ORIGINAL ARTICLE

Ghrelin Level in Type2 Diabetes Mellitus and Obesity

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Abstract

Ghrelin, a peptide hormone is responsible for change in energy homeostasis affected by food intake and growth hormone (GH) secretion. Moreover, it also modulates glucose metabolism. Studies have suggested that levels of ghrelin fluctuates in different physiological conditions like obesity and diabetes mellitus.

Objective: The objective of the study was to compare the ghrelin levels in these three groups and to investigate whether there is any association of ghrelin with type 2 diabetes and obesity.

Material and Method; A total number of 90 subjects were enrolled in the study after considering inclusion and exclusion criteria ,among them 30 subjects were suffering from Type2DM, 30 were obese with BMI >30 and 30 were normal without Type2DM and Obesity. A comprehensive questionnaire was used to collect the data from 90 volunteers. Body height, weight, waist and hip circumference were measured according to procedure described by WHO and BMI was calculated. Glucose and ghrelin levels were measured by using Ghrelin measurement kits.

Results; All parameters of study of obese subjects were higher as compared to diabetic and control subjects. Blood glucose levels of diabetics were towards the higher side as compared with obese and control subjects. Fasting serum ghrelin was significantly lower in type 2 diabetic and obese as compared to control subjects [34.63±6.86 (diabetic), 46.97± 12.04 (obese), 171.56±28.0 (control)] $P < 0.005$. There was a negative correlation of serum ghrelin levels with fasting glucose ($r = - 0.40$, $p = 0.024$*). This study demonstrated that hyperglycemia may result in suppression of ghrelin levels in type 2 diabetic and obese subjects

Conclusion; serum Ghrelin level is decreased in T2DM and Obesity

Key words: *ghrelin, Type2DM,obesity, BMI*

Introduction

Diabetes mellitus(DM) a multi-system metabolic disorder characterized by increased serum glucose level resulting from deficiency of insulin. Diabetes is a silent killer disease affecting millions of people worldwide by causing serious health complications[(1)].

There are several types of diabetes i.e.; type 1 diabetes (T1DM), Type 2 diabetes (T2DM), gestational diabetes etc. Most common are T1DM, T2DM, and gestational diabetes. Type 1 diabetes is also called juvenile diabetes and also named as insulin-dependent diabetes mellitus (IDDM). Patients with type 1 diabetes must take insulin on regular basis[(2)].

T2DM is due to a progressive insulin secretion defect on the background of insulin resistance.

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Insulin resistance is the state of decreased actions of insulin in the body in spite of normal concentration in blood[3]". T2DM is a metabolic disorder, in which body initially makes insulin, but this glucose-controlling hormone is not properly utilized by the body. Eventually enough insulin is not produced by the pancreas to meet the body's requirement. In T2DM the body's cells become insulin resistant and muscle, fats cells do not respond properly to insulin. As a result, blood glucose cannot enter the cells to be stored for energy and results in the increase in glucose level that is called hyperglycemia[4]. T2DM is usually preceded by Pre diabetes, in which levels of glucose (blood sugar) are above normal but not high enough yet for a diagnosis of diabetes. Genetically predisposed obesity in people is considered to be one of the main causes of T2DM. As in pre diabetes, it has no classical symptoms of diabetes and remains undiagnosed for many years because hyperglycemia develops gradually at an early stage. Insufficient and imperfect insulin secretion in these patients makes them insulin resistant.

According to WHO diabetes is going to be the 7th leading cause of mortality in 2030[5]. In 2013, globally estimated prevalence of diabetes was 8.3%[6]. T2DM, a poorly understood disease is characterized by poor response to insulin. This ultimately causes insulin resistance in later stage and is mostly accompanied with inactivity and weight gain [7].

There are several early symptoms ofT2DM that includes increased urination and an increase in appetite, thirst, blurred vision, pain or numbness in the feet or hands. T2DM causes microvascular diseases such as nephropathy, retinopathy and neuropathy and also causesmacro vascular diseases such as coronary artery disease and stroke [8].

It has been reported that there is a very strong relation between diabetes and obesity. The increasing global prevalence of T2DM is tied to rising rates of obesity [9].

