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Visfatin, Liptin and Resistin Levels in Sera Patients with Type 2 Diabetes Mellitus

A Thesis

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((الْحَمْدُ لِلَّهِ الَّذِي لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ وَلَهُ الْحَمْدُ فِي الْآخِرَةِ وَهُوَ الْحَكِيمُ الْخَبِيرُ))

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

[سورة سبا : الآية 1]

Dedication

To… My dear father …

The advisor of my life..

To… My precious mother …

The source of my support Goodness and charity..

To… My sister

Appreciation and respect..

All of you I dedicate this work …

To… My friends especially…

Rafah Raheem, Niran Allawi, Roqaya Mohammed, Yusif hadi.

whose love and interest supported me in my study trip …

Wafa 2023

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Summary

Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose and affects 415 million adults around the world. Raised levels of blood glucose result from the insufficient secretion of insulin or an imperviousness to the impacts of insulin, a hormone framed by the pancreas. DM lead to complications in most organs of the human body such as heart, eye, kidney, and nervous system which have resulted in high cost and burden, therefore, diagnosis of disease in early stages is very essential.

The aim of the study is measurement of visfatin in sera patient with type 2 diabetes mellitus and DM with atherosclerosis Also Study some cytokine like liptin and Resistin in sera patients with DM type 2 and DM with atherosclerosis

Blood samples participants were collected from first of (October 2022 till November 2022). They were enrolled to Babylon Center for Diabetes and Endocrinology in Marjan Teaching Hospital in Hilla city Babylon province and second group enrolled to Iraqi Center for Cardiac Surgery Baghdad Governorate.

This case-control study includes (90) persons divided into three groups the first group includes (30) patients previously diagnosed with T2DM and the second group includes (30) apparently healthy control and three group includes (30) patients diagnosed with T2DM and atherosclerosis.

Visfatin ,liptin ,resistin concentration were determined by enzyme linked immunosorbent assay (ELISA) method .

Lipid Profiles concentration were determined by Spectrophotometer .

Serum cholesterol, LDL-C, TG were significantly high in type 2 DM patients group compared control group, while HDL level was low in type 2 DM compared control group.

Result of the present study show that visfatin was significantly high in serum of type 2 diabetic patients group and DM with atherosclerosis more than control (p-value ≤ 0.05).

Result of the present study show that liptin was significantly high in serum of type 2 diabetic patients group more than control while significant high in Liptin level in patients have DM with atherosclerosis (p-value ≤ 0.05).

Result of the present study show that resistin was significantly high in serum of type 2 diabetic patients group more than control and while significant high in patients have DM with atherosclerosis(p-value ≤ 0.05).

Finally visfatin appears a high sensitive molecule with clinical significance and diagnoatic ,prognostic and therapeutic applications in many cardiovascular metabolic disorders ,leptin associated with IR and lipid profile resistin is associated with over weight and T2DM .Serum cholesterol ,TG ,LDL-c w ere significantly high in type 2 DM group compared with normal control group ($p<0.05$) ,while serum HDL-c level low level in DM patients and patients have DM with atherosclerosis more than control group .

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List of Abbreviations

Abbreviations	Details
AHA	American Heart Association
AMPK	Adenosine monophosphate activated protein kinase
Ab	Antibodies
BMI	Body mass index
CVD	cardiovascular disease
ELISA	Enzyme linked immunosorbent assay
FPG	Fasting plasms glucose
HGP	hepatic glucose production
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance
HDL	High Density Lipoprotein
HRP	Horse Radish Peroxidase
Hb1C	Glycated hemoglobin
IDF	Iraqi Diabetes Federation
IR	insulin resistance
IDL	Intermediate Density Lipoprotein
JNK	N-terminal Kinases
LDL	Low Density Lipoprotein
LRP	lipoprotein receptor –related protein
NIDDM	Non-insulin dependent diabetes mellitus
NHANES	National Health and Nutrition Examination Survey
NAD	nicotinamide adeninedinucleotide
Pg	Pico gram
PCOS	Polycystic Ovarian Syndrome
PRPP	phosphoribosyl pyrophosphate
PBEF	pre-B enhancing factor
SOCS-3	suppressor of cytokine signaling -3
TMB	Tetra Methyl Benzidine
VLDL	Very Low Density Lipoprotein
WHO	World health organization

CHAPTER ONE

Introduction and Literature Survey

1. INTRODUCTION

1.1. Diabetes Mellitus

Diabetes mellitus DM is epidemic allow the world characterized by is in increase all over the world short period and onset a relatively young age and low body mass index (BMI) [1]. In parallel with even in development and nutrition transition the rate of overweight and obesity has been increasing rapidly. Abdominal or central adiposity particularly detrimental to type 2 diabetes mellitus or non –insulin dependent diabetes mellitus (NIDDM) [2]. The increased risk of gestational diabetes (GDM), childhood obesity, over nutrition in later life may contribute substantially to the increasing diabetes epidemic in world [3].

1.2. Definition

Diabetes mellitus (DM) is a chronic disease described by elevated of blood glucose. The raised concentration of blood glucose result from the insufficient creation of insulin or an imperviousness to the impacts of insulin, a hormone framed by the pancreas [4]. According to previous medical records there is still no cure for diabetes but the effects can be well balanced with adequate health management and regular medical check-ups [5]. Frequent urination increased thirst and increased hunger are all signs of elevated blood sugar. Diabetes if left untreated can lead to a slew of consequences, cardiovascular disease stroke, chronic kidney failure, foot ulcers, and eye impairment are all serious long-term complications [6]. Ketoacidosis or hyperosmolar hyperglycemia condition are the most serious clinical complications which can lead to dehydration, unconsciousness, and, in the absence of adequate treatment death [7].

1.3. Epidemiology

Diabetes comes from the Greek "to pass through" and mellitus term from the Latin word meaning "sweetened with honey", Ancient Egyptians described characteristic similar to DM around 3 thousand years ago but the actual term „diabetes" was only primarily used by the physician Aretaeus of Cappadocia in the second century AD. Later, in 1675, mellitus" was added by Thomas Willis, a physician who rediscovered the urine"s sweet taste [8]. The rise in diabetes cases in developing countries coincides with the trend of urbanization and lifestyle changes most notably a "Western-style" diet According to the National Center for Chronic Disease Prevention and Health Promotion (Centers for Disease Control and Prevention one out of every three Americans born after the year 2000 will get diabetes throughout their life time according to the National Health and Nutrition Examination Survey (NHANES III) in the United States 18-20% of people over 65 years old have diabetes, with 40% having diabetes or its precursor impaired glucose tolerance. Diabetes is one of the top ten if not the top five, most important diseases in the developed world and it is growing in importance there and abroad [9].

1.4. Classification of Diabetes Mellitus

The only classification system that could help with this right now is one that uses clinical criteria to identify diabetes subtypes [10].

1- Diabetes Mellitus type 1 includes diabetes primarily caused by the destruction of pancreatic beta cells, and is likely to develop ketoacidosis. This form includes auto-immune beta-cell destruction cases unknown for their etiology.

2- Diabetes Mellitus type 2 (Impaired insulin secretion through a dysfunction of the pancreatic β-cell, Impaired insulin action through insulin resistance) [10].

3- Gestational diabetes mellitus is a type of glucose intolerance that develops or it is discovered during pregnancy (non-overt diabetes diagnosed in the second or third trimester of pregnancy) [11].

4- Other Specific Types of Diabetes Mellitus According (ADA): Maturity Onset Diabetes of the Young (MODY) 3 (Chromosome 12, Hepatocyte Nuclear Factors (HNF -1α) • MODY 1 (Chromosome 20, HNF-4α) • MODY 2 (Chromosome 7, glucokinase) [11].

Table (1-1): Classification and observations on types of DM [12]

Feature	Type 1	Type 2	Gestational
Age of onset	Usually during childhood or puberty	Frequently after the age of 35	2^{O} or 3^{O} trimester of pregnancy
Pattern of onset	Abrupt – Symptoms develop rapidly	Slow – Symptoms appear gradually	Aggressive clinical progress
Prevalence	10% of diagnosed cases	90% of diagnosed cases	2-5% of pregnant women
Genetic predisposition	Moderate	Very strong	
Nutrition	Undernourished	Mostly obese	
Biochemical defect	Autoimmune destruction of ß-cells (90%, Type 1a), or unknown cause (10%, Type 1b)	Insulin resistance and inability of ß-cells to produce enough amount of insulin	ß-cells are no able to compensate for the increased insulin resistance
Plasma insulin	Low to absent	High in the early stage, low in the disease of long duration	
Comments	Association with other		May persist after

Classification and observations on types of DM.

1.5. Diabetes Diagnostic Criteria

1- Fasting plasms glucose (FPG) ≥7.0 mmol/L.

Fasting = no caloric intake for at least 8 hours.

2- Glycated hemoglobin (HbA1C) ≥6.5% (in adults).

Using a standardized validated assay in the absence of factors that [12].

affect the accuracy of the A1C and not for suspected type 1 diabetes.

3- Two-hour plasma glucose in a 75 g oral glucose tolerance test ≥11.1 mmol/L.

4- Random plasma glucose ≥11.1 mmol/L.

Random = any time of day regardless of the time since the last meal [12].

1.6. Type 2 Diabetes

The rising global incidence of type 2 diabetes is a major source of concern in the medical community. Type 2 diabetes trends for all ages were compiled globally and regionally from 1990 to 2017 [10]. Type 2 diabetes impacted approximately 462 million people worldwide in 2017 accounting for 6.28 percent of the global population (4.4 percent of those aged 15–49 15% of those aged 50–69, and 22% of those aged 70+) or a prevalence rate of 6059 cases per 100,000 [11]. Diabetes alone is responsible for over 1 million deaths each year making it the ninth leading cause of death. The global burden of diabetes mellitus is increasing and in industrialized places such as Western Europe it is increasing at a considerably quicker rate [12]. The incidence peaks at roughly 55 years of age and the gender distribution is equal by 2030 the global prevalence of type 2 diabetes is expected to climb to 7079 people per 100,000 exhibiting an upward trend in all regions of the globe [13]. In low-income countries there are alarming tendencies of increased prevalence. Preventive public health and therapeutic actions are required immediately [14]. Diabetes affects approximately 1.4 million Iraqis. T2DM prevalence in Iraq has been reported between 8.5% (Iraqi Diabetes Federation (IDF)—age-adjusted) to 13.9%. A local survey of almost 5400 people in Basrah Southern Iraq found a 19.7% age-adjusted prevalence of diabetes in people aged 19–94 [15]. There are few epidemiological research and randomized controlled trials on diabetes in Iraq, making it difficult to completely comprehend the incidence of diabetes in Iraq and the most effective treatments for Iraqis type 2 diabetes often known as adult-onset diabetes can strike anyone at any age, including children. Type 2

diabetes, on the other hand is more common in middle-aged and older persons [16]. Obese and sedentary people are also more likely to develop type 2 diabetes Insulin resistance, which arises when fat, muscle, and liver cells do not use insulin to transport glucose into the body's cells for use as energy [16].IR is the most common cause of type 2 diabetes .As a result more insulin is required to help glucose enter cells [17]. At initially the pancreas responds by producing more insulin to meet the increased demand. When blood sugar levels rise such as after meals the pancreas produces insufficient insulin over time [18].

1.6.1. Pathophysiology of Type 2 Diabetes Mellitus

The patients with T_2DM are thought to be born with a genetic susceptibility to insulin resistance [19]. Insulin suppresses hepatic glucose production (HGP) in people with normal insulin sensitivity. In the case of hepatic insulin resistance (IR) however gluconeogensis persists even when the fasting insulin level is high resulting inhyperglycemia [20]. In the fed state HGP suppression in response to insulin is also impaired post-meal glucose uptake occurs as a result of peripheral tissue insulin resistance and postprandial hyperglycemia develops [21]. Obesity and physical inactivity are insulin resistant situations that reveal the pancreatic β-cell dysfunction when they fail to increase insulin production to compensate for insulin resistancecon sequences [22]. Glucose tolerance is maintained as long as the β-cells are able to increase their insulin secretion to compensate for the influence of insulin resistance [22]. However, as β-cells lose their ability to compensate for the insulin resistance, postprandial plasma glucose (PPG) and then fasting plasma glucose (FPG) levels begin to rise, leading to overt diabetes The following are the main physiological defects that go into causing type 2 diabetes [23].

1.6.2. The Risk Factors of Type 2 Diabetes Mellitus

1- **Age:** Diabetes is more common as people get older type 2 diabetes is uncommon in most populations before the age of 30 but it rises rapidly and steadily as people get older age has been found to be a substantial risk factor in prospective observational studies [24].

2- **Gender:** The European Prospective Investigation into Cancer and Nutrition (EPIC) found that men have a higher risk of diabetes than women across all European countries [25].

3- **Genetic component:** There is a significant genetic component to the condition. Higher concordance rates are indicating that T2DM has a major genetic component. Furthermore 40 percent of first-degree relatives of T2DM patients are at risk of developing diabetes compared to only 6% in the general population [26].

4- **Lifestyle variables:** Sedentary lifestyles, physical inactivity, smoking, and alcohol use are all important contributors in the development of T2DM [27].

5- **Vitamins and type 2 diabetes:** There is growing evidence that vitamin D may have a role in the regulation of T2DM as there is seasonal change in the glycemic status of T2DM patients and hypovitaminosis D, which is more common in the winter is likely to be linked to T2DM aggravation [28]. Phylloquinone (vitamin K1) and menaquinones are two naturally occurring forms of vitamin K. Menaquinone-4 (vitamin K2) is the active form of vitamin K in bone tissue and is involved in bone quality maintenance. Furthermore a recent study found that vitamin K1 aids glucose homeostasis, as higher vitamin K1 intake is linked to improved insulin sensitivity and glycemic control. Because vitamin K deficiency can lead to impaired glycemic management and bone quality [29].

6- **Obesity:** The period and the time-course to develop obesity in addition to total adiposity of the human body and the central fat distribution are all

alerting factors for the possibility of precipitating T2DM In particular subjects with genetic predisposition [30]. With this regards it is found that about 80% of the patients are obese. This is attributed to the fact that adipose tissues are nor inert neither they are limited to the function of energy storage but they are the largest endocrine organ with a significant participation in the pathogenesis of T2DM[31]. About 5–10% weight loss is related to major health effects including enhanced lipid parameters glycemic regulation and blood pressure [32]. In May 2022 the World Health Assembly endorsed five global diabetes coverage and treatment targets to be achieved by 2030 identifying metabolic syndrome as a cluster of risk factors for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) which include raised blood pressure dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol) raised fasting glucose, and central obesity [33].

1.6.3. Signs and Symptoms of Type 2 Diabetes

Most common feature of diabetes type 2 is asymptomatic but the following signs and symptoms could appear: thirst, polyuria, polyphagia frequent hunger, feeling very fatigued, , sores that heal slowly, dry, itchy skin, pins and needles in feet, loosing feeling in feet, impaired eyesight are all signs and symptoms of diabetes [34].

1.6.4. Complications of Type 2 Diabetes

1.6.4.1. Acute Complications of Type 2 Diabetes

Patients with type 2 diabetes can present with various acute crises that impart a substantial short-term risk of morbidity and mortality, hyperglycaemic hyperthermia-like syndrome with rhabdomyolysis [35].

1.6.4.2. Chronic Complications of Type 2 Diabetes

A-The one or more of the following are caused by the damage of small blood vessels that bring about to a micro angiopathy [36].

1.6.4.2.a. Micro-angiopathy:

1- Diabetic nephropathy.

2- Diabetic neuropathy.

3- Diabetic retinopathy.

B) The one or more of the following are caused by macrovascular disease [37].

1- Cardiovascular disease to which accelerated atherosclerosis is a contributor coronary artery disease leading to angina or myocardial infarction.

2- Diabetic myonecrosis ("muscle wasting").

3- Intermittent claudication which is exertion-related leg and foot pain and diabetic foot are contributed by peripheral vascular disease.

4- Stroke (mainly the ischemic type).

5- Diabetic foot which is caused by a combination of sensory neuropathy (numbness or sensitivity) and vascular damage raises the risk of skin ulcers (diabetic foot ulcers) infection, necrosis, and gangrene in serious cases. People with diabetics are more prone to leg and foot infections and why wounds on their legs and feet take longer to heal. In the industrialized world it is the most prevalent cause of nontraumatic adult amputation mainly of the toes and/or feet[38] .

1.6.4.2.b. Macro-angiopathy:

Cause cardiovascular disease and peripheral artery disease.

1- Cardio-vascular disease (CVD): the name of CVD is used to describe of cardiovascular diseases and vessel disorders including: coronary heart disease and Hypertension. One of the most devastating consequences of DM is its effect on cardiovascular disease .In T2DM, fasting blood glucose of more than 100 mg/dL significantly contributes to the risk of ASCVD and cardiovascular risk can develop before frank hyperglycemia [39]. The United States Diabetic retinopathy contributes to 12000 to 24000 new cases of blindness annually and treatments generally consist of laser surgery and

glucose control [40]. 19% percent had cerebrovascular disease and 23% had peripheral vascular disease. As expected diabetes and smoking were strongly associated with cardiovascular diseases. Increasing age was also an important contributor especially in the group less than 55 years and in non diabetic patients [41]. The major macrovascular complications include accelerated cardiovascular disease resulting in myocardial infarction and cerebrovascular disease manifesting as strokes. Although the underlying etiology remains controversial there is also myocardial dysfunction associated with diabetes which appears at least in part to be independent of atherosclerosis. These complex atherosclerotic plaques may then destabilize and rupture, resulting in myocardial infarction, unstable angina, or strokes[42].

