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STUDY OF SERUM GHRELIN LEVEL IN PATIENTS WITH DIABETES MELLITUS TYPE 2: IN RELATION WITH INSULIN LEVEL

ABSTRACT

Ghrelin is an orexigenic hormone. It has a suppressive effect on insulin release from pancreatic islets. In T2DM patients, hyperinsulinemia, lower ghrelin levels are observed. Total ghrelin levels in fasting plasma were lower in T2DM patients than in non-diabetic patients. It was aimed to consider serum ghrelin windows in the T2DM patient and control. It was observed that ghrelin diabetes experiments in general patient groups were estimated to be lower (262.37±129.29pg/mL), (487.40±53.81pg/mL) compared to the control group ($p<0.001$). Patient group insulin (27.39±11.06µU/mL) and control settings (12.07±5.49µU/mL) ($p<0.001$). It also shows high yield positive rates with ghrelin and insulin history ($p<0.001$, $r=-0.699$). Significantly improved performance with serum ghrelin and insulin, culprit glucose and HbA1C. A significant relationship between ghrelin and insulin and T2DM has been investigated in this comprehensive context, and there is a content relationship between ghrelin and insulin.

Keywords: Ghrelin, Insulin, Type 2 Diabetes, Plasma, Serum

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most common diseases in the world. It damages many organs. While it can cause death, it also causes coronary artery diseases such as eye diseases and kidney diseases [1 and 2]. DM is characterized by hyperglycemia and is a dangerous disease [3 and 4]. Insulin deficiency of DM is known to contribute to disease increase in other pathological conditions [6]. The body produces insulin (the hormone that regulates blood sugar) in pancreatic β cells [5]. Lack of insulin in the body, Type 2 diabetes (T2DM) patients need regular insulin therapy to control their blood sugar levels. Without insulin, their life is in danger and it can be fatal. The main symptoms of T2DM are excessive urination and dry skin, vision changes and fatigue [7 and 8]. Hyperglycemia is the main symptom of T2DM [10] It is a rapidly increasing health problem closely related to obesity. Hyperglycemia is a high risk due to individual elements of the insulin resistance syndrome. Various pathophysiological disorders, environmental factors (obesity, unhealthy diet, and physical inactivity) contribute to deficiencies in glucose homeostasis. The most harmful risk factors for T2DM are over nutrition, sedentary lifestyle and obesity [9]. The glucose plasma criterion is often used in the diagnosis of diabetes. The HbA1c test is an excellent diagnostic tool. The diagnostic test is widely used to diagnose both FPG (126mg/dL or 7.0mmol/L for diabetic glucose) and 2hour OGTT (200mg/dL or 11.1mmol/L for plasma glucose) [11]. It is

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important to measure HbA1c at any given time and plan the subjects studied. Unpredictable glycemic and psychological stress changes do not affect HbA1c. The average blood sugar levels over the last three months are important in this. Therefore, HbA1c assessment is done approximately every 3 months [12]. In 2010, HbA1c was considered by the American Diabetes Association (ADA) as the diagnostic criterion for diabetes [13].

2. RESEARCH SIGNIFICANCE

The aim of the study was to evaluate serum ghrelin levels in T2DM patients and healthy control group. The relationship between fasting blood glucose, serum fasting metabolism and serum ghrelin was investigated. The results were determined to be quite remarkable and important.

Highlights:

- Hyperinsulinemia and ghrelin levels should be known in T2DM patients.
- It is important how the total ghrelin levels in fasting plasma compare cells with T2DM to non-diabetic cells.
- Considered to have an effect with serum ghrelin and insulin, glucose and HbA1C.

