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Study of Serum Leptin Level in Patients Diabetes Mellitus Type 2: in Relation with Insulin Level

MateenBahjat SADEQ[1,a], Aysel SARI[2,b*]

[1]*Graduate School of Natural and Applied Sciences Department of Chemistry Biochemistry,* [2]*Department of Biochemistry, Faculty of Science, Fırat University, Elazığ, Turkey*

**Corresponding author*

Research Article

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ABSTRACT

Leptin is a food intake hormone. Informed leptin diet provides adequate nutrition. Leptin plays an important role in T2DM. Having high leptin and being in love is not a reason for preference. Evaluation of serum and ptin levels in T2DM patients and healthy patients, and the relationship between serum fasting insulin and leptin in T2DM. In the endocrine and market cross-sectional education, a total of 92 serums were studied, including 70 patients with T2DM and 22 patients who were not healthy. Serum leptin was measured by ELISA, serum insulin autoanalyzer Cobas E411. Hemoglobin was studied with HPLC D10. Posting regarding serum leptin and insulin, fasting and BMI. In this study, a significant relationship was observed between leptin and insulin and T2DM, and a significant positive relationship between leptin and insulin ($p<0.001$, $r=0.479$) was observed.

Keywords: Leptin, Insulin, Type 2 Diabetes Mellitus, Serum

Diabetes Mellitus Tip 2 Hastalarında Serum Leptin Durumu ve İnsülin Düzeyiyle İlişkisinin Araştırılması

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ÖZ

Leptin, gıda alımını düzenleyen bir hormondur. Alınan diyetin leptin direncini etkilediği bilinir. T2DM'te leptin önemli bir rol oynar. Leptinin yüksek olması diyabet ve obezitede herhangi bir hastalığın nedeni değildir. T2DM hastalarında ve sağlıklı hastada serum leptin düzeyini değerlendirmek ve T2DM'de serum açlık insulin ve leptin arasındaki ilişki araştırıldı. Endokrin ve diyabet ünitesinde kesitsel olarak alınan örneklemde T2DM'li 70 hasta ve sağlıklı diyabetik olmayan 22 hasta olmak üzere toplam 92 kişi üzerinde serumunda çalışıldı. Serum leptin, ELISA kullanılarak, serum insülini autoanalyzer Cobas E411 ile ölçüldü. Hemoglobin HPLC D10 ile çalışıldı. Serum leptin ve insülin, açlık glukozu ve BMI ile önemli ölçüde ilişkilidir. Bu çalışmada leptin ile insülin ve T2DM hastalarda leptin ile insülin arasında istatistiksel olarak ($p<0,001$, $r=0,479$) pozitif yüksek anlamlı ilişki gözlendi.

Anahtar sözcükler: Leptin, İnsülin, Tip 2 Diyabet Mellitus, Serum

[a] *amedimateen@yahoo.com* *https://orcid.org/ 0000-0002-7902-1511* | [b] *ayselsari@hotmail.com* *https://orcid.org/ 0000-0002-4966-2254*

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Introduction

Diabetic Mellitus

Diabetic Mellitus (DM) is a dangerous disease affected by the β-cells of the pancreas and characterized by hyperglycemia as a result of poor insulin in the body [1,2]. Glucose levels in the blood are regulated by a peptide hormone called insulin, which is secreted in pancreatic β-cells in the islets of langerhans [3]. reported that DM occurs in the presence of irregular excess dietary sugar and fat [4,5]. Insulin plays a role in preventing blood sugar level rise (hyperglycemia) or glucose level fall (hypoglycemia). The hormone insulin is bound to plasma membrane-bound receptors on target cells [6]. While many bodily cell types have insulin receptors, insulin plays a major role in glucose homeostasis through its direct effects on skeletal muscle, liver and white adipocytes [7]. Insulin hormone has the main role as a regulator of all properties of biological adipose tissues. Adipose tissues are the cell types most responsive to insulin. Insulin stimulates glucose transport and triglyceride synthesis (lipogenesis) [8]. Currently, approximately 90% of patients diagnosed with DM, the most common disease in the world, cause morbidity and mortality, while Type 2 DM (T2DM) is the most common type [9,10].

Insulin

Insulin, the hormone that regulates blood sugar, caused German scientists Minkowski and von Mering to develop severe diabetes in animals in 1889 [11]. It was discovered to be a polypeptide in 1928. In 1952, the amino acid sequence was defined. It was considered a dipeptide containing the A and B chain, respectively. It consists of 51 disulfide-linked amino acids with a molecular weight of 582 [12]. Normal or high levels of insulin production, which is an attenuated biological response, is known as insulin resistance [13]. Hyperinsulinemia is a consequence of the phenomenon of insulin resistance in adipocytes and muscles and the secretion of insulin from the β-cell to maintain blood glucose levels. When glucose levels in the blood increase, insulin leads to secretion [3].