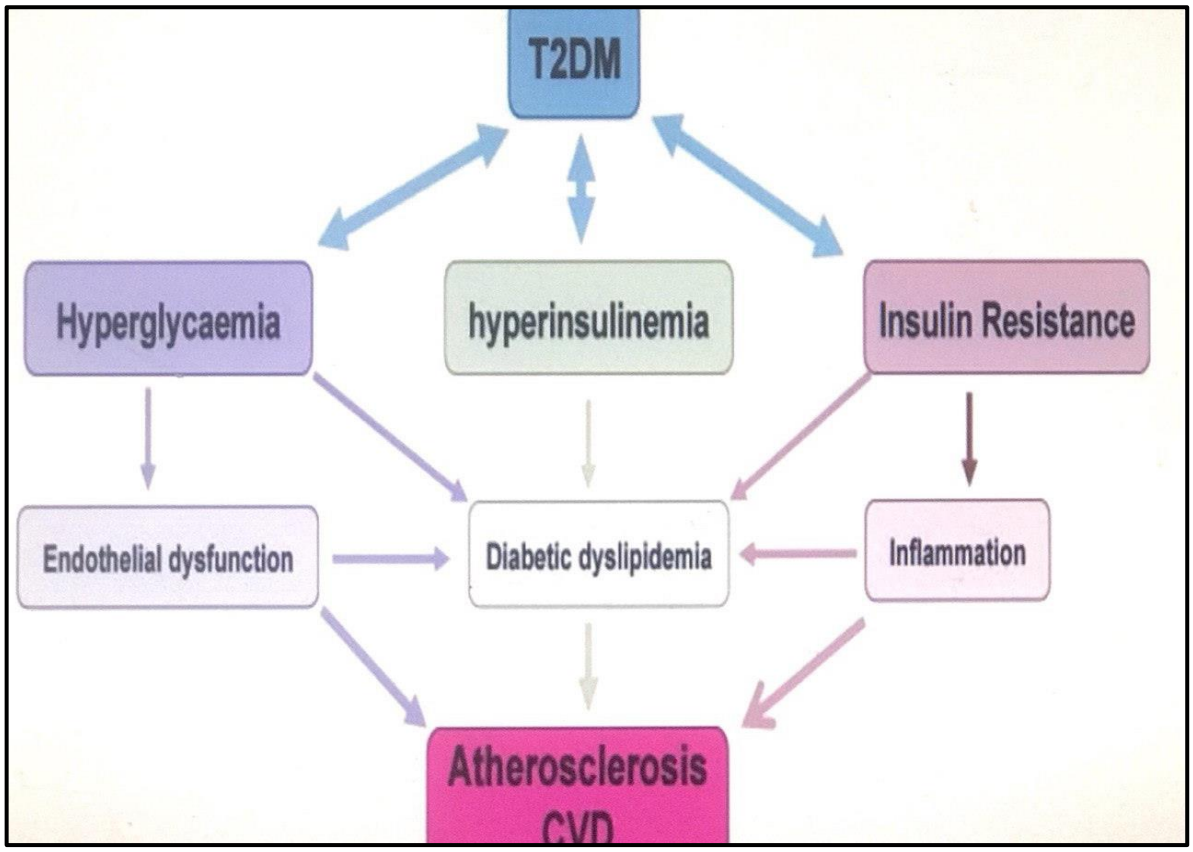

Figure 1-1: Factors implicated in cardiovascular risk outcomes from T2DM and the interactions between them. T2DM derived hyperglycemia, hyperinsulinemia and IR causes endothelial dysfunction, diabetic dyslipidemia and inflammation leading to CVD. The flowchart illustrates the multiple interactions among the implicated factors[43].

As described in the previous sections T2DM is a multisystem disease with a strong correlation with CVD development T2DM [44]. leads to a two-to four-fold increase in the mortality rate of adults from heart disease and stroke and is associated with both micro-and macrovascular complications the latter consisting of accelerated atherosclerosis leading to severe peripheral vascular disease [45]. Premature coronary artery disease (CAD) and increased risk of cerebrovascular diseases these factors lead to T2DM being considered risk factor for CVD [46].

1.7. Insulin Resistance (IR)

Insulin resistance is defined as insulin's reduced capacity to exert biological activity over a wide range of levels [47]IR is a common sign of T2D and it can emerge years before signs appear. It's also a well-known link between obesity and T2D with many fat people developing the disease [48]. Even in women with adequate glucose homeostasis insulin resistance rises during the second half of pregnancy due to the development of placentally derived hormones such as human placental lactogen [49].

If the maternal β-cells are unable to produce enough insulin to offset the insulin resistance, gestational diabetes mellitus (GDM) develops. Insulin resistance affects the liver, muscle, and adipose tissue, among other organs [50].

1.7.1. Hepatic Insulin Resistance

Insulin resistance in the liver is shown in the baseline state by glucose overproduction despite fasting hyperinsulinemia and reduced insulin suppression of HGP after a meal [51]. The brain consumes more than half of the glucose produced due to its compulsion for glucose. The liver and to a lesser extent the kidneys are responsible for meeting this glucose need [52]. In healthy people the liver produces 2 mg/kg per minute of glucose however in people with diabetes this rate is increased to 2.5 mg/kg per minute even when fasting

plasma insulin levels are increased 2.5 to 3-fold, this elevated HGP occurs demonstrating substantial resistance to the suppressive impact of insulin [52].

1.7.2. Muscle Insulin Resistance

After carbohydrate administration insulin resistance causes decreased glucose absorption in the muscle resulting in postprandial hyperglycemia [53]. Skeletal muscle provides for more than 75% of excess glucose absorption in the insulin-stimulated postprandial stateand it accounts for the majority of glucose disposal impairment in patients with diabetes [54]. Muscle insulin resistance contributes for more than 85–90% of the total body glucose disposal deficit in T2D [54].

Type 2 diabetes mellitus (T2DM) when glucose levels become higher throughout the day as the resistance increases and compensatory insulin secretion fails the most common type of IR is associated with obesity in metabolic syndrome. IR progresses to full NIDDM seen when hyperglycemia [55].

1.8. Parameters

1.8.1. Visfatin

1.8.1.1. Definition

Visfatin is also called nicotinamide phosphoribosyl transferase NAMPT gene because of its significant sequence and functional homology with enzyme nicotinamide phosphoribosyl transferase an enzyme involved in nicotinamide adeninedinucleotide (NAD) biosynthesis from nicotinamide. It is produced by the visceral adipose tissue. The expression of visfatin is increased in individuals with abdominal obesity and type 2 diabetes [56].

Visfatin was originally identified as a pre-B cell colony enhancing factor (PBEF) and is thought to play roles in immune response and inflammation. Many studies have emphasized its role as an adipose hormone that mediates

pro-inflammatory actions in peripheral tissues invarious metabolic diseases like obesity type 2 diabetes and cardiovascular disease [57].

Visfatin was first described in 2005. They showed that visfatin is expressed mainly by visceral adipose tissue with insulin-like effects[58]. The same molecule was previously identified as Pre-B colony enhancing factor (PBEF), growth factor involved in the early development of B lymphocytes PBEF was previously shown to be synthesized in several tissues including liver, bone marrow and skeletal muscle [59]. Because of the role of visfatin in inflammation and in obesity (a low-grade inflammatory process) it has been postulated that visfatin may play a role in innate immunity. Although visfatin was previously identified as PBEF its expression in visceral adipose tissue and insulin like effects are novel [59].

Visfatin possesses enzyme nicotinamide phosphoribosyl transferase (NAMPRTase) activity . This means that visfatin is able to catalyze the reaction between nicotinamide and phosphoribosyl pyrophosphate (PRPP) to produce nicotinamide mononucleotide. In addition it also possesses cytokine activity property as well as adipocytokine properties [60] has been identified as a novel and multifaceted protein which plays an important role in regulating a variety of physiological and pathological functions. In the context of metabolic diseases elevated circulating levels of visfatin have been proposed as markers of inflammation and endothelial dysfunction. The association between circulating visfatin with cardiovascular disease has also been extensively analyzed [61]. High visfatinplasma levels may promote vascular inflammation and atherosclerotic plaque destabilization [62]. Increased serum visfatin levels have been associated with carotid atherosclerosis in patients with type 2 diabetes or metabolic syndrome[63]. However, circulating visfatin levels in atherosclerosis plaque progression in patients with type 2 diabetes [64]. or its association with the vascular territory affected remain unclear [65].

1.8.1.2. Visfatin and Obesity

It was observed that plasma concentration of visfatin was two-fold higher in non-diabetic obese children compared to age matched controls [66]. Moreover it has also been shown that the subcutaneous as well as visceral adipose tissues of obese patients contain significant high levels of visfatin [67]. Observed a decrease in plasma visfatin concentration after massive weight loss involving bilio-pancreatic diversion. In addition other recent reports showed that the increased plasma visfatin level in obese patients was significantly reduced after gastric banding The differences observed in the above-mentioned reports may be due to the differences in the experimental setting as well as the cohorts of patients [68]. Studies performed in a relatively large population of subject in Chennai India showed a strong correlation between serum visfatin in spite of the controversy surrounding the correlation of visfatin with obesity it appears that there is more evidence to support a positive correlation of plasma and or tissue visfatin with obesity and obesity which is derived mainly from visceral but not subcutaneous fat [69]. Polycystic Ovarian Syndrome (PCOS) is a multifaceted metabolic disease associated with insulin resistance and obesity. Recent studies showed that women with PCOS have significantly higher plasma levels of visfatin compared to normal controls [70]. In addition the level of visfatin in the subcutaneous as well as omental (visceral) adipose tissues was markedly higher in PCOS women. All of these observaions point to a strong role for visfatin in the metabolism of adipocytes, which may influence the way that glucose is handled [71]. **factors that stimulate (+) or inhibit (-) visfatin expression in visceral adipose tissue Some physiological effects of visfatin** in **Figure 1-2**.

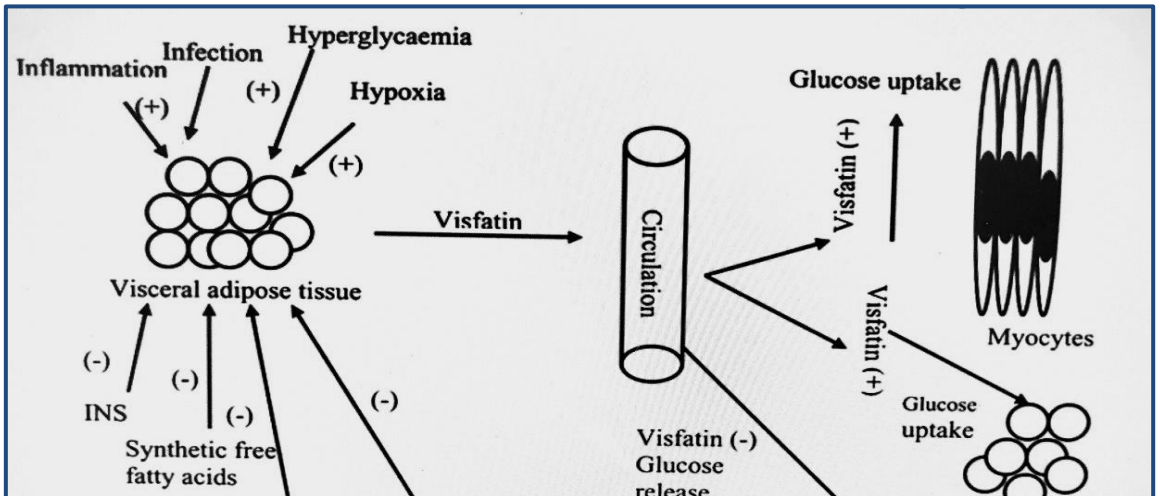

Figure 1-2: Schematic diagram showing factors that stimulate (+) or inhibit (-) visfatin expression in visceral adipose tissue Some physiological effects of visfatin are also indicated. INS=insulin [72].

1.8.2. Leptin

The adipose tissue is considered as major store for fat that plays a passive role in energy metabolism, it is widely accepted the adipose tissue the largest ever endocrine organ [73]. It secretes a lot of hormone adipokines that control feeding immunity reproductive hormones and neuroendocrine function The adipose tissue has emerged after the discovery of the liptin [74]. Leptin is mainly synthesized and secreted by adiopocyte (the major source of leptin) can also be produced by brown adipose tissue placenta, ovaries, stomach, mammary epithelial cell, bone marrow, pituitary and liver [75]. Is a messenger of satiety from that fat cell to the brain regulator of insulin and glucose metabolism and plays role in energy balance and body weight by neuroendocrine mechanism [76]. Leptin circulates in the plasma in a free or bound to leptin–binding proteins is produced larger quantities in subcutaneous adipose tissue than visceral adipose tissue [77]. A fall in leptin mediates weight gain through hypothalamus to increase appetite decrease energy expenditure modify neuroendocrine functions [78].

1.8.2.1. Structure of Leptin

The recently cloned obese (ob) gene encodes 167 amino acid polypeptide (leptin) is specifically from the adipose tissue humans [79]. has been reported endogenous leptin human serum has 145-146 amino acid sequence (16kDa)a C-terminal intermolecular disulfide bond consists a loop structure and without predicted N-terminal signal peptide [80].

To which of these structures the disulfide bound N-terminal sequence or C- terminal sequence is most important the biological activates of leptin [81].

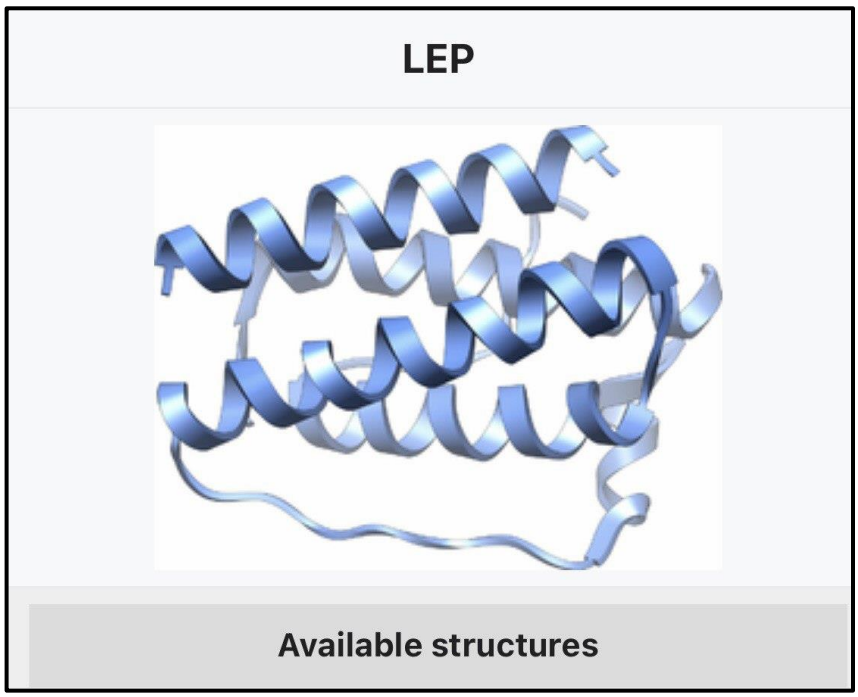

Figure 1-3: Agraphic depiction of leptin molecule the crystal structure of leptin reveals a four– helix– bundle similar to that of long chain helical cytokine family [82].

1.8.2.2. Function of Leptin

Leptin amulti functional protein involved physiological processes include energy regulation inflammation fetal development puberty and digestion [83].leptin is primarily viewed as being involved energy balance and mediator of the adaptation to fasting increased levels in the blood positively correlate with fat stores in many species [83]. When secreted binds to its long isoform receptor lipoprotein receptor–related protein (LRP) hypothalamic neurons to inhibit feeding behavior and increase sympathetically–mediated thermogenesis [84]. As an adipostat hormone that should maintain stable adipose tissue mass a classical negative feedback mechanism has multiple in addition to anorexia and thermogenesis in particular can increase sympathetic nerve activity to non–thermogenic tissues and increase blood pressure [85]. Leptin bind to changes the conformation of LRP the process promotes intracellular activation of Janus

Kinasa 2 (JAK2) and phosphorylation of SH-2domains the signal and activator of transcription protein type 3 (STAT3) phosphorylated dimerized and bind to nuclear promoter regions [86]. The JAK-STAT pathway binding of leptin to the LRb activates phosphatidylinositol-3-kinase mitogen activated protein kinase[87].

Leptin suppresses appetite increases thermogenesis induces weight loss as commonly found in obesity characterized leptin resistance hyper leptinemia is found in obesity and used index of leptin resistance and adipose tissue mass [88].

1.8.3. Resistin

Resistin is distinctive cysteine-rich signaling molecule obtained fromadipocytes, which mainly consists of 114 amino acids and is initially recognized in obese mice. Resistin was cloned in human at 2001 and was shown to be a thiazolidinedione (TZD)-regulated cytokine expressed in adipose tissue The effect of resistin on insulin action has been extensively investigated in laboratory models [89].

Tissue resistin in mice is expressed in higher adipose tissue while resistin in humans is expressed in lower adipose tissue [90]. Yet is primarily expressed in human macrophages. It is considered to be a molecule of pro- inflammatory cytokines that also have a critical role in the diabetes and its complications of pathogens is a recurring release [91].

It was seen as evoking 'high' bad cholesterol (low density lipoprotein) and contributing to heart disease and resistin promotes the development of LDL in human liver cells as well as adversely affects liver LDL receptors .The liver may be less capable of removing 'poor' cholesterol from the body [92].

1.8.3.1-Resistin and Obesity

The concentration of serum resistin was positively correlated with changes of BMI and body adipose mass. Circulating resistin levels increase with age probably reflecting the increase in the body fat content [93]. With human

obesity elevated serum resistin levels were observed when compared with humans in lean condition [94]. Resistin is involved in the proliferation of adiposities and angiogenesis. Obesity is associated with abnormally elevated JNK activity predominantly provided by JNK1. It is a vital component of the obesity-induced insulin resistance pathway in vivo [95].

Scientists have suggested that resistin is a hormone that links obesity to diabetes. Experiments in humans have shown no differences in resistin expression among norma linsulin-resistant and type 2 diabetic samples [96].

1.8.3.2. Resistin and Diabetes

The role of adipocyte hormones in modulating insulin sensitivity and glucose tolerance are of common interest and importance in studies of type 2 diabetes mellitus. Recently resistin has been proposed to play an important role in the pathogenesis of obesity related insulin resistance [97]. An elevated expression of resistin in circulation leads to glucose intolerance hyper insulinemia related with impaired insulin signaling in skeletal muscle liver and adipose tissue. The important role of enzyme adenosine monophosphate activated protein kinase (AMPK) in the liver is to stimulate the fatty acid oxidation that cholesterol synthesis and intonate insulin secretion by pancreaticβ cells [98]. Resistin inhibits the phosphorylation of the hepatic AMPK pathway that down regulates β oxidation to lipid accumulations subsequently resistin stimulates suppressor of cytokine signaling -3 SOCS-3 in mice adipose The stimulated of suppressor of cytokine signaling (SOCS-3) inhibits the insulin signaling pathway in tissues. Moreover, resistin affects glycogen metabolism, leading to type 2diabetes [99].

An elevated level of circulating resistin was detected in obesity and diabetes. This discovery suggests that deregulation of resistin induces insulin resistance in genetic models and in a diet-induced model of diabetes and obesity[100]. In humans resistin is primarily released by monocytes/macrophages

suggesting that soluble levels may be associated with macrophage activation. Here systemic and monocyte-released resistin levels were found to be similar in type 2 diabetic [101]. Human resistin in the development of insulin resistance and inflammation human resistin may be linked from insulin resistance to inflammatory diseases such as obesity type 2 diabetes, and atherosclerosis [102].