3. GHRELIN

Ghrelin is a powerful hunger inducing factor in the bloodstream. It is a regulator for energy balance in metabolism [14]. Since its discovery, ghrelin has been explored as a treatment option for many diseases [15]. Ghrelin is the hormone whose main function is to regulate appetite and energy homeostasis directly at the stomach level via an endocrine or paracrine signal. It metabolizes certain neuronal circuits in the hypothalamus [16]. Kojima et al found the hormone ghrelin in 1999 [17]. Soon after, ghrelin was called hunger. Neuropeptide Y (NPY) expressing GHSR has been reported to promote food intake by acting on hypothalamic arcuate (ARC) neurons in the nucleus [18]. Many researchers hypothesized that it could treat obesity by suppressing or reducing ghrelin [19 and 20]. As a result of the first studies showing that plasma ghrelin concentrations are sensitive to metabolic status, fasting was reported to play a strikingly important role in obese patients [21]. An increased role of GH plasma was observed after fast or calorie limit (CR). It has been suggested that ghrelin plays an important role in the energy deficit. It is now widely accepted that the benefit of CR on blood glucose neurotechnological, neurogenesis, and mood regulation is mediated by the effect of ghrelin and is a marker of hunger [22 and 23]. Moreover, its various functions increase the possibility of clinical application [24 and 25]. Diagnosing and treating ghrelin deficiency is an important avenue because of its potent ghrelin releasing activity and specificity. In ghrelin deficiency, insulin-induced hypoglycemia is the most common ghrelin stimulant, with blood glucose levels falling to 40mg/dl [26 and 27]

The treatment included ghrelin deficiency in adults and children [28 and 29]. Moreover, co-administration of ghrelin and GHRH is the most potent inducer of ghrelin release [30]. Chronic obesity can be reversed by blocking or neutralizing the effect of ghrelin [31]. On the other hand, it may be useful in the treatment of food disorders as an orexigenic agent [32]. Ghrelin injection can stimulate the appetite and improve nutritional status of these patients [33 and 34]. Central and intraperitoneal administration of ghrelin dose-dependently reduced ethanol-induced gastric ulcers [35 and 36]. Ghrelin has positive

cardiovascular effects. It is known to regulate energy metabolism [37, 38, 39, 40 and 41]. Nutrition is the most important factor for the regulation of ghrelin secretion. Plasma ghrelin concentration increases during fasting and decreases after food consumption [42 and 43]. Plasma ghrelin concentration is administered orally or intravenously [44 and 45].

In one study, it was reported that ghrelin levels increase during sleep. Plasma ghrelin concentrations have been found to be high in lean people [46 and 47]. It is known that plasma ghrelin concentration decreases due to tissue disorder [48 and 49]. Leptin administration does not alter ghrelin levels [50 and 51]. There was no statistically significant relationship between serum levels of leptin and ghrelin in patients with obstructive sleep apnea [52]. Obesity plays a very important role not only mechanically but also metabolically. The importance of sleep and ghrelin is very important in obesity. Plasma ghrelin levels are lower in obese patients than in non-obese participants [53].

4. GHRELIN IN TYPE 2 DIABETES MELLITUS

It has been suggested that ghrelin affects glucose tolerance. It was stated in the study that it plays a decisive role in pancreatic islets [54]. Stabilization of T2DM patients is the insulin resistance observed at low ghrelin level, that is, ghrelin plasma concentrations [55]. Diabetes was developed by animal experiments in 1889 [56]. One study also found discordant results in the management and isolation of pancreatic islet "insulin" [57]. In 1921, insulin was isolated, insulin was purified. Insulin experiments with subjects began to be performed [58]. Animal insulin was developed in 1923 [59]. It was determined in 1928 that insulin was a polypeptide and a dipeptide with A and B chains, respectively, in 51 amino acid sequences in 1952 [60]. Insulin is commonly synthesized in the β cells of pancreatic islets of Langerhans [61]. Insulin helps prevent blood sugar from rising (hyperglycemia) or falling (hypoglycemia) [62]. It plays an important role in glucose homeostasis, typically through direct effects on skeletal muscle, liver and white adipocytes [63]. Insulin stimulates adipocyte triglyceride stores by many mechanisms. Glucose levels in the blood are regulated by pancreatic β-cells known as insulin. Normal or high insulin production is known as insulin resistance, which is a reduced biological response [64]. It plays an important role in glucose homeostasis, typically through direct effects on skeletal muscle, liver and white adipocytes [63]. Insulin stimulates adipocyte triglyceride stores by many mechanisms. Glucose levels in the blood are regulated by pancreatic β-cells known as insulin. Normal or high insulin production is known as insulin resistance, which is a reduced biological response [64].

