FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

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

Mateen Bahjat SADEQ

Master's Thesis
Department of Chemistry
Biochemistry
AUGUST -2020

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THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled " Study Of Serum Leptin Level In Patients Diabetes Mellitus Type 2: In Relation With Insulin Level" in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

10 January 2020

Mateen Bahjat SADEQ

PREFACE

This study was carried out on 92 participants 70 of them had diabetes mellitus Type 2 and 22 subjects were no diabetes which is control. In our study, we found some difficulty in sample collection because some of the patients did not accept to give as there samples and some times we did not get the inclosing subjects that we need it in our study, but in general, we did not have any problem in our work.

At first, thanks to God who gave me strength and health and facilitated the ways for me to carry on my study.

It is my privilege to express my deepest thankfulness and indebtedness to my supervisor Dr. Öğrt.Üyesi Aysel SARI for her interest, close supervision, constant encouragement, and generous devotion of her time and advice, which guided me throughout my study and enable me to complete it.

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Mateen Bahjat SADEQ
ELAZIG, 2020

TABLE OF CONTENTS

ABSTRACT

Study Of Serum Leptin Level In Patients Diabetes Mellitus Type 2: In Relation With Insulin Level

Mateen Bahjat SADEQ

Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences
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August 2020, Page: xii + 47

Background: Leptin is a hormone that regulates food intake. The leptin secreted in adipocytes is a protein consisting of 167 amino acids. It is known that the diet taken affects leptin resistance. In diabetes mellitus Type 2, leptin plays an important role. However, although leptin is elevated in diabetes and obesity, it is not the cause of any disease.

Objective: This study aims to evaluate the level of serum leptin in Type 2 diabetes mellitus and healthy patients and to investigate the relationship between serum fasting insulin and leptin in T2DM.

Material and method: Material and method: This study was carried out in cross-section, Azadi Education Hospital Endocrine and Diabetes Unit in Duhok, Iraq. This study was performed in 92 subjects, 70 patients with T2DM, and 22 patients without healthy diabetic subjects. Serum leptin was measured using ELISA. Serum insulin was measured with autoanalyzer Cobas E411 and made with glycated hemoglobin (HPLC D10) automated analyzer.

Results: In our study, we investigate leptin significantly in the diabetic patient group was higher than in control subjects group, leptin in the patient group (23.42±9.79 ng/mL) against (17.01±6.84 ng/mL) with ($p<0.05$), and insulin in the patient group (20.66±11.06 μU/mL) against (12.07±5.49 μU/mL) in the control group with ($p<0.01$). also, leptin and insulin positively correlated with high significantly with each other($p<0.001$, $r= 0.479$). Serum leptin and insulin are significantly related to fasting glucose and BMI.

Conclusion: In this study, a significant relationship between leptin and insulin and T2DM was investigated and a significant relationship was found between leptin and insulin.

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

ÖZET

Diabetes Mellitus Tip 2 Hastalarında Serum Leptin Durumunun ve İnsülinDüzeyiyle İlişkisinin Araştırılması

Mateen Bahjat SADEQ

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Amaç: Leptin gıda alımını düzenleyen bir hormondur. Adpositlerde salgılanan leptin 167 amino asitten oluşan bir proteindir. Alınan diyetin leptin direncini etkilediği bilinir. Diabetes mellitus Tip 2' de de leptin önemli role sahiptir. Bununla beraber leptin diyabet ve obezitede yükselse de, herhangi bir hastalığın nedeni değildir. Bu çalışmanın amacı tip 2 diyabetes mellitus ve sağlıklı olan hastada serum leptin düzeyini değerlendirmek ve T2DM 'de serum açlık insulin ile leptin arasındaki ilişkiyi araştırmaktır.

Gereç ve yöntem: Bu çalışma kesitsel, Irak'ın Duhok şehrinde bulunan Azadi Eğitim Hastanesi Endokrin ve Diyabet Ünitesinde gerçekleştirildi. Bu çalışma 92 denekte, 70'i T2DM hastası ve 22'si diyabetik olmayan sağlıklı deneklerde gerçekleştirilmiştir. Serum leptin ELISA kullanılarak ölçüldü. Serum insulin otoanalizör Cobas E411 ile ölçüldü ve Glikatlı hemoglobin (HPLC D10) otomati kanaliz cihazı ile yapıldı.

Sonuç: Çalışmamızda, diyabetik hasta grubunda leptinsan, control grubu ile karşılaştırıldığında, hasta grubunda ve hasta grubunda (20.66±11.06 μU) leptine (23.42±9.79ng/mL) ($p<0.05$) gore daha yüksekti. İnsüline karşı control grubuyla (12.07±5.49 μU/mL) ($p <0.01$). Ayrıca leptin ve insulin birbirleri ile yüksek pozitif korelasyon gösterdi ($p<0.001$, $r= 0.479$). Serum leptin ve insülin, açlık glikozu ve BMI ile önemli ölçüde ilişkilidir.

Bulgular: Bu çalışmada leptin ve insülinile T2DM arasında anlamlı bir ilişki araştırılmış ve leptin ile insulin arasında da anlamlı bir ilişki bulunmuştur.

Anahtar Kelimeler: Leptin, İnsülin, Tip 2 Diabetes Mellitus, Serum,

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF APPENDICES

Page

ABBREVIATIONS

ADA	American Diabetes Association
AMP	Adenosine Mono Phosphate
ATP	Adenosine Tri Phosphate
BMI	Body Mass Index
DIO	Diet İnduced Obesity
DM	Diabetes Mellitus
EAE	Experimental Autoimmune Encephalomyelitis
EDTA	Ethylene Diamine Tetra Acetic Acid
FBS(FPG)	Fasting Blood (Plasma) Sugar (Glucose)
G6P	Glucose 6 Phosphate
GDM	Gestational Diabetes Mellitus
GIP	Glucose Dependent Insulin Tropic Polypeptide
GLP-1	Glucagon-Like Peptide 1
HbA1c	Glycated Hemoglobin
HDL-C	High-Density Lipoprotein Cholesterol
I.V.	Intravenous
IDF	International Diabetes Federation
kDa	Kilo Dalton
LDL-C	ow-Density Lipoprotein Cholesterol
mRNA	Message rRibo Nucleic Acid
NPY	Neuropeptide Y
OGTT	Oral Glucose Tolerance Test
PACAP	Pituitary Adenyl Atecyclase Activating Polypeptide
RER	Rough Endoplasmic Reticulum
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TG	Triglyceride
VEGF	Vascular Endothelial Growth Factor
VIP	Vasoactive Intestinal Peptide

1. INTRODUCTION

Below we will briefly introduce diabetes mellitus leptin and insulin

1.1. Diabetic Mellitus

The most prevalent disease in the world currently is diabetes mellitus. About 90% of patients diagnosed are Type 2 diabetes mellitus, the most public type. It has a significant characteristic, in peripheral tissues which are insulin resistance, precisely: adipose tissue, liver, and skeletal muscle also insulin deficiency and defective in pancreatic beta-cell function, which leads to hyperglycemia. Many diseases are caused because of the Diabetes that make damages in different organ and cause coronary artery disease, (ophthalmologic and kidney diseases), That may cause considerable morbidity and mortality in DM patients [1, 2]. According to the estimation of IDF (The International Diabetes Federation), there are one of 11 adults aged (20-79 years) had DM (415 million adults) internationally in 2015. This number estimated probably rises to 642 million by 2040, in the region in which its economic evolution translates from low incomes to middle-income levels more increased in diabetes [3]. Some studies from the World Health Organization show that thepatientnumberswith DMareforetoldrising from 171 million in 2000 to 366 million in 2030 [4]. Overfeeding, senior, inactive lifestyle and one of the most harmful risk factors for T2DM is. As well as to the above risk factors there are some micronutrients such as zinc in the occurrence of diabetes that has been proposed [5, 6].

Lately, it was noted that simply in 2012 as a minimum of 1.5 million deaths persuaded from diabetes [7]. The expressions of diabetes and Mellitus both come from the Greek language. Diabetes means a perimeter over, a siphon while the sweet means Mellitus. It is considered that Greeks enabled it such a method, in line for the overstated urine amounts yield through diabetic patients which appealed hovers and bees [8, 9]. From the exact principal labeled case of DM 3000 years before through the Egyptians ancient (81-133) AD to 1675 after English doctor Thomas Willis finds again the urine and blood sweetness in patients, then information for DM enormously has been developing [10,11].

Several ideas care that economic and insurance rank would show a key part happening to the expression of DM Type 2. Furthermore, a new study expressed that race would similarly have a significant factor in DM occurrence types 1 and 2 [12].

1.2. Burden of diabetes

DM is a dangerous disease described by hyperglycemia that effected by the pancreatic β-cells make poor insulin (the hormone to regulates blood glucose) after the body unable competently custom the insulin or together [13,14]. The Organization of World Health has classified DM as the Seventh 4s foremost cause in united states America whereas it was guessed nearly 422 million current diabetes in 2014, higher than the reported cases in the eighties for four times [7]. Clinicians have been considered that DM might be happened by the sugars and fat presence in regular diet assumed that starch digestion in mammals is abled by a-amylase and a-glucosidase. Reticence in enzymes of starch digestive or glucose transporters can decrease glucose-free and absorption in the slight intestine. This decrement could assistance to accomplish DM [15, 16].

DM is unique to the extreme health problems of the recent century the common of departments and public health authorities save existence unaware of the present autoimmune influence of this illness and its problems. In many countries, the qualified cases of two types of diabetes haven't been studied in great detail. However, it appears that Type 1 diabetes is not common like Type 2 diabetes, in addition to it is growing by 3 percentage every year internationally. It has been related that in maximum industrialized countries, the better skill of DM suitcases in children and teenagers is associated with Type 1 diabetes while Type 2 diabetes is showed as a further corporate condition.