1.8.4. Insulin

Insulin is polypeptide hormone composed of 51 amino acids and has molecular weight of 5808 Dalton secreted by pancreatic B-cells of Langerhans the pancreas consists of clusters of endocrine tissue called the islets of Langerhans [103]. Insulin are embedded in exocrine tissue islets are composed of four major phenotypically specific hormone producing cell (α β, and δ, or cells produce glucagen, insulin, somatostatin and pancreatic poly peptide respectively[104]. Islets are innevated by autonomic nervous system and hightly vascularized with perfusion rate similar to that of the brain and can respond to changes of nutrient content in the blood [104]. The body's metabolism of carbohydrates and fats is controlled by insulin which allows cells in the liver, muscles, and fat tissue to absorb glucose from the blood and store it as glycogen[105]. Insulin was detected in the cerebro spinal fluid and insulin receptors demonstrated in the brain. These result suggested circulating insulin enters the brain to regulate neuronal function not related to glucose uptake . Insulin enters the human brain, enhances learning and memory and benefits memory in particular brain insulin signaling by means of unsulin administration suggesting that central nervous insulin to the control of whole body energy homeostasis in humans [106]. TIDM and/or the metabolic syndrome can develop if there is insulin resistance which necessitates the pancreas secreting more insulin. If this compensatory increase is absent blood glucose levels rise[107]. Insulin resistance is defined inability of insulin to reduce plasma glucose level due to impairment of target organs to respond normally to the biological action

of insulin, it considered a physiological state in which cells, particularly those of muscle, liver, tissue and fat, exhibit resistance to insulin by failing to take up and utilize glucose for energy and metabolism [108].

1.9. Lipid Profile

1.9.1. Cholesterol

Cholesterol is present in tissue and plasma lipoprotein as free or combined with along chain fatty acid as cholesteryl ester. Sources of cholesterol are dietary cholesterol *de novo* synthesis from acetyl-CoA[109]. Cholesterol synthesized in many tissues from acetyl-CoA and ultimately eliminated from the body in the bile as cholesterol or bile salt [110].

Cholesterol is the precursor of other steroid in the body such as corticosteroids, sex hormones, bile acid vitamin D. typically a product of animal metabolism and occurs in foods of animal origin such as meat, egg yolk, liver, brain [111]. Found as cholestery lester the hydroxyl group on position 3 is esterified with along chain fatty acid. **The structure of cholesterol, the most common animal steroid** in **Figure 1-4**

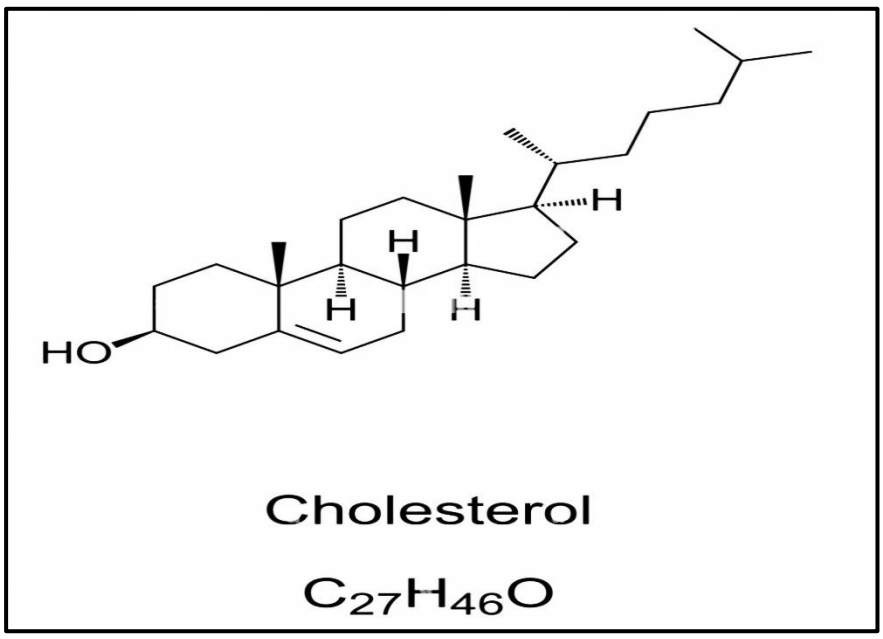

Figure 1-4: The structure of cholesterol, the most common animal steroid [112].

Increased cholesterol is found in high fat diets hypercholesterolemia hypothyroidism primary biliary cirrhosis and some cases of diabetes. Low levels have been found in malabsorption severe liver disease polycythemia [113].

1.9.2. Triacylglycerol

Triacylglycerols store metabolic energy in organisms and have industrial uses as foods and fuels. Excessive accumulation of triacylglycerols in humans causes obesity and is associated with metabolic diseases [114]. Triacylglycerol synthesis is catalysed by acyl-CoA diacylglycerol acyltransferase (DGAT) enzymes [115]. A structure obtained with oleoyl-CoA substrate resolved at approximately 3.2 Å shows that the CoA moiety binds DGAT1 on the cytosolic side and the acyl group lies deep within a hydrophobic channel, positioning the acyl-CoA thioester bond near an invariant catalytic histidine residue [116]. Within the reaction center in a direction opposite from that of acyl-CoA is a lipid-like density that may represent an acyl-acceptor molecule. The basis for a model of DGAT's catalysis of triacylglycerol synthesis is provided by the insights offered by the DGAT1 structures along with mutagenesis and functional research [117].

1.9.3. Plasma Lipoproteins

Total plasma lipids range between 400-600mg/dl. One third is cholesterol one third is triacylgycerol and other include phospholipid lipid are insoluble in water they help to be carried in plasma they are complexed with protein to from lipoprotein the protein part of lipoprotein is called apo lipoprotein [118]. Plasma lipoprotein are spherical macromolecular complexes of lipid and specific protein (apo Lipoprotein) [118]. They include chylomicrons very low density lipoprotein (VLDL). low density lipoprotein (LDL) intermediate density lipoprotein (IDL) high density lipoprotein (HDL). They differ in lipid and protein composition, size, density, site of origin. Function both to keep their component lipid soluble they transport in the plasma to provide efficient mechanism for transporting lipid contents to the tissue [119].

Lipoprotein are composed of neutral lipid core (triacylglycerol, cholesteryl esters) surrounded by shell of amphipathic apo lipoprotein, phospholipid, non esterified cholesterol, depending on density or electrophoretic mobility are classified five major type [120].

1.9.3.1. Chylomicron

Lipoprotein particles of the lowest density a largest in size and contain the hightest percentage of lipid and smallest percentage of protein [121]. It represent triacylglycerol and ester of cholesterol with coating of phosphpolipids, cholesterol and protein enter the lymphatic system and transported via the thoracic duct to the blood stream [122]. Main sites for removal of chylomicrons are muscle and liver lipoprotein lipase enzyme bound to the capillary endothelium of extra hepatic tissues hydrolyzes triacylglycerol in chylomicrons VLDL in free fatty acide and glycerol [123]. After entering adipose tissue or muscle thesis compound are esterified and stored. Smaller remnant particles contain mainly cholesterol and pass to the liver they are metabolized further [124].

1.9.3.2. Very Low Density Lipoprotein (VLDL-C)

VLDL-C are produced by the liver and serve vehicle for delivery of endogenous lipid to peripheral tissue. Nascent VLDL-C formed within the hepatocyte from the fusion of partially lapidated [125].

Synthesized apoB-100 with triacylglycerol–rich lipid droplet follow by addition of apo E, apoC-I, apoC-II. triacylglycerol and cholesterolester used by hepatocytes incorporation into VLDL generated by the enzyme diglycerol acyltransferase (DGAT)and acyl-COA cholesterol acyltransferase (ACAT) [126].

1.9.3.3. Low density lipoprotein

IDL is converted to LDL largely by the liver by removal of additional TGs in addition to its formation from VLDL some LDL, is produced and released by the liver LDL is a major transport form of cholesterol and cholesteryl esters[127] The relative rates of VLDL and LDL release by the liver

depend ,the availability of cholesterol regulatory pathway signal the liver to increase cholesterol output, the liver increases is LDL production, is internalized by receptor mediated endocytosis the receptor LDL complex is transported to lysosomes the LDL receptors are recycled LDL is created from VLDL after being degraded by lipoprotein lipase From the liver to peripheral tissue LDLs carry cholesterol [128]. High levels of LDL show that there is considerably more cholesterol in the bloodstream than is essential ,since LDL causes atherosclerosis and is hence referred to as bad cholesterol It is therefore crucial to keep LDL levels low [129].

1.9.3.4. Intermediate Density Lipoproteins

IDL as intermediate during the metabolism between VLDL and LDL are synthesized from VLDL after losing part of triacylglycerol amount [128]. The compound either converted to lipoprotein or transport to liver the concentration in blood are very small or seemed to be transition step [129].

1.9.3.5. High Density Lipoproteins

High Density Lipoprotein HDL are synthesized *de novo* in the liver and small intestine as primarily protin rich disc shaped particles newly formed HDL are nealy devoid of cholesterol and cholesteryl esters. The apoprotein of HDL are apo A-I, apo-C-I,apo-C-II and apo-E.HDL are converted into spherical lipoprotein particles the accumulation of cholesteryl esters. Any free cholesterol present in chylomicron remnants and VLDL remnant can be esterified through the action of the HDL-associated enzyme lecithin cholesterol acyltransferase LCAT is synthesized in the liver because transfers a fatty acid from the C-2 position of lecithin to the C-3OH of cholesterol generating a cholesteryl ester and lysolecithin. The activity of LCAT required interaction with apo –A-I is found on the surface of HDL.

HDL is the main transport from cholesterol from peripheral tissue to liver, is later excreted through bile .contain the highest protein concentration it was known protective against heart attacks [130].

Objectives

Aims of this study are:

1- Study the role of visfatin in sera patient with type 2 diabetes mellitus with and without atherosclerosis.

2- Study the hormone liptin and resistin in sera of patients with DM type 2 and DM with atherosclerosis.

3- Study the sensitivity and specifity for adipokines and anther parameter in sera of patient group.

CHAPTER TWO

Materials and Methods

2. Materials AND METHODS

2.1. Material

2.1.1. Chemicals and Kits

The chemical and kits in the present study were used as supplied from purchases without additional purification .Kits and chemicals used in the present study are show in Table 2-1.

Table 2-1: The chemical and kits

No.	Chemicals	Company and country
1	Glucose Kit	Linear (Spain)
2	HDL-cholesterol Kit	Linear (Spain)
3	Resisten ELISA Kit	Mybiosource (USA)
4	Leptin ELISA Kit	Mybiosource(USA)
5	Insulin ELISA Kit	Mybiosource (USA)
6	Triglyceride Kit	Linear (Spain)
7	Total cholesterol Kit	Linear (Spain)
8	Visfatin ELISA Kit	Mybiosource (USA)

2.1.2. Instrument and Equipments

The instruments and Equipments used in this study are listed in the Table 2-2.

No.	Instrument and Equipments	Company, Origin
1	Deep Freeze	Samsung\Korea
2	Centrifuge	Hettich
3	ELISA Reader	Biotek, (USA)
4	ELISA Washer	Biotek, (USA)
5	Disposable syringes (5ml)	Medical jet (Syria)
6	Disposable test tube (10 ml)	Meheco (China)
7	Eppindorf tube (0.5ml)	China
8	Distiller	GFL, (Germany)
9	Spectrophotometer CECIL7200	CECIL. (England)
10	Blue and yellow tips	JRL, (Lebanon)
11	Incubator	Fisher Cient, (Germany)
12	Water bath	Grant, (Germany)
13	Micropipettes (5-50m), (2-20m), 20-200m), (100-1000 m)	Slamed, (Germany)
14	Multichannel micropipette(0-250ul)	Watson Nexty (Japan)
15	Filter papers	AFCO (Jorden)
16	Plain tube	ASL (Jorden)
17	Printer	Epson (Indonesia)

2.2. Subjects

This is a case-control study includes (90) people divided into three groups the first group includes (30) patients previously diagnosed with T2DM .The seconed group includes (30) apparently healthy control and third group includes (30) patients diagnosed with T2DM and atherosclerosis according to cardiac catheterisation. Blood samples participants were collected from (October 2022 till November 2022). Questionnaires was filled by participants and to get the agreement was gotten to participants in this study to collect the information of control and patients group.

The Patients group involved (two) group they were diagnosed with T2DM patient and T2DM with atherosclerosis with a mean age of (35-75) years. All patients were diagnosed by physicians and according to ADA criteria. They were enrolled to Babylon Center for Diabetes and Endocrinology in Marjan Teaching Hospital in Hilla city Babylon province. The second sub group enrolled to Iraqi Center for Cardiac Surgery Baghdad Governorate . All patients were diagnosed by physicians and according to cardiac catheterisation. all patients with the following problems were excluded from the current study.

1- Smokers.
2- Pregnant women.
3- Type 1 diabetes patient
4- Patient with kidney disease.
5- Thyroid disease
6- Chronic liver disease,

Control group involved appereutly healthy subjects . All individual do not have any signs and symptoms of diseases. Controls subjects consist of (15 males and 15 females). The blood samples were collected from relatives and medical staff of Medical city of Baghdad the patients and control groups age ranged between (35-75) years.

2.3. Methods

2.3.1. Blood Sampling

Blood samples were collected from control and patients group. Three to five milliliters of blood were obtained from diabetic patients and control then collected. In tube without anticoagulants tubes were left for 15 minutes at room temperature to clot. After that the blood samples were centrifuged at 2000 (xg) for 10 minutes . Then the sera were aspirated and stored at (-20 ºC) until use determination of (serum insulin, liptin, visfatin, resistin).

2.3.2. Body Mass Index (BMI)

Body mass index was calculated in all subjects which is calculated the weight in kilogram divided by the square height in meters and the results were considered as follows:

Underweight ≤ 18.5.

Normal weight between 18.5 - 24.9.

Overweight between 25-29.9

Obese ≥ 30

BMI (kg/m2) = weight (kg) / height (m2) [131].

2.3.3. Measurement the Concentration of Fasting Blood Glucose

2.3.3.1. Principle:

The first step was oxidized of glucose to produce gluconate by glucose oxidase (GOD) . In this interaction hydrogen peroxide (H_2O_2) is liberate . In the presence of peroxidase (POD) enzyme .Phenol and 4-amino antipyrine (4-AA) The latter is oxidized by H_2O_2 to produce a red quinonimine dye. This dye is equivalent to the level of glucose in sample the reactions illustrated in following: [131]

$$\text{Glucose} + O_2 + H_2O \text{ -GOD} \longrightarrow H_2O_2 + \text{Gluconate}$$

$$2H_2O_2 + \text{Phenol} + \text{4-AA POD} \longrightarrow 4H_2O + \text{Quinonimine}$$

2.3.3.2. Reagents

1- Reagent 1 (Buffer):- Consist of 100 mmol/L of Tris HCl buffer pH7 and 0.3 mmol/L Phenol.

2- Reagent 2 (Enzymes):- Consist of 10000 U/L of glucose oxidase, 1000 U/L of peroxidase and 2.6 mmol/L of 4-amino-antipyrine.

3- Reagent 3 (Standard):- Consist of 100 mg/dl or 5.56 mmol/L of glucose.

2.3.3.3. Preparation of Reagents

Working reagents was prepared by adding the substance in vial Reagent 2 which contains (Enzymes) to a vial of reagent 1 which contains (Buffer). The mixture was mixed gently to complete dissolving of all components. The procedure is illustrated in the following Table:

Reagents	**Blank**	**Standard**	**Sample**
Working reagent	1ml	1m	1 m
Standard (Std.)	-	0.01ml	-
Sample	-	-	0.01mI

After addition the tube was allowed to stand for 5 minutes at a temperature (37˚C.). Then the absorbance was read at 500 nm using cuvette of 1 cm light path.

2.3.3.4. Calculation

Result was calculated from the following formula:-

Glucose (mmol/L) $\frac{Sample\ absorbance}{Standard\ absorbance}$ × C standard

2. 4. Measurement of Lipid Profiles

2. 4.1 Measurement of Total Cholesterol

2. 4.1.1 Principle

As show in the following Cholesterol concentration was determined enzymatic reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten 4 one 3} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{PAP} \xrightarrow{\text{peroxidase}} \text{Quinonimine (pink)} + 4H_2O$$

2. 4.1.2 Reagents

Reagents	Composition	
Reagent 1 (Buffer)	Phosphate buffer	100 mmol/L
	Chloro-4-phenol	5.0 mmol/L
	Sodium chloride	2.3 mmol/L
	Triton ×100	1.5 mmol/L
	Preservative	
Reagent 2 (Enzymes)	Cholesterol oxidase	100 IU/L
	Cholesterol esterase	170 IU/L
	Peroxidase	1200 IU/L
Reagent 3 (Standard)	Cholesterol 200 mg\Dl	

2. 4.1.3. Procedure

Working reagents was prepared by adding the substance in vial Reagent 2 which contains (Enzymes) to a vial of reagent 1,which contains (Buffer) .The mixture was mixed gently to complete dissolving of all components .The procedure is illustrated in the following Table :

Reagents	Blank	Standard	Sample
Reagent	1mL	1mL	1mL
Demineralized water	10mL	-	-
Standard	-	10mL	
Sample	-	-	10ml

The tube were mixed and then let stands for 5 minutes at 37°C record absorbance at 500 nm the color is stable for 1 hour.

2.3.4.1.4. Calculation

Cholesterol (mmol/L)= $\frac{sample\ absorbance\ (at\ 500\ nm)}{standard\ absorbance}$ ×5.17 (mmol\L)

2.3.4.2. Measurement of Triglyceride (TG)

2.3.4.2.1. Principle

TG concentration was determined enzymatically splitting with lipoprotein lipase, as show in the following reactions:

Triglycerides $\xrightarrow{LPL}$ Glycerol +free fatty acids

Glycerol + ATP $\xrightleftharpoons{G\ K}$ Glycerol-3-phosphate +ADP

Glycerol-3-p+O_2 $\xrightleftharpoons{GPO}$ Dihydroxyacetone –p+H_2O_2

H_2O_2+4-chlorophenol+PAP $\xrightarrow{POD}$ Quinonimine pink)+4H_2O

2.3.4.2.2. Reagents

Reagents	Composition
Reagent 1(Buffer)	100 mmol/L Magnesium chloride 9.8 mmol/L Chloro-4-phenol 3.5 mmol/L Preservative
Reagent 2 (Enzymes)	Lipase 1000IU/L Peroxidase 1700IU/L Glycerol-3-p-oxidase 3000IU/L Glycerol kinase 660IU/L PAP 0.5mmol/L ATP 1.3mmol/L
Reagent 3 (Standard)	Glycerol equivalent to triglycerides 200 mg/dl or 2.28 mmol/L

2.3.4.2.3. Procedure

The content of vial reagent 2 Enzymes was added to vial reagent 1 Buffer, mixed gently until complete dissolution 2 minutes to prepare work reagent was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1mL	1mL	1mL
Demineralized water	10mL	-	-
Standard	-	10m	
Sample	-	-	10m

The tube mixed, and let stands for 5 minutes at 37°C or 10 minutes at room temperature absorbance at 500 nm against blank.

2.3.4.2.4. Calculation

Triglyceride (mmol) = $\frac{Sample\ absorbance}{Standard\ absorbance}$ × 2.28 (mmol/L)

2.3.4.3. Measurement of High Density Lipoprotein–Cholesterol (HDL-C)

2.3.4.3.1. Principle

LDL,VLDL and chylomicron from specimens were precipitated by phosphortungstic acid and magnesium chloride . HDL-cholesterol obtained in supernatant after centrifugation then measured with total cholesterol reagent.

