

Clinical Study of Obestatin Hormone and its Relation to Diabetes Mellitus

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(Received 23 / 9 / 2012 ; Accepted 28 / 1 / 2013)

ABSTRACT

The research included an estimation of the concentration of obestatin hormone level in diabetic patients (Type I and Type II) and control.

The results demonstrated that the normal mean of obestatin hormone in serum was (306.54 ± 3.0 pg/ml) in control for both sexes, with the ages range between (less or equal to 15 - more or equal to 55) year.

The results demonstrated a significant decrease in the level of obestatin hormone in serum of type I and type II diabetic patients compared to control, and between types I & II diabetic patients.

The data showed that the level of obestatin hormone was not affected by age and sex, in control and diabetic patients, while showed a significant decrease according to body mass index (BMI) in control and diabetic patients (Type I and II). A significant gradual decrease in the concentration of obestatin as glucose concentration increases in diabetic patients was also observed.

The results showed a significant increase of adiponectin concentration between type I diabetic patient compared to type II, control, and a significant decrease in the concentration of adiponectin in diabetic patients (type II) compared with control.

Correlation coefficients of obestatin hormone with some biochemical parameters of control and diabetic patients showed that obestatin hormone has a significant negative correlation with glucose, HOMA-IR, total cholesterol and LDL-C.

Also, there was a significant positive correlation with HDL-C and adiponectin in control and diabetic patients (type I and II).

The result showed that obestatin hormone has a significant negative correlation with insulin, VLDL-C, total lipids in type II diabetic patients. Also, with triglyceride in control and type II diabetic patient.

It was concluded that low level of obestatin hormone index is a serious risk of diabetes mellitus and a major role for obestatin in diabetes mellitus type II. Moreover, the level could be used as a marker for diabetes mellitus and its role in obesity.

Keywords: Obestatin hormone, adeponectin hormone, diabetes, biochemical parameters, insulin resistance.

دراسة سريرية لهرمون الأوبستاتين وعلاقته بداء السكر
الملخص

(306.54±3.0 pg/ml)

(55 -15)

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INTRODUCTION

Diabetes mellitus is a systemic, chronic metabolic disease characterized by hyperglycemia, dyslipidemia, glucosuria and various concomitant clinical and biochemical symptoms, as well as a high risk of early death (Turk *et al.*, 2012). There are three major types of diabetes: type 1, type 2 and gestational diabetes (American Diabetes Association, 2012). Type II diabetes is characterized by a progressive worsening in secretion functions of the produced insulin and the development of peripheral resistance to insulin, rather than a deficit in insulin production (Turk *et al.*, 2012).

Recent studies showed that there might be a relationship between diabetes mellitus and newly discovered hormone obestatin (Ren *et al.*, 2009).

Obestatin is a 23 – amino acids amidated peptide hormone, and was identified recently as a product of the posttranslational processing of preproghrelin encoded by the ghrelin (GHRL) gene (Zhang *et al.*, 2005). It is predicted to yield two peptides, ghrelin and obestatin. The word obestatin is a contraction of obese, and it is derived from Latin "obedere", meaning "to devour" and "statin", denoting "suppression" (Zhang *et al.*, 2005). It was originally reported to be the ligand for the G-protein coupled receptor 39 (GPR-39) (Zhang *et al.*, 2005, 2008; Dong *et al.*, 2009).

Obestatin is mainly produced in the stomach (Zhang *et al.*, 2005; Stengel and Tache, 2012), plasma (Bang *et al.*, 2007), adipose tissue (Granata *et al.*, 2012) and different peripheral tissues (Gronberg *et al.*, 2008; Ren *et al.*, 2009).

Obestatin has been reported to reduce food intake, body weight gain, gastric emptying, jejunal and duodenum motility (Lagaud *et al.*, 2007; Fujimiya *et al.*, 2010; Lacquaniti *et al.*, 2011; Fujimiya *et al.*, 2012).