Type 2 diabetes existence has been raised together with enhanced socio-cultural changes: of advanced years populations, growing individuals living in cities, small physical action, greater than before sugar consumption in addition to little fruit and vegetable consumption [7].

The particular cause of DM is indeterminate until nowadays. Yet, researchers believe that genetic factor, environmental influences and extra pathological circumstances for example annihilation of the pancreatic β-cells which irritate insulin deficiency and other oddities which cause confrontation to insulin action appears to include in the increase of the disease [17].

1.3. Types of diabetes mellitus

Three types of DM are recognized Type 1 related with occupied insulin deficiency, Type 2 advanced insulin deficiency [18], and gestational DM which is identified in the 2nd or 3rd semester of pregnancy. Currently, while Type 1 cannot be banned, Type 2 is avoidable with normal health, physical exercise, and healthy food. Primary identification is the main to manage diabetes. However, Type 2 has touched a high population and prime to problems in

numerous parts of the body, heart, nerves, eyes, and kidney [19]. Diabetes is classified into three common classes.

1.3.1. Diabetes mellitus Type 1

Diabetes Type 1 is a product of β-cell destruction which normally irritates whole insulin deficiency. It was previously recognized as insulin-dependent, young, or child diabetes and which the autoimmune reaction is one of its causals, in which the resistant system occupied against the 4insulin generating pancreatic ß-cells.

T1DM is famed by incomplete insulin construction in the body. In such type of DM, the patients need regular management of insulin to glucose level controlling in the blood cell. Have not accepted the insulin, their life is existence endangered and can be deadly.

The aim of Type 1 DM is not known up until now being currently not avoidable. Although the details for Type 1 diabetes are static uncertain, deviations in environmental hazard factors and virus-related contaminations may influence the presence of DM. Risky urination and dryness, starvation, wrong diet, vision changes, and exhaustion are the chief indications of this type of DM. Additionally, the number of individuals who identified with Type 1diabetes is being more than pervious at the last of time[20, 22].

1.3.2. Diabetes mellitus Type 2

T2DM which previously called noninsulin-dependent expected to be a result of an incessant insulin secretory deficiency on the contextual of insulin struggle on the version of the body's incompetent use of insulin. Type 2 diabetes is the most characteristic DM

The body is adept at making insulin but develops so unaffected that the insulin is unproductive. Up to now, insulin levels could then turned out deficient. Deficiency and resistance of insulin are the main cause of high blood glucose levels. Assumed that the symptoms are usually less visible or absent, the disease could be discharged and be undiagnosed for many years, and not up to problems have already risen.

Aimed at several years, Type 2 DM was detected individual in adults, currently, it has surprised to be realized in children. In anticipation of extant, the exact causes for the change of type2 diabetes are unidentified, various important threat factors being pointed out. The noteworthy ones take account of the extra weight of the body, physical laziness, and bad diet. Additional factors that compressed are background, family history of DM, oldness, and gestational diabetes history in previous [20, 22].

1.3.3. Gestational diabetes mellitus

Gestational Diabetes Mellitus (GDM) a type of DM determined in pregnancy stages that are not obvious diabetes. GDM is a temporary illness that occurs in pregnancy and takes the continuing risk of Type 2 diabetes. Females with little raised the level of blood glucose are identified as having GDM, at the same time as females with considerably raised levels of glucose the blood is categorized as having DM in pregnancy.

GDM has a habit of arising from the 24th week of pregnancy. Showing by incomes of an oral glucose acceptance examination is so suggested and necessity is mannered primary for high danger woman in pregnancy and between the week 24^{th} and the week 28^{th} of pregnancy in entire females. Females with hyperglycemia identified throughout pregnancy are at a superior risk of opposing pregnancy results for instance: actual high blood pressure and fetal macrosomia, with the vaginal birth being problematic and unsafe. In various cases, clinicians recommend insulin to regulator the levels of blood glucose. Nevertheless, gestational diabetes generally vanishes when distribution however the earlier identified women are at risk of giving GDM in following pregnancy and T2DM in the future. Furthermore, chilled born by GDM mothers have more risk of rising Type 2 diabetes throughout the teenage years [8].

1.4. Diagnostic Tests for Diabetes Mellitus

The major symptom of T2DM is Hyperglycemia. These diseases include many common T2DM signs consist of polyuria, polydipsia, weakness, weight loss, and glucose to the urine. The most commonly known diagnostic tests for T2DM is plasma fasting glucose and oralGTT. Duo to the popularity of available automated laboratory machines and the low cost the FPG test consider as an important test. While FPG, in clinical settings is more practice than the OGTT test, while the OGTT identified as longest as FPG which is considered as one of diabetes diagnostic modalities. Actuality, the WHO has hindered the usage of OGTT for diabetes diagnosis due to its inconvenience, poor reproducibility, and high cost. Diagnostic tests are widely used for 2-hour OGTT (plasma glucose levels diagnosis of 200 mg/dL or 11.1mmol/L) and also FPG (diabetes diagnosis at plasma glucose levels of 126 mg/dL or 7.0 mmol/L). Diabetes is usually diagnosed using the criterion for plasma glucose [23]. wherefore, a good diagnostic tool is an HbA1c test. Firstly, measurements of HbA1c can be performed at every time, and don't need planning by studied subjects. Secondly, it has a low biological variation intra-individual, with high reproducibility. HbA1c isn't affected by unexpected psychological stress and glycemic variation. Thirdly, HbA1c is considered as a description of the means levels of glucose in the blood along the last three months. Therefore, every three months

approximately the level of HbA1c can be measured to evaluate whether the target of glycemic control of the patient has been maintained and achieved. Fourthly, according to of estimation of epidemiological analyses that 25% reduced diabetes-associated deaths, reducing about 35% in the microvascular complication risks, and with each decreasing in a percentage point in the HbA1c level it leads to a reduction in 18% in combined nonfatal and fatal myocardial infarction [23]. According to the proposal of (ADA) the American Diabetes Association, the HbA1c considered as a diagnostic criterion for DM in 2010, while the ADA recommended the cut-off value for the diagnosis of T2DM is about 6.5 %, the production of diabetic retinopathy is the way of determination, which steeply increases at ≥ 6.5 %[23]. DM could be established on analysis mostly made on A1C standards or plasma glucose Measures, the fasting plasma glucose (FPG) after 75 gr (OGTT). Similar analytics are used to shade for and identify DM in addition to identify persons with pre diabetes[21, 22].

1.5. Leptin

The hormone which called leptin it is a protein include 167 amino acids peptide that is generally generated in white adipose tissue, placenta as well as, mammary glands, ovary, brown adipose tissues, stomach, skeletal muscles, bone marrow, pituitary gland, in humans also in lymphoid tissues, the visceral adipose tissues produce less amount of leptin than subcutaneous adipose tissues. Leptin shows an essential part of the regulation of neuroendocrine, immune function, energy homeostasis, lipid, glucose, and metabolism of bone [24].

There is a significant relationship between the leptin's concentration in the blood with the quantity of body fat, however, according to calorie consumption variability (reduced quickly throughout fasting) and daily measure at midafternoon with lowest concentrations, and after the middle night, the concentration is highest [24]. The action of the sympathetic nervous system decreases leptin levels, mostly by β3ARs [25]. The circulating levels of leptin are high, in obesity, then after that, it fails to decrease (leptin resistance) weight gaining; besides leptin exogenous direction doesn't reduce the body fat content in obese humans beings. The leptin in high levels expects to fade of the syndrome of metabolism obesity freely. The fluid cerebrospinal on the road to the leptin in the plasma part is reduced, telling leptin transport decreased through the blood-brain wall. In the obesity pre-clinical copies, intrathecal leptin direction straight to the fluid cerebrospinal the food conception is reduced. Leptin [68Ga] injection into the baboons lumbers space evidenced that leptin intrathecal administration can stretch the hypothalamus in concentrations therapeutic.

Leptin controls immune (adaptive and essential) roles by neutrophil chemotaxis stimulation, assisting phagocytosis macrophage, and the pro-inflammatory cytokines production stimulation, for instance, IL-6, IL12, and TNF-ɒ.

Leptin can add to the improvement of resistant diseases: such as leptin potentiates treatment the EAE animal typical of several cases of sclerosis. Low leptin levels in malnourished persons may well give to an increased hazard of lacking cell-mediated resistant and infections roles [26].

Kidneys clear the leptin from the movement. In renal failure patients, the blood concentration is prominent. Leptin in glomeruli renal moved through filters and is activated by proximal convoluted tubules in megalin- as well as endocytosis mediated clathrin saturable. The later parts of the structure of renal tubular are establishing by Lepusandducts assembling, where may leptin raise diuresis and natriuretic. Once reuptake, it remains degraded in a saturable development [27].

Metreleptin is a similarity of leptin of human, that can be used for handling the leptin lack. Leptin was determined in 1994. Leptin is a hormone that is secreted by adipose tissues and has been establishing to control the intake of food [28]. Leptin benefits in the instruction of intake conduct through essential neuroendocrine mechanisms [29]. It is structurally like cytokines and contains a disulfide bond that has practical importance. Leptin is formed mainly by white adipose tissues and freed equally by a 16 kilo Dalton [kDa] protein. The circulating of leptin relates to using leptin mRNA and protein levels in adipose tissue [23].

The hypothalamus sanities the relating to diet state of the body concluded gesturing delivered via the leptin hormone. Leptin reductions the eating of food over the up directive of neuropeptides, for example, α-d melanocyte-stimulating hormone, which is recognized to remain anorexigenic. It alongside down adjusts orexigenic influences for instance neuropeptide Y. Obesity can be produced by genetic weaknesses in anorexigenic gesturings, such as changes in the leptin receptors [30]. The direction of leptin in humans makes a decrease in extreme intake and obesity [31].