Obesity is a condition in which there is an excess of body fat contents the term means that the person's body weight is greater than what's considered healthy for his or her height. The weight of a body is a sum weight of body fat, muscle, bone and body water [10].

Obesity is the condition when Body Mass Index (BMI) of ≥ 30 or greater. Obesity is a growing global health problem. WHO says that obesity has nearly doubled since 1980 worldwide [11].

It is Body mass index (BMI) a parameter to asses obesity and it is calculated by measuring weight and height of the person then weight in kilograms is divided by the square of the height in meters (kg/m^2).

According to WHO an adult who has a BMI between 25 and 29.9 is in category of overweight – a person who has a BMI of 30 or higher is labelled as obese. A BMI<18.5 is considered underweight and between 18.5 to 24.9 a normal as far as weight is considered. The standard range of BMI for Asian population is given in table 1.1:

Other methods of estimating body fat and its distribution includes waist/hip circumference ratios calculation, skin fold thickness measurement etc.[12]

Table 1.1: *BMI chart for Asian population (12)*

BMI	Weight Status
Below 18.5	Underweight
18.5 – 24.9	Normal
25.0 – 29.9	Overweight
30.0 and Above	Obese

Excess body fats accumulation is the primary cause of obesity. As obesity is associated with numbers of physiological changes resulting into diseases like insulin resistance, cardiac diseases and hypertension numerous studies have reported a strong association of obesity with diabetes.

Diabetes and obesity often go hand in hand. The incidence of diabetes in 2000 was 171 million. According to WHO T2DM is on a major rise in the world and studies estimated that these numbers are set to rise to 366 million by 2030. This is probably because in developing countries there is a lack of

physical activities and people are switching more to affluent lifestyle of unhealthy diet. In obese individuals the amount of insulin produced is normal but this may not meet the requirement of the body due to insulin resistance. Insulin resistance is the hallmark of T2DM seen among obese individuals[13].

Free fatty acid (FFA) provides the most important source of energy to kidneys, liver and muscles. Adipose tissue stores FFA in triglyceride form and in periods of prolonged fasting these triglycerides are broken down by the process of lipolysis and provides energy. These fatty acids present in muscles oxidize to provide energy. Breakdown of fats into usable glucose is processed by hormone insulin. Due to insulin resistance in diabetes mellitus there is lack of FFA breakdown (lipolysis) causing an increase in free fatty acid (FFA). This is the major impact of type 2 diabetes mellitus.

In obesity, there is disturbance in the amount of hormones produced by the adipose tissue. The major hormone affected in obese state is adipokines; increased secretion of adipokines causes insulin resistance. Increase in chemokines by adipose tissue is also a feature associated with obesity. This leads to the activation of inflammatory cells like macrophages that produce cytokines. Cytokines affects insulin sensitivity by making cells insulin resistant [14].

Ghrelin is a hormone produced predominantly by specialized cells of stomach called P/D1 cells, present in the lining of stomach and is secreted in small amount from epsilon cells of pancreas. In Pituitary gland, hypothalamus, kidney, placenta and brain also have smaller amount of ghrelin. Ghrelin increases appetite by acting on the hypothalamus which is a part of the brain control appetite and promotes fat storage [15].

Another important role of hormone ghrelin is that it encourages the growth hormones (GH) secretion from the anterior pituitary gland by binding to its specialized receptors GHSR present in the anterior pituitary and theses receptors have also been found in adipose tissue, heart and hypothalamus[16]. Ghrelin is appetite-stimulating and GH-releasing peptide [17].

Initially it was believed that GH-releasing hormone (GHRH) is main factor the secretion of growth hormone (GH) and is inhibited by somatostatin a hypothalamic hormone. The discovery of ghrelin has introduced a novel regulatory pathway of GH release; however studies have proved other physiological functions of ghrelin i.e.; in controlling glucose metabolism and insulin secretion other than regulating GH release and energy homeostasis [18].

The objective of the study was to investigate the association of ghrelin with type 2 diabetes and obesity.

Method

It was a Case Control study. The study was carried out at Sakina Institute of Diabetes and Endocrinology Research (SIDER) Centre at Shalamar Institute of Health Sciences Lahore and Pakistan Medical and Research Council (PMRC) Sir Ganga Ram Hospital, Lahore.