Some studies showed that obestatin promotes the survival of β -cells and stimulates the expression of the main regulatory β -cell genes, and insulin biosynthesis (Granata *et al.*, 2008). It inhibits glucose induced insulin secretion (Ren *et al.*, 2008), prevents lypolysis and acts similarly to insulin, reduced insulin resistance and reduced adipocyte, inflammation in metabolic tissue. These functions provide evidence of a noval role for obestatin in adipocyte function and glucose metabolism which suggest potential therapeutic perspectives in insulin resistance and metabolic dysfunctions (Granata *et al.*, 2012). Also, obestatin increases adiponectin hormone. Adiponectin is an adipokine specially secreted by adipocytes (Chen *et al.*, 2012 ; Granata *et al.*, 2012) known to improve insulin sensitivity by increasing energy expenditure and fatty acid oxidation. Individuals with type II diabetes mellitus often exhibited decreased adiponectin levels, and insulin sensitivity is improved by adeponectin therapy (Kadowaki *et al.*, 2006).

Aim of the research

Since there are no previous studies in Iraq about obestatin hormone, so it was suggested to study control and diabetic patients (Type I and II) and the relationship between obestatin hormone and some biochemical parameters.

MATERIALS AND METHODS

This study included (70) healthy subjects (34 female, 36 male), with age matching to the patients group as control. Seventy five patients (41 female, 34 male) with diabetes

mellitus from al-waffaa center for diabetic patients in Mosul city; were included in this study and divided into two subgroups:

Sub group I: (25) patients from both sexes and their ages range between (less or equal to 15) years old and (16-34) years old with type I diabetes mellitus.

Sub group II: (50) patients from both sexes and their ages range (35-44) years old, (45-54) years old and (equal or over than 55) years old with type II diabetes mellitus.

Fasting blood samples were taken and the serum was separated and used to estimate the following biochemical parameters.

1-Obestatin hormone was measured by enzyme linked immunosorbent assay (ELISA) technique (Cupping and Dongmei, 2009) using CUSABIO kit (China).

The analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city using BIO-TEK INSTRUMENTS, INC, USA.

2- Adiponectin hormone was measured by enzyme linked immunosorbent assay (ELISA) technique (Ouchi *et al.*, 2004), using USBIOLOGICAL kit (USA). This analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city.

3- Insulin hormone was measured By ELISA technique (Kahn and Rosenthal, 1979), using Monobind kit (USA). The analysis was performed in the immunity laboratory in the department of biology, College of Science, University of Mosul using (Labtech), UK.

4-Blood glucose was determined by the enzymatic colorimetric method (Trinder, 1969), using BIOLABO kit (France).

5-Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting insulin and glucose by the following equation:

$$\text{HOMA - IR} = \text{Insulin } (\mu\text{U/ml}) \times \text{Glucose (mmol/L)} / 22.5 \text{ (Matthews } et al., 1985).$$

6-Total lipids was determined manually by colorimetric method (Chabrol and Chardonnet, 1937).

7-Total proteins was determined by biuret method (Grandall, 1983), using BIOLABO kit (France).

8-Albumin was determined by Bromocresol Green Method (Doumas *et al.*, 1971), using BIOLABO kit (France).

9-Globulin was calculated by following the equation:

$$\text{Globulin conc.} = \text{Total protein conc.} - \text{Albumin conc. (Richterich, 1969).}$$

10- Total cholesterol was determined by the enzymatic colorimetric method (Allain *et al.*, 1974), using BIOLABO kit (France).

11- Triglycerides was determined by enzymatic colorimetric method (Fossati and Prencipe, 1982) using BIOLABO kit (France).

12- High density lipoprotein-cholesterol (HDL-C) was determined by precipitation method (Lopez-Virella *et al.*, 1977), using BIOLABO kit (France).