Leptin works indifference toward ghrelin, a peptide mostly formed in the stomach which motivates the appetite. An upsurge in starvation is straightly relational to a rise in the percentage of ghrelin to leptin [32].

Clinical examinations appear that leptin levels rise with sleep Study also established that ghrelin levels are contrariwise connected to sleep period [33].

1.6. Mechanism action of leptin

No specific studies are showing the ability of leptin to cross the blood-brain barrier to reach neurons receptors, as the blood-brain barrier in the intermediate eminence area is fairly inattentive, it remains close to where the nucleus NPY neurons are. If this occurs by an aggressive or passive.

The mechanism is unknown where it crosses the blood-brain barrier. Leptin can reach at the choroid plexus to the brain, where there is an intense expression of leptin receptor molecule that could serve as the transport mechanism. When leptin has been linked to the receptor Ob-Rb, it stimulates the stat 3 molecule, which is actions to the center and phosphorylated, it is believed, to influence fluctuations in the appearance of the gene. An individual of the principal special effects on the appearance of the genesis the regulating down of the appearance of endocannabinoid, accountable between their many other purposes for increasing hunger. There are other intracellular paths started by leptin, nonetheless, a smaller amount was recognized around in what way they purpose in this classification. Neuronal receptors adapt in comeback to leptin to have a dissimilar number and diverse sorts of synapses [34].

While leptin is a sign of decreased appetite, people fat typically have a remarkably high leptin concentration circulating. These people are supposed to be uninfluenced by the leptin effects, in the same way as people with T2DM are immune to the insulin effects. Consequently, obesity is increased when personal income demands extra calories for a prolonged period and, despite the anti-appetite signals of leptin circulation, this food intake excess is not driven by desire. The high leptin sustained concentration from the expanded storage fat results in the cells that in the leptin response being desensitized. Also in rodents, leptin is needed to be aimed at the fertility of females and males. Usually in mammals, in humans also in individually, adolescence in females is bonded to a dangerous body fat level, (Fig 1.1.) While levels of the fat drop below this starting point for example in anorexia, the cycle of ovarian halts and females halt menstruating. Besides leptin is toughly tied using angiogenesis, rising VEGF levels [13].

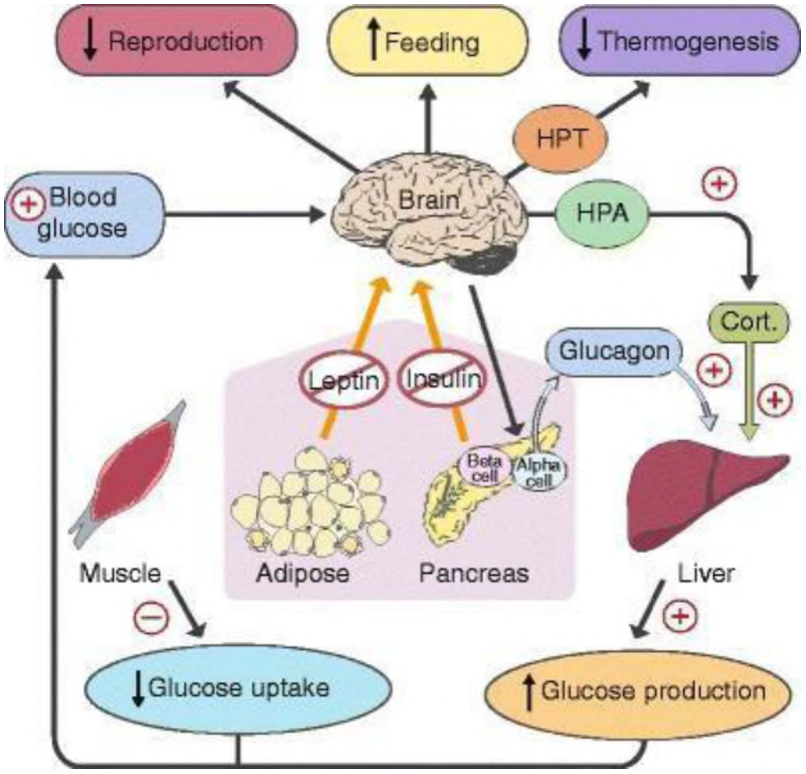

Figure 1.1. Leptin action direction [13]

1.7. The receptors of leptin

The Six leptin receptor is alternatively linked isoforms (LepR, ObR) and recognized, LepRa-LepRf. Lepus is severely glycosylated; unlike leptin. The (LepRb) isoform extension is directed extremely in the brain, in the hypothalamus particularly, after leptin quashes energy motivates and food intake overheads. The expression of (LepRb) is extraordinary as well on immune cells and hematopoietic cells. The soluble form of Lepre (Obie) is produced by ectodomain shedding in humans.

The leptin receptor (LepRd, LepRa, LepRf, LepRc) isoforms have intracellular shorter domains, besides deficiency the gesturing domain intracellular. These piece variations have an extensive distribution of tissue, counting the spleen, lungs, liver, and kidneys [27].

While on choroid plexus and cerebral microvessels the (LepRa) is showed and has been supposed toward that leptin referee transport through the BBB, on the other hand, the brain further mechanism admission of leptin can be current also, megalin (LRP2) [27, 35]. The role of leptin or else leptin receptor that changing in it's the cost causes consequence in to sever early beginning obesity, nevertheless, besides function abnormalities of immune, remaking, bone formation, and angiogenesis. Simply 10 to 20 percent of receptors of leptin are placed at the cell

membrane, as a consequence of fractional protein retention in the synthesis process, and owing to immanent receptor endocytosis, free arranged ligand. Similar to numerous other receptors of cytokine. Lepus can procedure inactive oligomers or dimmers.

Leptin impacts the mesolimbic dopamine system both alone or jointly, decreasing the hedonic motivation to intake. Short leptin levels through fasting overwhelm thyroid and growth hormone levels. Energy expenses are improved over leptin achievement proceeding with the sympathetic nervous system [35].

Leptin in mice excites in brown adipocytes. By nervous system sympathetic, it also excites the lipolysis process in the liver with adipocyte. The receptors of leptin are showed in both adipocytes (white and brown) at least in rodents, and it prevents the insulin movement during these tissues, causing decreasing the uptake of glucose. Leptin decreases the secretion of glucagon from ά-cells of the pancreas (maybe through mechanisms indirect) and prevents the production of insulin and β-cells secret it. The expression of Lepe is larger in somatostatin freeing δ-cells of the pancreas than in β-cells and ά-cells. Insulin excites leptin creation plus in adipocyte secretion. In muscle-skeletal, it raises glucose acceptance and oxidation of fatty acid. Glucose creation overturns by leptin, in the liver. While leptin performance, in bone, toward a center and outside; production of osteoblast, mineralization, and variation is motivated, and adipose tissue of bone marrow is reserved. Receptors of leptin are current in the lesion of atherosclerotic. İt helps monocyte's enrollment and cell foam formation [36].

1.8. Leptin and Diabetic Mellitus

Leptin is a hormone that is derived from adipose tissues, there is some tissues product it, these tissues are the intestine, stomach, as well as the human placenta, and it acts on the hypothalamus receptors to decrease the intake of food and energy consumption increasing [37]. According to several studies, suggestions refer to leptin also there is the main effect of leptin on the glucose homeostasis regulation of whole-body [38]. Some studies showed a positive relationship between direct and indirect measures of the concentration of plasma leptin and adiposity leptin [39].

Two decades ago, from the discovery of leptin, it changed the biologic opinion of metabolic diseases and diabetes radically. Originally, the leptin hormone is known as an anti-obesity hormone, later leptin suggested protecting nonadipocytes, like the endocrine pancreas, liver, and heart, from lipotoxicity often related with (DIO) Diet-Induced Obesity. Certainly, a phenotype of lip toxic disease of these organs appears, whenever the action of leptin imperfect or congenitally of leptin is absent. Now, we evaluate tow areas in which the actions of leptin have classic views

replacement of metabolic disease or force revision. Firstly, we consider the connection of the physiologic anti-lip toxic between non-adipocytes and leptin; secondly, we inspect the pharmacologic and physiologic interactions between β-cells sun covered through the suppression of leptin of hyper glucagonemia diabetes; thirdly, this new prudence can be applied for diabetes treatment improving [40].

Recently investigate in new potential mediators of insulin resistance, containing leptin and anti-obesity hormone [41].

Cytokine adipose tissues drive a leptin hormone, mainly was known to be participatory in energy expenditure and food intake regulation [42]

Obesity is an established T2DM risk factor, yet the physiology of obesity is only partially understood [43].

1.9. Leptin with insulin resistance

Insulin resistance in T2DM which is lead to the high level of insulin in the blood, as a result, it is related to overexpression of mRNA for the ob leptin in rodent models of genetic obesity, and insulin has been reported to directly stimulate leptin mRNA in rat adipose tissues [44]. Giving some doses of leptin without any effect on body weight modified obesity-related diabetes in the ob/ob mice, most likely through the improvement of insulin resistance [45]. Lately, after food intake in rat adipocyte ob mRNA levels have been recounted to be stimulated by two days of insulin casting as well as hyperinsulinemia by postprandial and a single injection of insulin [46, 47]. But in studies had been done in mice by leptin dosing daily normalize blood glucose concentrations [45, 48]. The studies in ob/ob mice pair-fed mice with an injection of leptin produces a dramatic reduction in plasma insulin concentration than in the pair-fed mice [49]. The notion is increasing levels of both insulin and leptin are common characteristics of obesity, but in the same time's leptin inhibits the food intake which inhibits the insulin secretion, but here we can recognize that it has been suggested that obesity is related with resistance to the biological effects of both leptin and insulin [50]. In vivo studies, injection leptin in central or I .v. to rats improves hepatic and peripheral sensitivity of insulin and rises whole-body glucose utilization [51,53]

The adipocytes, skeletal muscle, and liver considered as leptin receptor propose that leptin might affect the function of these three classical insulin objective tissues, and could influence the response to insulin in these tissues [54].