5. MATERIALS AND METHODS

5.1. Subjects and Study Design

This study was carried out in the Education Hospital Endocrine and Diabetes Unit. Appropriate sampling techniques were used in the study. The control group was selected by personal request from the hospital staff. Each participant was informed. Blood samples were taken from T2DM patients (70 patients, 22 healthy individuals (control group)) diagnosed according to the WHO protocol. Individuals fasted for 12 to 14 hours overnight. Venous blood samples (6ml) were drawn between 8:30 and 11:30 in the morning. The questionnaire included questions such as name, age, gender, smoking habit, type of medication used for diabetes (only for patients), family history of diabetes, physical activity, duration of diabetes, waist circumference

measurement, and body calculation. Patients who received any treatment related to mass index and diabetes were divided into two groups according to age (equal or under 40 years old, over 40 years old). The patients were divided into two groups according to their diabetes duration as 5 years and less than 5 years. BMI was calculated as weight (in kg) for each participant divided by height in square meters. 18.5g/m^2 and 24.9kg/m^2 were accepted as normal weight. 25kg/m^2 and 29.9kg/m^2were considered as overweight, and 30kg/m^2 and above were considered obese. Body Mass Index (BMI)uses weight and height to determine if an adult is in the healthy weight range, underweight.

BMI=Weight (kg)÷[height]^2(m)^2

Glycemic HbA1c below 6.5% was accepted as control. Made according to national cholesterol education program guidelines for lipids. Total serum cholesterol less than 200mg/dL, less than 200-239mg/dL, equal to or greater than 240mg/dL, serum triglyceride level less than 150mg/dL normal, 150-199mg/dL high, 200-499mg/dL hypertriglyceridemia and 500mg/dL as excessive and high-risk factor serum high-density lipoprotein cholesterol less than 40mg/dL was considered low level. Normal levels were considered greater than or equal to 40mg/dL. Serum low-density lipoprotein cholesterol above 100mg/dL was considered optimal, 100-129mg/dL close to optimal, 130-159mg/dL as a borderline high-risk factor, 160-189mg/dL as high, equal to 190mg/dL or a greater risk factor was considered a very high-risk factor. The cases were divided into two groups according to their smoking habits. Serum insulin level (2.6-24.9µU/mL) was accepted as normal range, ≥25µU/mL as high range, and ≥21.9ng/mL as very high range. Serum leptin level (2.5-21.8ng/mL) was accepted as the normal range, and ≥21.9 ng/mL was considered as the high range. For the analysis of HbA1c, 2ml was collected in a vacuum tube containing the anticoagulant K3 Edta, and the remaining 4ml was collected in vacutainer system gel separator tubes. Serum was separated from whole blood after coagulation using centrifugation (Hitachi, model O5P-21) for 10 min at 5000rmp. Serum glucose, total cholesterol, triglycerides, HDL, LDL, ghrelin and insulin were determined. Serum ghrelin was measured using Elisa. Serum insulin was measured with the auto analyzer cobas E411 and glycated hemoglobin (HPLC D10) autoanalyzer device.

5.2. Statistical Analysis

Worked with statistical package for social science (SPSS) version 23 for data analysis. The t-test was used for the comparison ratio. A p-value of $p \leq 0.05$ was considered statistically significant, while a p-value of $p<0.001$ was considered statistically highly significant. One-way Anova was used to compare more than two groups. Pearson correlation was used to determine the correlation coefficient between study parameters.

6. RESULTS

6.1. General Characteristic of Studied Participants

In this study, we take 92 samples in which 70 diabetic patients compared with 22 healthy controls. The samples were considered as 43 males and 49 females. They were divided as 77 subjects 84% of whom non-smokers, in comparison with 15 smokers 16%, and out of 68 subjects, 74% did not do exercise while 24 subjects 26% were physically active. Furthermore, the study showed that (33, 36%) of participants had a family history of diabetes. The mean age of the total subjects was 49.52±9.93 years (Table1). The BMI mean ±SD was 29.72±4.79kg/m^2. According to the duration of disease, the patients

that have diabetes mellitus during ≤5 years were in the mean (32,45.7%), and (38,54.3%) in the case that has in >5 years (Table 1).