2.3.4.3.2. Reagents

Reagents	**Composition**
Reagent 1(precipitant)	Phosphotungstic acid 13.9 mmol/L Magnesium chloride 490 mmol/L
Reagent 2 (Standard)	CholesterolEsterase 100mg/dl Cholesterol Oxidase 200 mg/dl

2.3.4.3.3. Procedure

The procedure was carried as in the following:

Reagent	**Volume**
Serum	0.5 ml
Precipitant	50Ul

The tubes were mixed vigorously let stand for 10 minutes at room temperature centrifiuge 15 minutes at 1400-1800×g. Apply next procedure include measurement of cholesterol

The tube mixed then let stands 5 minutes at 37°C. Absorbance at 500 nm againt blank the color is stable for 1 hour.

2.3.4.3.4. Calculation

HDL-cholesterol mmol\L=$\frac{Sample\ absorbance\ (at\ 500\ nm)}{Standard\ absorbance}$×2.58 (mmol\L)

2.3.4.4. Measurement of Low Density Lipoprotein –Cholesterol (LDL-C)

LDL-cholesterol concentration calculated by using Friedewald equation[132]

LDL-cholesterol (mmol\L)=Total cholesterol - HDL-cholesterol +$\frac{TG}{5}$ (VLDL)

2.3.5. Determination of Serum Visfatin concentration

2.3.5.1. Principle

VF ELISA Kit applies the competitive enzyme immunoassay technique utilizing polyclonal anti–VF antibodyand an VF-HRP conjugate the assay sample and buffer are incubated together with VF-HRP conjugate in pre-coated plate for one hour . After the incubation period the wells are decanted and washed five time the wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex finally a stop solution is added to stop the reaction will then turn the solution yellow the intensity of color is measured spectrophotometrically at 450nm in amicroplate reader. The intensity of the color is inversely proportional to the to the VF concentration since VF from sample and VF-HRP conjugate compete for the anti-VF antibody binding site. The number of sites is limited fewer sites are left to bind VF-HRP conjugate. Standard curve is plotted relating the intensity of the color (O.D.) The concentration of standards the VF concentration in each sample is interpolated form standard curve [132].

Figure 2-1: ELISA instrument used in the study.

2.3.5.2. Reagents preparation

1- Constitutes of concentrated wash solution were diluted to 1000ml with DW in a suitable container .
2- Each vial of the standards solution was reconstituted with 2 ml of DW.

2.3.5.3. Procedure

1- A volume of (100µl) was taken from, controls, calibrators and samples, and were added to the wells of a microplate.
2- A volume of (100µl) was taken from Visfatin Enzyme Reagent and it was added to each well.
3- The plate was shaken gently for (20–30) sec for mixing. Then the plate was covered by using plate seal and incubated for (60) min at the room temperature.
4- Microplate was washed three times with 350µl of wash buffer for each well per wash.
5- A volume of 100µl of substrate solution was added to each well.
6- Reaction waited for 15 min at room temperature.
7- The reaction was stopped by using 50µl of stop solution addition.
8- The absorbance was read within (15) minutes at 450nm.

2.3.5.4. Calculation of Results:

A dose response standard curve was used to evaluate concentration of in serum as shown in figure 2-2.

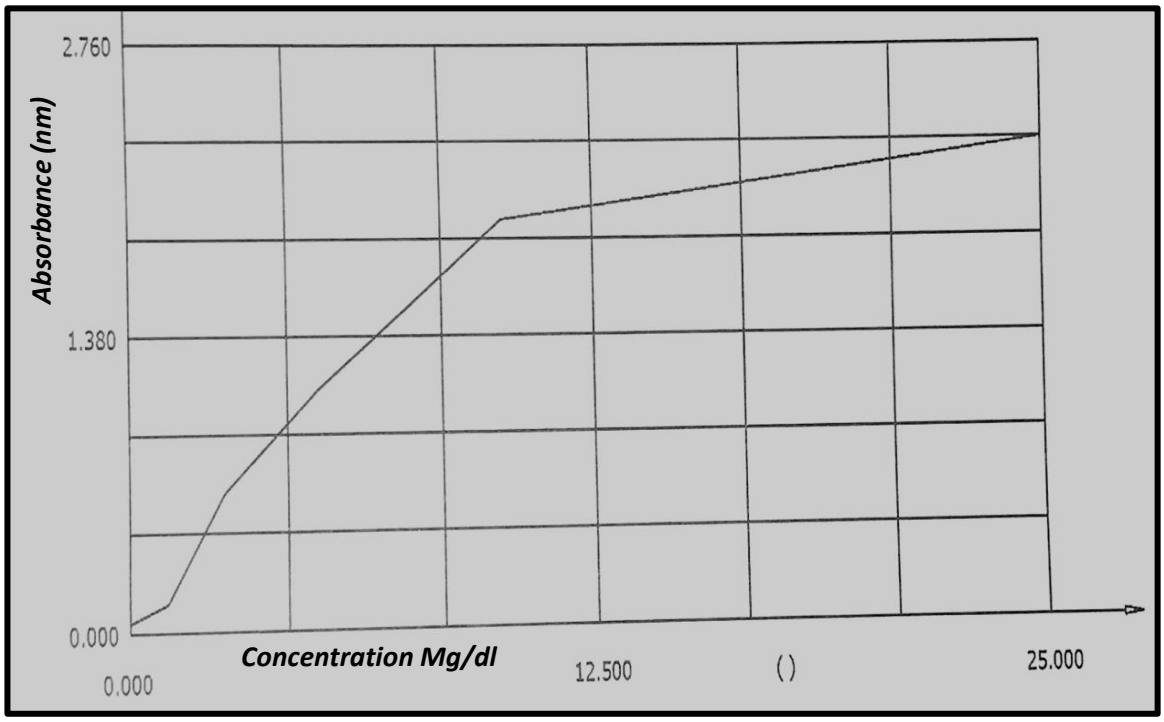

Figure 2-2: Standard Curve of Human Visfatin.

2.3.6. Measurement of Human Leptin

2.3.6.1. Principle

This assay is based on a sandwich ELISA formal leptin present in sample or standards bind to the anti-leptin antibodies pre-adsorbed on the microtiter plate next biotinylated anti-leptin antibody is added to the plate well and binds to the captured laptin . A streptavidin enzyme conjugate is added binds to the biotin of the second antibody unbound streptavidin enzyme conjugate is removed during awash step and substrate solution is added to the wells acolored product is formed in proportion to the amount of leptin present in the sample the reaction is terminated by addition of acide and sbsorbance is measured at 450 nm sample concentration is then determined by comparing to the known values of the standard curve [133].

2.3.6.2. Reagents

1-Constitutes of concentrated wash solution were diluted to 1000ml with DW in a suitable container .

2-Each vial of the standards solution was reconstituted with 2 ml of DW

2.3.6.3. Procedure

1-A volume of (100µl) was taken from, controls, calibrators and samples, and were added to the wells of a microplate.

2-A volume of (100µl) was taken from Liptin Enzyme Reagent and it was added to each well.

3-The plate was shaken gently for (20–30) sec for mixing. Then the plate was covered by using plate seal and incubated for (60) min at the room temperature.

4-Microplate was washed three times with 350µl of wash buffer for each well per wash.

5-A volume of 100µl of substrate solution was added to each well.

6-Reaction waited for 15 min at room temperature.

7-The reaction was stopped by using 50µl of stop solution addition.

8-The absorbance was read within (15) minutes at 450nm

2.3.6.5 Calculation of Results

A dose response standard curve was used to evaluate concentration leptin in serum as shown in figure (2-3).

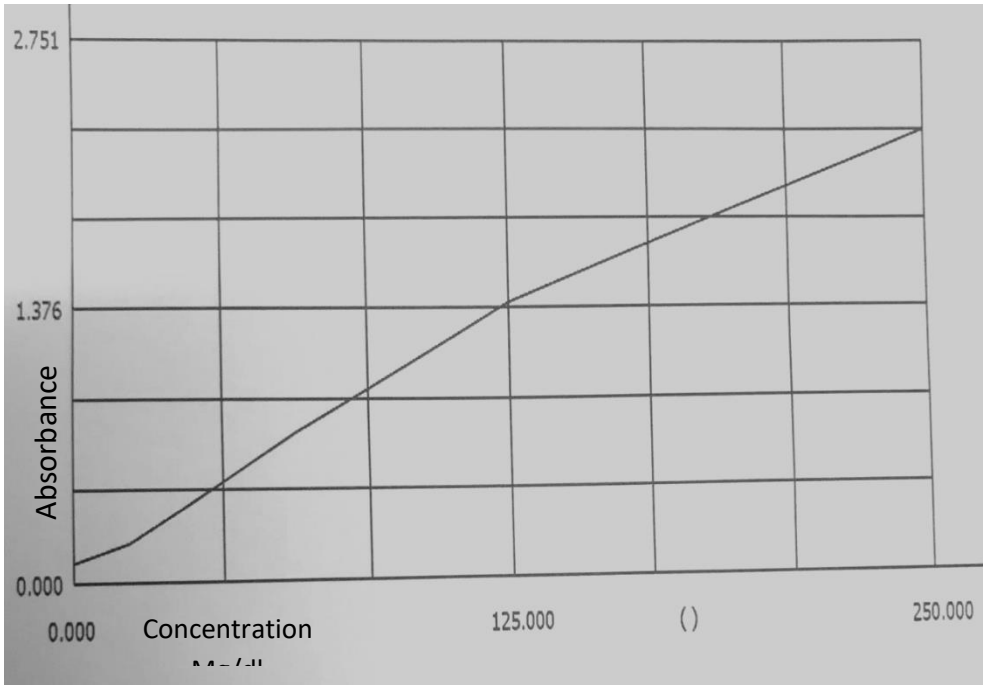

Figure (2-3): Standard Curve of Human leptin.

2.3.7.7. Measurement of Human Resistin

2.3.7.1. Principle:

This assay is based on a sandwich ELISA formal resistin present in sample or standards bind to the anti-resistin antibodies pre-adsorbed on the microtiter plate next biotinylated anti-resistin antibody is added to the plate well and binds to the captured resistin. A streptavidin enzyme conjugate is added binds to the biotin of the second antibody unbound streptavidin enzyme conjugate is removed during awash step and substrate solution is added to the wells acolored product is formed in proportion to the amount of resistin present in the sample the reaction is terminated by addition of acide and sbsorbance is measured at 450 nm sample concentration is then determined by comparing to the known values of the standard curve[134].

2.3.7.2. Reagents

1- Microelisa strip plate with 96 wells.
2- Standard Solution: Consist of 1 vial contains 0.5ml of 2700 pg/ml resistin.
3- Standard Diluents: Consist of 1 vial contains 1.5ml of diluents.
4- HRP-Conjugate Reagent: Consist of 1 vial contains 6ml of HRP reagent.
5- Sample Diluents: Consist of 1 vial contains 6ml of diluents.
6- Chromogen Solution A: Consist of 1 vial contains 6ml of solution.
7- Chromogen Solution B: Consist of 1 vial contains 6ml of stop solution.
8- Stop Solution: Consist of 1 vial contains 6ml of stop solution.
9- Wash Solution: Consist of 1 vial contains 20ml of wash solution.

2.3.7.3. Reagents Preparation

Standards Preparation:

The preparations of the standards were done by use seriesdilution process as described in standard and as shown in figure 2-4.

After this process the concentrations of standard solution will be 1800 pg/ml 1200 pg/ml, 600 pg/ml, 300pg/ml and 150 pg/ml, respectively.

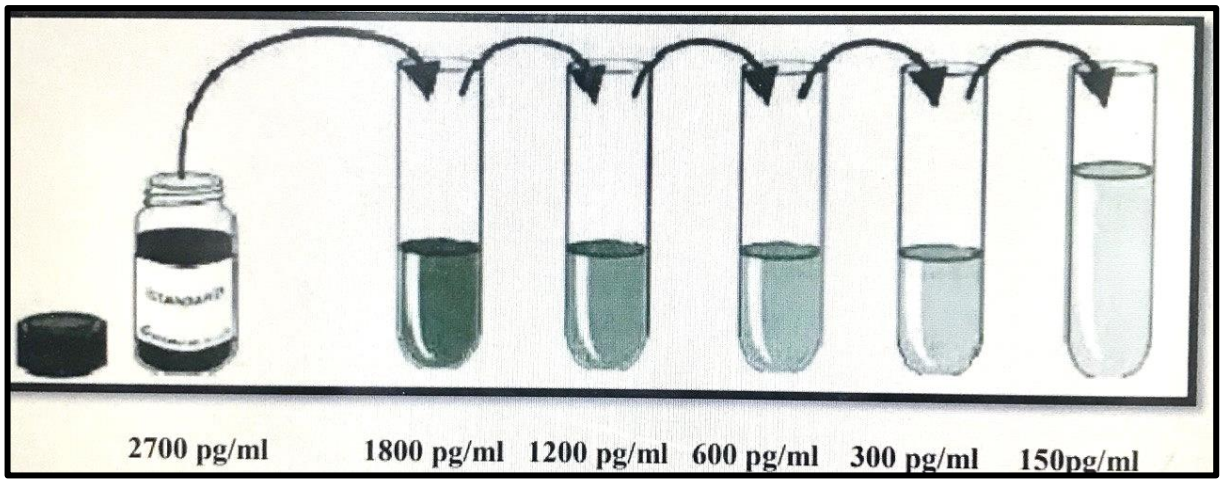

Figure 2-4 Series Dilution of Standards Solution of Resistin.

2.3.7.4. Procedure

1- In the plate, a well was left empty as a blank control, in sample wells 40µl of sample dilution buffer and 10µl of the sample were added and mixed well with gentle shaking.
2- The plate was incubated for thirty min at 37℃ after covering with closure plate membrane.
3- The cover on a plate was removed and wells were washed with 400µl of wash solution by use auto washer for 5 times.
4- A volume of 50 µl (HRP-Conjugate) reagent was added to each well in the plate except the well of blank control.
5- Incubation was done as described in step two.
6- Washing was done as described in step three.
7- A volume of 50 µl of chromogen solution A and 50 µl chromogen solution B were added to each well and mixed with gentle shaking and plate was incubated at 37 ℃ for 15 min the plate should be protected from light during the process of chromogen addition.
8- A volume of 50 µl of stop solution was added to each well to stop the reaction.

9- A microtiter plate reader was used to read the absorption at 450 nm within 15 minutes the absorption value of the blank control well was set as zero.

2.3.7.5. Calculation of Results

A dose response standard curve was used to evaluate the concentration of resistin in serum as shown in figure 2-5.

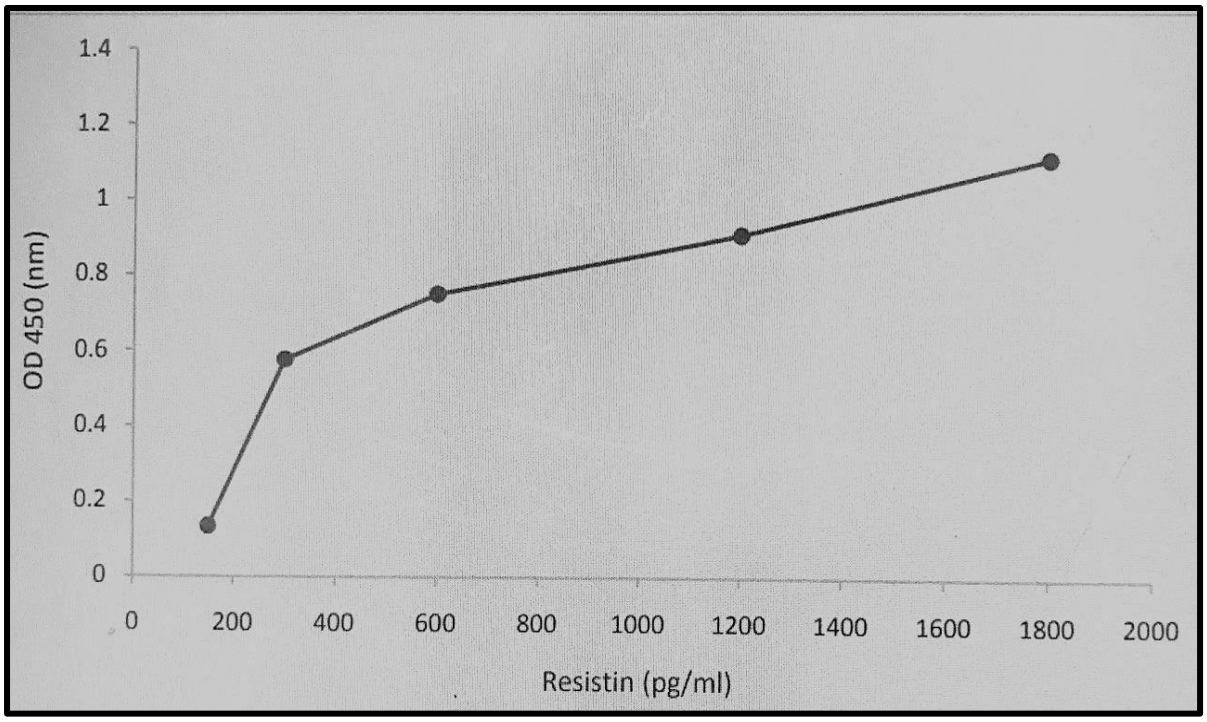

Figure 2-5: Standard Curve of Human Resistin.

2.3.8. Measurement of Human Insulin

2.3.8.1. Principle

This kit was based on sandwich enzyme linked immune sorbent assay technology Anti INS antibody was pre coated onto 96 well plates. and the biotin conjugated antibody was used as detection antibody the standards test sample and biotin conjugated detection antibody were added to the wells subsequently and washed with wash buffer HRP-streptavidin was added and unbound conjugates were washed away with wash buffer TMB substrates were used to

visualize HRP enzymatic reaction TMP was catalyzed by HRP to produce blue color product that changed into yellow after add acidic stop solution the density of yellow is proportional to the INS amount of sample captured in plate read the O.D absorbance at 450 nm in microplate reader than the concentration of INS can be calculated [135].

2.3.8.2. Reagent preparation

1-Constitutes of concentrated wash solution were diluted to 1000ml with DW in a suitable container .

2-Each vial of the standards solution was reconstituted with 2 ml of DW

2.3.8.2.2. Procedure

1-A volume of (100µl) was taken from, controls, calibrators and samples, and were added to the wells of a microplate.

2-A volume of (100µl) was taken from Liptin Enzyme Reagent and it was added to each well.

3-The plate was shaken gently for (20–30) sec for mixing. Then the plate was covered by using plate seal and incubated for (60) min at the room temperature.

4-Microplate was washed three times with 350µl of wash buffer for each well per wash.

5-A volume of 100µl of substrate solution was added to each well.

6-Reaction waited for 15 min at room temperature.

7-The reaction was stopped by using 50µl of stop solution addition.

8-The absorbance was read within (15) minutes at 450nm

2.3.8.2.3. Calculation of Results

A dose response standard curve was used to evaluate the concentration of INS in serum as shown in figure 2-6.