13- Low density lipoprotein-cholesterol (LDL-C) was calculated using the following equation:

$$\text{LDL-C (mmol/L)} = \text{Total Cholesterol conc.} - \text{HDL-C} - \text{TG conc.} / 2.2 \text{ (Burtis and Ashwood, 1982).}$$

14- Very low density lipoprotein-cholesterol (VLDL-C) was calculated using the following equation:

$$\text{VLDL conc. (mmol/L)} = \text{TG conc. (mmol/L)} / 2.2 \text{ (Fischbach, 2000).}$$

The weight and height were also measured and the body mass index (BMI) calculated as weight in kilogram divided by the square height in meters (Harvey *et al.*, 2011).

DATA ANALYSIS

The data obtained in the current study were analyzed using Statistical Package for Social Sciences (SPSS)

1. Standard statistical methods were used to determine the mean and standard error.
2. T-Test was used to compare between two parameters.
3. One way Anova (Duncan-test) is used to compare between more than two parameters.
4. Linear regression analysis [Pearson correlation coefficient (r)] was performed to identify the relationship between different biochemical parameters.
5. P-Value ≤ 0.05 was considered to be statistically significant (Kirkwood, 1988)

RESULTS AND DISCUSSION

The results (Table 1) showed that the normal mean level of obestatin hormone is (306.54 \pm 3.0 pg/ml) in control. This was approximate to the results showed by others (Lippl *et al.*, 2008; Zamrazilova *et al.*, 2008), where they showed that the concentration of obestatin in normal subjects was (298.7 pg/ml) and (267.9 pg/ml) respectively.

Table 1: The concentration of obestatin hormone in control and diabetic patients according to age and sex

Age (year)	Concentration of obestatin hormone (pg/ml) mean \pm S.E					
	Control group		Type I Diabetes		Type II Diabetes	
	Male	Female	Male	Female	Male	Female
≤ 15	293.31 \pm 16.06	329.69 \pm 17.89	257.67 \pm 7.02	268.41 \pm 8.03		
16-34	306.84 \pm 7.93	326.54 \pm 10.96	258.27 \pm 6.22	255.5 \pm 7.48		
35-44	295.53 \pm 8.07	305.66 \pm 5.58			223.82 \pm 11.14	238.81 \pm 10.97
45-54	298.65 \pm 7.11	308.70 \pm 4.62			221.50 \pm 9.64	226.28 \pm 11.21
≥ 55	294.40 \pm 13.2	307.55 \pm 5.44			229.03 \pm 11.31	236.23 \pm 11.08
Total mean \pm S.E	306.54 \pm 3.0		*259.897 \pm 3.49		**229.56 \pm 4.34	

*Significant difference at $p \leq 0.05$,

** Significant difference at $p \leq 0.01$

1. Obestatin concentration level in diabetic patients compared to control:

The data in Table (1) showed that diabetic patients (Type I and II) have a significantly lower obestatin level compared to control. The table also showed that there was a significant lower of obestatin concentration in type II diabetic patients compared to type I diabetic patients. This indicates the major role of obestatin in type II diabetic patients. These results

were in agreement with those obtained hormone by others (Qi *et al.*, 2007; Lippl *et al.*, 2008). The cause of these decreases in type II diabetic patients might due to the obestatin role in appetite regulation (Qi *et al.*, 2007) or might be also due to the increase of BMI and insulin resistance in type II diabetic patients (Cuiping and Dongmei, 2009). The cause might be due to the decrease of GPR-39 level in obese type II diabetic patients (Catalan *etal.*, 2007), but the cause of the decreases of obestatin in type I compared to control might be due to the insulin and the whole body insulin sensitivity could be regulate the obestatin secretion (Anderwald *et al.*, 2007). Furthermore, obestatin positively correlated to insulin concentrations and its actions (Chanoine *et al.*, 2006).

2. Obestatin concentration level in diabetic patients compared to control group according to the age and sex:

The results in (Table 1) showed that there were no significant differences in obestatin between males and females among diabetic patients (type I and II) groups and control group. These results were in agreement with those found by others (Guo *et al.*, 2007). The table also demonstrates that there was no significant difference in obestatin according to age groups in diabetic patient (Type I and II) and control. These results were in agreement with those obtained by (Lippl *et al.*, 2008).