1.10. Insulin

The beta-cell in the pancreas secrete a peptide hormone named Insulin that permits your body to use glucose from carbohydrates in the diet that you eat. Insulin helps keeps your blood sugar level from increasing sugar level (hyperglycemia) or decreasing glucose level(hypoglycemia). The insulin hormone is bound plasma membrane-bound receptors in target cells to coordinate an integrated anabolic response to nutrient availability [55]. While many corporal cell types evident insulin receptors, insulin plays a main role in glucose homeostasis by typified insulin's direct effects on skeletal muscle, liver, and white adipocytes [56]. The insulin hormone has the main role in a virtually critical regulator all features of biologic adipose tissues, and adipose tissues are cell types that are the most highly insulin-responsive. Insulin by several mechanisms can boost adipocyte triglyceride stores, including fostering the differentiation of preadipocytes to adipocytes and, ingrown adipose tissues, stimulating glucose transport, and triglyceride synthesis (lipogenesis), as well as inhibiting lipolysis [57].

The levels of glucose in the blood are regulated by a peptide hormone which is secreted by the pancreatic β-cells in islets of Langerhans called insulin, these phenomena occur by simplifying uptake of cellular glucose, this hormone also responsible for the regulation of the metabolism of protein, lipid, and carbohydrate and promoting growth and division of cells through its effects of mutagenic [58].

The normal or high level of insulin production which is an attenuated biological response is known as Insulin resistance [59].

Hyper insulinemia is a result of a phenomenon of insulin resistance in adipocytes and muscle and insulin secretion from β-cell to maintain glucose levels in the blood [58].

1.11. The Insulin Discovery

Minkowski and von Mering the German scientists investigate In 1889, they developed severe diabetes from their experimental work with animals [60]. In their experiment, they suggested that the pancreas secrete a substance that is responsible for metabolic control. This hypothesis was refined later by others, observing the relationship between diabetes with the destruction of the islets of Langerhans. The attempt of two scientists in the America Zuelzer in Scott, and Minkowski in Germany, these two scientists detected maladjusted results, in administration and isolation the missing substance of pancreatic islet "insulin" was proposed by Belgian investigator de Meyer in 1909, as British researcher Schaeferdid in 1916 [58].

Finally, insulin was isolated, in 1921, hence the purification of insulin occurred and it had been available in a capable form of therapeutic administration. Insulin was begun experiments in

dogs, these experiments occurred by the scientist Toronto Surgeon Banting, there was a number of the best medical students helps him, and the prof. McLeod supervising them, Carbohydrate metabolism professor, in May 1921. They observed the lowering of blood glucose when they give cool pancreatic saline extracts through intravenous to diabetic dogs through pancreatectomy. The USA physiological association, and collie of biochemist, In December 1921 had joined the team, hence this work was presented to them, this extract also additionally demonstrated that returned glycogen of hepatic mobilization and ketones clearing capacity. The first experiments were done on a human it began on a boy with diabetes who was in 14 years old, in 1922 whose administration of the pancreatic isolate was essentially reversed the clinical symptoms and biochemical abnormalities. Hence, in May 1922, and the results of these experiments presented to the Association of American Physicians. porcine insulin produced subsequently by Eli Lilly, the principle isoelectric precipitation for purification was enhanced in 1923, made by commercial quantities. In the same year, 1923 Banting and McLeod were awarded by the Nobel prize [60].

1.12. Insulin Structure and Chemical Properties

In 1928 it discovered that Insulin is a polypeptide, while in 1952 sequence oh amino acid of insulin identified. It is considered as a dipeptide, including a chain of A and B respectively, a disulfide bound connect it, it consists of 51 amino acids, with 582 molecular weight. Its isoelectric point pH is 5.5 [61]. The 51 amino acids distributed as 21 amino acids in A chain and chain B include 30 amino acids. In chain A, the linked occupied between N-terminal helix to an anti-parallel C-terminal helix; while chain B has a segment of central helical. The 2 disulfide bonds linked the two chains (A and B), which the helices of N- and C- terminal of the chain A joined to the central helix of the chain B. the chain A joining peptide bounds the N-terminal of the chain A to the C-terminal of the chain B that occurs in the pro-Insulin [62].

1.13. Insulin Synthesis and Release

Insulin synthesized in the β-cells in the Langerhans islets of the pancreas and it is as its precursor on the short arm chromosome 11 is coded, pro-Insulin [63]. The ribosomes of the RER (Rough Endoplasmic Reticulum) synthesize Pro-insulin from mRNA as pre-pro-insulin. sequential synthesis of a signal peptide forms Pre-pro-insulin. The signal peptide Removal forms pro-insulin, which obtains its 3D characteristic structure in the reticulum endoplasma. The transformation of pro-insulin from Rough Endoplasmic Reticulum to Golgi apparatus occurs by a secretory vesicle, the environment of aqueous zinc, and calcium-rich of which favors the development of zinc-containing soluble of pro-Insulin hexamers [62]. Conversion of pro-insulin

to insulin and C-peptide by enzymes acting outside the Golgi, when immature storage vesicles form from the Golgi [58]. Hexamers of zinc-containing which are insoluble, are formed through Insulin, at pH 5.5 as chemically stable crystals they precipitate. Into circulation when the mature granules released, the insulin, exocytosis, and equimolar ratio C-peptide released. Exocytosis, insulin, and an equimolar ratio of C-peptide are released when mature granules are released into circulation. Islet cell secretion of Pro-insulin and zinc typically is not more than 6% [62].

The summation of secretory coordination bursts from one million islet cells is a reflection of characteristically pulsatile by insulin secretion from the islet cells into the portal veins [64]. In addition to post-meal variability, an ultradian oscillatory pattern of insulin release has been identified. Insulin secretion is characteristically biphasic in glucose stimulation response, insulin hormone after initial rapid period secretion is followed by less intense of the hormone but more hormone prolonged releases [65].

1.14. Insulin Biosynthesis and Release Influencing factors

The changes in the gene transcription synthesis, modification of translation, and post-transition in the Golgi can be the effect on insulin secretion in addition to the influencing on insulin release factors from secretory granules. Effect on the β-cells mass and differentiation can consequence in longer-term alteration [66]. Glucose has more than effects on biosynthesis and its secretion, insulin plays a pivotal role in the metabolism of glucose and its utilization. However, these processes are also affected by different influences like acetylcholine, fatty acids, amino acids, and Pituitary AdenylateCyclase-Activating Polypeptide (PACAP), GLP-1 (Glucagon-Like Peptide), GIP (Glucose-dependent Insulin topic polypeptide, and other multiple agonists, in combination [65].

1.15. Mechanisms of insulin secretion

When the glucose levels in the blood increased it leads to insulin secretion "First Phase" of glucose-mediated this occupied by insulin-releasing from secretory granules in the β-cell is resultant of increased levels of glucose. The entry of glucose is sensed into β-cell through glucokinase, which generating ATP by glucose phosphorylation converting to (G6P) Glucose-6-Phosphate [58]. The pulsatile insulin secretion occurs when the closing of the channel of K^+-ATP dependent consequences in the membrane activation and depolarization of voltage-dependent calcium channels leading to concentration increasing of intracellular calcium [66]. Both pathway, K^+-ATP-independent channel calcium-independent, and K^+-ATP-independent channel calcium-dependent pathways of the action of glucose lead to an augmentation of this response [65]. Many

releasing of insulin mediators contains phospholipases activation and protein kinase C (e.g, by acetylcholine) and the activity of adenylyl cyclase stimulation and activation of β-cell protein kinase A, which insulin secretion potentiates. The activation of this last mechanism can be happened by hormones, like Vasoactive Intestinal Peptide (VIP), PACAP, GLP-1, GIP. In the second step of insulin secretion mediated by glucose, these factors tend to play a significant role, after trans granules secretory filling again from reserve pools [65].

1.16. Insulin secretion mechanisms and regulation at the cellular level

The regulation of insulin secretion and synthesis is occurred by the tow secretagogues nutrient and non-nutrient, in the situation of the interplay of other hormones and stimuli of the environment [62]. Nutrient secretagogues like glucose tend to activate the secretion of insulin from β-cells by intracellular ATP increasing and K^+-ATP channels closing as above described [62]. Enhancing insulin release occur also by the cyclic AMP generation and augmentation of cellular energy intermediates. The insulin action doesn't need to cross the glucose to the β-cells (also doesn't mannose, fructose, or galactose) [58].

1.17. Aim and objective

1. This study aims to evaluate the levels of serum leptin in a patient with diabetic Mellitus Type 2 and healthy control.

2. We also investigate the relation between fasting blood sugar, serum fasting insulin with serum leptin.

2. MATERIALS AND METHODS

This cross-sectional study was carried out in the Endocrine and Diabetes Unit at Azadi Teaching Hospital and Emergency Teaching Hospital in the Duhok city in Iraq. The study spanned the period from September 2019 to September 2020 using convenient sampling techniques.

2.1. Study design and subjects

A total number of 70 of diabetic outpatients visiting the endocrine and diabetic unit were selected. Based on the diabetes record for each diabetic patient, the following conditions were excluded: Patients with chronic liver diseases, patients with chronic kidney diseases, patients who are taking injector insulin therapy, leptin hormone as supplements, Pregnant women, Inflammatory conditions, And for T1DM patients. In this study we take 92 cases, 70 cases are patients in T2DM (case group). And other 22 individual healthy (control group) were recruited by personal request from the staff of Azadi Teaching Hospital and Emergency Teaching Hospital in Duhok city, the blood samples will be collected after an overnight fast for the test.