Table 1. General characteristic of studied participants

Subject Characteristics (n=92)	Frequency Distribution	
	Frequency or Mean, Median	Percentage or S.D, IQ
Subject Categorizes		
Control	22	24%
Diabetic	70	76%
Age (Years)*	49.52	9.93
Gender* **		
Male	43	47%
Female	49	53%
BMI *		
kg/m^2	29.72	4.79
Duration of Diabetes (Years)***		
≤5 years	54	59%
>5 years	38	41%
Family History of Diabetes* **		
Positive	33	36%
Negative	59	64%
Exercise* **		
Yes	24	26%
No	68	74%
Smoking* **		
Yes	15	16%
No	77	84%
*Mean and S.D for normal, **Median, and interquartile for non-normal data and ***Frequency and percentage were performed in the calculations		

In addition, the mean ±SD of biochemical indicators of glucose, HbA1c, insulin, ghrelin, cholesterol, triglyceride, HDL and LDL levels were 156.72±63.38mg/dl, 7.96±2.13%, 23.72±11.95µU/mL, 316.18±152.52pg/mL, 176.93±38.49mg/dl, 185.38±82.47mg/dl, 44.21±10.43mg/dl, 102.50±36.78mg/dl, respectively (Table 2).

Table 2. General characteristics of biochemical indicators

Biochemical Indicators	Frequency Distribution	
	Frequency or Mean, Median	Percentage or S.D,IQ
Glucose (mg/dl)*	156.72	63.38
HbA1c (%) **	7.96	2.13
Insulin *	23.72	11.95
Ghrelin**	316.18	152.52
Cholesterol (mg/dl)*	176.93	38.49
Triglyceride (mg/dl)**	185.38	82.47
HDL (mg/dl)*	44.21	10.43
LDL (mg/dl)**	102.50	36.78
*Mean and S.D for normal, **Median, and interquartile for non-normal data and ***Frequency and percentage were performed in the calculations		

In this study when stratification of patients, and subject characteristic based on the study group, we recognized that the majority of the sample's patients and controls, were females (35.50%) diabetic patients and (14.64%) were non-patients and males (35.50%) diabetic patients and (8.36%) in the control group there was no significant statistical difference between the subject genders (p=0.398). Besides, we found that the study detected that most of the participants were non-smokers in the two studied group, 82%, 84% control, and diabetic groups respectively (p=0.785), whereas the majority of the controls (64%), and diabetic patients (54%) were non-exercisers (p=0.208). Concerning the family history, the study revealed that most of both the control group and diabetic patients' group have a positive family history with (82%), (59%) respectively (p<0.05). The mean ±SD of age was found in the controls (41.22±7.83) and diabetic patients (52.12±9.09) with a statistically substantial

difference (p<0.001). The mean ±SD of BMI, glucose, HbA1c, ghrelin, insulin and triglyceride, in diabetic patients, was greater than in the control subjects, BMI (kg/m^2) 30.50±4.70 (p<0.01), glucose (mg/dl) 176.64±59.90 (p<0.001), HbA1c (%) 8.81±1.71 (p<0.001) ghrelin (pg/mL) 262.37±129.29 (p<0.001), insulin (µU/mL) 27.39±11.06 (p<0.001), and triglyceride (mg/dl) 203.24±80.88 (p<0.001), as shown in (Table 3). On the other hand, the study showed the mean ±SD of HDL, in diabetic patients to be lower than in control subjects, HDL (mg/dl) 42.3±8.59 (p<0.01). Other characteristics were not different in a substantial way, cholesterol (mg/dl) (p=0.405), LDL (mg/dl) (p=0.320) (Table 3).