Leptin

Leptin was determined in 1994. Leptin is a hormone secreted by adipose tissues and established to control food intake [14]. Leptin is a protein produced in humans generally in white adipose tissue, placenta, as well as mammary glands, ovary, brown adipose tissues, stomach, skeletal muscles, bone marrow, pituitary gland, and contains 167 amino acid peptide. It also produces less leptin in lymphoid tissues than in subcutaneous adipose tissues. Leptin shows an important part in the regulation of neuroendocrine, immune function, energy homeostasis, lipid, glucose and bone metabolism [15]. Leptin is useful in teaching intake behavior through basic neuroendocrine mechanisms [16]. It contains a disulfide bond that is structurally similar to cytokines and is of practical importance. Leptin is composed mainly of white adipose tissues and is equally released by 16 kilo daltons [kDa] of protein. The circulation of leptin is related to the utilization of leptin mRNA and protein levels in adipose tissue [17]. There is a significant relationship between the concentration of leptin in the blood and the amount of body fat, but according to the variability of caloric consumption (which decreases rapidly during fasting) and daily measurement, the concentration was highest in the mid-afternoon and after midnight with the lowest concentrations [15]. The effect of the sympathetic nervous system lowers leptin levels [18]. Circulating leptin levels are high in obesity, weight gain does not reduce with leptin resistance. Leptin contributes to the healing of resistant diseases. Low leptin levels in malnourished individuals may result in an increased risk of lacking the roles of cell-mediated resistance and infection [19]. The hypothalamus argues that body movements mediated by the hormone leptin are linked to dietary status [20]. In humans, the direction of leptin leads to a reduction in excess intake and obesity [21]. Leptin works unrelated to ghrelin, a peptide that mostly forms in the stomach and stimulates the appetite. An increase in hunger is directly related to an increase in the percentage of ghrelin to leptin [22]. Leptin levels increase with sleep [23]. Leptin acts as a sign of decreased appetite. This sense of influence is not channeled by excess food intake for appetite suppressant signals of the leptin circulation [1]. Leptin affects the mesolimbic dopamine system both alone and in combination, reducing hedonic motivation for intake. Progressive energy expenditure by the sympathetic nervous system improves according to leptin success [24]. Leptin receptors inhibit insulin action, resulting in reduced glucose uptake [25].

Leptin and Diabetic Mellitus

Leptin is a hormone derived from adipose tissue, some tissues it affects are the intestine, stomach and human placenta. It reduces food intake and energy expenditure by acting on hypothalamus receptors [26]. Various studies indicate that leptin has a major effect on glucose homeostasis regulation of the whole body [27]. A positive association was expressed between direct and indirect measurements of plasma leptin concentration and adiposity leptin [28]. Leptin was originally known as an antiobesity hormone. Pharmacological and physiological interactions between β-cells through suppression of leptin were studied. It has been observed to play a role in improving diabetes management [29]. Recently, new potential mediators of insulin resistance including leptin and the antiobesity hormone have been investigated [30]. The cytokine activates a leptin hormone in adipose tissues. It is known that the hormone mainly participates in the regulation of energy expenditure and food intake [31]. Obesity is an established risk factor for T2DM, but the physiology of obesity is only partially understood [32].

Leptin with insulin resistance

Insulin resistance in blood T2DM is associated with over expression of mRNA for obleptin in genetic models of obesity. Insulin has been reported to directly

stimulate leptin mRNA in the rat [33]. Increased levels of both insulin and leptin are common features of obesity. At the same time, leptin inhibits food intake, which inhibits insulin secretion. It has been suggested to be associated with resistance to obesity. It is expressed as the biological effects of both leptin and insulin [34]. In vivo studies have shown that leptin injection improves central and peripheral responsiveness and increases whole-body glucose utilization [35,36]. Adipocytes, skeletal muscle, and liver, which are considered leptin receptors, can affect insulin objective tissue, namely its function, and the response to insulin in these tissues [37]. T2DM disease increases with advanced socio-cultural changes. Elderly populations, increasing individuals living in cities, less physical activity, less fruit and vegetable consumption in addition to increased sugar consumption [38]. While T2DM is detected in adults, it is noteworthy that it is now noticed in children. Body weight gain, decreased physical activity, and malnutrition were observed to be highly effective [39,40]. This study aims to evaluate serum leptin levels in a patient with T2DM and a healthy control. This is involved in the relationship between fasting blood glucose, serum fasting insulin, and serum leptin.

Materials and Methods

Study design and subjects

This cross-sectional study was conducted with blood samples taken from patients hospitalized in the endocrine and diabetes service. Appropriate sampling techniques were used (September 2019-2020). In this study, out of 92 cases (35-75 years old), 70 cases were T2DM patients (case group). Other 22 healthy individuals (control group-hospital worker) were informed. Based on the diabetes record for each diabetes patient, the following conditions were excluded: patients with chronic liver disease, patients with chronic kidney disease, patients on injecting insulin therapy, supplemental leptin hormone, pregnant, inflammatory conditions, and T1DM patients. Patients were fasted overnight for testing before taking blood samples. Each participant was informed and consent was obtained. 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 less than 40 years old, more than 40 years). The patients were divided into two groups according to the duration of diabetes, 5 years and less than 5 years. BMI was calculated by dividing weight (in kg) by height per square meter for each participant. 18.5 and 24.9kg/m^2 were accepted as normal weight. 25 and 29.9kg/m^2 were considered as overweight, equal to or more than 30 kg/m^2 were considered obese. BMI uses weight and height to determine if an adult is in the healthy weight range, underweight. Overweight or obese. *BMI=Weight (kg)÷ [height]2(m)2. For glycemic control, HbA1c less than 6.5% was accepted as control. HbA1c greater than or equal to 6.5% was considered fair control. Cut-off points for lipids were made according to the following national cholesterol education program guidelines. Total serum cholesterol was considered to be less than 200mg/dL, 200-239mg/dL at the borderline, equal to or greater than 240mg/dL. Serum triglyceride lower than 150mg/dL was considered normal, 150-199mg/dL was high, 200-499mg/dL was hypertriglyceridemia, and 500mg/dL was considered as excessive. High risk factor. Serum high-density lipoprotein cholesterol less than 40mg/dL was considered low level, normal levels considered equal to or greater than 40mg/dL. Serum low-density lipoprotein cholesterol above 100mg/dL was considered optimal, 100-129mg/dL was considered close to optimal, 130-159mg/dL was considered a borderline high risk factor, 160-189mg/dL was considered high. A risk factor equal to or greater than 190mg/dL was considered a very high risk factor. Subjects were divided into two groups according to their smoking habits: daily non-smoker, smokers. Smoker (light or heavy smoker) when more than ten cigarettes are smoked per day. Serum insulin level was accepted as 2.6-24.9µU/mL normal range. Insulin ≥25µU/mL was considered high range. ≥21.9ng/mL was considered high range. Serum leptin level 2.5-21.8ng/mL was considered as the normal range.leptin ≥21.9 ng/mL was considered as the high range.