The study group ages were between 35 and 75 years. It had obtained informed consent from each participant commenced before the study and the recruited one were approved by the Ethics Committee of the Directorate of health, of Duhok city-Governate.

A meeting interview was conducted for filling a questionnaire form that was designed to match the study needs. The questionnaire form was similar to the diabetic clinic questions at the endocrine and diabetic unit with some modifications.

The questionnaire form includes questions such as name, age, sex, smoking habit, type of drugs taken for diabetes mellitus (only for patients), family history of diabetes, physical activity, duration of diabetes mellitus, with measurement waist circumference and calculation of body mass index and any therapy was taken related with diabetes disease.

2.2. Assessment of studied variables

In our study we get aprotocol that can inclusion the partici pants to get the results that exlpain our aims.

2.2.1. Ages groups

The subjects were classified according to their age into two groups:

1. Equal to, or Less than 40 years.

2. More than 40 years.

2.2.2. Family history of diabetes mellitus

Subjects were classified according to the family history of the disease into two groups:

1. Negative diabetic family history.

2. Positive diabetic family history.

2.2.3. Duration of diabetes mellitus

Patients were classified depending on the duration of diabetes into two groups:

1. Equal to or less than 5 years.

2. More than 5 years.

2.2.4. Physical activity

According to physical activity subjects were classified into two groups:

1. Inert physical activity when the patient did not exercise or for less than 15 minutes daily.

2. Active physical activity when the patient did exercise for 15 minutes or more daily.

2.2.5. Body mass index (BMI)

BMI was calculated by measuring the weight in (kg) divided by the hight in the square meter for each participant.

1. BMI between 18.5 and 24.9 kg/m^2 were considered normal weight.
2. BMI between 25 and 29.9 kg/m^2 were considered overweight
3. BMI equal to, or more than 30 kg/m^2 were considered obese.

BMI uses weight and hight to determine whether an adult is within the healthy weight range, underweight. Overweight or obese.

*BMI=Weight (kg) ÷ $[hight]^2(m)^2$

2.2.6. Smoking habit

Based on smoking habits, the subjects were classified into two groups:

1. Non-smoker, when no cigarette was smoked daily.

2. Smoker (either light or heavy smoker) when more than ten cigarettes were smoked daily.

2.2.7. Diabetes status (glycemic control)

For glycemic control, glycated hemoglobin was measured for all diabetic patients:

1. HbA1c less than 6.5% was considered good control.
2. HbA1c equal to, or more than 6.5% was considered fair control.

2.2.8. Lipid profile status

1. Total serum cholesterol

Cut points of lipids were done based on national cholesterol education program guidelines below:

1. Total serum cholesterol less than 200 mg/dL were considered as normal.
2. Total serum cholesterol between 200 and 239 mg/dLwas considered borderline.
3. Total serum cholesterol equal to, or more than 240 mg/dl was considered as hypercholesterolemia.

2. Serum triglyceride (S.TG)

1. S.TG less than 150 mg/dl was considered as normal.
2. S.TG between 150 and 199 mg/dL was considered as high.
3. S.TG between 200 and 499 mg/dL was considered as hypertriglyceridemia.
4. S.TG equal to, or more than 500 mg/dl was considered as a very high-risk factor.

3. Serum high-density lipoprotein cholesterol (S.HDL-C)

1. S.HDL-C less than 40 mg/dl was considered as a low level.
2. S.HDL-C equal to, or more than 40 mg/dL considered normal levels.

4. Serum low-density lipoprotein cholesterol (S.LDL-C)

1. S.LDL-C less than 100 mg/dL was considered optimal.
2. S.LDL-C between 100 and 129 mg/dLwas considered as near-optimal.
3. S.LDL between 130 and 159 mg/dLwas considered as a borderline high-risk factor.
4. S.LDL between 160 and 189 mg/dLwere considered as a high-risk factor.

5. S.LDL-C equal to, or more than 190 mg/dl was considered as a very high-risk factor.

2.2.9. Serum insulin level

1.S.Insulin 2.6-24.9 μU/mL was considered as a normal range.

2.S.Insulin ≥25 μU/mL was considered as the high range.

2.2.10. Serum leptin level

1.S.Leptin 2.5-21.8 ng/mL was considered as the normal range.

2.S.Leptin ≥ 21.9 ng/mL was considered as the high range.

2.3. Methods

70 blood samples were collected from T2DM patients diagnosed according to the WHO protocol. 22 blood samples were also collected from apparently healthy individuals (control group).

2.3.1. Processing of blood sample and collection

Participants who attended the endocrine and diabetic unit in the morning were fasting overnight for 12 to 14 hours. Venous blood samples (6 ml) were collected between 8:30 -11:30 AM, withdrawn by venipuncture using vacutainer from the antecubital vein. Two ml were collected immediately into a vacuum tube containing K3 EDTA as an anticoagulant for estimation of HbA1c. The remainder 4 ml was collected in vacutainer system gel separator tubes.

The serum was separated from the whole blood after clotting using the centrifugation process (HITACHI centrifuge, model O5P-21) for 10 min at 5000 rpm, the serum was processed immediately for measuring glucose, total cholesterol, triglycerides, HDL, LDL, leptin, and insulin.

2.3.2. Biochemical analysis

Most of the tests were performed by the Cobas c 311, Cobas E 411, and ELISA devices for laboratory analyzes, and here we review all these tests. **Appendix (1,2)**

Fasting blood sugar

The sugar level is detected by using glucose HK Gen.3 300 tests Roche/Hitachi Cobas c311, Cobas c501/502 with reference number 04404483 190.

Cholesterol

The cholesterol level is detected by using cholesterol Gen.2 400 tests Roche/Hitachi Cobas c311, Cobas c501/502 with reference number 03039773 190.

Triglyceride

The Triglyceride level is detected by using Triglyceride. 250 tests Roche/Hitachi Cobas c311, Cobas c 501/502 with reference number 20767107 322.

HDL

The HDL level is detected by using HDL-Cholesterol Gen.4 tests Roche/Hitachi Cobas c311, Cobas c 501/502with reference number 07528566 190.

LDL

The LDL level is detected by using LDL-Cholesterol Gen.3 tests Roche/Hitachi Cobas c311, Cobas c 501/502 with the reference number 07005717 190.

Insulin

The insulin level is detected by using elecsys insulin by Cobas E411 with reference number 12017547122

Leptin

Leptin is detected by using ELISA kit

HbA1c

The determination of the HbA1c level was done by (HPLC D10) auto analyzer device. **Appendix (3)**

2.4. Statistical analysis

Statistical Package for Social Science (SPSS) was used for data analysis version 23. For comparison proportion t-test was used. A P value of$\leq$0.05 was considered statistically significant,

while the p-value was 0.01 it considered statistically as a highly significant. One Way ANOVA was used to compare among more than two groups. The person correlation was used to determine the correlation coefficient between study parameters was determined by using the Pearson correlation.

3. RESULTS AND DISCUSSION

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, a ccording tot our tests, we get the below results.

3.1. General characteristic of studied participants

They 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 diabetic family history. The mean age of the total subjects was (49.52±9.93) years (Table 3.1). The BMI Mean±SD was (29.72±4.79 kg/m^2. According to the duration of disease, the patients that have diabetes mellitus during ≤5 years was in the mean (32,45.7%), and (38,54.3%) in the case that has in >5 years. Also, the mean ±SD of biochemical indicators of glucose HbA1c, insulin, leptin, cholesterol, triglyceride, HDL, LDL levels were 156.72±63.38 mg/dl, 7.96±2.13%, 18.60±10.64 µU/ mL, 21.89±9.54 ng/mL,

176.93±38.49 mg/dl, 185.38±82.47 mg/dl, 44.21±10.43 mg/dl, 102.50±36.78 mg/dl, respectively (Table 3.2).

Table 3.1. General characteristic of studied participants

	Frequency Distribution	
Subject characteristics (n=92)	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 *	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%**

Table 3.2. General characteristics of biochemical indicators:

Biochemical indicators	Frequency Distribution	
	Frequency or Mean, Median	Percentage of S.D,
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 and interquartile for non-normal data and ***frequency and percentage were performed in the calculations.**

3.2. Patients and subjects characteristics according to control, and diabetic patients

In this study when stratification of patients, and subjects characteristic based on the study group, we recognized that the majority of the samples 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 subjects 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 controls group and diabetic patients group have a positive family history with (82%), (59%) respectively with ($p<0.05$). The means ±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 Means ±SD BMI, llucose, HbA1c, leptin, insulin, 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 ($p<0.001$), Leptin (ng/mL) 23.42±9.79 ($p<0.05$), Insulin (µU/mL) 20.66 ±11.06, and triglyceride (mg/dl) 203.24±80.88 ($p<0.001$), as shown in (Table 3.3). 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 3.3).

Table 3.3. Patients and subjects 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	8(36%)	35(50%)	
Female	14(64%)	35(50%)	**0.398**
BMI ** kg/m2	27.22±4.55	30.50±4.70	**<0.01**
Family history of diabetes* Positive**	4(18%)	29(41%)	**<0.05**
Duration of diabetes (years)*≤5**		38(54%)	
Exercise* **			
Yes	8(36%)	16(23%)	
No	14(64%)	54(77%)	
Smoking* **			
Yes	4(18%)	11(16%)	
No	18(82%)	59(84%)	
Glucose (mg/dl)**	93.32±9.18	176.64±59.9	**<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±26.4	178.44±41.6	**0.405**
Triglyceride (mg/dl)*	128.54±59.4	203.24±80.8	**<0.001**
HDL (mg/dl)*	50.31±13.35	42.3±8.59	**<0.01**
LDL (mg/dl)*	108.36±26.7	100.65±39.4	**0.320**

***mean and S.D for normal, **median and interquartile for non-normal data and ***frequency and percentagewere performed in the calculations.**

3.3. Leptin 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 insulin and leptin in T2DM and healthy subjects with age group, therefore, serum leptin and insulin statistically no significant with age. In (Table 3.4).