3. Obestatin concentration level in diabetic patients compared to control according to BMI:

The data in (Table 2) showed that there was a significant gradual decrease of obestatin in control and type II diabetic patients as BMI increases. These results were in agreement with those performed by others (Zamrazilova *et al.*, 2008; Zhang *et al.*, 2011), which they found that obestatin decreases in obese groups compared to normal ones. Furthermore, obestatin negatively correlated with BMI (Guo *et al.*, 2007; Lippl *et al.*, 2008), which might be due to biopsy studies which reduced the number of obestatin cells in the gastric body mucosa of these individuals (Gao *et al.*, 2010). Also might be supported the hypothesis that obestatin and ghrelin interact with each other, and the balance between them might play a role in the regulation of body weight (Zhang *et al.*, 2011).

On the other hand, other investigators found that obestatin and ghrelin release is differentially regulated by meal intake in lean individuals. This regulation is impaired in obesity (Maier *et al.*, 2010).

The results in (Table 2) also showed that type II diabetic patients have a significantly lower obestatin as compared to type I diabetic patients and control when matched for BMI. These results were in agreement with those found by other investigators (Qi *et al.*, 2007; Cuiping and Dongmci, 2009). The results also demonstrate that type I diabetic patients have a significantly lower obestatin concentration compared to control.

Table 2: The concentration of obestatin hormone in control and diabetic patients according to BMI

BMI kg/m ²	Concentration of obestatin (pg/ml) mean ± S.E		
	Control group	Type I Diabetes	Type II Diabetes
≤25 normal weight	329.30 ± 4.55	*267.91 ± 1.93	*248.83 ± 2.93
26-29 over weight	*309.83 ± 1.02	*242.95 ± 6.229	*216.26 ± 1.97
≥30 obese	*281.21 ± 3.69		**198.33 ± 1.52

*Significant difference at $p \leq 0.05$.** Significant difference at $p \leq 0.01$.**4. Obestatin concentration level in diabetic patients according to the blood glucose concentration:**

In order to find relationship between obestatin concentration and glucose concentration, the groups of diabetic patients were divided according to the concentration of glucose into three levels as shown in (Table 3).

Table 3: The concentration of obestatin hormone in diabetic patients according to glucose concentration

Glucose conc. mmol/L	Concentration of obestatin (pg/ml) mean ± S.E	
	Type I Diabetes	Type II Diabetes
6.2-10	268.06 ± 3.65	260.03 ± 3.41
10.1-14	246.44 ± 3.67	219.79 ± 3.65
14.1-20	*212.40 ± 5.45	*194.31 ± 0.93

*Significant difference at $p \leq 0.05$.

The results in Table (3) demonstrate that there was a significant gradual decrease of obestatin in type I and II diabetic patients as the concentration of glucose increases. This might be due to the role of obestatin in enhancing glucose uptake in either the absence or presence of insulin and also due to the effect of obestatin in reducing insulin resistance, increasing insulin secretion from pancreatic islets (Granata *et al.*, 2012) and upregulating glucokinase expression (Granata *et al.*, 2008).

The concentration of some biochemical parameters in control and diabetic patients:

The research also involves an estimation of some biochemical parameters in diabetic patients compared with control to find the relationship between obestatin and some biochemical parameters as shown in Table (4).

1. Adiponectin concentration level in diabetic patients compared to control

The data in Table (4) demonstrate that type I diabetic patients have a significantly higher concentration of adiponectin compared to type II diabetic patients and control. These results were in agreement with results found by others (Leth *et al.*, 2008). The cause might be due to insulin's effect on adiponectin synthesis and secretion, where the insulin regulates adiponectin synthesis in vitro (Fasshauer *et al.*, 2002). Therefore, it is possible that insulin deficiency in patients with type I diabetes might be associated with an increase in adiponectin synthesis.