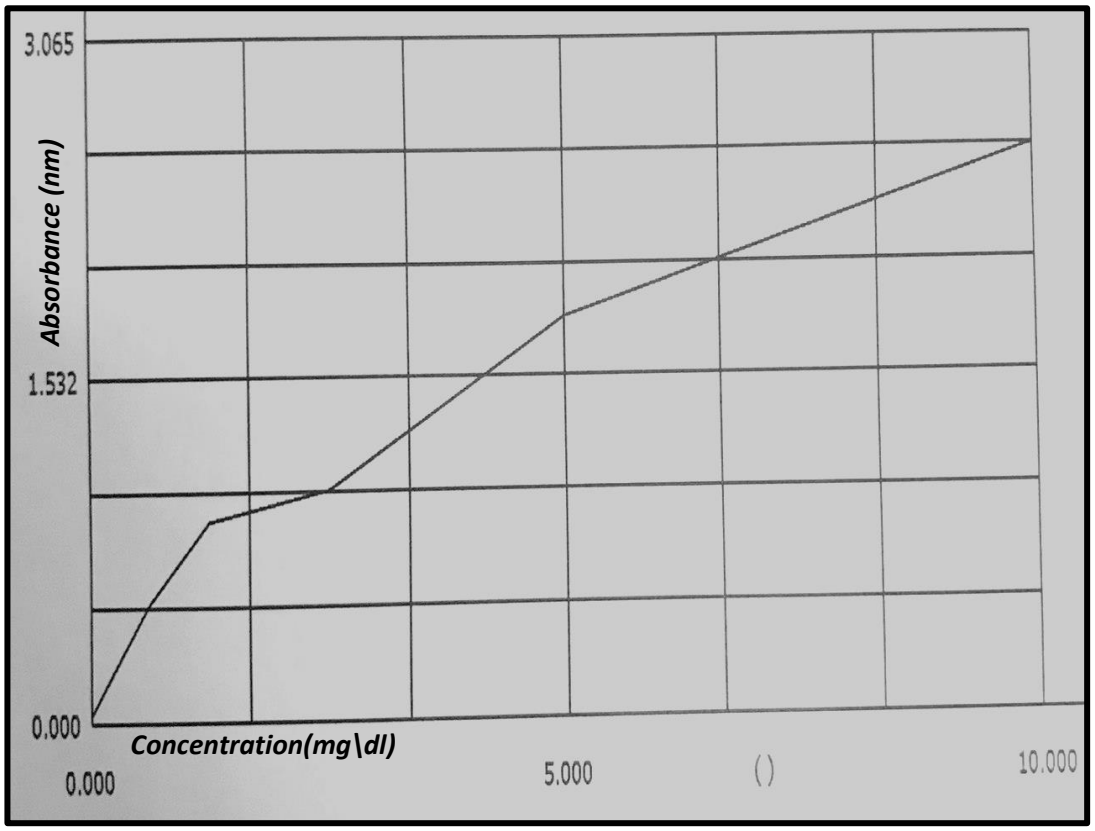

Figure 2-6: Standard Curve of Human INS.

2.4. Statistical Analysis

Statistical analysis done by SPSS, frequency and percentage used for categorical data, mean, median and SD for continuous data. Pearson correlation show the correlation between continuous data. T test used for evaluation differences between mean and median of continues variables. ROC curve also used to show more specific and sensitive cutoff point. P-value less or equal to 0.05 is consider significant.

CHAPTER THREE

Results and Discussion

3. Results and Discussion

3.1. General characteristics of studied groups:

3.1.1. Gender

The global prevalence of diabetes is higher in men, with type 2 diabetes [136]. More males get diabetes before puberty, whereas more women have diabetes after menopause and in old age, this reversal in the gender gap in diabetes prevalence coincides with the reproductive life stage [137]. studies in Sweden have shown that a male predominance was found in all age groups, with a male-to-female ratio of 1.8:1 for Type 1 diabetes and 1.3:1 male > female for Type 2 diabetes [138]. and that agree with study.

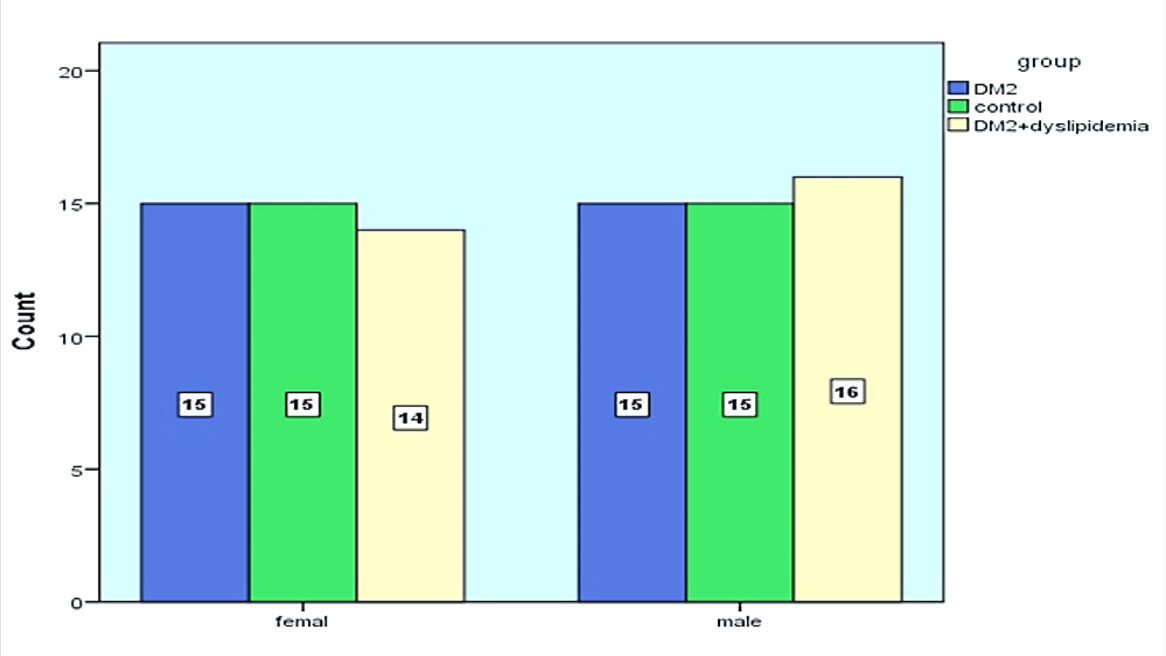

Figure 3-1: Distribution of gender according to study groups.

Concerning atherosclerosis, the disease process is influenced by several factors, including genetics, lifestyle factors (such as diet, physical activity, and smoking), and the presence of underlying un health conditions (such as high blood pressure, high cholesterol, and diabetes). While the exact causes of atherosclerosis are not yet fully understood, it is well-established that the risk of

developing the disease increases with age and is higher in men than in women[139].

3.1.2. BMI

BMI is a measure of body fat based on height and weight, and individuals with a high BMI are more likely to develop type 2diabetes, which is the most common form of diabetes. This is because excess body fat, especially around the waist, can increase insulin resistance, which is a significant factor in developing type 2 diabetes. There is a strong correlation between body mass index (BMI) and the risk of developing diabetes mellitus (D.M.) and atherosclerosis[140]. The Table 3.1 a significant increase in BMI in D.M. patients and patients with D.M. with atherosclerosis more than in control persons. At the same time, no significant difference in mean BMI between D.M. patients and patients with D.M. with atherosclerosis [141].

Table 3.1: Difference in mean BMI between groups.

Group	NO	Mean ± SD		P-value
DM	30	30.46	1.1	**0.0001**
Control	30	20.30	1.34	
DM+ atherosclerosis	30	26.10	0.80	**0.0001**
Control	30	20.30	1.34	
DM+ atherosclerosis	30	26.10	0.80	**0.15**
DM	30	30.46	1.10	

P-value ≤0.05 (significant).

Similarly, a high BMI is also a significant risk factor for the development of atherosclerosis, which is a disease characterized by the buildup of fatty deposits in the arteries. This buildup can lead to blockages in the blood vassals, increasing the risk of heart attack, stroke, and other cardiovascular problems. The link between high BMI and atherosclerosis is likely because obesity is associated with elevated cholesterol levels, high blood pressure, and insulin

resistance, all known risk factors for developing the disease and that agree with our study[142].

3.2. Biochemical characteristic

3.2.1. Fasting blood sugar

Individuals with D.M., high blood sugar levels can lead to several long-term complications, including cardiovascular disease, nerve damage, and eye problems. Therefore, regular monitoring of FBS levels is vital for managing the condition and reducing the risk of these complications [144]. In the current study, we found the mean concentration of (FBS) in D.M. patients and patients with D.M. with atherosclerosis more than in control persons. Also, there is a significant increase in FBS levels in D.M. patients more than in patients who have D.M. with atherosclerosis[143] .

Table 3.2: Fasting level in the serum sample of the study group

Group	NO	Mean ± SD		P-value
DM	30	183.6	52.7	**0.0001**
Control	30	96.5	10.2	
DM+ atherosclerosis	30	181.3	49.8	**0.0001**
Control	30	96.5	10.2	
DM+ atherosclerosis	30	181.3	49.8	**0.0001**
DM	30	183.6	52.7	

P-value ≤0.05 (significant).

It is important to note that several factors, such as age, weight, physical activity, diet, and medications, can also influence FBS levels. Therefore, it is recommended that FBS tests be performed under consistent conditions and at regular intervals to provide an accurate picture of a person's glucose control over time[144].

3.2.2. Triglycerides

The Table 3.3; is shows a significant increase in TG levels in D.M. patients and patients have D.M. with atherosclerosis more than control persons. Also, there is a substantial decrease in TG levels in D.M. patients less than those with D.M. with atherosclerosis.

Table 3.3: Levels of Triglycerides in the serum sample of the study group

Group	NO	Mean ± SD		P-value
DM	30	2.202	0.948	**0.0001**
Control	30	1.606	0.391	
DM+ atherosclerosis	30	2.73	1.68	**0.0001**
Control	30	1.606	0.391	
DM+ atherosclerosis	30	2.73	1.68	**0.024**
DM	30	2.202	0.948	

P-value ≤0.05 (significant).

triglyceride levels can increase in individuals with diabetes mellitus (D.M.) due to several factors:

1. Insulin resistance: D.M. is characterized by high blood sugar levels and insulin resistance, which can cause the liver to produce and store more triglycerides.
2. Poor glucose control: When blood sugar levels are poorly controlled, the liver produces more glucose, which can also increase triglycerides.
3. Obesity: Individuals with D.M. who are also overweight or obese may have higher triglyceride levels, as excess body fat can lead to insulin resistance and increase triglyceride production .
4. Poor diet: A diet high in refined carbohydrates and saturated fats can increase triglyceride levels and contribute to the development of insulin resistance.
5. Certain medications: Some medications used to treat D.M., such as

6. thiazolidinediones, can increase triglyceride The cholesterol content of the remnant rather than the triglycerides is suspected of causing atherosclerosis since the human cells degraded triglycerides and still have cholesterol inside them. Then[146]. the rest penetrates quickly the arterial intima and may preferably be trapped in subendothelial space. Inside intimal macrophages, these cells are converted into foam cells filled with cholesterol, resulting in fatty streak formation and ultimately developing atherosclerosis, ischemic stroke, myocardial infarction, and ischemic heart disease[147]. .

3.2. 3. Total cholesterol (TC)

Table 3.4; is show significant increase in cholesterol level in D.M patients and patients have D.M with atherosclerosis more than control persons. While no significant difference in mean of cholesterol between D.M patients and patients have D.M with atherosclerosis.

Table 3.4: cholesterol level in the study group

Group	NO	Mean ± SD		P-value
D.M	30	4.06	1.52	0.0001
Control	30	2.54	0.43	
D.M with atherosclerosis	30	4.04	1.06	0.0001
Control	30	2.54	0.43	
D.M with atherosclerosis	30	4.04	1.06	0.96
D.M	30	4.06	1.52	

The total cholesterol test measures the combined sum of all cholesterol molecules found in the blood. This test alone does not specify the breakdown of different types of cholesterol; however, it is often combined with other tests that include measurements of HDL-c, LDL-c, and triglycerides [148].

Patients with Diabetes mellitus can have many lipid abnormalities, including elevated levels of total serum cholesterol, total cholesterol level reflects the risk

of heart disease. In general, the higher the level, the higher your risk. An increase in total cholesterol as among patient with T2DM group may be related to decrease lipoprotein lipase LPL level[149], they reported that several factors are related to diabetic dyslipidemia including insulin effects on liver. Apoprotein production, regulation of lipoprotein lipase, and peripheral actions of insulin on adipose and muscle tissue, in an insulin-resistant, which is low-density lipoprotein that is deposited in the arteries, which can lead to a heart attack if it is not remedied with the appropriate treatment unless it is remedied with appropriate treatment and that's agree with our study according to (Langlois and Sniderman,) [150].

3.2. 4. High Density Lipoprotein

The mean value of serum blood HDL level is illustration in [Table 3.5], that shows significant decrease in HDL level in D.M. patients and patients have D.M. with atherosclerosis more than control persons. While no significant difference in mean of HDL between D.M. patients and patients have D.M. with atherosclerosis.

Table 3.5: Difference in mean of HDL-c mmol\L between groups

Group	NO	Mean ± SD		P-value
D.M	30	0.766	0.282	**0.579**
Control	30	0.795	0.252	
D.M with atherosclerosis	30	0.394	0.231	**0.0001**
Control	30	0.795	0.252	
D.M with atherosclerosis	30	0.494	0.231	0.467
D.M	30	0.766	0.282	

P-value ≤0.05 (significant).

HDL reduced in patients with type 2 diabetes mellitus by increasing plasma insulin and activating adenosine monophosphate AMP-activated protein kinase in skeletal muscle[151]. Type 2 of DM and the cluster of pathologies including glucose intolerance/ insulin resistance obesity and high plasma triglycerides that constitute the metabolic syndrome are associated with low and dysfunctional HDL[152], although the mechanisms liking low HDL to atherosclerosis are well characterized the link between low HDL and disordered energy metabolism remain relatively unexplored [153].The lipoprotein abnormalities commonly present in type 2 diabetes include an abnormally high level of triglycerides (TG), a high proportion of small dense low density lipoprotein cholesterol (LDL -c), low high density lipoprotein cholesterol (HDL). This pattern of lipid profile in type 2 diabetes mellitus is termed diabetic dyslipidemia. [154].

3.2. 5. Low-Density Lipoprotein

Table [3.6]; show significant increase in LDL-c level in D.M. patients and patients have D.M. with atherosclerosis more than control persons. While no significant difference in mean of LDL between D.M. patients and patients have D.M. with atherosclerosis.

Table 3.6: Difference in mean of LDL mmol/Lbetween groups

Group	NO	Mean ± SD		P-value
DM	30	1.62	0.39	**0.0001**
Control	30	1.2	0.47	
DM+ atherosclerosis	30	2.05	1.07	**0.001**
Control	30	1.2	0.47	
DM+ atherosclerosis	30	2.05	1.07	0.09
DM	30	1.62	0.39	

P-value ≤0.05 (significant).

Hyperglycemia promotes increased enteral cholesterol absorption and reduces LDL Receptor (LDLR) expression in the liver. The subsequent increase in LDL-C plasma concentration stimulates glucose-mediated insulin secretion. Thus, in the short term, variations in plasma LDL-C concentration are expected when there is a disruption in glucose homeostasis In contrast, in T2DM, chronic exposure to hyperglycemia and insulin resistance triggers a wide range of changes in LDL, in particular, the formation of LDL, which potentiate the pro-atherogenic action LDLs. In addition, LDL have reduced affinity for LDLR and, therefore, exhibit prolonged residence time in plasma, during which they a re subject to constant oxidation and glycation, making them more atherogenic[155].

Furthermore addition of LDL at physiological concentrations to cultured islets decreases glucose-stimulated insulin secretion. Interestingly, this seems to be dependent on the presence of the LDL receptor (LDLr) suggesting a major role for the LDLr in LDL uptake and cholesterol-induced B-cell dysfunction. Indeed, we found that high circulating cholesterol levels, as seen in ApoE, increase islet cholesterol and decrease B-cell function,lack of the LDL receptor leads to increased circulating cholesterol levels without affecting islets cholesterol levels or B-cell function],suggesting that the lack of LDL receptor protects b-cells from cholesterol-induced b-cell dysfunction in a hypercholesterolaemic environment[156]. Notably a lack of the LDL receptor did not prevent cholesterol accumulation in B-cells indicating that cholesterol efflux is the rate-limiting event in maintaining cellular cholesterol levels in B-cells[157] .

Regular monitoring and management of LDL cholesterol levels can help reduce the risk of cardiovascular disease and other complications in individuals with D.M. [158].

3.2.2.6. Visfatin

Table 3.7; shows significant increase in visfatin level in D.M. patients and patients have D.M. with atherosclerosis more than control persons. Also, there is a significant increase in visfatin level in D.M. patients more than patients have D.M. with atherosclerosis.

Table 3.7: Difference in mean of Visfatin ng/ml between groups

Group	NO	Mean ± SD		P-value
DM	30	2.59	0.63	**0.0001**
Control	30	1.50	0.11	
DM+ atherosclerosis	30	2.26	0.32	**0.0001**
Control	30	1.50	0.11	
DM+ atherosclerosis	30	2.26	0.32	**0.015**
DM	30	2.59	0.63	

P-value ≤0.05 (significant).

Visfatin has been suggested to play a role in the development of diabetes mellitus some studies have found that visfatin levels are elevated in individuals with obesity and type 2 diabetes mellitus (T2DM) [159]., which is the most common form of diabetes. Increased levels of visfatin have been associated with insulin resistance, suggesting that visfatin may contribute to the development of T2DM[160]. It has been observed that there is a connection between being obese and having a higher level of visfatin.The more severe the obesity, the higher the level of visfatin. Moreover, it has also been shown that the subcutaneous as well as visceral adipose tissues of obese patients contain significant high levels of visfatin [161]. In a comparison of the serum levels of visfatin in normal and type 2 diabetic patients, several investigators showed that visfatin was significantly elevated in the serum of type 2 D.M. patients compared to controls and the current study agreement [162]. The results on the visfatin levels did not reveal any significant gender-based differences, which is consistent with earlier observations[163]. Significantly higher levels of visfatin were observed in the

T2DM patients, irrespective of their level of obesity, although higher values were observed in the obese and highly obese patients, as reported previously. Several reports suggest a positive correlation of visfatin with insulin resistance whereas others do not suggest a direct role for visfatin in insulin resistance. Fioravanti et al[164]. reported that visfatin levels were substantially reduced in non-diabetic individuals after three weeks of weight-loss therapy, but the reverse was observed with regard to the serum visfatin levels in T2DM patients. This can be explained on the basis of the hypothesis that such a phenomenon developed as a compensatory response to impaired insulin action, thereby confirming that the plasma visfatin levels were a consequence of the degree of insulin resistance. . Nevertheless, the exact relationship between serum visfatin levels and insulin resistance remains unclear, and relevant studies have reported conflicting results . [165]. Elevated visfatin level in patients with T2DM in this study may suggest the impairment of visfatin signaling in targets tissues or the dysregulation in biosynthesis or in response to hyperglycemia, hyperinsulinemia, or adipocytokines in state of diabetes. These need to be clarified by further studies. Plasma visfatin level was likewise significantly associated with $HOMA_{IR}$ in simple regression analysis but not in multiple regression analysis. Only WHR remained significantly associated with plasma visfatin level. On the other hand, plasma visfatin did not correlate with BMI and other biomarkers, such as blood pressure and lipid profile. [166].