Methods

Blood samples were obtained from T2DM patients diagnosed according to the WHO protocol and from healthy-appearing individuals. Participants in the morning endocrine and diabetes unit fasted for 12-14 hours overnight. Venous blood samples (6 ml) were collected between 8:30 and 11:30 in the morning. Two ml were immediately collected into a vacuum tube containing K3 EDTA as an anticoagulant for HbA1c estimation. The remaining 4 ml were collected in vacuum system gel separator tubes. Serum was separated from whole blood after coagulation using centrifugation (HITACHI centrifuge, model O5P-21) for 10 min at 5000 rpm, serum glucose, total cholesterol, triglycerides, HDL, LDL, leptin, and insulin.

Statistical analysis

Statistical package for social science (SPSD) version 23 was used for data analysis. Proportion t-test was used for comparison. A p-value of ≤0.05 was considered statistically significant, while a p-value of <0.001 was considered statistically significant. One way ANOVA was used to compare more than two groups. Pearson correlation was used to determine the correlation coefficient between the research parameters, determined using the pearson correlation.

Results and Discussion

General characteristic of studied participants

Twenty-two healthy controls and 70 diabetic patients, 92 specimens (43 men and 49 women) were recruited. 15 smokers, and 74% of 68 subjects who did not exercise, 24 subjects were divided into 77 individuals, 26% of whom were physically active. Diabetic family history of the participants (33%, 36%) was taken. The mean age of the individuals (49.52±9.93) (Table 1). BMI mean±SD was 29.72±4.79kg/m^2. According to the duration of the disease, those with diabetes for ≤5 years average (32,45.7%) and those with diabetes for >5 years (38,54.3%). Mean±SD of biochemical indicators of glucose HbA1c, insulin, leptin, cholesterol, triglyceride, HDL, LDL levels 156.72±63.38mg/dL, 7.96±2.13, 18.60±10.64µU/mL, 21.89±9.54ng/mL, 176.93± 38.49mg/dL, 185.38±82.47mg/dL, 44.21±10.43mg/dL, 102.50±36.78mg/dL (Table 2).

Table 1. General characteristic of studied participants

Subject characteristics (n=92)	Frequency Distribution	
	Frequency or Mean, Median	Percentage of 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 *		4.79
kg/m^2	29.72	
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%

Table 2. General characteristics of biochemical indicators

Biochemical indicators	Frequency Distribution	
	Frequency or Mean, Median	Percentage of S.D, IQ
Glucose (mg/dl)*	156.72	63.38
HbA1c (%) **	7.96	2.13
Insulin *	18.60	10.64
Leptin*	21.89	9.54
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, interquartile for non-normal data and ***frequency, percentage were performed in the calculations.

Patients and subjects characteristics according to control, and diabetic patients

Classification of patients and subjects according to the study group, the sample consisted of female patients and controls (35.50%) with diabetes mellitus and (14.64%) non-patients. There was no statistically significant difference between the genders of the subjects in the diabetic patients (35.50%) and the control group (8.36%) in men (p=0.398). In the two study groups, 82%, 84%, control and diabetic groups did not smoke (p=0.785), and controls (64%) did not smoke. Diabetic patients (54%) were not exercising (p=0.208). The mean±SD age in controls (41.22±7.83) and diabetic patients (52.12±9.09) was found with a statistically significant difference (p<0.001). Mean±SD BMI, glucose, HbA1c, leptin, insulin, triglyceride were higher in diabetic patients than control subjects, BMI 30.50±4.70kg/m^2 (p<0.01), glucose 176.64±59.90 mg/dL (p<0.001), HbA1c (%) 8.81±1.71 (p<0.001), leptin 23.42±9.79ng/mL (p<0.05), insulin 20.66±11.06µU/mL and triglyceride 203.24±80.88mg/dL (p<0.001), (Table 3. 3). It was observed that the mean±SD of HDL was lower in diabetic patients than in controls, and HDL was 42.3±8.59mg/dL (p<0.01). Other features were not significantly different, cholesterol (mg/dL) (p=0.405), LDL (mg/dL) (p=0.320) (Table 3.).

Leptin and Insulin level in T2DM patients, and healthy subjects in relations with age group

In a cross-sectional study, the mean age was 49.52±9.93 years. It was observed that there was no relationship between insulin and leptin in T2DM and healthy individuals in the age group, serum leptin and insulin were not statistically significant with age (Table 3).