The data also demonstrate that type II diabetic patients have a significantly lower concentration of adiponectin compared to control. These results were in agreement with those obtained by others (Kadowaki *et al.*, 2006). The cause might be due to insulin that regulates adiponectin mRNA expression. It has been proposed that hyperinsulinemia, which is associated with insulin resistance in type II diabetic patients, might be responsible for the decrease in serum adiponectin.

2. Homeostasis model assessment insulin resistance (HOMA-IR) in diabetic patients compared to control

There was no significant difference of HOMA-IR between type I diabetic patients and control, but there was a significant difference between type II diabetic patients compared with control and type I diabetic patients as shown in Table (4). These results were in agreement with those found by others (Bakari and Onyemelukwe, 2005). The cause of that might be attributed to the increase of glucose and insulin levels in type II diabetic patients which is related to obesity.

The values of insulin conc., glucose, cholesterol, triglyceride, HDL-C, VLDL-C, LDL-C, total lipids conc., and blood proteins were listed in (Table 4). These values were similar to what were obtained in literature and can be used for correlation studies with obestatin.

Correlation between the concentration of obestatin hormone and some biochemical parameters in control and diabetic patients.

The results in (Table 5) demonstrated that there was a positive correlation between obestatin and adiponectin in control and diabetic patients (type I and II). The cause might be attributed to that the obestatin induced adiponectin in adipose tissue because obestatin reduced insulin resistance and improved insulin sensitivity (Granata *et al.*, 2012). On the other hand, there was a negative correlation between obestatin and insulin in type II diabetic patients. These results were in agreement with the results found by others (Lippl *et al.*, 2008; Zamrazilova *et al.*, 2008; Cuiping and Dongmei *et al.*, 2009). The cause might be due to that obestatin inversely correlated to insulin resistance in type II diabetic patients and to increasing BMI.

Table 4: The concentration of some biochemical parameters in control and diabetic patients

Biochemical Parameters	Control mean \pm S.E	Diabetic Patients	
		Type I Mean \pm S.E	Type II Mean \pm S.E
Adiponectin $\mu\text{g/ml}$	11.38 \pm 0.31	**19.04 \pm 0.38	**4.57 \pm 0.23
Insulin $\mu\text{u/ml}$	10.01 \pm 0.16	**4.11 \pm 0.16	9.88 \pm 0.42
Glucose mmol/L	5.07 \pm 0.25	**11.69 \pm 0.81	**10.54 \pm 0.44
HOMA-IR	2.16 \pm 0.05	1.88 \pm 0.1	**4.53 \pm 0.27
Cholesterol mmol/L	4.87 \pm 0.13	*5.96 \pm 0.16	*6.58 \pm 0.08
Triglyceride mmol/L	1.42 \pm 0.05	*2.28 \pm 0.05	**2.92 \pm 0.06
VLDL-C mmol/L	0.28 \pm 0.01	*0.46 \pm 0.02	**0.58 \pm 0.01
LDL-C mmol/L	3.32 \pm 0.13	*4.59 \pm 0.24	**5.12 \pm 1.31
HDL-C mmol/L	1.21 \pm 0.02	*0.88 \pm 0.06	*0.91 \pm 0.04
Total Lipids mg/dL	688.4 \pm 8.04	*777.88 \pm 11.82	**841.78 \pm 8.28
Total proteins g/L	71.75 \pm 0.97	**62.87 \pm 0.72	68.1 \pm 0.72
Albumin g/L	41.33 \pm 0.42	39.91 \pm 0.37	*36.26 \pm 0.36
Globulin g/L	30.44 \pm 0.46	**23.33 \pm 0.45	31.84 \pm 0.37

*Significant difference at $p \leq 0.05$.** Significant difference at $p \leq 0.01$.