3.2.2.7. Leptin

Table 3.8; show significant increase in liptin level in DM patients more than control persons. While significant decrease in liptin level in patients have DM with atherosclerosis than in control persons. Also, there is a significant increase in Liptin level in DM patients more than patients have DM with atherosclerosis.

Table 3.8: Difference in mean of Liptin ng/ml between groups

Group	NO	Mean ± SD		P-value
DM	30	68.68	9.67	**0.0001**
Control	30	42.18	4.38	
DM+ atherosclerosis	30	61.69	2.61	**0.0001**
Control	30	42.18	4.38	
DM+ atherosclerosis	30	61.69	2.61	**0.0001**
DM	30	68.68	9.67	

P-value ≤0.05 (significant).

The exact reason for the increase in leptin levels in individuals with diabetes is not completely understood, but there are several theories. One theory is that the increased body fat in individuals with obesity, including those with type 2 diabetes, leads to increased production of leptin by fat cells [167].

Another theory is that insulin resistance, which is a hallmark of type 2 diabetes, may lead to alterations in the regulation of leptin, causing an increase in its levels. Additionally, high blood glucose levels, which are common in individuals with diabetes, may also contribute to the increase in leptin level duo the data we mentioned above our study is in agreement with a study [168].

However, it is important to note that while there may be an increase in leptin levels in individuals with diabetes, this does not necessarily mean that they are not also experiencing leptin resistance [169].

Leptin resistance can impair the ability of the brain to respond to leptin signals, leading to decreased energy expenditure and increased appetite, and contributing to the development and progression of obesity and type 2 diabetes. Therefore, understanding the mechanisms underlying both the increase in leptin levels and the development of leptin resistance is crucial for the effective management of diabetes[169].

It is important to note that the specific reasons for hypoleptinemia can vary depending on the individual and the underlying health conditions they may have. Further research is needed to fully understand the mechanisms underlying changes in leptin levels. In some cases, treatments to increase leptin levels, such as weight gain or hormonal therapy, may be necessary to manage the symptoms of hypoleptinemia and improve overall health[170].

3.2.2.8. Resistin

Table 3.9; show significant increase in resistin level in DM patients more than control persons. Also, there is a significant increase in Resistin level in DM patients more than patients have DM with atherosclerosis, while significant decrease in patients have DM with atherosclerosis less than patients has DM.

Table 3.9: Difference in mean of Resistin ng/ml between groups

Group	NO	Mean ± SD		P-value
DM	30	98.9	112.3	**0.0001**
Control	30	82.3	53.6	
DM+ atherosclerosis	30	93.5	98.3	**0.0001**
Control	30	82.2	53.6	
DM+ atherosclerosis	30	93.5	98.3	**0.0001**
DM	30	98.9	112.3	

P-value ≤0.05 (significant).

Increased resistin levels have been observed in individuals with type 2 diabetes and obesity. Resistin is a hormone produced by fat cells and has been implicated in the regulation of glucose metabolism and insulin resistance[171]. It is believed that increased resistin levels may contribute to insulin resistance and the development of type 2 diabetes. In individuals with insulin resistance, the body's cells become less sensitive to insulin, leading to elevated blood glucose levels[172]. Some studies have also suggested an association between increased

resistin levels and insulin resistance, diabetes and cardiovascular disease. Genetic studies have provided additional evidence for a role of resistin in insulin resistance and inflammation. Resistin appears to mediate the pathogenesis of atherosclerosis by promoting endothelial dysfunction, vascular smooth muscle cell proliferation, arterial inflammation, and formation of foam cells[173]. Indeed, resistin is predictive of atherosclerosis and poor clinical outcomes in patients with coronary artery disease and ischemic stroke. There is also growing evidence that elevated resistin is associated with the development of heart failure[174].

3.2.2.9 Insulin

Table 3.10: show significant increase in insulin level in DM patients and patients have DM wit atherosclerosis more than control persons. Also, there is a significant decrease in Insulin level in DM patients less than patients have DM with atherosclerosis.

Table 3.10: Difference in mean of Insulin (U/ml) between groups

Group	NO	Mean ± SD		P-value
DM	30	0.85	0.14	**0.0001**
Control	30	0.38	0.05	
DM+ atherosclerosis	30	0.95	0.22	**0.0001**
Control	30	0.38	0.05	
DM+ atherosclerosis	30	0.95	0.22	**0.037**
DM	30	0.85	0.14	

P-value ≤0.05 (significant).

In type 2 diabetes, insulin levels can vary. Initially, in the early stages of type 2 diabetes, the body tries to compensate for insulin resistance by producing more insulin. This results in elevated insulin levels in the blood, also known as hyperinsulinemia, the results of our study agree with [175].

In addition, certain factors such as poor diet, physical inactivity, and weight gain can contribute to the progression of insulin resistance and the

decline of insulin production in type 2 diabetes. Proper management, including lifestyle modifications, weight loss, and medications, can help regulate insulin levels and manage the condition [176].

3.2.2.10. Insulin resistance

Table 3.11; show significant increase in insulin resistance level in DM patients and patients have DM with atherosclerosis more than control persons. Also, there is a significant increase in insulin resistance level in DM patients more than patients have DM with atherosclerosis.

Table 3.11: Difference in mean of insulin resistance between groups

Group	NO	Mean ± SD		P-value
DM	30	0.26	0.12	**0.0001**
Control	30	0.04	0.01	
DM+ atherosclerosis	30	0.15	0.04	**0.0001**
Control	30	0.04	0.01	
DM+ atherosclerosis	30	0.15	0.04	**0.0001**
DM	30	0.26	0.12	

P-value ≤0.05 (significant).

Several factors could increase the likelihood to develop insulin resistance. For instance, age increases the risk of having insulin resistance due to the high proportion of visceral fat, oxidative stress and mitochondrial dysfunction [177]. Abdominal adiposity and increased body fat are other risk factors for insulin resistance and this is due to the high amount of free fatty acids and pro-inflammatory cytokines released from visceral fat tissue into the portal vein of obese subjects, causing the development of hepatic insulin resistance and type 2 diabetes[178]. Other risk factors include gender, physical inactivity. Further, diet has been shown to be effective in improving insulin resistance and reducing the incidence of type 2 diabetes [179]. A study conducted among subjects with

impaired glucose tolerance showed that diet was able to reduce the incidence of type 2 diabetes by 33% after a follow up period of 6 years [180].

HOMA-IR=[fasting insulin (uU/ml)] × fasti[ng glucose (mmol/L]/22.5

3.3. Relationships and correlation coefficients

3.3.1. Correlation of Cholesterol and TG And Another Parameter in Patients Have DM Type 2 Only.

There is significant **positive** correlation between cholesterol and (TG, LDL-c and vasfatin), and also there is significant **positive** correlation between TG and vasfatin, HDL-c and insulin, LDL-c and vasfatin, insulin and vasfatin, age (years) and BMI (kg/m^2). As show in Table 3.12: correlation between parameters with each other in patients have DM type 2 only.

Table 3.12: Correlation of evaluated parameters in patients have DM type 2 only

		CHOL	TG	HDL-c	LDL-c	visfatin	Liptin	Insulin	BMI	R	IR	Age
F.B. S	**R**	0.21	0.25	0.11	0.1	0.13	0.27	0.06	-0.03	0.14	-0.18	-0.19
	P	0.25	0.16	0.53	0.58	0.47	0.13	0.71	0.87	0.44	0.31	0.30
CHOL	**R**	-	**0.47**	0.26	**0.85**	**0.57**	0.12	0.33	-0.1	0.41	0.05	0.12
	P		**0.008**	0.16	**0.0001**	**0.001**	0.50	0.06	0.57	0.02	0.78	0.51
TG	**R**	-	--	0.004	0.25	**0.49**	-0.03	0.27	-0.02	0.15	0.03	0.02
	P			0.982	0.17	**0.006**	0.85	0.14	0.91	0.41	0.83	0.91
HDL	**R**	-	-	-	0.12	0.04	0.29	**0.40**	0.02	0.05	0.1	0.17
	P				0.51	0.806	0.11	**0.03**	0.9	0.78	0.59	0.34
LDL	**R**	-	-	-	-	**0.48**	-0.005	0.24	-0.22	0.24	-0.02	0.02
	P					**0.007**	0.98	0.185	0.23	0.18	0.88	0.89
visfatin	**R**	-	-	-	-	-	0.34	**0.55**	0.07	0.09	-0.08	0.16
	P						0.06	**0.001**	0.7	0.6	0.66	0.39
Liptin	**R**	-	-	-	-	-	-	0.1	0.27	0.07	-0.27	0.31
	P							0.59	0.14	0.68	0.14	0.09
Insulin	**R**	-	-	-	-	-	-	-	0.04	-0.03	-0.01	0.06
	P								0.8	0.84	0.93	0.73
BMI	**R**	-	-	-	-	-	-	-	-	0.04	0.15	**0.58**
	P									0.82	0.42	**0.001**
R	**R**	-	-	-	-	-	-	-	-	-	0.36	-0.09
	P										0.05	0.6
IR	**R**	-	-	-	-	-	-	-	-	-	-	0.01
	P											0.95

P-value ≤0.05 (significant). R= pearson correlation, p= p.value

Regarding triglycerides and cholesterol in Type 2 diabetes is associated with decreased plasma HDL cholesterol levels related to reduction of the HDL2 subfraction. Reduced HDL level, in type 2 diabetes, has been shown to be correlated with both hypertriglyceridemia and obesity. The decrease in HDL cholesterol, noted in patients with type 2 diabetes, is due to increased catabolism of HDL particles, which has been demonstrated by in vivo kinetic studies using radioisotopes [181].

Regarding triglycerides and visfatin, as an adipocytokine, has insulin-mimetic effects including inhibition of hepatic glucose release, and as a result of the increase in total cholesterol, triglycerides, LDL cholesterol rises, augmentation of glucose uptake in adipocytes and myocytes and increase in triglyceride synthesis and its accumulation in pre-adipocytes [182].

3.3.2. The relationship between insulin and visfatin in Patients Have D.M Type 2 only

Visfatin was originally called Pre-B colony Enhancing Factor (PBEF) and was noted to be involved in the maturation of B cell precursors [183]. More recently, VF was characterized as an adipokine which was highly expressed in visceral fat and exhibited insulin-like functions. These insulin mimetic functions were mediated through stimulation of the insulin signal transduction pathway through induction of phosphorylation of signal transduction proteins in the insulin signaling pathway, and also through binding to the insulin receptor at a site distinct from that of Insulin (Hug and Lodish)[184]. There is no significant relationship between Visfatin and Age and BMI in our study.

3.3.3. Correlation between BMI and Age

Table (3.13): Association between age groups and groups of related study

		Group		
		DM2	Control	DM2+ atherosclerosis
Age Groups (years)	30-39	2	3	0
		40.0%	60.0%	0.0%
	40-49	4	13	3
		20.0%	65.0%	15.0%
	50-59	8	9	5
		36.4%	40.9%	22.7%
	≥ 60	16	5	22
		37.2%	11.6%	51.2%

Insulin resistance or inadequate insulin synthesis causes the high blood sugar levels characteristic of type 2 diabetes, a chronic metabolic condition. The chance of acquiring type 2 diabetes increases with both age and BMI (body mass index). Type 2 diabetes is more likely to occur as people get older. The American Diabetes Association reports that those above the age of 35 have an increased chance of having diabetes [185]. This is because, as people become older, their metabolism slows and they become more likely to be overweight or obese. Body mass index[186]. Obesity is a major contributor to the development of type 2 diabetes. The body mass index (BMI) is an indicator of overall body fatness. Obesity is defined as a body mass index (BMI) of 30 or higher, whereas overweight is defined as a BMI of 25 or higher. Having a higher body mass index (BMI), and especially abdominal obesity, has been linked to an increased risk of acquiring type 2 diabetes (central obesity) those facts agree with our study [187].

3.3.4. Correlation between parameters with each other in patients have DM 2 and atherosclerosis

Table 3.14: Correlation between parameters with each other in patients have DM 2 and atherosclerosis

		CHOL	TG	HDL	LDL	visfatin	Liptin	Insulin	BMI	R	IR	Age
F.B. S	**R**	0.33	-0.21	0.093	0.366	**0.378**	-0.118	-0.286	0.20	0.20	0.16	0.19
	P	0.07	0.25	0.625	0.047	**0.040**	0.536	0.125	0.28	0.27	0.37	0.29
CHOL	**R**	-	-0.02	-0.052	0.913	-0.063	0.016	0.013	-0.14	0.20	**0.41**	0.03
	P		0.91	0.787	0.12	0.743	0.935	0.945	0.45	0.28	**0.02**	0.85
TG	**R**	-	--	-0.225	-0.351	-0.177	-0.056	**0.378**	-0.29	0.11	-0.07	0.12
	P			0.232	0.057	0.348	0.769	**0.039**	0.11	0.56	0.71	0.52
HDL	**R**	-	-	-	-0.117	0.027	**0.408**	-0.165	**0.40**	0.27	-0.01	0.29
	P				0.539	0.887	**0.025**	0.384	**0.02**	0.13	0.92	0.11
LDL	**R**	-	-	-	-	-0.032	-0.002	-0.179	-0.1	0.13	**0.46**	-0.04
	P					0.868	0.993	0.345	0.59	0.48	**0.01**	0.81
visfatin	**R**	-	-	-	-	-	-0.262	-0.258	0.28	-0.04	-0.07	0.06
	P						0.162	0.169	0.13	0.83	0.70	0.72
Liptin	**R**	-	-	-	-	-	-	0.083	0.18	-0.01	-0.04	0.32
	P							0.664	0.32	0.93	0.82	0.07
Insulin	**R**	-	-	-	-	-	-	-	-0.20	0.008	-0.29	0.03
	P								0.27	0.96	0.11	0.86
BMI	**R**	-	-	-	-	-	-	-	-	-0.04	0.09	0.33
	P									0.82	0.62	0.07
R	**R**	-	-	-	-	-	-	-	-	-	0.003	0.36
	P										0.98	0.04
IR	**R**	-	-	-	-	-	-	-	-	-	-	-0.02
	P											0.88

P-value ≤0.05 (significant). R=pearson correlation, p= p.value

the figure [3.2]: Patients with diabetes and atherosclerosis had higher levels of lipid profiles (LDL-C, total cholesterol (TC). Low-density lipoprotein (LDL) is the most common lipoprotein that is associated with atherosclerosis [188]. An important component of these atherogenic lipoproteins is Apolipoprotein B (Apo B) that promotes the accumulation of LDL in the intima initiates atherosclerosis, this is mediated by increased endothelial permeability and raised intimal retention of LDL[188]. Moreover, diabetes (T1DM and T2DM) is associated with increased hepatic production of triglyceride-rich lipoproteins, which leads to increased formation of atherogenic VLDL. Silent atherosclerosis and cardiovascular complications start to commence during the pre-diabetic period in genetically susceptible people [189]. In other hand, Elevated total cholesterol, and high triglyceride levels make up the condition known as diabetic dyslipidemia so patients with diabetic dyslipidemia had a worse prognosis than did those with isolated elevated LDL levels this is with agreement of our study and with agree (Lorenzatti and Toth, 2020) [190].

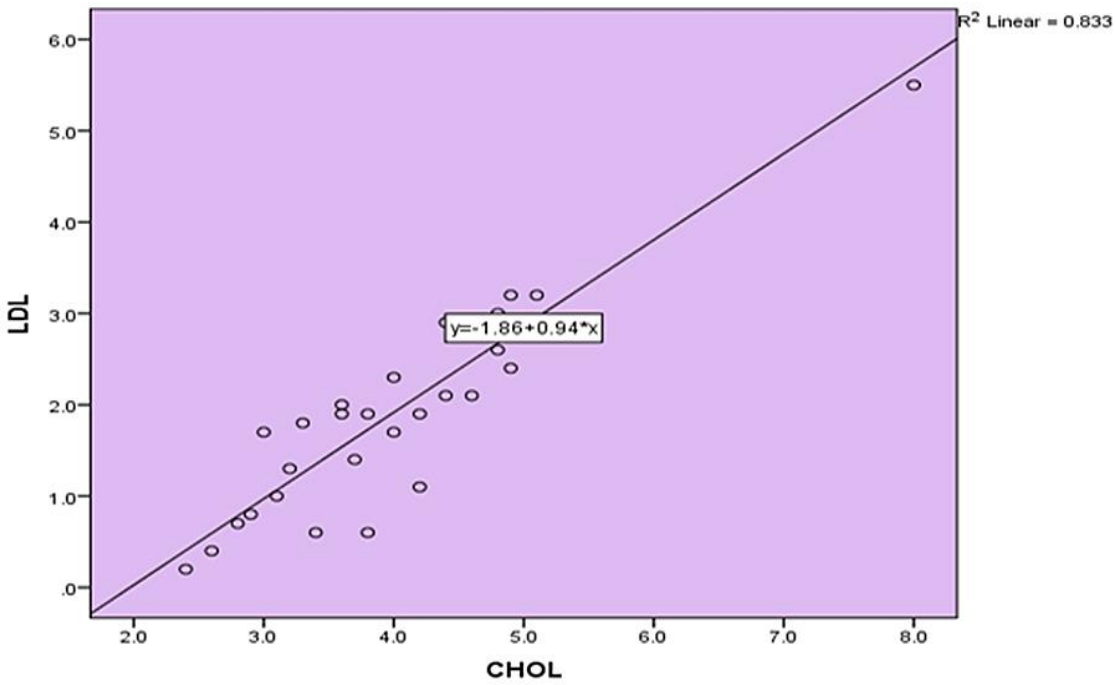

Figure 3.2: Significant positive correlation between LDL and cholesterol in diabetic patients with atherosclerosis.

the figure 3.3: In a comparison of the serum levels of visfatin in normal and type 2 diabetic patients, several investigators showed that visfatin was significantly elevated in the serum of type 2 DM patients compared to controls. The serum level of visfatin is also significantly elevated in patients with long-term type 2 DM compared to control. This increase in the level of serum visfatin in diabetic patients is considered to be a sign of beta cell deterioration [191]. Other studies performed on more than 150 patients in India showed a strong correlation between serum visfatin and type 2 diabetes [192].

Circulating visfatin concentrations are increased by hyperglycemia [193]. visfatin levels have been found to be positively correlated with (FBS) levels in diabetes.