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

Subjects, characteristics (n=92)	Frequency Distribution		p-value (twosides)
	Controls (n=22)	Diabetic (n=70)	
Age (years)*	41.22 ± 7.83	52.12 ±9.09	<0.001
Gender* **			
Male	8(36%)	35(50%)	0.398
Female	14(64%)	35(50%)	
BMI **	27.22±4.55	30.50±4.70	<0.01
kg/m^2			
Duration of diabetes (years)***			
≤5	No Diabetic	32(46%)	
>5		38(54%)	
Exercise* **			
Yes	8(36%)	16(23%)	0.208
No	14(64%)	54(77%)	
Smoking* **			
Yes	4(18%)	11(16%)	0.785
No	18(82%)	59(84%)	
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	20.66±11.06	<0.01
Leptin	17.01±6.84	23.42±9.79	<0.05
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, interquartile for non-normal data and ***frequency and percentage were performed in the calculations.

Leptin and insulin level in T2DM patients, and healthy subjects with BMI group.

In the patient group, the relationship between insulin and BMI was statistically significant. The mean±SD of serum leptin was positively correlated with BMI by increasing BMI, BMI<25kg/m^2. Leptin mean±SD 15.44±6.52ng/mL (p<0.05), BMI 25-29.9kg/m^2, leptin mean±SD 21.01±7.43ng/mL (p<0.05). BMI>30kg/m^2, leptin mean±SD 23.42±10.77ng/mL, (p<0.05). In the control group, with the increase in BMI insulin level, mean±SD of serum insulin positively related to BMI increased, BMI<25, kg/m2 In the control group with insulin mean values, it was 7.79±4.05µU/mL, (p<0.05), BMI 25-29.9kg/m^2, insulin mean±SD 8.27±1.42µU/mL, (p<0.05), BMI>30kg/m^2 means mean±SD of insulin 15.48±4.64µU/mL (p<0.05), also leptin means mean±SD in the control group that increased with increasing BMI, control subject with BMI <25kg/m^2 Leptin mean±SD 10.94±6.81ng/mL (p<0.05), BMI 25-29.9kg/m^2 leptin mean±SD 17.56±2.57ng/mL (p<0.05), BMI>30kg/m^2, leptin mean±SD 19.87±6.10ng/mL (p<0.05) (Table 4).

Table 4. Leptin and insulin level in T2DM patients, and healthy subjects with BMI group.

Biochemical Parameters	Diabetic (n=70)			
	<25 kg/m^2 (n=6)	25-29.9 kg/m^2(n=30)	>=30 kg/m^2(n=34)	p-value
Insulin	16.11±5.62	20.38±13.71	21.07±8.99	NS
Leptin	15.44±6.52	21.01±7.43	23.42±10.77	<0.05
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
Leptin	10.94±6.81	17.56±2.57	19.87±6.10	<0.05

Mean ±SD, NS: Statistically No Significan. p-value<0.05 is considered significant, p>0.05 is considered no significant. An independent t-test was performed for statistical analysis.

Leptin and insulin level in T2DM patients, and healthy subjects with gender group.

It was observed that the mean±SD of serum insulin by gender was not statistically significant in the patient and control groups, however, there was a significant relationship between serum leptin levels and gender. The mean±SD of female leptin in the control and patient groups was higher than the mean±SD of male serum leptin. While the mean±SD mean of serum leptin in the control group was 20.06±3.91ng/mL in women, it was 11.68±7.79ng/mL in men and p<0.001 in the patient group, mean±SD of leptin in women was 28.66±9.42ng in men. /mL 18.17±7.03ng/mL (Table 5).

Table 5. Leptin and insulin level in T2DM patients, and healthy subjects concerning gender group.

Biochemical Parameters	Diabetic (n=70)			Controls (n=22)		
	Male (n=35)	Female (n=35)	p-value	Male (n=8)	Femal (n=14)	p-value
Insulin	22.12±13.36	19.19±8.07	NS	12.36±6.24	11.91±5.26	NS
Leptin	18.17±7.03	28.66±9.42	<0.001	11.68±7.79	20.06±3.91	<0.001

Mean ±SD, NS: Statistically No Significant. p-value<0.05 is considered significant, p-value>0.05 is considered No significant. An independent t-test performed for statistical analysis.

Leptin and insulin levels in T2DM patients, and healthy subjects to a physical activity group.

In the study, while the relationship between insulin and physical activity was not statistically significant in both groups (patient and control), it was observed that the mean±SD of serum leptin was negatively associated with physical activity by increasing physical activity. The mean ± SD of serum leptin in the patient group who did moderate physical activity was 17.97±7.38ng/mL (p<0.05), and the mean±SD of serum leptin in the patient group who did not do physical activity was 25.03±9.89ng/mL (p<0.05). In the control group, mean±SD of serum leptin was 13.37±7.40ng/mL, (p<0.05) at moderate physical activity, mean±SD of serum leptin was 19.10±5.76 ng/mL, (p<0.05) in those who did not do physical activity (p<0.05) (Table 6).

Table 3.6. Leptin and insulin level in T2DM patients, and healthy subjects with physical activity group.

Biochemical Parameters	Diabetic (n=70)			Controls (n=22)		
	Yes (n=16)	No (n=54)	p-value	Yes (n=8)	No (n=14)	p-value
Insulin	17.08±4.81	21.71±12.15	NS	10.05±5.65	13.23±5.25	NS
Leptin	17.97±7.38	25.03±9.89	<0.05	13.37±7.40	19.10±5.76	<0.05

Mean ±SD, NS: Statistically No Significant. p-value< 0.05 is considered significant, while p-value>0.05 is considered no significant. An independent t-test performed for statistical analysis.

Leptin and insulin level in T2DM patients with the duration group.