Table 3. Patients and subject characteristics according to control, and diabetic patients

Subjects, Characteristics (n=92)		Frequency Distribution		p-value (Two Sides)
		Controls(n=22)	Diabetic (n=70)	
Age (Years)*		41.22±7.83	52.12±9.09	<0.001
Gender***	Male Female	8(36%) 14(64%)	35(50%) 35(50%)	0.398
BMI ** kg/m^2		27.22±4.55	30.50±4.70	<0.01
Family History of Diabetes*** Positive Negative		18(82%) 4(18%)	41(59%) 29(41%)	<0.05
Duration of Diabetes (Years)*** ≤5 >5		No Diabetic	32(46%) 38(54%)	
Exercise***	Yes No	8(36%) 14(64%)	16(23%) 54(77%)	0.208
Smoking***	Yes No	4(18%) 18(82%)	11(16%) 59(84%)	0.785
Glucose (mg/dl)**		93.32±9.18	176.64±59.90	<0.001
HbA1c (%) **		5.25±0.17	8.81±1.71	<0.001
Insulin**		12.07±5.49	27.39±11.06	<0.001
Ghrelin**		487.40±53.81	262.37±129.29	<0.001
Cholesterol (mg/dl)*		172.13±26.45	178.44±41.61	0.405
Triglyceride (mg/dl)*		128.54±59.45	203.24±80.88	<0.001
HDL (mg/dl)*		50.31±13.35	42.3±8.59	<0.01
LDL (mg/dl)*		108.36±26.71	100.65±39.40	0.320
*Mean and S.D for normal, **Median, and inter quartile for non-normal data and ***Frequency and percentage were performed in the calculations				

6.2. Ghrelin and Insulin level in T2DM Patients, and Healthy Subjects in Relations with Age Group

In a cross-sectional study, we take a mean age of 49.52±9.93 we found in this range that there was no relationship between Insulin and ghrelin in type 2 diabetes mellitus and healthy subjects with age group, therefore, serum ghrelin and insulin statistically no significant with age (Table 4).

Tablo 4. Ghrelin and insulin in T2DM patients, and healthy subjects with age group

Biochemical Parameters	Mean ±SD			Mean ±SD		
	Diabetic (n=70)			Controls (n=22)		
	<=40Year(n=4)	>40 Year(n=66)	p-value	<=40 Year(n=13)	40 Year(n=9)	p-value
Insulin	26.65±9.48	27.43±11.21	NS	12.95±5.11	10.81±6.08	NS
Ghrelin	214.75±73.40	265.25±131.71	NS	497.23±63.09	473.22±35.30	NS
NS:Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.						

6.3. Ghrelin and Insulin Level in T2DM Patients, and Healthy Subjects with BMI Group

In this study, in the patient group the relation of insulin and ghrelin with BMI were statistically no significant, on the other hand in the control group we found that the means ±SD of serum insulin

positively related to BMI by increasing the BMI insulin level increased, the control group with BMI <25 (kg/m^2) insulin means was 7.79±4.05 (µU/mL) (p<0.05), BMI 25-29.9 (kg/m^2) insulin means was 8.27±1.42 (µU/mL) (p<0.05), BMI >30 (kg/m^2) insulin means was 15.48±4.64 (µU/mL) (p<0.05), but ghrelin in the control group was statistically no significant (Table 5).

Table 5. Ghrelin and insulin level in T2DM patients, and healthy subjects with BMI group

Biochemical Parameters	Mean±SD			Mean±SD		
	Diabetic (n=70)			Controls (n=22)		
	Male(n=35)	Female(n=35)	p-value	Male(n=8)	Female(n=14)	p-value
Insulin	29.57±12.24	25.20±9.43	NS	12.36±6.24	11.91±5.26	NS
Ghrelin	258.94±132.70	265.80±127.62	NS	499.25±76.97	480.64±36.76	NS
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.						

6.4. Ghrelin and Insulin Level in T2DM Patients, and Healthy Subjects With Gender Group

In this study, as a result, it showed that the means ±SD of serum insulin and GH related to gender statistically no significant in the patient and control group (Table 6).