A total of ninety subjects were included in the present study thirty were non obese diabetics, thirty were non diabetic obese. The remaining thirty were controls. A written informed consent was taken from each subject.

As far as inclusion criteria is concerned 90 subjects of both gender between age of 45-60 years with history of non-obese and T2DM (fasting blood glucose $\geq$ 126mg/dl) was confirmed from past medical record BMI $\leq$ 29.9), the other group of non-diabetic with BMI $\geq$ 29.9 and third group of Healthy normal control subjects with BMI 18.5-24.9 with equal number in all groups and according to standard value of glucose level and BMI set by WHO[19].

Ninety subjects between 45-60 years of age from those visiting Sir Ganga Ram Hospital, Lahore and SIDER centre at Shalamar Institute of Health Sciences Lahore were registered to participate in the study after their permission and consent after explaining the whole procedure..

A research proforma containing data, physical measurement like height, weight, BMI, waist circumference and family history,) was filled by the

subject of study. .

5 ml of fasting blood was taken from subjects. 2ml of blood sample was taken in gel vials for glucose estimation and 3ml was taken in gel vials and allowed to clot for serum separation. The gel vials were centrifuged at 4000 revolution per minute (rpm) for 5 minutes at 37oC to separate it from blood cells. Finally clear serum was then carefully transferred to Eppendorf tubes using micropipettes and stored at –20°C for further analyses.

Glucose and ghrelin level was estimated using commercially available diagnostic.

Determination of human ghrelin concentration was done by ELISA technique using Glory Science Kit.

Serum and reagents were brought to room temperature by gently thawing.

50µl of standard diluent was added to 6 tubes. Secondly 100µl of conc standard was pipetted in the first tube mixed gently, shifted 100µl from the first tube to the second and mixed it well. 50µl of standard dilution from the second tube was added to the third tube and same step was repeated to produce dilution series so that each 6 tubes contain 50µl. First well of the microtitre plate contain blank and 6 prepared standard dilution was pipetted to next six wells.

40µl of sample diluent and 10µl of testing sample (serum) was pipetted to each testing well and mixed gently. Sealed the microtitre plate with the adhesive strip and incubated it for 30 minutes at 37°C.

Wash solution was diluted 30 times by adding 600ml of distilled water to 20ml of wash solution. Uncovered the adhesive strip, discarded the liquid and washing buffer was added to every well washed for 30 seconds and drained. The process was repeated for 5 times through an automated Bio- Rad micro plate washer.50µl of Horseradish peroxidase (HRP) conjugated reagent was added to each well except blank and incubated it for 30 minutes at 370C. After incubation repeated the same washing step.50µl of Chromogen A and Chromogen B was pipetted to each well and kept at 37C for 15 minutes.50µl of stop solution was added to each well to stop the reaction .the blue color changed to yellow. Blank well was taken zero. Measured absorbance at 450 nm within 15 minutes after adding stop solution.

Detection range for ghrelin is between 80 – 3000 pg/ml (20)

All data was analyzed by SPSS). All the results are presented in frequency, percentage and mean ± SEM. Statistical difference analyzed by analysis of variance (ANOVA). Serum concentration of glucose, ghrelin, and biochemical profiles were analyzed by ANOVA followed by Tukeys post Hoc test. Pearson Correlation was performed to test the relationship between metabolic & anthropometric parameters.

Results

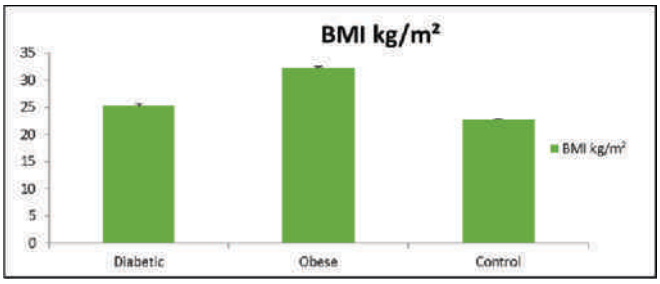
Characteristics of study population

- A total of 90 subjects (type 2 diabetic = 30, non-diabetic obese = 30 & control = 30) of both genders between 45-60 years of age were included in the study. Data were arranged on basis of the age and gender and medical updates of patients into different groups; the details of all which and the characteristics of study are given in Table 4.1.
- Whole data were taken for age and gender, the percentage of male and female was 50% in each group and in all three groups. Family history of diabetes (FHD) in diabetic subjects was 63% and family history of obesity (FHO) in obese subjects was 33% respectively. Subjects of control group were normal healthy individuals.
- Frequencies and percentages in parenthesis are given