The mean±SD of serum insulin associated with the duration of diabetic diagnosis, mean±SD of serum insulin diagnosed for ≤5 years in the diabetic patient group, 23.70±13.87µU/mL, (p<0.05), 18.08±7.21µU/mL in the group diagnosed for >5 years while (p<0.05), however, the relationship between mean±SD of serum leptin and the duration of disease diagnosis was not statistically significant (Table 7).

Table 7. Leptin and insulin level in T2DM patients with the duration group.

Biochemical Parameters	Diabetic (n=70)		
	≤5 year(n=32)	>5 year(n=38)	p-value
Insulin	**23.70±13.87**	**18.08±7.21**	**<0.05**
Leptin	23.77±9.75	23.12±9.95	**NS**

Mean ±SD, NS: Statistically No Significant p-value<0.05 is considered significant, while p-value>0.05 is considered No significant. An independent T-test was performed for statistical analysis

Leptin and levels of insulin in the whole study population with a fasting blood glucose level group.

A positive correlation was observed between fasting blood glucose level and serum insulin mean±SD. In the group with FPG level ≤110mg/dL, the mean serum insulin was 14.19±6.79µU/mL, (p<0.05). In those with fasting blood glucose >110mg/dL, mean serum insulin was 21.07±11.61µU/mL, (p<0.05), and a negative correlation was found between serum leptin mean±SD and fasting blood glucose. In the group with fasting blood glucose ≤110mg/dL, mean serum leptin was 17.89±7.75ng/mL, (p<0.01). In patients with fasting blood glucose >110mg/dL, the mean serum leptin was 24.12±9.78ng/mL, (p<0.01) (Table 8).

Table 8. Leptin and insulin level in the whole study population with a fasting blood glucose level group.

Biochemical Parameters	Whole study population (n=92)		
	Fasting blood glucose levels ≤110 mg/dl(n=33)	Fasting blood glucose levels >110 mg/dl(n=59)	p-value
Insulin	14.19±6.79	21.07±11.61	<0.05
Leptin	17.89±7.75	24.12±9.78	<0.01

Mean ±SD, NS: Statistically No Significant. p-value < 0.05 is considered significant, while p-value > 0.05 is considered No significant. An independent T-test was performed in the statistical analysis.

Leptin and insulin levels in the whole study population with the HbA1C level group.

Insulin levels in the entire study population relative to the HbA1C level group were statistically lower in subjects with serum insulin concentration <6.5% HbA1c, serum mean±SD insulin in subjects ≥6.5% of HbA1c at p<0.05, respectively It was 14.18±7.21, 20.74±11.40µU/mL, (p<0.05). However, the leptin level was not statistically significant in the HbA1C group and the entire study population (Table 9).

Table 9. Leptin and insulin level in the whole study population with the HbA1C level group.

Biochemical Parameters	Whole study population (n=92)		
	HbA1c levels < 6.05(n=30)	HbA1C level≥ 6.5(n=62)	p-value
Insulin	14.18±7.21	20.74±11.40	<0.05
Leptin	19.36±9.11	23.11±9.58	NS

Mean ±SD, NS: Statistically No Significant p-value< 0.05 is considered significant, while p-value > 0.05 is considered No significant.
An independent T-test was performed for statistical analysis.

Leptin level in whole study population subjects with the insulin group.

A positive relationship between the leptin level and the insulin group was statistically significant, when the serum insulin concentration level increased, the serum concentration level increased, in the subjects with the insulin concentration <25µU/mL, the mean±SD of serum leptin was 20.46±9.30ng/mL, (p<0.01) (Table 10).

Table 10. Leptin level in whole study population subjects with the insulin group.

Biochemical Parameters	Whole study population (n=92)		
	Insulin level <25 (n=78)	Insulin level ≥25 (n=14)	p-value
Leptin	**20.46±9.30**	**29.80±6.79**	**<0.01**

Mean ±SD, NS: Statistically No Significant. p-value< 0.05 is considered significant, while p-value > 0.05 is considered No significant.
An independent T-test was performed for statistical analysis.

Insulin levels in the whole study with the leptin group.

In association with the leptin group, insulin levels in the entire study population gave a positive association, 55 subjects with serum leptin concentration <21ng/mL mean±SD serum insulin 13.78±4.08µU/mL (p<0.01), serum leptin concentration ≥21ng/mL In 37 individuals, mean±SD of serum insulin was 25.77±13.06µU/mL (p<0.01) (Table 11).

Table 11. Insulin level in the whole study population with the leptin group.

Biochemical Parameters	Whole study population (n=92)		
	Leptin level <21 (n=55)	Leptin level ≥21(n=37)	p-value
Insulin	13.78±4.08	25.77±13.06	<0.001

Mean ±SD, NS: Statistically No Significant. p-value< 0.05 is considered significant, while p-value > 0.05 is considered No significant.

An independent T-test was performed for statistical analysis.

Pearson Correlation (r) between parameter (correlation analysis).

According to the correlation coefficient (r), serum insulin level [age, BMI, cholesterol, LDL, (r=0.055, p=0.606), (r=0.055, p=0.606), (r=0.201, p=0.054), (r=0.051, p=0.631), (r=0.048, p=0.647), respectively, (Table 3.13.). Serum insulin level was positive with high significant associations with [Triglyceride and Leptin (r=0.459, p<0.01, respectively), (r=0.479, p<0.01, respectively)]. It was observed that serum insulin level was negative and showed statistically significant correlation with HDL level (r=-0.220, p< 0.05). Serum insulin level was positive with low significant associations with each of [FSB and Hb1c (r=0.239, p<0.05, r=0.218, p<0.05), respectively) in (Table 12).

