenomena in other diseases, altered ghrelin levels have also been observed in Cushing's syndrome and thyroid disease probably due to the secondary IR in these subjects [28].

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Table (1): Serum biochemical parameters in patients and control groups.

	Patients	Control
FBS (mmol/l)	12.01 ± 4.88	5.48 ± 1.35*
HbA1c (%)	8.51±2.23	5.28±1.14*
Cholesterol (mmol/l)	5.57 ± 1.23	4.13 ± 1.21*
TG (mmol/l)	3.17 ± 1.66	1.79 ± 0.64*
VLDL-C (mmol/l)	1.45 ± 0.76	0.82 ± 0.29*
HDL-C (mmol/l)	1.02 ± 0.35	0.98 ± 0.48
LDL-C (mmol/l)	3.16 ± 1.28	2.31 ± 1.11*
LDL-C/HDL-C	3.44 ± 1.91	3.03 ± 1.96
TG/HDL-C	3.40 ± 1.92	2.34 ± 1.72*
Chol/HDL-C	5.99 ± 2.13	5.08 ± 2.32
Insulin (μIU/ml)	18.58±15.68	6.85±1.49*
Insulin/Glucose	1.90±1.71	0.75±0.18*
HOMA2-IR	4.48±7.28	0.89±0.19*
HOMA2-%S	66.61±71.61	118.76 ±26.94*
HOMA2-%B	59.87±61.07	94.19±23.30*

(*): Significantly different (p<0.05).

	IR Diabetic Patients	Non-IR Diabetic patients	Control	Significance
Insulin	27.63 ± 16.30	9.22 ± 6.79	6.85±1.49	a,b,c
Insulin/Glucose	2.63 ± 1.93	1.14±0.92	0.75±0.18	a,b,c
HOMA2-IR	6.30 ± 4.48	1.24±0.71	0.89±0.19	a,b,c
HOMA2-%S	20.84 ± 8.96	113.96±77.26	118.76±26.94	a,b



HOMA2-%B	76.04 ± 74.86	43.15±33.95	94.19±23.30	a,b,c
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Table (2): Results of fasting insulin, Insulin/Glucose, HOMA2-IR, HOMA2-%S, and HOMA2-%B in diabetic with IR and without IR (Non-IR) subgroups and control group.

(a): Significant difference ($p < 0.05$) between Diabetes IR and Diabetes (Non-IR) groups

(b): Significant difference ($p < 0.05$) between Diabetes IR and Control groups

(c): Significant difference ($p < 0.05$) between Diabetes (Non-IR) and Control groups

Table (3): Correlation coefficient (r) of the comparisons between Ghrelin level and different parameters in Patients and control group.

	Patients	Control
Ghrelin vs. FBS	-0.11	0.20
Ghrelin vs. BMI	-0.48	-0.38
Ghrelin vs. HbA1c	-0.43	-0.36
Ghrelin vs. Cholestrol	-0.41	-0.46
Ghrelin vs. TG	-0.36	-0.40
Ghrelin vs. VLDL-C	-0.36	-0.40
Ghrelin vs. LDL	-0.26	-0.18
Ghrelin vs. HDL	0.42	0.33
Ghrelin vs. LDL/HDL	-0.44	-0.29
Ghrelin vs. TG/HDL	-0.36	-0.08



Ghrelin vs. Chole/HDL	-0.53	-0.35
Ghrelin vs. Insulin	-0.12	0.02
Ghrelin vs. Insulin/Glucose	-0.07	0.24
Ghrelin vs. HOMA2-IR	0.56	0.49
Ghrelin vs. HOMA2-%S	-0.63	-0.52
Ghrelin vs. HOMA2-%B	0.28	0.40

Table (4): Correlation coefficient (r) of the comparisons between Ghrelin level and Insulin & insulin resistance parameters in IR and Non-IR Patients.

	IR Diabetes	Non- IR Diabetes
Ghrelin vs. Insulin	-0.140	0.079
Ghrelin vs. Insulin/Glucose	0.116	0.038
Ghrelin vs. HOMA2-IR	0.448	0.775
Ghrelin vs. HOMA2-%S	-0.570	-0.701
Ghrelin vs. HOMA2-%B	0.158	0.397