There was no significant difference in body height of diabetics, obese and control subjects (Table 4.2). The mean body weight (BW) and BMI of obese subjects was significantly higher ($p<0.05$) than the diabetic and control group (Table 4.2) .Hip and waist circumference of obese was significantly

Table 4.1: *Characteristics of Study Population*

Frequency	Diabetics (n= 30) Frequency (%)	Obese (n= 30) Frequency (%)	Control (n= 30) Frequency (%)
Sex	15 (50)	15 (50)	15 (50)
Male	15 (50)	15 (50)	15(50)
Female			
Marital Status	30(100)	30 (100)	30 (100)
Married	0 (0)	0 (0)	0 (0)
Un Married	19 (63)	0(0)	0 (0)
Family History of Diabetes			
Family History of Obesity	0 (0)	10(33)	0 (0)



higher (p<0.05) than other two groups. Waist/hip ratio of obese was higher than diabetic and control group. Diabetics had significantly higher body weight and BMI than control group (Table 4.2).

There is a significant difference (p<0.05) in the mean fasting blood glucose (FBG) in three groups (table 4.3) . FBG of diabetic and obese subjects were significantly higher as compared to controls. There was also significant difference in FBG of obese and diabetics. The FBG level of diabetic was significantly higher as compared to obese (table 4.3). Serum ghrelin levels of diabetic and obese subjects were significantly lower as compared to control group (Table 4.3).

Weight showed a highly significant correlation with BMI and waist circumference in diabetic and obese subjects (Table 4.4). Hip and waist circumference of obese was significantly higher (p<0.05) than other two groups. There is a significant correlation of waist with hip in all three groups. (Table 4.4). Serum ghrelin level of diabetic subjects was significantly lower and showed an inverse correlation with weight and waist circumference (Table 4.4). Waist and hip circumference of obese subjects

Table 4.2: *Anthropometric variables of type 2 diabetic, obese and healthy subjects*

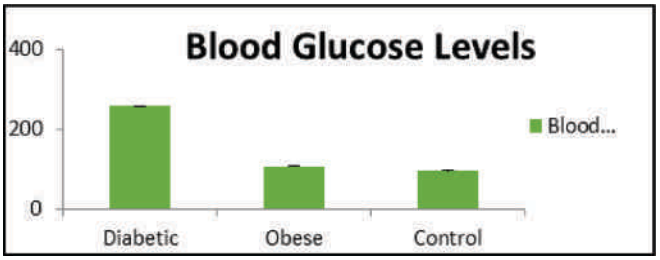
Parameters	Obese (n= 30)	Diabetics (n= 30)	Control (n= 30)	p-value
Age (years)	48 ± 0.61	51± 1.06	49 ±0.71	0.083
Height (m)	1.5 ± 0.01	.6± 0.01	1.5 ±0.01	0.088
Weight (kg)	95.7 ± 1.55$^{(a)(b)}$	74.7 ± 1.78$^{(a)}$	70.4± 1.57	**0.000***
Waist circumference (cms)	115 ± 2.35$^{(a)(b)}$	99.8 ± 1.16$^{(a)}$	89 ±2.02	**0.000***
Hip circumference (cms)	119 ± 2.18$^{(a)(b)}$	104 ± 1.47$^{(a)}$	93.5 ± 2.37	**0.000***
Wait/Hip ratio	0.95± 0.09	0.88 ± 0.08	0.84 ± 0.06	0.105
BMI (kg/m²)	32.3 ± 0.30$^{(a)(b)}$	25.4 ± 0.30$^{(a)}$	22.8± 0.10	**0.000**