Table 6. Ghrelin and insulin level in T2DM patients, and healthy subjects concerning gender group

Biochemical Parameters	Mean ±SD			Mean ±SD		
	Diabetic (n=70)			Controls (n=22)		
	Yes(n=16)	No(n=54)	p-value	Yes(n=8)	No(n=14)	p-value
Insulin	22.70±6.13	28.77±11.84	<0.05	10.05±5.65	13.23±5.25	NS
Ghrelin	349.62±133.28	236.51±9.89	<0.05	519.37±70.73	469.14±31.54	NS
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.						

6.5. Ghrelin and Insulin Level in T2DM Patients, and Healthy Subjects To a Physical Activity Group

In this study, as a result, it showed that the means ±SD of serum insulin and ghrelin related to the physical activity group statistically no significant in the control group, while we found that the insulin was related to the physical activity of patients group and means ±SD of serum ghrelin and inversely related to physical activity by increasing the physical activity ghrelin level decreased in patients' group (Table 7).

Table 7. Ghrelin and insulin level in T2DM patients, and healthy subjects with physical activity group

Biochemical Parameters	Mean±SD			
	Diabetic (n=70)			
	<25kg/m^2(n=6)	25-29.9kg/m^2(n=30)	>=30kg/m^2(n=34)	p-value
Insulin	28.93±4.56	28.90±13.60	25.78±9.22	NS
Ghrelin	194.16±109.59	261.23±124.21	275.41±136.20	NS
Biochemical Parameters	Controls (n=22)			
	(n=6)	(n=4)	(n=12)	p-value
Insulin	7.79±4.05	8.27±1.42	15.48±4.64	<0.05
Ghrelin	519.50±70.60	458.25±25.65	481.08±46.56	NS
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.				

6.6. Ghrelin and Insulin Level in T2DM Patients with The Duration Group

In our study, we found that the means ±SD of serum ghrelin-related with a duration of diabetic diagnosing, in the diabetic patient group the means ±SD of serum who ≤5years diagnosed was 301.87±141.42pg/mL ($p<0.05$), while in the group who >5years diagnosed was 229.10±109.22pg/mL ($p<0.05$), but the relationship between the means ±SD of serum insulin with a duration of disease diagnosing was statistically no significant (Table 8).

Table 8. Ghrelin and insulin level in T2DM patients with the duration group

Biochemical Parameters	Mean±SD		
	Diabetic (n=70)		
	<=5 Year(n=32)	>5 Year(n=38)	p-value
Insulin	27.30±14.06	27.45±7.92	NS
Ghrelin	301.87±141.42	229.10±109.22	<0.05
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.			

6.7. Ghrelin and Insulin Level in The Whole Study Population With

In this study, there was a positive relationship between means ±SD of serum insulin with fasting blood glucose level (statistically significant), in the group that had fasting glucose level ≤110mg/dL the means of serum insulin was 13.65±5.40µU/mL ($p<0.001$), while who had fasting glucose level >110mg/dL the means of serum insulin was 29.36±10.87µU/mL ($p<0.001$), on the other hand, we found an inverse relationship between means ±SD of serum ghrelin with fasting blood glucose level (statistically significant), in the group that had fasting glucose level ≤110mg/dL the means of serum ghrelin was 479.12±57.22pg/mL ($p<0.001$), while who had fasting glucose level >110mg/dL the means of serum ghrelin was 225.05±100.94pg/mL ($p<0.001$) (Table 9).

Table 9. Ghrelin and insulin level in the whole studypopulation with a fasting blood glucose level group

Biochemical Parameters	Mean±SD		
	Whole study population (n=92)		
	Fasting Blood Glucose Level <=110mg/dl(n=33)	Fasting Blood Glucose Level >110 g/dl(n=59)	p-value
Insulin	13.65±5.40	29.36±10.87	<0.001
Ghrelin	479.12±57.22	225.05±100.94	<0.001
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.			