Table 12. Correlation between insulin and other parameters in the study population.

Parameter	(r)	p-value
Age (year)	**0.055**	**0.606**
BMI (kg/m^2)	0.201	0.054
Cholestrole	0.051	0.631
Triglycerides	**0.459****	**< 0.001**
HDL	**-0.210***	**< 0.05**
LDL	0.048	0.647
FBS	**0.239***	**<0.05**
HbA1c	**0.218***	**<0.05**
Leptin	**0.479****	**<0.001**

*represent low significant correlation, **represent High significant correlation

Correlation between leptin and other parameters in the study population.

According to the pearson correlation coefficient(r), the results in the entire study population showed that serum leptin level was not positively correlated with age (r= 0.070, p=0.505), with a highly significant positive correlation between serum leptin level and BMI (kg/m^2. presence (r=0.576, p<0.01), serum leptin level was found to be positively correlated with cholesterol and low significant correlation (r=0.235, p<0.01). Serum leptin level did not have a positive significant relationship with [Triglycerides and HDL (r=0.108, p=0.304), (r=0.031, p=0.772), respectively, but serum leptin level was positively correlated with each of LDL and HbA1c, (r=0.234, p<0.01), (r=0.234, p<0.05). Serum leptin level had a highly significant positive correlation with each of the FBS and insulin levels, respectively (r=0.300, p<0.05), (r=0.479, (p<0.05) (Table 13).

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

Parameter	(r)	p-value
Age (year)	0.070	0.505
BMI (kg/m^2)	**0.576****	**<0.001**
Cholestrole	**0.235***	**< 0.01**
Triglycerides	0.108	0.304
HDL	0.031	0.772
LDL	**0.234***	**< 0.01**
FBS	**0.300****	**<0.05**
HbA1c	**0.234***	**<0.05**
Insulin	**0.479****	**<0.001**

*represent low significant correlation,**represent High significant correlation

Many recent studies report that DM is one of the most common epidemic diseases worldwide [41]. Due to the important role of insulin in carbohydrate and lipid metabolism, it has a clear relationship with the hormone leptin. It is known to have an important role in the balance of nutrient intake and energy expenditure [42]. In conclusion, it has been reported that insulin resistance in T2DM, which leads to high insulin levels in the blood, is associated with overexpression of mRNA for the obesity gene obleptin hormone and mRNA in adipose tissues [33]. Leptin regulates the balance of energy expenditure and food intake. Similarly, in the same evaluation study, a highly significant correlation was observed between the concentration of leptin in the blood and the amount of body fat. In another study, it is assumed that the regulation of whole body glucose homeostasis is also affected by the hormone leptin [27]. Obesity is an established risk factor for T2DM, but the physiology of obesity is only partially understood [43]. In our study, a positive highly significant correlation was observed between leptin and insulin (p<0.001, r=0.479). In the study, mean±SD of 23.42±9.79ng/mL serum leptin level (p<0.05) in 70 diabetic patients had a significant relationship when compared to 17.01±6.84ng/mL in 22 control subjects (p<0.05) (Table3.4). This elevation in serum leptin levels in diabetic patients may be due to increased blood insulin levels [33]. These results in the study are consistent with many previous studies [43,44,45,46]. Contrasted with the reported result in leptin level for the patient with control subjects [47]. When we look at the relationship of insulin with diabetes patients and controls, it was observed that the mean±SD of serum insulin was higher in diabetic patients than in

control subjects, with 20.66±11.06, 12.07±5.49µU/mL (p<0.01), which is consistent with other studies [48,49]. 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 [49]. A highly significant positive correlation was observed between serum leptin level and serum insulin level (p<0.001, r=0.479). This elevation in serum leptin levels in diabetic patients may be due to increased blood insulin levels [33]. Its result in a recent study was in agreement with other studies [44,49]. A diabetic patient had a BMI of 30.50±4.70, 27.22±4.5kg/m^2 higher than the control group, and it was significant from the main risk factors for DM (p<0.01) [44.50]. Many previous studies support the results. A highly significant positive correlation between leptin and BMI (p<0.01, r=0.576), leptin level by increasing BMI in the patient group (p<0.05), and this increase in leptin with increasing BMI may be due to leptin resistance in the obese body. The results are supported by other studies [44,45,51,52]. It was observed that the mean±SD of serum leptin in females was higher than in male patients (p<0.001) according to gender. The mean±SD of serum leptin in women was 20.06±3.91ng/mL, and mean±SD in men was 11.68±7.79ng/mL. There were also significant differences in the control group, mean serum leptin levels of the female group were 28.66±9.42,18.17±7.03ng/mL, respectively (p<0.001) higher than the male group. According to these results, it was concluded that the mean±SD serum leptin level in women in two groups (patient and control subjects) was higher than in men. It was observed that the results were significant with other studies [45,52,53]. However, there was no statistically significant difference in the mean±SD of insulin level according to the gender group. The lack of difference is also supported by another study [54]. Mean glucose mean±SD in diabetic patients was higher than that of healthy controls, 176.64±59.90mg/dL (p<0.001), leptin levels in the entire study population were positively correlated with fasting blood glucose (p<0.001). This result has been reported by other studies such as [43]. These elevations may be due to insulin resistance [41,55].