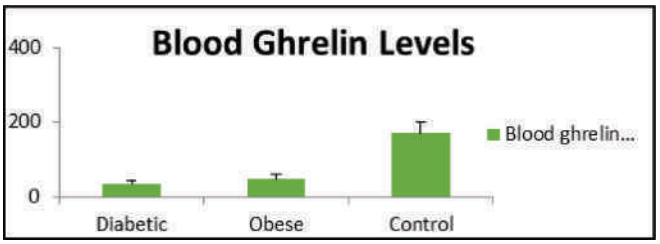
Data are presented as mean ± SEM a, b significantly different from appropriate group (P*< 0.05 Tukey Post hoc test)
a = Obese vs. control, diabetic vs. control
b= Obese vs. diabetic

Table 4.3: *Biochemical variables of type 2 diabetic, obese and healthy subjects*

Parameters	Obese (n= 30)	Diabetics (n= 30)	Control (n= 30)	p-value
Glucose (mg/dl)	107 ± 1.40$^{(a)(b)}$	257 ± 14.00$^{(a)}$	95 ± 1.70	**0.000***
Ghrelin (pg/ml)	46.97± 12.04$^{(a)(b)}$	34.63± 6.86$^{(a)}$	171.56±28.0	**0.000***

Data are presented as mean ± SEM a, b significantly different from appropriate group (P*< 0.05 Tukey Post hoc test)
a = Obese vs. control, diabetic vs. control
b= Obese vs. diabetic





correlated inversely with ghrelin ($p<0.05$) (Table 4.4) whereas no significant correlation was found between waist/hip ratio and ghrelin in three groups. There was significantly inverse correlation of serum ghrelin with glucose in diabetic subjects (Table 4.4), however no significant correlation ($p >0.05$) of ghrelin with glucose was found in obese and control subjects (Table 4.4).

Discussion

Total 90 subjects between 40 to 60 years were included in the study and divided into three groups as follows: 30 subjects with T2DM, 30 subjects with obesity and 30 controls subjects. Demographic data showed that the mean age of diabetic group was 51±1.0 years, obese and control group mean age was 48 ±0.6 years and 49 ± 0.7 years respectively.

Table 4.4: *Pearson Correlation of Anthropometric and Biochemical parameters of type 2 diabetic, obese and healthy subjects*

Parameters	Diabetics		Obese		Control	
	r	P-value	r	P-value	r	P-value
BMI – Weight	0.73	**0.000**	0.26	**0.007***	0.17	0.188
BMI –Waist	0.43	**0.008****	-0.05	0.393	0.08	0.340
BMI – Glucose	-0.26	**0.052***	-0.10	0.286	0.08	0.329
Ghrelin – Weight	-0.31	**0.047***	-0.10	0.327	0.11	0.281
Ghrelin – BMI	-0.20	0.174	-0.07	0.366	0.16	0.205
Ghrelin – Waist	-0.30	0.056	-0.35	**0.042***	0.18	0.179
Circumference						
Ghrelin – Hip	0.06	0.374	-0.44	**0.020***	0.26	0.099
Circumference						
Waist/Hip	0.96	0.31	0.341	0.20	0.16	0.191
Ratio – Ghrelin						
Ghrelin– Glucose	-0.40	**0.024***	-0.04	0.425	-0.17	0.192

Bi-variate correlation was determined by applying Pearson test.
p *< 0.05 was considered statistically significant

Although no significant difference was found between the ages of the three groups (table 4.1). Each group consists of 50% male and 50% female. The family history of participants showed that 63% of the diabetic subjects had family history of diabetes whereas in obese subjects 33% had family history of obesity (table 4.1). Mean duration of diabetes was 9.2 years. There was no significant difference ($p > 0.05$) in the mean height of the diabetics, obese and control subjects.

There was a significant difference ($p < 0.05$) in the mean weight of three groups, obese subjects had mean body weight of (95.7 ± 1.5 kg) which was significantly higher than diabetics (74.7 ± 1.7 kg) ($p < 0.05$) and control subjects (70.4 ± 1.5 kg) ($p < 0.05$). Waist circumference of three groups was significantly different and obese participants had waist circumference 115±2.3 cms which was higher than diabetics (99.8 ± 1.1 cms) and control (89 ± 2.0 cms), similarly hip circumference of obese was higher (119±2.1 cms) than diabetics (104 ± 1.4 cms) and control subjects(93.5 ± 2.3cms) (Table 4.2). Waist/Hip ratio of obese was higher (0.95± 0.09) as compared to diabetic (0.88 ± 0.08) and control (0.84 ± 0.06). Mean BMI of three groups were significantly different ($p < 0.05$) and obese group had higher mean value which was (32.3 ± 0.3) than diabetics (25.4 ± 0.3) and control participants (22.8 ± 0.1) (Table 4.2). The result was consistent with the previous investigation of Shiyaet al. where it was reported that the BMI of obese was greater than diabetics and healthy controls and BMI plays an important role in decreased ghrelin levels. The mean fasting blood glucose levels of diabetics were significantly higher than other two groups (table 4.3). Mean FBG level of diabetics was (257 ± 14 mg/dl), obese (107 ± 1.4 mg/dl) and control was (95 ± 1.7mg/dl).