Table 3.4. Leptin and insulin in T2DM patients, and healthy subjects to age group.

Biochemical Parameters	Mean ±SD Diabetic (n=70)			Mean ±SD Controls (n=22)		
	≤40 (n=4)	>40 (n=66)	p	≤40 (n=1	>40 (n=9)	p
Insulin	19.13±	20.75±	N	12.95	10.81	N
Leptin	22.48±	23.47±	N	16.52	17.73	N

NS: Statistically No Significant

P-value < 0.05 is considered significant, P-value > 0.05 is considered No significant. An

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

In this study, in the patient group, the relation of insulin with BMI was statistically no significant, while we found that the means ±SD of serum leptin positively related to BMI by increasing the BMI leptin level increased inpatient diabetics group, the patient group with BMI <25 (kg/m^{20}) Leptin means ±SD was 15.44±6.52 (ng/mL) ($P < 0.05$), BMI 25-29.9 (kg/m^2) leptin means ±SD was 21.01±7.43 (ng/mL) ($p<0.05$), BMI >30 (kg/m^2) leptin means ±SD was 23.42±10.77 (ng/mL) ($p<0.05$), on the other hand in 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^{20}) 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) ($p<0.05$), also leptin means ±SD in control group increased by increasing BMI, the control subject with BMI <25 (kg/m^{20}). Leptin means ±SD was 10.94±6.81 (ng/mL) ($p<0.05$), BMI 25-29.9 (kg/m^2) leptin means±SD was 17.56±2.57 (ng/mL) ($p<0.05$), BMI >30 (kg/m^2) leptin means ±SD was 19.87±6.10 (ng/mL) ($p<0.05$) in (Table 3.5).

Table 3.5. Leptin and insulin level in T2DM patients, and healthy subjects with BMI group.

Biochemical Parameters	**Mean ±SD**		**Mean ±SD**	
	Diabetic (n=70)			
	<25 kg/m2 (n=6)	25-29.9 kg/m2 (n=30)	>=30 kg/m2 (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**

NS: Statistically No Significant

p-value<0.05 is considered significant, p>0.05 is considered no significant. An independent T-test was performed for statistical analysis.

3.5. Leptin 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 related to gender statistically no significant in the patient and control group, on the other hand, there was an obvious relation shape between levels of serum Leptin and the gender in the tow groups.in females in the control group and patient group the Means±SD of leptin is higher than the male means ±SD of serum leptin. The ±SD means of serum leptin in the control group for female 20.06±3.91 ng/mL while in the male 11.68±7.79 ng/mL with (p <0.001), in the patient group the Means±SD of leptin in the female 28.66±9.42 ng/mL while in the male 18.17±7.03 ng/mL.in (Table 3.6).

Table 3.6. Leptin and insulin level in T2DM patients, and healthy subjects concerning gender group.

Biochemical	**Mean ±SD**			**Mean ±SD**		
	Diabetic (n=70)			**Controls (n=22)**		
Parameters	Male	Female	p-value	Male	Female	**p-value**
	(n=35)	(n=35)		(n=8)	(n=14)	
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**

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.

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

In our study, in the either (patient and control) group the relation of insulin with physical activity was statistically no significant, while we found that the means ±SD of serum leptin negatively related to physical activity by increasing the physical activity leptin level decreased, the patient group which does a medium physical activity the means of serum leptin was 17.97 ±7.38 ng/mL ($p<0.05$), while who didn't do the physical activity the means of serum leptin was 25.03±9.89 ng/mL ($p<0.05$), in the control subjects group the means of serum leptin for whom in medium physical activity was 13.37±7.40 ng/mL ($p<0.05$), while who didn't do the physical activity the means of serum leptin was 19.10±5.76 ng/mL ($p < 0.05$) in (Table 3.7).

Table 3.7. Leptin and insulin level in T2DM patients, and healthy subjects with physical activity 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	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**

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.

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

In our study, we found that the means ±SD of serum insulin-related with a duration of diabetic diagnosing, in the diabetic patient group the means ±SD of serum insulin who ≤ 5years diagnosed was 23.70±13.87 µU/mL ($p<0.05$), while in the group who >5 years diagnosed was 18.08±7.21 µU/mL ($p<0.05$), but the relationship between the means ±SD of serum leptin with a duration of disease diagnosing was statistically no significant. In (Table 3.8).

Table 3.8. Leptin 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	23.70±13.87	18.08±7.21	**<0.05**
Leptin	23.77±9.75	23.12±9.95	**NS**

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.

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

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 FBG levels ≤ 110 mg/dL the means of serum insulin was 14.19±6.79 µU/mL ($p<0.05$), while who had fasting blood glucose levels > 110 mg/dL the means of serum insulin was 21.07±11.61 µU/mL ($p< 0.05$), on the other hand, we found the negative relationship between Means±SD of serum leptin with fasting blood glucose level (statistically significant), in the group that had fasting blood glucose levels ≤110 mg/dL the means of serum leptin was 17.89±7.75 ng/mL ($p<0.01$), While who had fasting blood glucose level>110 mg/dL the means of serum leptin was 24.12±9.78 ng/mL ($p<0.01$) in (Table 3.9).

Table 3.9. Leptin and insulin level in the whole study population with a fasting blood glucose level group.

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

NS: Statistically No Significant

p-value < 0.05 is considered significant, while p-value > 0.05 is considered No significant

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

Levels of insulin in the whole study population in relations with HbA1C level group showed that the serum insulin concentration was statistically lower in subjects those have < 6.5% HbA1c levels, than those of ≥ 6.5% HbA1c in p-value(<0.05), means ±SD of serum insulin was (14.18±7.21, 20.74±11.40) µU/mL ($p<0.05$) respectively. But the leptin level in the whole study population with HbA1C level group showed that there was statistically no significant. In (Table 3.10).

Table 3.10. Leptin and insulin level in the whole study population with the HbA1C level group.

Biochemical	Mean ±SD		
	Whole study population (n=92)		
Parameters	hbA1c levels	HbA1C level	
	< 6.05	≥ 6.5	
	(n=30)	(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

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.

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

Leptin level in whole study population subjects in relations with Insulin group revealed a positive relationship (statistically significant), when the level of serum insulin concentration increase the level of serum concentration will increase, the subjects who were at insulin concentration < 25 µU/mL had a means of serum leptin 20.46±9.30 ng/mL (p<0.01) in (Table 3.11).

Table 3.11. Leptin level in whole study population subjects with the insulin group.

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

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

3.11. Insulin levels in the whole study with the leptin group.

Levels of insulin in the whole study population in relations with Leptin group gave as a positive relationship (statistically significant), the 55 subjects who were in serum leptin concentration < 21 ng/mL had a means of serum insulin 13.78±4.08 µU/mL ($p<0.01$), while other 37 subjects who were in serum leptin concentration ≥ 21 ng/mL had a means of serum insulin 25.77±13.06 µU/mL ($p<0.01$) in (Table 3.12).

Table 3.12. Insulin level in the whole study population with the leptin group.

Biochemical Parameters	Mean ±SD		
	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

NS: Statistically No Significant

p-valu< 0.05 is considered significant, while p-value > 0.05 is considered No significant. An

3.12. 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=0.055, p=0.606), (r=0.201, p=0.054), (r=0.051, p=0.631), (r=0.048,p

=0.647), respectively], showing in (Table 3.13.). Serum insulin level had positively with high significantly relations with each of [Triglyceride and Leptin (r = 0.459, p< 0.01), (r = 0.479, p <

0.01) respectively]. And serum insulin level had negatively with statistically significantly relations with HDL level (r=-0.220, p< 0.05) showing. Serum insulin level had positively with low significantly relations with each of [FSB and Hb1c (r=0.239, p<0.05, r=0.218, p< 0.05) respectively] in (Table 3.13).

Table 3.13. Correlation between insulin and other parameters in the study population.

Parameter	(r)	P-value
Age (year)	0.055	**0.606**
BMI (Kg/m2)	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**		

3.13. Correlation between leptin and other parameters in the study population.

According to the Pearson correlation coefficient (r), our results in the whole study population showed that serum leptin level had positively no significantly relations with age (r = 0.070, p=0.505), serum leptin level had positively with high significantly relations with BMI (Kg/m^2) (r= 0.576, p<0.01) serum leptin level had positively low significantly relations with cholesterol (r=0.235, p<0.01) while serum leptin level had positively no significantly relations with each of [Triglycerides and HDL (r=0.108, p=0.304), (r=0.031, p=0.772) respectively, serum leptin level had positively low significantly relations with each of LDL and HbA1c level (r = 0.234, p <0.01), (r=0.234, p< 0.05), but serum leptin level had a high significant positively relation with each of FBS and Insulin level (r=0.300, p< 0.05), (r =0.479, p<0.05) respectively, that shown in (Table 3.14).

Table 3.14. Correlation between leptin and other parameters in the study population.