Conclusion

When looking at serum leptin levels in T2DM, it showed a significant increase compared to those without diabetes. One of the reasons for this increase in leptin levels in diabetic patients may be leptin resistance. Serum leptin level is positively correlated with serum insulin level.

References

1. care, A.D.A.J.D., 2004. Diagnosis and claSDification of diabetes mellitus, 27, S5.
2. care, A.D.A.J.D., 2013. Diagnosis and claSDification of diabetes mellitus, 36(Supplement 1), S67-S74.
3. Wilcox, G.J.C.b.r., 2005. Insulin and insulin resistance, 26(2), 19.
4. Tan, Y., Chang, S.K., Zhang, Y.F. 2017. Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera, 214, 259-268.
5. RoSDi, E.J., Sim, L., Kuntz, D.A., Hahn, D., Johnston, B.D., Ghavami, A., Szczepina, M.G., Kumar, N.S., Sterchi, E.E., and Nichols, B.L.J.T.F.j., 2006. Inhibition of recombinant human maltase glucoamylase by salacinol and derivatives, 273(12), 2673-2683.
6. Jin Chan, S. Steiner, D.F.J.A.Z., 2000. Insulin through the ages: phylogeny of a growth promoting and metabolic regulatory hormone, 40(2), 213-222.
7. Petersen, M.C. Shulman, G.I.J.P.r., 2018. Mechanisms of insulin action and insulin resistance, 98(4), 2133-2223.
8. Kahn, B.B. Flier, J.S.J.T.J.o.c.i., 2000. Obesity and insulin resistance, 106(4), 473-481.
9. Zimmet, P., Alberti, K., Shaw, J., 2001. Global and societal implications of the diabetes epidemic, *Nature*, 414(6865), 782.
10. Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030, *Diabetes care*, 27(5), 1047-1053.
11. BliSD, M.J.D.C., 1993. The history of insulin, 16(Supplement 3), 4-7.
12. Alberti, K.G.M.M., Zimmet, P., and DeFronzo, R.A., 1997. International textbook of DM, J. Wiley.
13. Cefalu, W.T.J.E., and medicine, 2001. Insulin resistance: cellular and clinical concepts, 226(1), 13-26.
14. Minokoshi, Y., Kim, Y.-B., Peroni, O.D., Fryer, L.G., Müller, C., Carling, D., Kahn, B.B.J.N., 2002. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase, 415(6869), 339.
15. Park, H.-K. Ahima, R.S., 2015. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism, *Metabolism*, 64(1), 24-34.
16. Grinspoon, S., Gulick, T., Askari, H., Landt, M., Lee, K., Anderson, E., Ma, Z., Vignati, L., Bowsher, R., Herzog, D.J.T.J.o.C.E.,1996. Serum leptin levels in women with anorexia nervosa, Metabolism 81(11), 3861-3863.
17. Ahima, R.S. Flier, J.S.J.A.r.o.p., 2000. Leptin, 62(1), 413-437.
18. Nicholson, T., Church, C., Baker, D.J., Jones, S.W., 2018. The role of adipokines in skeletal muscle inflammation and insulin sensitivity, *Journal of Inflammation*, 15(1), 9.
19. Tyson, P., Cooper, G.R.J., McCarthy, T.J.I.J.o.C.A.J.o.t.R.M.S., 2002. Millennial to multi-decadal variability in the climate of southern Africa, 22(9), 1105-1117.