6.8. Ghrelin and Insulin Level in The Whole Study Population with The Hba1c Level Group

In the whole study population ghrelin level inversely but insulin positively in relations with HbA1C level group showed that the serum insulin concentration was statistically lower but ghrelin was higher in subjects those have <6.5% HbA1c levels, than those of ≥6.5% HbA1c in p-value (<0.001), means±SD of serum insulin was 14.02±6.35, 28.42±11.19µU/mL ($p<0.001$), serum ghrelin was 464.900±89.46, 244.22±117.71pg mlp-value (<0.001) (Table 10).

Table 10. Ghrelin and insulin level in the whole study population with HbA1C level group

Biochemical Parameters	Mean ±SD		
	Whole Study Population (n=92)		
	HbA1C Level<6.5(n=30)	HbA1C Level>=6.5(n=62)	p-value
Insulin	14.02±6.35	28.42±11.19	<0.001
Ghrelin	464.900±89.46	244.22±117.71	<0.001
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.			

6.9. Ghrelin Level in Whole Study Population Subjects with The Insulin Group

Ghrelin level in whole study population subjects in relations with Insulin group revealed an inverse relationship (statistically significant), when the level of serum insulin concentration increases the level of serum concentration will decrease, the subjects who were at insulin concentration <25μU/mL had a means of serum ghrelin 327.82±152.36pg/mL (p<0.05), insulin concentration ≥25μU/mL had a means of serum ghrelin 238.58±114.76pg/mL (p<0.05) (Table 11).

Table 11. Ghrelin level in whole study population subjectswith the Insulin group

Biochemical Parameters	Mean±SD		
	Whole Study Population (n=92)		
	Insulin Level <25(n=80)	Insulin Level≥25(n=12)	p-value
Ghrelin	327.82±152.36	238.58±114.76	<0.05
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.			

6.10. Insulin Level in The Whole Study Population with The Ghrelin Group

Insulin level in the whole study population in relations with ghrelin group gave as a n inverse relationship (statistically significant), the 59 subjects who were in serum ghrelin concentration <520pg/mL had a means of serum insulin 29.36±10.87μU/mL (p<0.001), while other 33 subjects those who was in serum ghrelin concentration >520pg/mL had a means of serum insulin 13.65±5.40μU/mL (p<0.001) (Table 12).

Table 12. Insulin level in the whole study population with the ghrelin group

BiochemicalParameters	Mean±SD		
	Whole Study Population (n=92)		
	Ghrelin Level <520(n=59)	Ghrelin Level>520(n=33)	p-value
Insulin	29.36±10.87	13.65±5.40	<0.001
NS: Statistically No Significant p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test was performed for statistical analysis.			

6.11. Pearson Correlation (r) Between Parameter (Correlation Analysis)

According to Pearson correlation coefficient (r), our results in the whole study population, showed that serum insulin level had positively no significantly relations with each of age, BMI, cholesterol, LDL, (r=0.192, p=0.606), (r=0.101, p=0.337), (r=0.180, p=0.086), (r=0.174, p=0.097) (Table 13). Serum insulin level had positively with high significantly relations with each of (Triglyceride, FBS and HbA1c, (r=0.512**, p<0.001), (r=0.681**, p<0.001), (r=0.603**, p<0.001), but had a negative correlation with

ghrelin and HDL, (r=-0.699**, p<0.001), (r=-0.319**, p<0.001) (Table 13).

Table 13. Correlation between Insulin and other parameters in the study population women

Parameter	(r)	p-value
Age (Year)	0.192	0.066
BMI(kg/m^2)	0.101	0.337
Cholesterol	0.180	0.086
Triglycerides	0.512**	<0.001
HDL	-0.319**	<0.001
LDL	0.174	0.097
FBS	0.681**	<0.001
HbA1c	0.603**	<0.001
Ghrelin	-0.699**	<0.001
*Represent low significant correlation **Represent high significant correlation		

7. DISCUSSION

T2DM is a chronic condition [65]. T2DM is the leading cause of non-traumatic blindness and kidney failure. It is rapidly becoming more common. It has also been stated to be effective in dementia that occurs as a result of diseases such as Alzheimer's and vascular dementia [66]. In addition, hyperpigmentation of the skin, sexual dysfunction and recurrent infections are among other complications. There is also a link between T2DM and mild hearing loss [67]. Insulin is a peptide hormone found in pancreatic islets [68]. It controls carbohydrate, fat and protein metabolism by promoting glucose absorption from the blood to the liver and fat [69]. The common pathology of T2DM is a pathological condition in which cells become resistant to insulin and lead to high blood sugar [70]. Ghrelin is a peptide that is an endogenous natural ligand for growth hormone (GH) [17].