Parameter	(r)	P-value
Age (year)	0.070	0.505
BMI (Kg/m2)	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		

Recently many studies revealed that diabetes mellitus (DM) is one of the most epidemic diseases worldwide in the world. By one estimation of IDF (The International Diabetes Federation) that between 20 -79 years there is have diabetes, according to the studies in 2015, 415 million adults had diabetes mellitus around the world [3]. The most diagnosed patient of diabetes is Type 2 around 90%. Hence insulin is a hormone in which β-cells of pancreas secreted it. Insulin action and secretion abnormality lead to diabetes mellitus and carbohydrate metabolism disorder [67]. Because of the important role of insulin in carbohydrate and lipid metabolism, it has an obvious relationship with leptin hormone, the tow hormones have an important role in food intake and energy expenditure balance [68]. As a result, we found that Insulin resistance in diabetes mellitus Type 2 which is lead to the high level of insulin in the blood, as a result, it is related to overexpression of mRNA for the ob leptin hormone of obesity gene, and it has been reported that mRNA of in adipose tissues [44]. Leptin is known as an obese hormone that adipocytes secrete it to energy expenditure and food intake balance regulation by inhibiting the hunger. Leptin shows an essential part of energy regulation homeostasis, immune function, neuro endocrine, lipid, glucose, and metabolism of the bone. There is an association in high significance between leptin concentration in the blood with a body fat amount. Supposed in several studies that homeostasis of whole-body glucose is regulation has also been affected by leptin hormone [38]. Obesity is an established risk factor for T2DM, yet the physiology of obesity is only partially understood[43]. In our study also we investigate positive high significant relation between leptin and insulin ($p<0.001$, $r=0.479$), and also this tow hormone directly related to diabetes mellitus and compared with healthy control subjects.

Leptin relation with diabetes patient and controls:

In our study, we investigate the means ±SD of serum leptin level significantly increased in 70 diabetes patients (23.42±9.79 ng/mL) compared with 22 control subjects which were (17.01 ±6.84 ng/mL) with (p-value <0.05) shown in (Table3.4). This elevated of serum leptin in diabetes patient may be due to the increase of insulin level in blood which is lead to overexpression of mRNA for the ob leptin hormone of genetic obesity, and insulin has been reported to directly stimulate leptin mRNA in adipose tissues [44]. This result in our studies is compatible with Al-Zubaidi. Et el. 2017 [69], Moonishaa, et al. [70], Diwan et al. 2018 [71], and Taheret el. 2017 [72]. While the study of Altawil, H.J.A.J.L.S. 2009. Investigate the inverted result to our result in the leptin level related to the patient with control subjects [73]. Maybe this difference in result due to the difference in exclusion and inclusion of subjects was taking.

Insulin relation with diabetes patient and controls:

We investigate in our study that the means±SD of serum insulin is higher inpatient diabetes than in control subjects (20.66±11.06, 12.07±5.49) μU/mL with (p-value <40.01), this results is compatible with the study of Czech, M.P.J.N.m. 2017[74], and Shebl. Et el. [75]. The higher insulin level in the patient group more than in the control subjects maybe because of insulin resistance, to reduce the high blood glucose in a diabetic patient [75].

In our study, we investigate a positive high significant relation between serum leptin level and serum insulin level ($p<0.001$, $r=0.479$), by increasing the serum insulin Means±SD ≥ 25 μU/mL the Means±SD of leptin is revealed 29.80±6.79 ng/mL while in the group of insulin means ±SD < 25 the leptin Means ±SD was 20.46±9.30ng/mL with (p value<0.01). Also when we state the leptin as a group (<21 and ≥21) we revealed the same positively high significant relation with ($p<0.001$, $r=0.479$). the Means±SD of serum insulin in the leptin group of <21 ng/mL was 13.78±4.08 μU/mL, while in leptin group ≥21 ng/mL serum insulin means ±SD was 25.77±13.06 μU/mL with (p-value <0.001). This elevated of serum leptin in diabetes patient may be due to the increase of insulin level in blood which is lead to overexpression of mRNA for the Ob leptin hormone of genetic obesity and insulin has been reported to directly stimulate leptin mRNA in adipose tissues[44]. Our result in a recent study was compatible with other studies like Shebl. Et el. [75] and Moonishaa, et al. [70].

In our study, we investigate that the Mean±SD of BMI in a diabetic patient was higher than in the control subject (30.50±4.70, 27.22±4.5) kg/m^2, with p-value <0.01, one of the main risk factors for diabetes mellitus Type 2 is Obesity [76]. Many studies support our results like Bhupathiraju, et al.[76], Moonishaa, et el.[70]. In a recent study, we investigate a positively high significant relation of leptin with BMI ($p<0.01$, $r=0.576$), in the patient group, means ±SD of leptin level increases by increasing BMI with p-value (<0.05) BMI<25 kg/m^2 leptin was 15.44±6.52 ng/mL, BMI 25-29.9 kg/m^2 leptin mean was 21.01±7.43 ng/mL, BMI ≥30 kg/m^2 leptin means was 23.42±10.77ng/mL, while in control subjects the means ±SD of leptin level with the same BMI group also directly increased with p-value <0.05 (10.94±6.81, 17.56±2.57, 19.87±6.10) ng/mL respectively. This elevation of leptin with increasing BMI may be due to leptin resistance in the obese body [77]. Our results are supported by other studies like Moonishaa, et al. [70], Diwan, et al. [71], and Antwi, et al. [78]. In our study, we revealed a low significant increase of serum insulin means ±SD level with BMI in control subjects with p-value (<0.05), the result was BMI<25 kg/m^2 insulin was 7.79 ±4.05 μU/mL, BMI 25-29.9 kg/m^2 insulin was (8.27±1.42 μU/mL), BMI ≥30 kg/m^2 insulin was (15.48±4.64 μU/mL) our results are supported by other studies like Zhao, et al. [79].

In our study we take 92 samples, 70 of them was diabetic patient, 35 males, and 35 females and it showed that the Mean ±SD of serum leptin in the patient females was higher than in male patient by (p-value<0.001) in concerning gender group, females had serum leptin Means ±SD 20.06±3.91ng/mL, the men mean ±SD was 11.68±7.79 ng/mL . in the control subject group also we revealed significant differences, the female's group means of serum leptin was higher than in the male's group (28.66±9.42, 18.17±7.03) ng/mL respectively by (p-value < 0.001). According to these results, we conclude that the Means ±SD serum leptin level is higher in females than the males in two groups (patient and control subject). Our results were supported by other studies like Diwan et al. 2018 [71] and Antwi, et el.v [78]. On the other hand in our study, we investigate statistically no significant differences in the Means±SD of insulin level concerning gender group. These results were supported by the study of Krag, et al. 2017 [80].

In our study, we examined that the mean ±SD of Glucose in diabetic patients is higher than healthy controls 176.64±59.90 (mg/dL), (p< 0.001), leptin levels in the whole study population positively in high significant correlated with fasting blood glucose (FBS) (p-value<0.05, r=0.300), leptin level in the whole study population concerning fasting blood glucose level group showed that serum of leptin level was considered significantly lower in the subjects those have ≤110 mg/dl FBS than those have >110 mg/dl FBS with (p-value<0.01), the means ±SD of serum leptin was (17.89±7.75, 24.12±9.78) mg/dl respectively (Table 3.10).

This result was supported by other studies like Zubaidi. Et el. 2017 [69]. Otherwise, the insulin level in the whole study population concerning fasting blood glucose (FBS) positively with low significant correlated (p-value <0.05, r=239), insulin means ±SD in the whole study population concerning fasting blood glucose level group showed that serum of insulin Means ±SD was considered significantly lower in the subjects those have ≤110 mg/dl FBS than those have >110 mg/dl FBS with (p-value<0.05), the means ±SD of serum insulin was (14.19±6.79, 21.07±11.61) μU/mL respectively (Table 3.10). These elevations may be due to insulin resistance [3]. Our result was supported by the study of Alzamil, et al. [81].

4. CONCLUSIONS

The study showed a significant increase in serum leptin levels in Type 2 diabetes than non-diabetes subjects, this elevation of leptin levels in diabetes patients, one of the reasons may be the leptin resistance, serum leptin level also positively related with the serum insulin level in two groups. Also, we investigate a positively significant relation of insulin with T2DM. This may be due to insulin resistance that is one of the main causatives of T2DM.

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APPENDICES

Appendix (1) Cobas C 311 Autoanalyzer

Appendix (2) Cobas E411 Autoanalyzer

Appendix (3) Hplc D10 Instrument Bio-Rad

Appendix (4) Research Questionnaire

Research Questionnaire

1. Patient's Name: ……
2. Patient's ID……
3. Age: ……
4. Job: ……..
5. Address: ……..
6. Tel.: ……

Diseases History

- Chronic kidney diseases: Yes No
- Chronic liver diseases: Yes No
- General inflammation: Yes No
- Smoker Yes No
- Types of diabetes therapy
- Duration of daisies ≤5 Years >5 Years

cal activity ɔ

ıg medications including:

i-Vitamins o

lin lo

ɡs Hormones lo

Physical examinations

1. Width circumferences (WC)…… ( )
2. Body mass index (BMI)…….. (kg/m^2)

Weight: …….. (Kg) Height: ……..