The results of present study showed that mean ghrelin levels of three groups were significantly different ($p < 0.05$). Serum Ghrelin level was lower in diabetics 34.63 ± 6.8 pg/ml and obese 46.97 ± 12.0 pg/ml than healthy control 171.56 ± 28 pg/ml. Similar findings have been found in a study conducted byCruz-Domínguezet al. in which control have higher level of ghrelin as compared to diabetics and obese thus showing that disturbance in glucose metabolism may be responsible for low ghrelin levels.

The correlation of biochemical and Anthropometric parameters are shown in Table 4.4. BMI showed statistically significant correlation ($p<0.05$) with weight in diabetic and obese group. Waist circumference showed significant correlation ($p < 0.05$) with weight and hip circumference in all three groups. BMI and glucose showed significantly

negative correlation (r = -0.26, p* < 0.05) in diabetics whereas no significant relation had been found in obese between two variables. These findings were consistent with the Stepienet al study in which no significant correlation (p > 0.05) was found between BMI and glucose. In diabetics ghrelin showed negative correlation (r=-0.31, p* < 0.05) with weight however no significant correlation of ghrelin with BMI was found in any of the three groups. These results were consistent with study conducted by Cruz-Domínguezet al in which diabetics with high BMI had low ghrelin levels another study conducted by Erdmann et al reported that possibility of having low ghrelin levels in high BMI subjects is may b due to the elevation of insulin levels. Ghrelin showed statistically negative correlation with waist circumference in diabetic and obese group. Similar results have been found in the study conducted by Sharifiet al in which increase in abdominal circumference and waist circumference showed decrease in ghrelin concentrations. However no significant relation (p > 0.05) had been found between BMI and ghrelin in three groups. These findings in accordance to the findings of McLaughlin in which no association of ghrelin was observed with BMI ranges 29-35 kg/m^2. This indicated that the relationship between obesity and ghrelin mediate via insulin resistance and another study of Erdmann et al reported that insulin is an inhibitor of ghrelin secretion and obesity related hyperinsulinemia could be responsible of low ghrelin levels. Alternatively, present study may have small sample size to identify the relationship between ghrelin and obesity. Ghrelin showed significantly negative correlation (r = -0.40, p* < 0.05) with fasting blood glucose in diabetic group whereas no significant correlation had been found with obese and control group. This was consistent with the findings of Seppo et al in their study they found that ghrelin levels and serum glucose level are inversely related to each other and further added that ghrelin levels is lower patients with type 2 diabetes mellitus. Poykkoet al also got an inverse relation between ghrelin level and insulin resistant in type 2 DM, showing that degree of association between ghrelin and blood glucose changes in relation to the different glycemic level. Previous study of Cruz-Domínguezet al established an inverse correlation between serum ghrelin and fasting glucose concentration (p=- 0.039) which is consistent with the findings of present study.

In summary, the present study demonstrates that there is a significant correlation between ghrelin and glucose due to insulin resistance in type 2 diabetic and obese subjects. However no association had been found between BMI and ghrelin. These findings suggest that hyperinsulinemia associated with insulin resistance decreases serum ghrelin levels in type 2 diabetic and obese subjects. Ghrelin a newly discovered gastric hormone plays an important role in the progression of type 2 diabetes and obesity and it has become increasingly important to understand the physiological processes of ghrelin to regulate body homeostasis.

Conclusion

The present study was in the favor that there is an inverse relationship between fasting glucose and Ghrelin level. The study also demonstrated that hyperglycemia due to disturbance in glucose metabolism may result in suppression of ghrelin level in T2DM and Obesity.

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