Ghrelin regulates nutrition, body weight and energy metabolism. Studies have shown that it plays a role in glucose metabolism as well as in the control of insulin secretion and sensitivity [71]. In our study, we investigated 70 diabetic patients (262.37±129.29pg/mL) with 22 control subjects (487.40±53.81pg/mL) with a mean ±SD (p<0.001) serum ghrelin level of serum ghrelin (Table 3). T2DM subjects had fewer fasting serum targets of total ghrelin compared to control subjects. The difference was differentiated by age, selection, and BMI in multi-talented models. Concerning the family history, the study revealed that most of both the control group and diabetic patients' group have a positive family history with (82%), (59%) respectively (p<0.05). The mean ±SD of age was found in the controls (41.22±7.83) and diabetic patients (52.12±9.09) with a statistically substantial difference (p<0.001). The mean ±SD of BMI, in diabetic patients, was greater than in the control subjects, BMI (kg/m^2) 30.50±4.70 (p<0.01). as shown in (Table 3). These results in our studies have been supported by studies [44 and 72]. The mean ±SD of serum insulin in inpatient diabetes was higher than the control group (27.39±11.06, 12.07±5.49) µU/mL (p<0.001), this result is consistent with the study below. The higher insulin level in the patient group compared to the control group may be due to insulin resistance to reduce high blood sugar in a diabetic patient [74 and 75].

In our study, an inverse, highly significant correlation was observed between serum ghrelin level and serum insulin level (p<0.001, r=-0.699), increasing mean±SD≥25µU/mL of serum insulin to obtain mean ±SD of ghrelin. (238.58±114.76pg/mL) was detected, while the mean of ghrelin was found to be ±SD 327.82±152.36pg/mL in the group with insulin mean±SD<25 (p<0.05). In addition, the same inverse

relationship was observed when the group with ghrelin was specified ($p<0.001$, $r=-0.699$). While the mean ±SD of serum insulin in the <520pg/mL ghrelin group was (29.36±10.87µU/mL), the mean ±SD of serum insulin was >520pg/mL in the ghrelin group (13.65±5.40µU/mL ($p-<0.001$) These results were in agreement with other studies, such as in a recent study [73 and 76].

Mean glucose ±SD in diabetic patients was found to be higher than in healthy controls (176.64±59.90mg/dL), ($p<0.001$), it was reported that ghrelin levels in the whole study population had a highly significant correlation inversely with fasting blood. Glucose (FBS) ($p<0.001$, $r=-0.934$**) showed that the serum ghrelin level was considered significantly higher in cases whose ghrelin level was ≤110mg in the fasting blood glucose group in the entire study population. FBS mg/dl, p-value (<0.001) mean ±SD of serum ghrelin (479.12±57.22), (225.05±100.94pg/dl), respectively (Table 9). This conclusion was supported by other study [72]. Fasting blood glucose (FBS) and insulin level in the entire study population had a highly significant positive correlation (p-value<0.001, $r=0.681$**). These elevations may be due to insulin resistance [77]. Ultimately, this study was supported by studies [73].

8. CONCLUSION

In this study, a significant decrease in serum ghrelin levels was observed in patients with T2DM and those without diabetes. Serum ghrelin levels were also inversely proportional to serum insulin levels in both groups, and it can be said that there is a positive and significant relationship between insulin and T2DM. This may be due to insulin resistance, which is one of the main causes of T2DM.

CONFLICT OF INTEREST

The authors have no conflicts of interest to be disclosed.

FINANCIAL DISCLOSURE

The authors declare that this study has not received any financial support.

DECLARATION OF ETHICAL STANDARDS

The authors of this article declare that the materials and methods used in this study do not require an ethical committee. Yes

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