On the other hand, the results also demonstrate that there was a significant negative correlation between obestatin hormone and glucose in type I and II diabetic patients and control. These results are in agreement with those found by other investigators (Guo *et al.*, 2007; Qi *et al.*, 2007; Cuiping and Dongmei, 2009). The cause might be due the effect of obestatin in improving the sensitivity and secretion of insulin and the enhancement of glucose uptake in either the absence or presence of insulin (Granata *et al.*, 2012). Also obestatin promotes β -cell and human islet cell survival and stimulates the expression of main regulatory β -cell genes (Granata *et al.*, 2008).

Moreover, there was a negative correlation between obestatin and insulin resistance (HOMA-IR) in diabetic patients (Type I and II) and control. These results are in agreement with those done by others (Cuiping and Dongmei, 2009). The cause might be due to the decrease of obestatin concentration when BMI and glucose level increases.

Table 5: The correlation between obestatin hormone and biochemical parameters in controls and type 1 and type 2 diabetes

	Obestatin	Adiponectin	Insulin	Glu.	HOMA-IR	TG	TC	VLDL-C	LDL-C	HDL-C	T.Lipid	TP	Alb.	Glob.
Control group	1.0	0.855**	-0.223	0.442*	-0.555**	0.268*	0.262*	-0.120	0.347**	0.620**	-0.216	0.233	0.277	-0.075
Type 1 Group	1.0	0.449*	0.069	0.477*	-0.568**	0.183	0.403*	-0.202	0.610**	0.572**	-0.217	0.183	0.218	0.057
Type 2 Group	1.0	0.564**	0.662**	0.521**	-0.869**	0.361*	0.347*	0.405**	0.442**	0.372**	-0.439**	0.200	0.346	0.154

*Correlation is significant at $p \leq 0.05$ **Correlation is significant at $p \leq 0.01$

Moreover, the results in (Table 5) showed that there was a significant negative correlation between obestatin and cholesterol in diabetic patients (type I and II) and control. The cause might be due to that obestatin increases the phosphorylation of AMP-activated protein kinase (Granata *et al.*, 2012) and this will inhibit the synthesis of 3-hydroxy-3methyl glutaryl-CoA reductase (HMG-CoA reductase) which contributes in the biosynthesis of cholesterol.

The results in Table (5) also, demonstrate that there was a significant negative correlation between obestatin and triglyceride in type II diabetic patients and control . The cause might be due to that obestatin promotes AMP-activated protein Kinase (Granata *et al.*, 2012) and this will inhibit acetyl CoA carboxylase which contribute in the biosynthesis of fatty acid and triglyceride (King, 2012) .

Furthermore, there was a significant positive correlation between obestatin and HDL-C in diabetic patients (Type I and II) and control.

The results of other investigators (Ander Wald-Stadler *et al.*, 2007) have supported our results. The cause might be due to that obestatin stimulates insulin secretion, so the activity of lipoprotein lipase will increase and lead to increase HDL-C, or the cause might be due to that obestatin promote adiponectin which correlated positively with HDL-C (Hsu *et al.*, 2012).

The data in (Table 5) also showed that there was a significant negative correlation between obestatin and LDL-C in control and diabetic patients (Type I and II). Also, the results showed that there was a significant negative correlation between obestatin and

VLDL-C in type II diabetic patients. The cause might be due to that obestatin increases the phosphorylation of AMPK (Granata *et al.*, 2012) and this inhibit acetyl CoA carboxylase which contribute in biosynthesis of fatty acid and lead to increase LDL-C and VLDL-C. Adinosine monophosphate activated protein kinase(AMPK) also increased fatty acid oxidation and inhibition of hepatic lipogenesis (Fogarty and Hardie, 2010).

On the other hand, the results showed that there was a significant negative correlation between obestatin and total lipid in type II diabetic patients. The cause might be due to that obestatin prevents lipolysis in adipose tissue and acts similarly to insulin which inhibits the activity of hormone sensitive lipase (Granata *et al.*, 2012).

CONCLUSIONS

It was concluded that obestatin hormone has a major role in diabetes mellitus (Type I and II), especially in type II diabetes there is relationship with BMI, and could be used as a marker for diabetes and obesity.

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