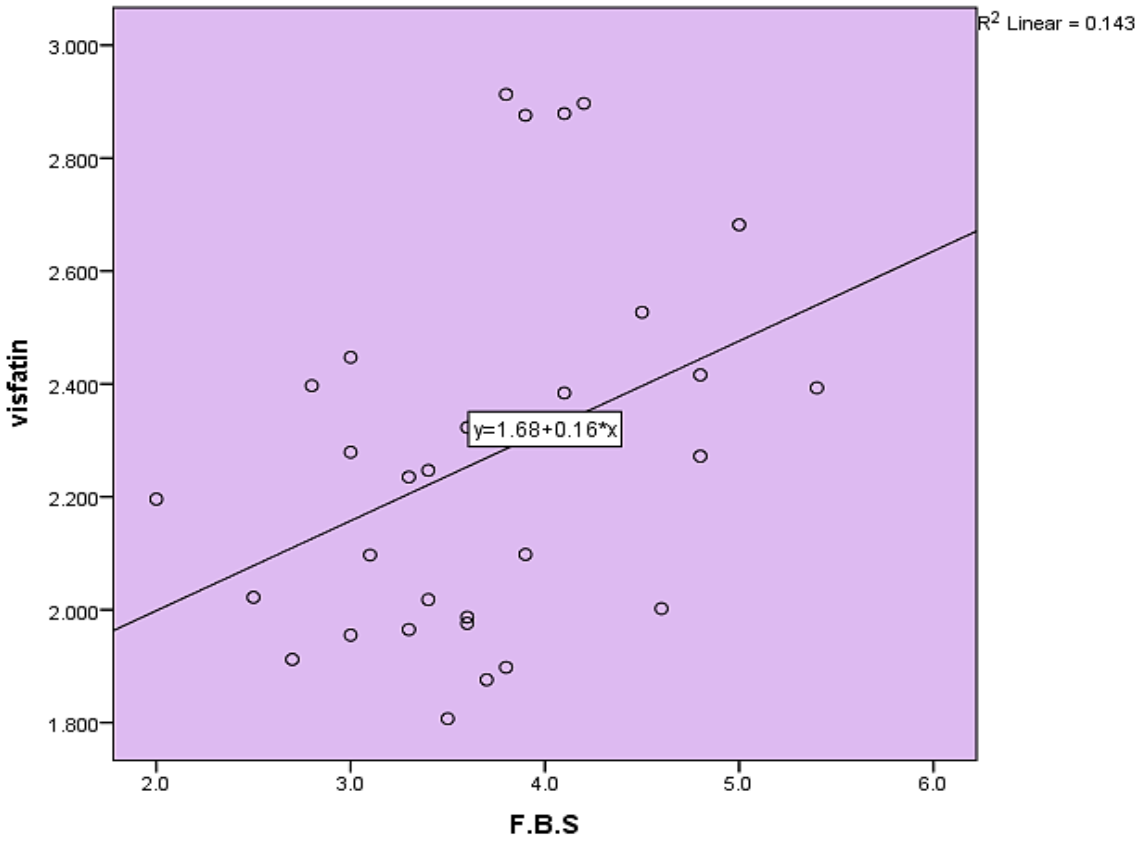

Figure 3.3: Significant positive correlation between Visfatin and FBS in diabetic patients with atherosclerosis.

the figure 3.4: in diabetic patients, there is evidence that an association between leptin and HDL levels. Specifically, studies have shown that higher levels of leptin may be associated with lower levels of HDL cholesterol in diabetic patients [194]. One possible explanation for this correlation is that leptin may contribute to the development of insulin resistance, which can lead to dyslipidemia (abnormal lipid levels) and a decrease in HDL cholesterol levels. Additionally, inflammation and oxidative stress, which are associated with diabetes, can also contribute to lower HDL levels [195].

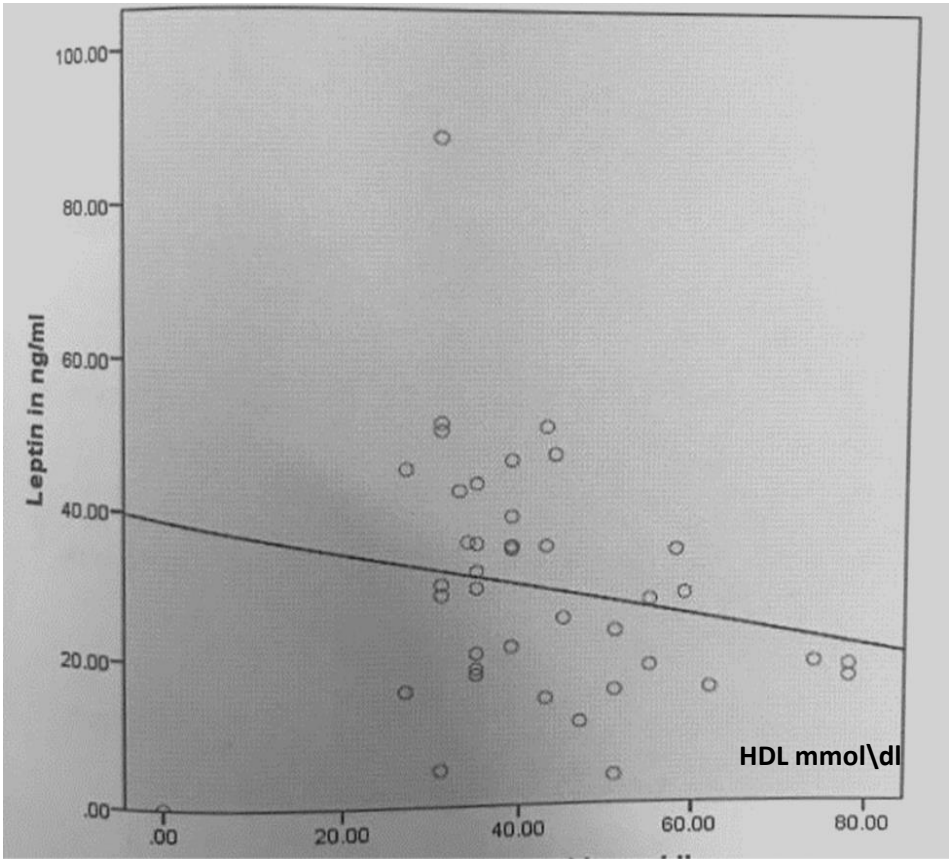

Figure 3.4 Significant negative correlation between leptin and HDL in diabetic patients with atherosclerosis.

the figure 3.5: Insulin resistance is strongly associated with atherosclerosis and frequently coexists with common proatherogenic disorders; increased concentrations of triglyceride-rich VLDL particles contribute to abnormal HDL metabolism in insulin resistance [196]. Cholesteryl ester transfer protein mediates the exchange of cholesteryl esters in HDL for triglycerides in VLDL, resulting in cholesteryl ester–enriched VLDL and triglyceride-enriched HDL. The latter particle a better substrate for hepatic lipase, which may be increased in insulin resistance, and HDL particles decrease because of enhanced metabolism[197].

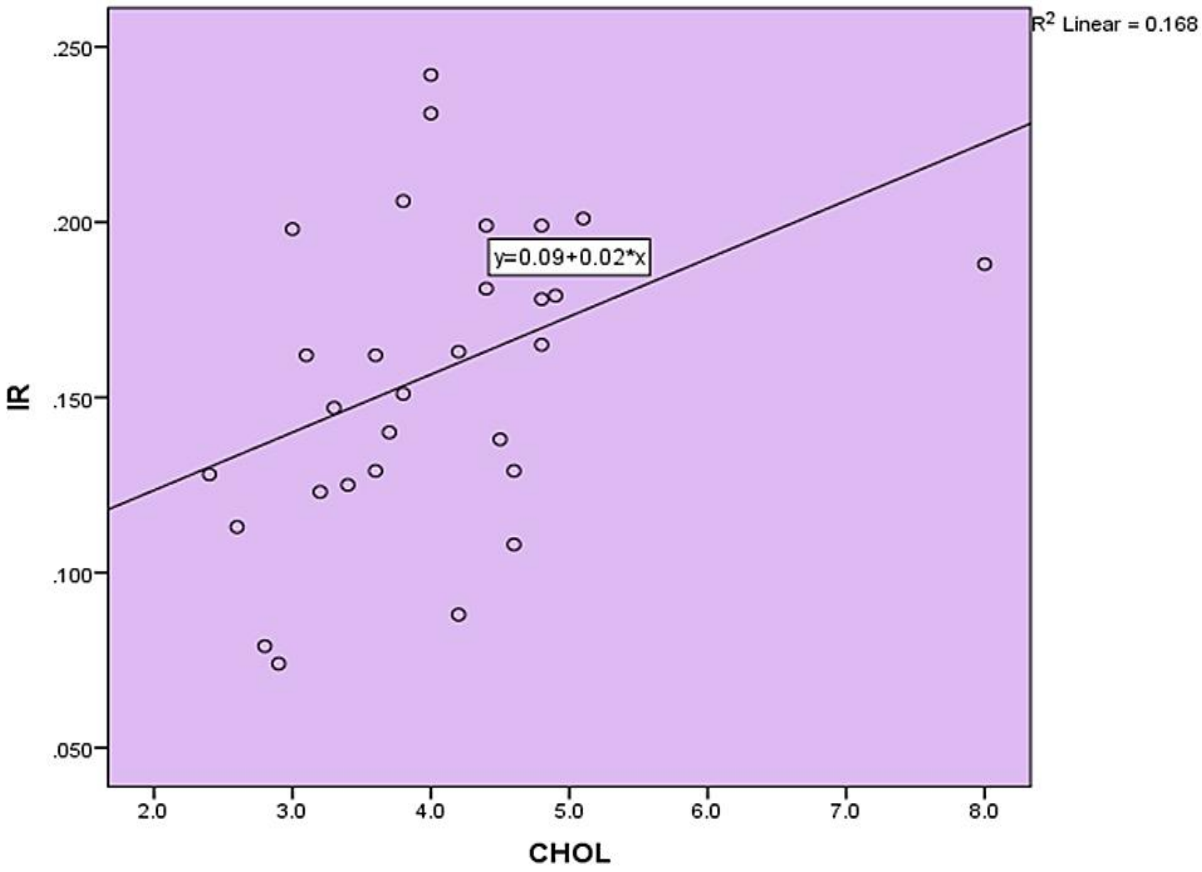

Figure 3.5: Significant positive correlation between insulin resistance and cholesterol in diabetic patients with atherosclerosis.

the figure 3.6 The lack of appropriate insulin signaling, especially in peripheral tissues such as adipose cells, results in abnormal lipid metabolism that consistently produces a proatherogenic phenotype in diabetic patients with atherosclerosis, there is evidence to suggest that there may be a correlation between insulin and triglyceride levels. Specifically, studies have shown that higher levels of insulin may be associated with higher levels of triglycerides in diabetic patients with atherosclerosis[198].

One possible explanation for this correlation is that insulin resistance, which is common in diabetic patients, can lead to an increase in triglyceride levels. Insulin resistance can cause the liver to produce more triglycerides and reduce the breakdown of triglycerides in adipose tissue[199]. Additionally, high levels of insulin can also stimulate the production of fatty acids, which can contribute to an increase in triglyceride levels, increased concentrations of triglyceride-rich VLDL particles contribute to abnormal HDL metabolism in insulin resistance [200].

A large body of evidence implicates the high-triglyceride, low-HDL phenotype in atherosclerosis. Accelerated atherosclerosis in the setting of insulin resistance could thus result from the direct entry of atherogenic VLDL-derived particles into the vasculature, or decreased availability of HDL particles to participate in unloading of cholesterol from the vasculature, known as reverse cholesterol transport [201].

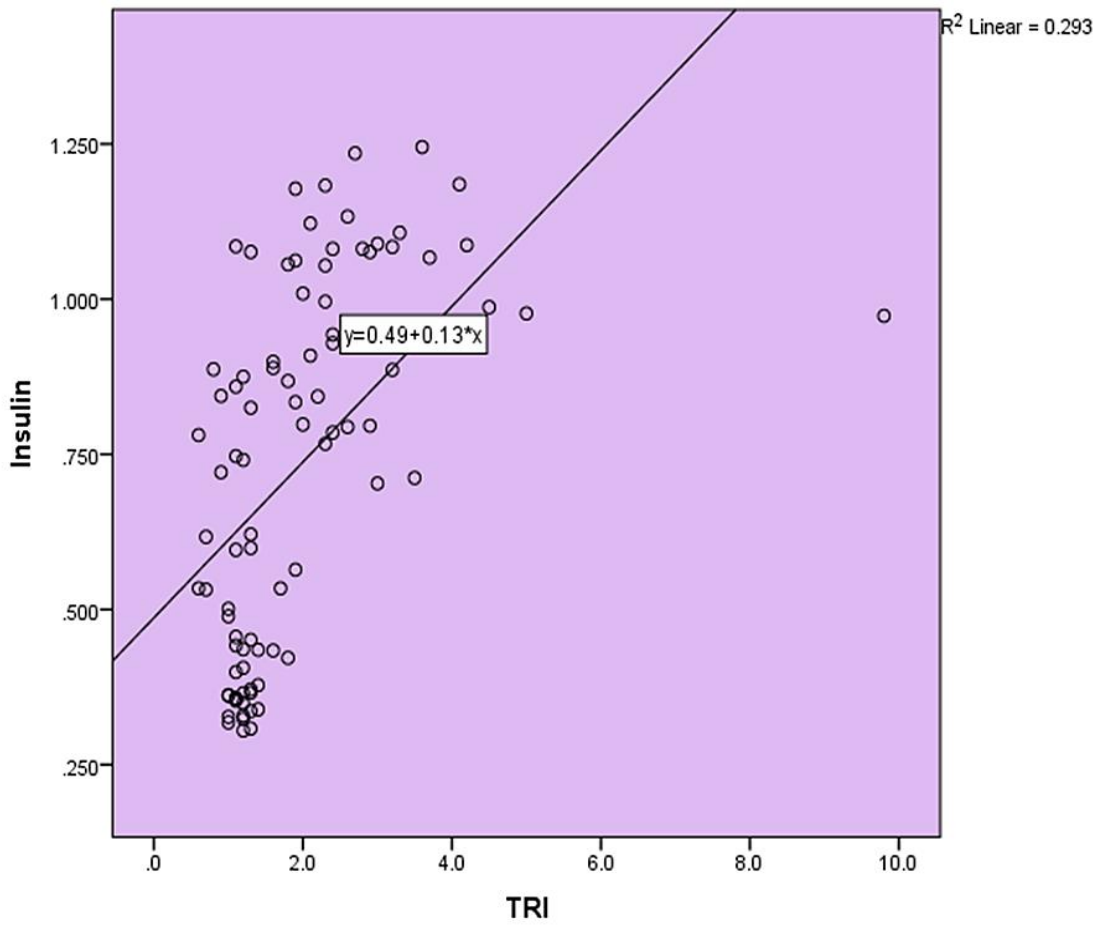

Figure 3.6: Significant positive correlation between insulin and triglyceride in diabetic patients with atherosclerosis.

the figure 3.7: positive correlation between insulin resistance and LDL in diabetic patients with atherosclerosis. During insulin resistance, several metabolic alterations induce the development of cardiovascular disease. For instance, insulin resistance can induce an imbalance in glucose metabolism that generates chronic hyperglycemia, which in turn triggers oxidative stress and causes an inflammatory response that leads to cell damage[202].

Insulin resistance can alter systemic lipid metabolism which then leads to the development of dyslipidemia and the well-known lipid triad: (1) high levels of plasma triglycerides, (2) low levels of high-density lipoprotein, and (3) the appearance of small dense low-density lipoproteins [203]. These triads, along

with endothelial dysfunction, which can also be induced by aberrant insulin signaling, contribute to atherosclerotic plaque formation [204].

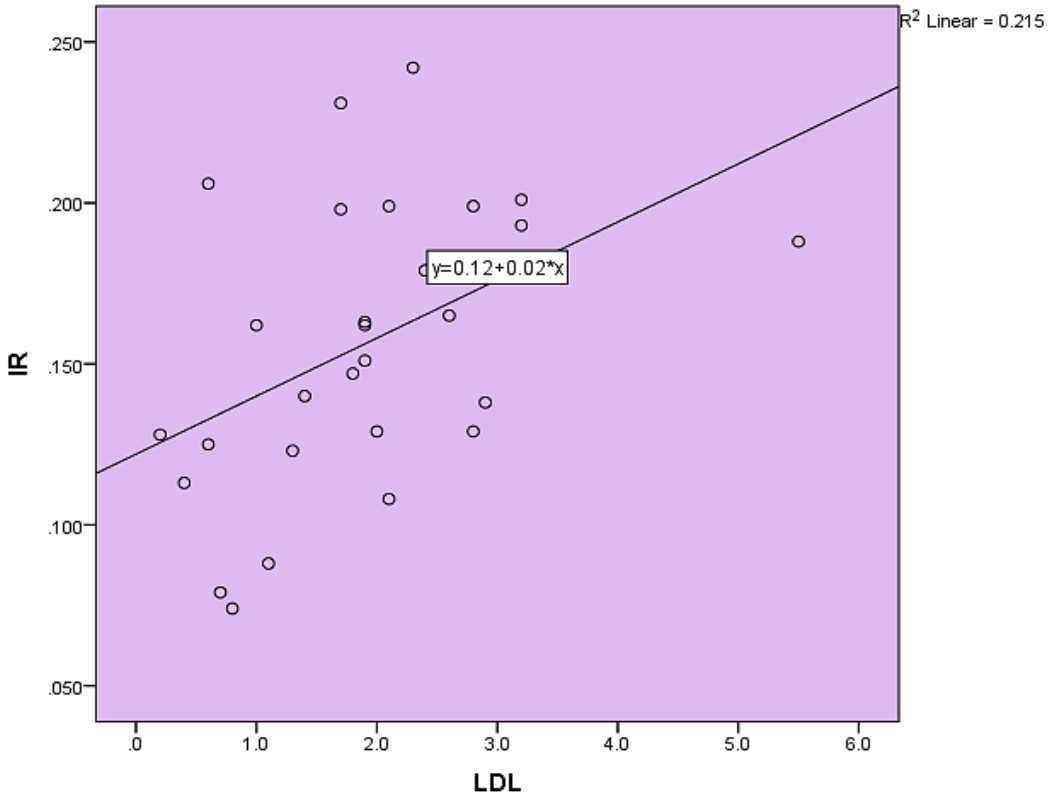

Figure 3.7: Significant positive correlation between insulin resistance and LDL in diabetic patients with atherosclerosis.

3.4. Receiver operating characteristic (ROC Test)

It is a special program that determines the sensitivity and specificity of the marker with patients. According to Table 3.15; the sensitive and specific cutoff points of FBS, CHOL, TRI more than these cutoff points mean positive when compare the group of patients have DM with atherosclerosis with control group.

Table 3.15: The sensitive and specific cutoff points of FBS, CHOL, TRI.