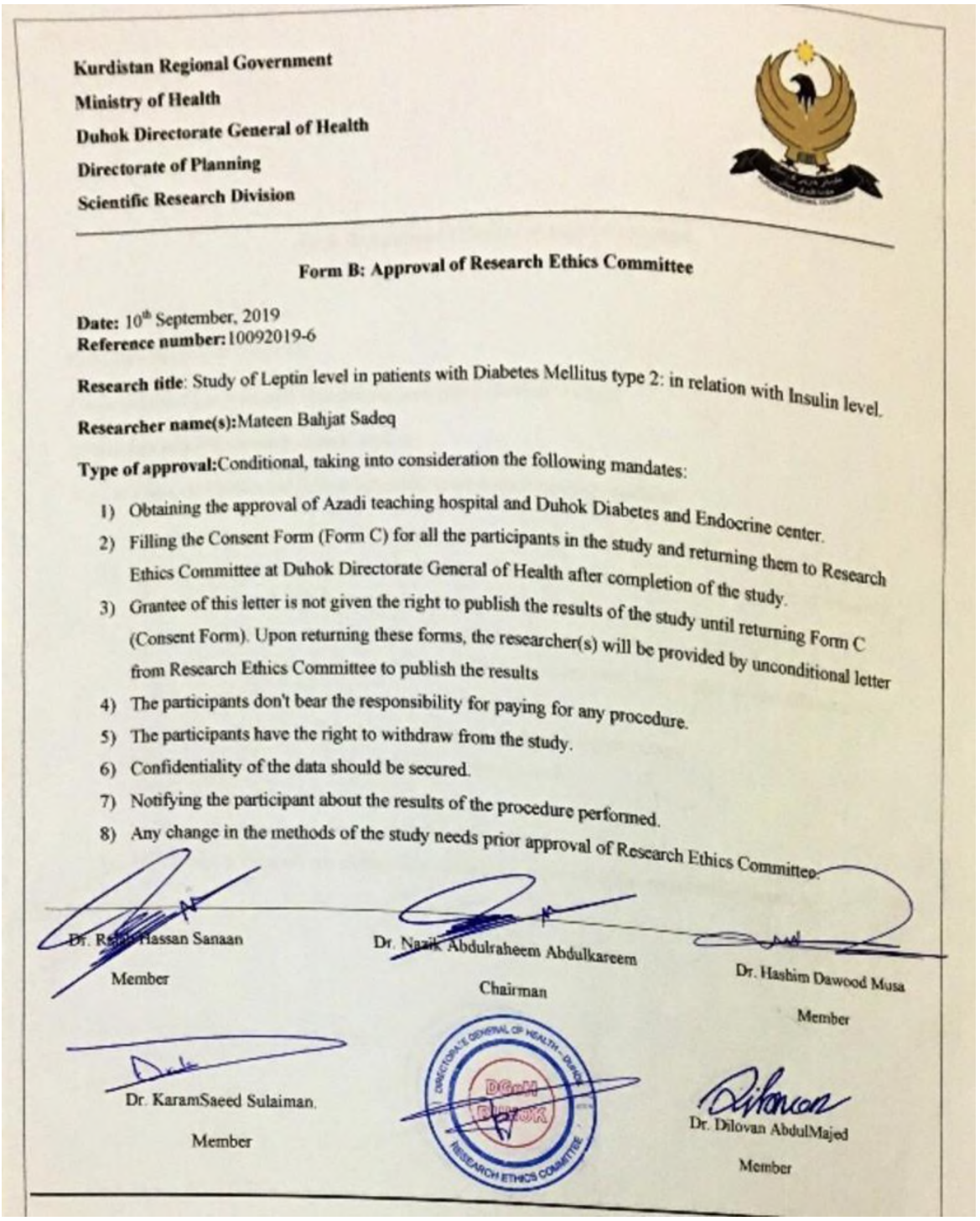
(cm) **Chemical investigations**

1. Serum Leptin:……....
2. Serum Insulin:…….
3. Serum Lipid Profile: ……

4. Fasting Blood Sugar:……

5. HbA1c:……

Appendix (5): Form (B) Approval Of Research Ethics Committee



Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 10th September, 2019
Reference number: 10092019-6

Research title: Study of Leptin level in patients with Diabetes Mellitus type 2: in relation with Insulin level.

Researcher name(s): Mateen Bahjat Sadeq

Type of approval: Conditional, taking into consideration the following mandates:

1) Obtaining the approval of Azadi teaching hospital and Duhok Diabetes and Endocrine center.
2) Filling the Consent Form (Form C) for all the participants in the study and returning them to Research Ethics Committee at Duhok Directorate General of Health after completion of the study.
3) Grantee of this letter is not given the right to publish the results of the study until returning Form C (Consent Form). Upon returning these forms, the researcher(s) will be provided by unconditional letter from Research Ethics Committee to publish the results
4) The participants don't bear the responsibility for paying for any procedure.
5) The participants have the right to withdraw from the study.
6) Confidentiality of the data should be secured.
7) Notifying the participant about the results of the procedure performed.
8) Any change in the methods of the study needs prior approval of Research Ethics Committee.

Dr. Rezan Hassan Sanaan
Member

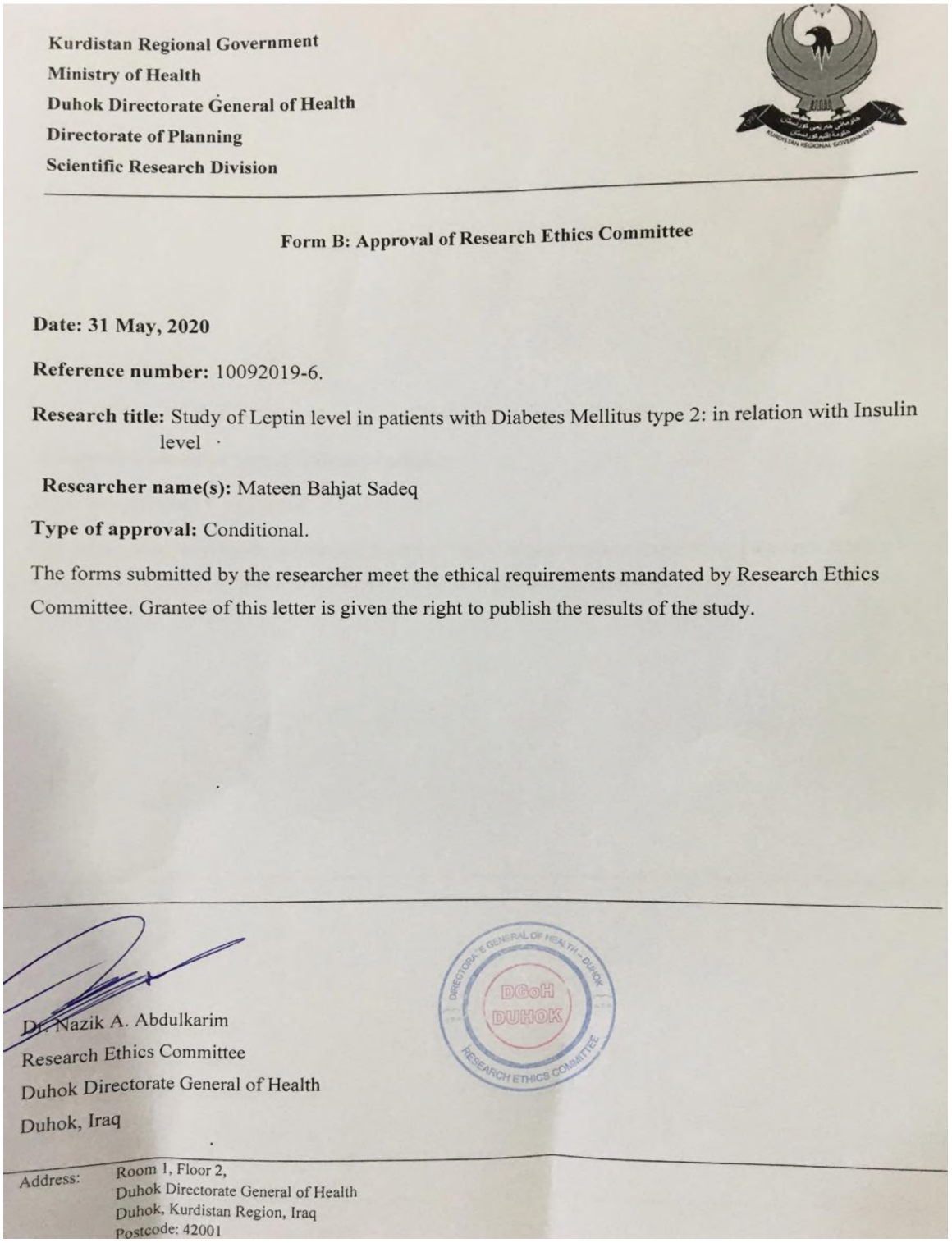
Dr. Nazik Abdulraheem Abdulkareem
Chairman

Dr. Hashim Dawood Musa
Member

Dr. KaramSaeed Sulaiman.
Member

Dr. Dilovan AbdulMajed
Member

Appendix (6): Form (B) Approval Of Research Ethics Committee



Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 31 May, 2020

Reference number: 10092019-6.

Research title: Study of Leptin level in patients with Diabetes Mellitus type 2: in relation with Insulin level

Researcher name(s): Mateen Bahjat Sadeq

Type of approval: Conditional.

The forms submitted by the researcher meet the ethical requirements mandated by Research Ethics Committee. Grantee of this letter is given the right to publish the results of the study.

Dr. Nazik A. Abdulkarim
Research Ethics Committee
Duhok Directorate General of Health
Duhok, Iraq

DIRECTORATE GENERAL OF HEALTH – DUHOK
DGoH DUHOK
RESEARCH ETHICS COMMITTEE

Address: Room 1, Floor 2,
Duhok Directorate General of Health
Duhok, Kurdistan Region, Iraq
Postcode: 42001

CURRICULUM VITAE

PERSONAL INFORMATIONS

Birth of Place	**: Duhok**
Birth of Date	**:** 1986/08/05
Nationalty	**:** IRAQ
Address	**:** Medıa- duhok-ıraq
E-mail	**:** amedımateen@yahoo.com
Languages	**:** Turkish (B1), English (B2)

EDUCATION

Bachelor	**:Duhok** University, .Science Faculty, Department of chemistry, 2011
High School	**:** KAWA, Duhok City, 2005

RESEARCH EXPERIENCES

- ✓ Instruments that I can work on it : COBAS C311, COBAS e411, Bıoles, HPLC,ELISE,
- ✓ Computer programs İ can use (wındows, world, Excel, net application.)

WORK EXPERIENCE

2013 – 2018 : hospital laboratory :

2018– 2019: quality control laboratory