20. Di Marzo V., Goparaju S.K., Wang L., Liu, J., Bátkai S., Járai Z., Fezza F., Miura G.I., Palmiter R.D., Sugiura T.J.N., 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake, 410(6830), 822.
21. Cowley, M.A., Smart, J.L., Rubinstein, M., Cerdán, M.G., Diano, S., Horvath, T.L., Cone, R.D., Low, M.J.J.N., 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus, 411(6836), 480.
22. Spiegel, K., Tasali, E., Penev, P., Van Cauter, E.J.A.o.i.m., 2004. Brief communication: sleep curtailment in healthy young men is aSDociated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite, 141(11), 846-850.
23. Taheri, S., Lin, L., Austin, D., Young, T., Mignot, E.J.P.m., 2004. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body maSD index, 1(3), e62.
24. Wauman, J., Zabeau, L.,Tavernier, J., 2017. The leptin receptor complex: heavier than expected?, *Frontiers in endocrinology*, 8, 30.
25. D'souza, A.M., Neumann, U.H., Glavas, M.M., Kieffer, T.J., 2017. The glucoregulatory actions of leptin, *Molecular metabolism*, 6(9), 1052-1065.
26. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue, *Nature*, 372(6505), 425.
27. Ceddia, R.B., Koistinen, H.A., Zierath, J.R., Sweeney, G., 2002. Analysis of paradoxical observations on the aSDociation between leptin and insulin resistance, *The FASEB journal*, 16(10), 1163-1176.
28. Havel, P.J., Kasim-Karakas, S., Mueller, W., Johnson, P.R., Gingerich, R.L., Stern, J.S., 1996. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loSD, *The Journal of Clinical Endocrinology & Metabolism*, 81(12), 4406-4413.
29. Unger R.H. Roth M.G., 2015. A new biology of diabetes revealed by leptin, *Cell metabolism*, 21(1), 15-20.
30. Masuzaki, H., Ogawa, Y., Sagawa, N., Hosoda, K., Matsumoto, T., Mise, H., Nishimura, H., Yoshimasa, Y., Tanaka, I., Mori, T., 1997. Nonadipose tiSDue production of leptin: leptin as a novel placenta-derived hormone in humans, *Nature medicine*, 3(9), 1029.
31. Scolaro, L., CaSDone, M., Kolaczynski, J.W., Otvos Jr, L., Surmacz, E., 2010. Leptin-based therapeutics, *Expert review of endocrinology & metabolism*, 5(6), 875-889.
32. Mcneely, M.J., Boyko, E.J., Weigle, D.S., Shofer, J.B., CheSDler, S.D., Leonnetti, D.L., Fujimoto, W.Y., 1999. ASDociation between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans, *Diabetes care*, 22(1), 65-70.
33. Dagogo-Jack S., Fanelli C., Paramore D., Brothers J., Landt, M.J.D., 1996. Plasma leptin and insulin relationships in obese and nonobese humans, 45(5), 695-698.
34. Wang J., Obici S., Morgan K., Barzilai N., Feng Z., RoSDetti, L.J.D., 2001. Overfeeding rapidly induces leptin and insulin resistance, 50(12), 2786-2791.
35. Harris, R.B.J.B. and communications, b.r., 1998. Acute and chronic effects of leptin on glucose utilization in lean mice, 245(2), 502-509.
36. Barzilai, N., She, L., Liu, L., Wang, J., Hu, M., Vuguin, P., RoSDetti, L.J.A.J.o.P.-E., Metabolism, 1999. Decreased visceral adiposity accounts for leptin effect on hepatic but not peripheral insulin action, 277(2), E291-E298.
37. Wauters, M., Considine, R.V., Van Gaal, L.F.J.E.j.o.e., 2000. Human leptin: from an adipocyte hormone to an endocrine mediator, 143(3), 293-311.
38. Chan, M.J.W.H.O., Geneva, Switzerland, 2016. Global report on diabetes, 1-88.
39. Olokoba A.B., Obateru O.A., Olokoba, L.B.J.O.m.j., 2012. T'DM: a review of current trends, 27(4), 269.
40. ASDociation, A.D.A.J.C.d.a.p.o.t.A.D., 2015. Standards of medical care in diabetes—2015 abridged for primary care providers, 33(2), 97.
41. Zheng, Y., Ley, S.H., Hu, F.B.J.N.R.E., 2018. Global aetiology and epidemiology of Type 2 diabetes mellitus and its complications, 14(2), 88.
42. Benoit, S.C., Clegg, D.J., Seeley, R.J., Woods, S.C.J.R.p.i.h.r., 2004. Insulin and leptin as adiposity signals, 59, 267-286.
43. Al-Zubaidi, R. Abbas, M.J.k.j.o.p.s., 2017. Association Between Total antioxidant Capacity and Leptin Levels in Type-2 Diabetic Patients, (12), 29-33.
44. Moonishaa, T.M., Nanda, S.K., Shamraj, M., Sivaa, R., Sivakumar, P., Ravichandran, K.J.I.J.o.A., and Research, B.M., 2017. Evaluation of leptin as a marker of insulin resistance in T2DM, 7(3), 176.
45. Diwan, A.G., Kuvalekar, A.A., Dharamsi, S., Vora, A.M., Nikam, V.A., Ghadge, A.A.J.I.j.o.e., and metabolism, 2018. Correlation of serum adiponectin and leptin levels in obesity and type 2 diabetes mellitus, 22(1), 93.
46. Taher, N.J.I.A.-H.J.F.P. and Science, A., 2017. The Effect of Leptin Hormone Levels In Type (II) Diabetic Nephropathy Patients, 22(3).
47. Altawil, H.J.A.J.L.S. and some Biochemical Parameters among T2DM Females in the Gaza Governorate, G.S., 2009. Leptin status and some biochemical parameters among T2DM females in the Gaza Governorate, Gaza Strip.
48. Czech, M.P.J.N.m., 2017. Insulin action and resistance in obesity and T2DM, 23(7), 804-814.
49. Shebl, T.H., Noor El Deen, A.A.,Younis, H.A., Soliman, A.M., Ashmawy, A.M., Ali, M.M.N.J.J.o.C.M.R., Practice, 2017. Relationship between serum leptin concentration and insulin resistance syndrome in patients with T2DM, 2(2), 125.

50. Bhupathiraju, S.N. Hu, F.B.J.C.r., 2016. Epidemiology of obesity and diabetes and their cardiovascular complications, 118(11), 1723-1735.
51. Hosoi, T. Maffei, M.J.F.i.e., 2017. leptin resistance in Metabolic disorders: PoSDible Mechanisms and treatments, 8, 300.
52. Antwi, J., Proulx, W., Sullivan, S., Lavin, R., and Bellavia, M.J.T.F.J., 2018. Serum Leptin is a Sociated with Fasting Plasma Glucose and Serum Insulin Levels independent of BMI in Haitian Americans with T2DM, 32(1_supplement), 670.9-670.9.
53. Zhao, Q., Laukkanen, J.A., Li, Q., Li, G.J.C.i.i.a., 2017. Body maSD index is a Sociated with T2DM in Chinese elderly, 12, 745.
54. Krag, M.Ø., HaSDelbalch, L., Siersma, V., Nielsen, A.B., Reventlow, S., Malterud, K., and de Fine Olivarius, N.J.D., 2016. The impact of gender on the long-term morbidity and mortality of patients with T2DM receiving structured personal care: a 13 year follow-up study, 59(2), 275-285.
55. Alzamil, H.J.J.o.O., 2020. Elevated Serum TNF-α Is Related to Obesity in Type 2 Diabetes Mellitus and Is ASDociated with Glycemic Control and Insulin Resistance, 2020