Cutoff point of FBS	Sensitivity	Specificity
3.150	73%	73%
3.250	**73%**	**77%**
3.350	66%	80%
Cutoff point of Cholesterol	**Sensitivity**	**Specificity**
3.050	**83%**	**90%**
3.150	80%	90%
3.250	76%	95%
Cutoff point of Triglyceride	**Sensitivity**	**Specificity**
1.350	83%	83%
1.500	**83%**	**98%**
1.650	83%	97%

Cutoff point of FBS was (3.250), Sensitivity (73%), Specificity (77%) Our results showed that FBS sounds reliable to separate diabetic from non-diabetic subjects, (FBS) is suggested as the best and the most common test in patients have DM with atherosclerosis. Regarding Cholesterol Cutoff point was (3.050), Sensitivity (83%), Specificity (90%) and Triglyceride Cutoff point was (1.500), Sensitivity (83%), Specificity (98%). Cholesterol and (TG) are important marker, are closely related to the occurrence and development of pre-DM and T2DM. Previous studies have shown triglyceride glucose (TG) index and triglyceride to HDL cholesterol (TG/HDL) ratio were suggested for useful surrogate indicators of development of type 2 diabetes using cohort data [205].

In addition, the excess cholesterol accumulation leads to β-cell dysfunction, thereby impairing glucose tolerance and affecting insulin secretion. In addition, islet cholesterol deposition may lead to increased islet amyloid polypeptide aggregation and increased islet amyloid formation, further deteriorating β-cell function and affecting glucose homeostasis [206].

In another hand, Triglyceride some studies found that there is no association between TG and the risk of CVD among T2DM. Elevated triglyceride levels are a common dyslipidemic feature accompanying type 2

diabetes and pre-diabetic state, some evidence suggests that fasting triglyceride levels can aid in predicting future type 2 diabetes [207].

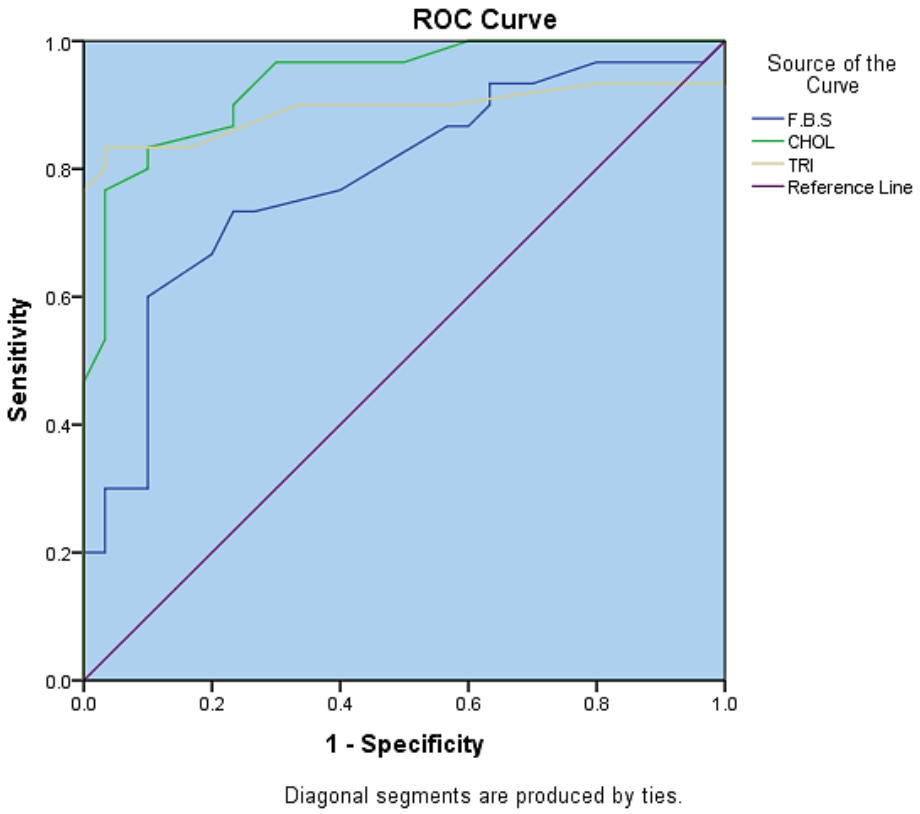

Figure 3.8: Sensitive and specific cutoff points of FBS, CHOL, TRI.

3.4.1. Vasfatin Sensitivity

Vasfatin Sensitivity and Specificity very high this indicates that it can be used to diagnose and predict diabetes type 2, Hyperglycemia also increases plasma visfatin level in type 2 D.M patients and becomes worse with increasing levels of hyperglycemia. Other studies showed that glucose concentrations of 8.3mmol/l caused a significant increase in plasma visfatin level in healthy males, and comparison of the serum levels of visfatin in normal and type 2 diabetic patients, several investigators showed that visfatin was significantly elevated in the serum of type 2 D.M patients compared to controls [208].

Table 3.16: The sensitive and specific cutoff points of vasfatin

Cutoff point of vastatin	Sensitivity	Specificity
1.778	100%	70%
1.805	**100%**	**100%**
1.841	97%	100%

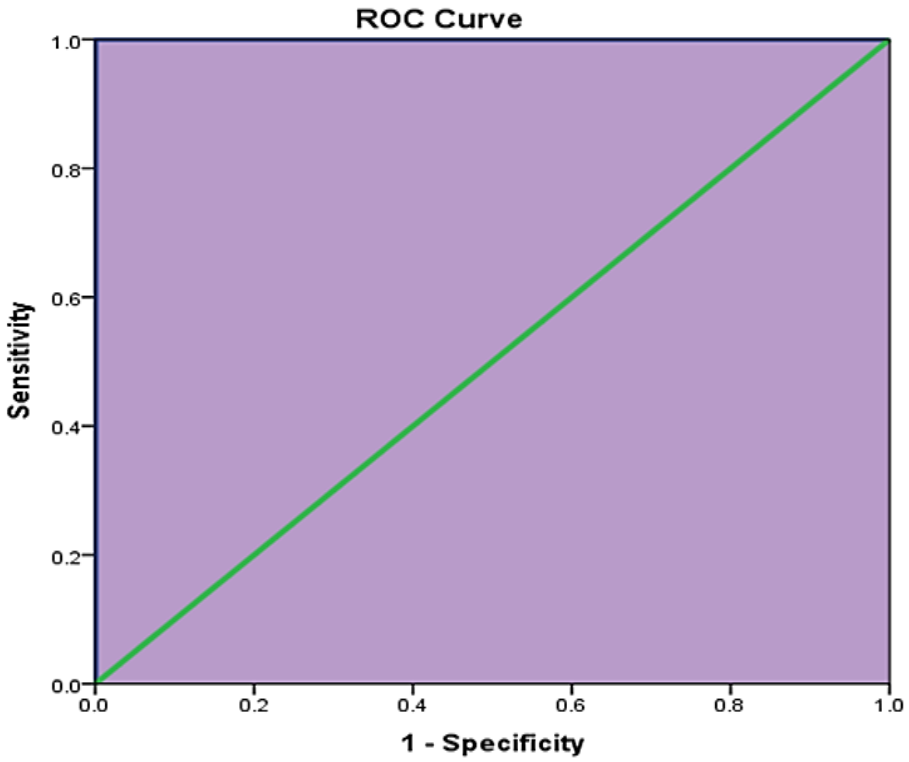

Figure 3.9: Visfatin Sensitivity and Specificity.

According to table Table 3.17; the sensitive and specific cutoff points of leptin more than these cutoff points mean positive when compare the group of patients have D.M with atherosclerosis with control group.

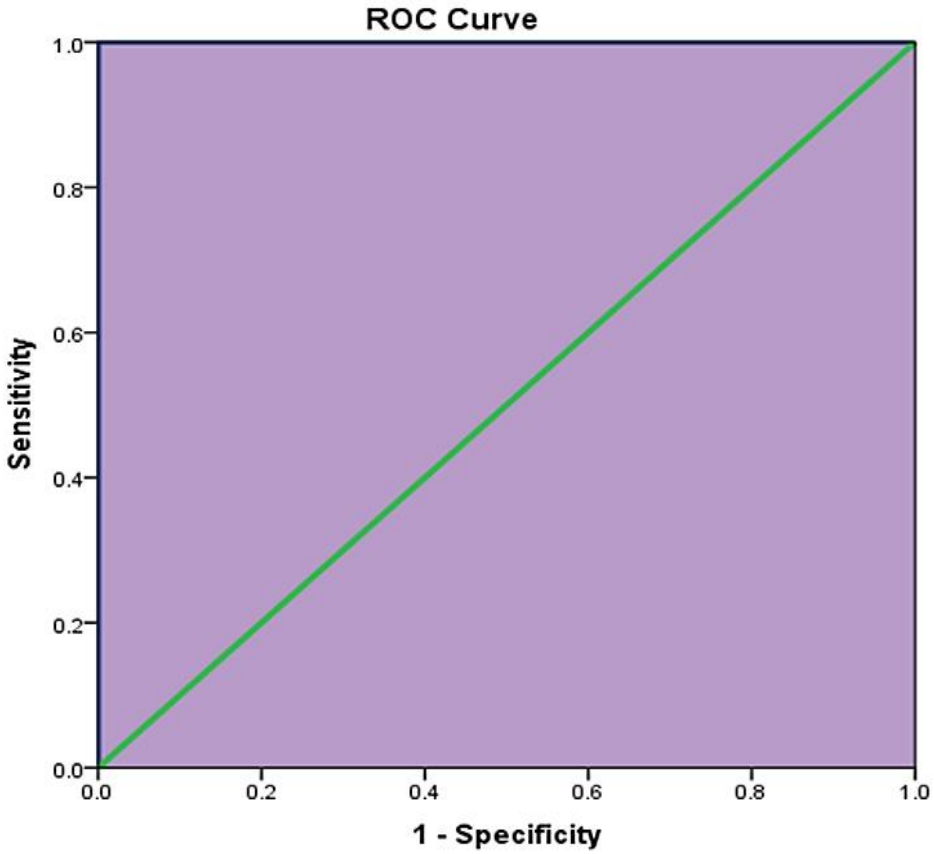

Figure 3.10: The sensitive and specific cutoff points of leptin.

Table 3.17: The sensitive and specific cutoff points of liptin

Cutoff point of Liptin	Sensitivity	Specificity
30.18	67%	100%
30.66	**97%**	**100%**

Leptin and insulin have a complex relationship in the body, as they can both affect each other's signaling pathways and have overlapping functions in regulating metabolism and energy balance. Research has shown that leptin can influence insulin sensitivity, which is the ability of cells to respond to insulin and take up glucose from the blood stream [209]. High levels of leptin have been linked to insulin resistance, which can lead to type 2 diabetes and other metabolic disorders. Conversely, insulin can affect leptin levels in the body.

Insulin can stimulate the production and release of leptin from fat cells, and high levels of insulin have been linked to increased leptin levels in the blood. In type 2 diabetes, the body becomes resistant to insulin, meaning that the cells in the body are less responsive to the hormone and are less able to take up glucose from the bloodstream [210].

This results in high levels of glucose in the bloodstream, which can lead to a range of health problems over time. Leptin also appears to be involved in the development of insulin resistance and type 2 diabetes. High levels of leptin have been linked to insulin resistance [211]. Which can contribute to the development of type 2 diabetes. In addition, leptin can affect insulin signaling pathways and impair insulin sensitivity which can contribute to the development of type 2 diabetes. [212].

Furthermore, excess body fat, which is the primary source of leptin, is strongly associated with insulin resistance [213]. People who are overweight or obese are at higher risk of developing type 2 diabetes, in part because of the effects of excess body fat on insulin and leptin signaling pathways [214].

According to table 3.18; the sensitive and specific cutoff points of resistin and insulin resistance more than these cutoff points mean positive when compare the group of patients have DM with atherosclerosis with control group.

Table 3.18: The sensitive and specific cutoff points of resistin and insulin resistance

Cutoff point of Resistine	Sensitivity	Specificity
1.48	67%	100%
1.51	**97%**	**100%**
1.87	**100%**	**100%**
Cutoff point IR	**Sensitivity**	**Specificity**
0.085	**93%**	**100%**
0.098	90%	100%
0.110	87%	100%

Table 3.20; the sensitive and specific cutoff points of resistin and insulin resistance. Adipose tissues secrete free fatty acids to meet energy demand. In addition to this, it also secretes several adipose tissue-specific small polypeptides, such as leptin, adiponectin, and resistin, whose levels is increasing in the obesity/prediabetic condition. Resistin, a hormone secreted from adipose tissue, resists insulin action and impairs glucose homeostasis [215].

Resistin is proinflammatory cytokine plays a key role in the pathogenesis of type 2 diabetes mellitus (T2DM). Many reports have previously identified changed serum resistin levels in patients with T2DM, in human individuals with severe insulin resistance had higher resistin levels than healthy individuals [216].

Resistin, an adipokine first discovered by Steppan in 2001, has been shown to induce insulin resistance In human studies, individuals with severe insulin resistance had higher resistin levels than individuals with normal insulin action Therefore, we hypothesized that resistin might also play a role in insulin resistance[217].

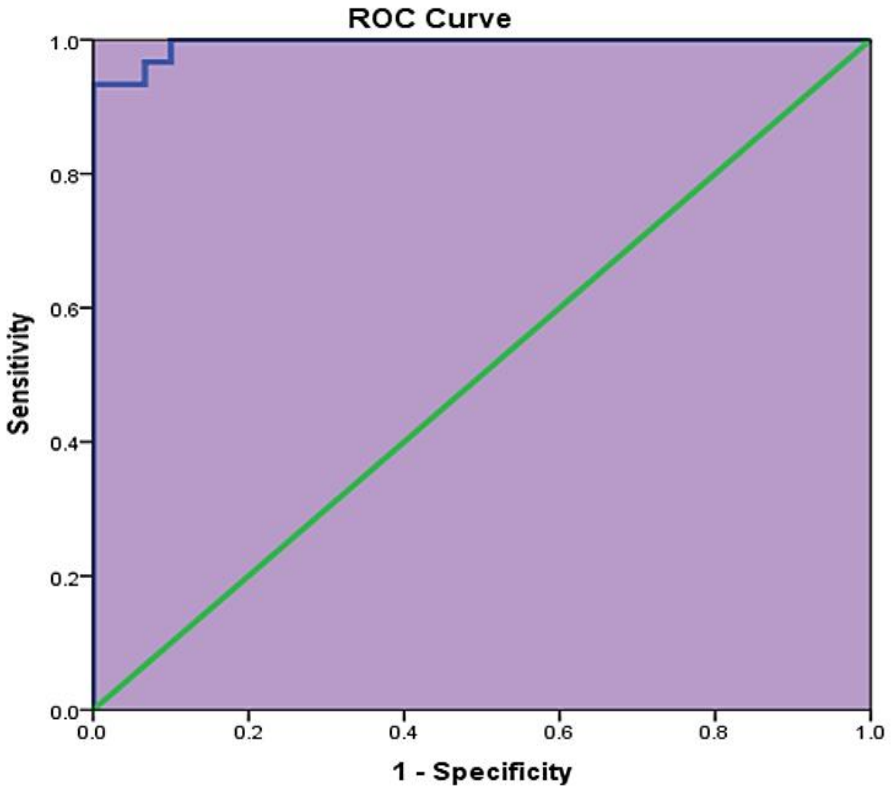

Figure 3.11: The sensitive and specific cutoff points of resistin and insulin resistance.

Conclusions and Recommendations

4. CONCLUSIONS

1- Visfatin appears to be a high sensitive molecule with a clinical significance and a prospective promising diagnostic, prognostic, and therapeutic applications in many cardiovascular-metabolic disorders.

2- Leptin hormone has a predictive ability to predict T2DM, as they are significantly associated with IR, and lipid profile.

3- Resistin is associated with overweight and T2DM, and may serve as a potential prognostic marker for overweight-related T2DM.

Recommondations

1- Preferably taken larger number of subjects.

2- The follow up study give a better result about the variability in the levels of resistin and visfatin and leptin.

3- T2DM, overweight patients should be monitored.

4- The present study advises to carry out a complete hormonal analysis as routine work to understand the hormonal status of patients with T2DM which may aid the physician to treat those patients and to prescribe them the drug of choice.

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الخلاصة

مرض السكري هو مرض مزمن يتميز بارتفاع نسبة السكر بالدم ويصيب اربعمائة وخمسة عشر مليون بالغ حول العالم ينتج ارتفاع مستويات الجلوكوز في الدم عن عدم كفاية افراز الانسولين او عدم القدرة على التاثير في تاثيرات الانسولين وهو هلرمون يفرز من البنكرياس يودي مرض السكري الى مضاعفات في معظم اعضاء الجسم مثل القلب والعين والكلى والجهاز العصبي مما ادى الى ارتفاع المرض وبالتالي فان تشخيص المرض في المراحل المبكرة امر ضروري للغاية الهدف من الدراسة هو قياس الفزفاتين في مصل الدم المصاب بداء السكري من النوع الثاني وتصلب الشرايين وكدلك دراسة بعض السيتوكين مثل اللبتين والرززستين في المرضى المصابين بداء السكري من نوع الثاني وتثلب الشرايين تم جمع عينات الدم من الاول من شهر اكتوبر حتى نومبر 2022 وتم سحب العينات في مركز الغدد والصم السكري في مستشفى مرجان التعليمي في محافظه بابل والمجموعة الثانية تم سحب العينات في مركز العراقي لجراحة القلب في مستشفى غازي الحريري في محافظة بغداد تضمنت دراسةالحالات 90 مريضا مقسمة الى ثلاث مجاميع الاولى مصابين بالسكر من نوع الثاني فقط والمجموعة الثانية مصابين بتصلب الشرايين وداء السكري والمجموعة الثالثة المقارنة باالاصحاء ظاهريا تم تحديد تركيز فزفاتين واللبتين والرززستين والانسولين عن طريق قياس باالاليزا وتم قياس نسبة السمر بالدم ونسبة دهون الجسم عن طريق قياس جهاز السبكتروفوتوميتر كان مستوى كولسترول والدهون الثلاثية والدهون الضاره مرتفعا بشكل ملحوظ في المجموعة الاولى مقارنة بمجموعة السيطرة وكان مستوى الدهون النافعة للجسم منخفضا بالنسبة لنفس مجموعة مقارنة بالسيطرة واظهرت نتائج ان الفسفاتين كان مرتفع معنويا في كلا المجموعتين اكثر من $P\leq0.05$ وكانت نتائج دراسة اللبتين كانت مرتفعة بشكل ملحوظ بالمجموعتين مقارنة بالسيطرة اكثر من $P\leq0.05$ وكانت نتائج الرززستين كانت مرتفعة بشكل ملحوظ بالمجموعتين مقارنة بالسيطرة اكثر من $P\leq0.05$ يظهر فيزفاتين جزء عالي من الحساسية للانسولين ويرتبط اللبتين بمقاومة الانسولين مع زيادة الوزن .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل - كلية الطب
قسم الكيمياء والكيمياء الحيوية

دراسة هرمونات الفزفاتين واللبتين والرزستين وعلاقتهم بمرضى السكري من نوع الثاني

رسالة مقدمة الى عمادة كلية الطب – جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في العلوم /الكيمياء الحياتية السريرية

من قبل:
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