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Relationships of Omentin-1 and YKL-40 with Some Biochemical Parameters in Men Patients with Type 2 Diabetes Mellitus in AL-Anbar Governorate

A dissertation Submitted to the Council of the College of Science at the University of Anbar, in Partial Fulfillment of Requirements for the Degree of Philosophy Doctor in the Science of Chemistry

By

Sarab Tariq Mahmood

Bachelor's degree, University of Anbar, College of Science, Department of Chemistry (2012-2013)

Master's degree, University of Anbar, College of Science, Department of Chemistry (2016-2017)

Supervised by

Prof. Dr. Mustafa Taha Mohammed **Prof. Dr. Khalid Farooq Abul Ghafoor**

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سُورَةُ طٰهٰ

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Supervisors Certifications

I certify that this dissertation was prepared under my supervision at University of Anbar as partial requirement for the Degree of Philosophy Doctor in the Science of Chemistry.

Signature:

Name: **Prof. Dr. Khaled Farouk Abul Ghafoor (Supervisor)**

Date:

Signature:

Name: **Prof. Dr. Mustafa Taha Mohammed (Supervisor)**

Date:

Chairman of the Postgraduate Studies Recommendations:

In view of the available recommendations, I forward this dissertation for debate by the examining committee.

Signature: S.R.M

Name: **Prof. Dr. Star Rajab Majid**

Date: 31/8/2022

COMMETEE CERTIFICATION

We the examining committee, certify that we have read this dissertation and examined the student in its content, and then in our opinion it is adequate as dissertation for the Philosophy Doctor degree (Excellent).

Head of committee
Signature:
Name: **Dr. Hanaa Addai Ali**
Scientific degree: Professor
Date:

Member
Signature:
Name: **Dr. Shakir Faris Tuleab**
Scientific degree: Professor
Date:

Member
Signature:
Name: **Dr. Hammeed Hussein Ali**
Scientific degree: Professor
Date: 1/9/2022

Member
Signature:
Name: **Dr. Amer Hasan Abullah**
Scientific degree: Assistant Professor
Date:

Member
Signature:
Name: **Dr. Hazim Ismael Ghazzay**
Scientific degree: Assistant Professor
Date: 4/9/2022

Member (Supervisor)
Signature:
Name: **Dr. Khaled F. Abul Ghafoor**
Scientific degree: Professor
Date:

Member (Supervisor)
Signature:
Name: **Dr. Mustafa Taha Mohammed**
Scientific degree: Professor
Date:

Approval by the Council of the College of Science.

Signiture:
Name: **Dr. Emad Abdul Rahman Mohammed**
Scientific title: Professor
Dean of the College of Science
Date: 11/9/2022

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College of Science

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Summary

Insulin shortage leads to a prolonged increase in blood glucose levels or diabetes as ß-cell activity declines or insulin resistance develops (or both). T2DM (Type-2 Diabetes Mellitus) is the most prevalent sort diabetes in the world.

This study aimed to determine Omentin-1 as an anti-inflammatory and YKL-40 as an inflammatory factor and their relationship with some biochemical parameters in male patients with T2DM. To achieve this aim, sixty of T2DM patients with ages range between 31-55 years and 30 apparently healthy control with ages range between 32-55 years were subjected to the study.

Where this study included measuring the body mass index BMI, determine glycemic factors, studying the lipid profile, oxidative stress, and inflammatory factors. The results were in the form of Mean ± SD.

The findings reveal a significant increase $p < 0.05$ in BMI $kg.m^{-2}$ of T2DM patients compared to control subject.

The results shown significant increase in glycemic factors in T2DM patients than control subject. In lipid profile there were strong higher in cholesterol CHO , triglyceride TGs, low density lipoprotein LDL-C and very low density lipoprotein VLDL-C levels in T2DM compared to control group. While there

was decrease in high density lipoprotein HDL-C levels in T2DM compared to control group.

The current study shown a significant increase in oxidants and decrease in antioxidants levels in T2DM patients compared to those control group, also increase oxidative stress index OSI levels in T2DM patients.

YKL-40 concentration 25.16 ± 8.7 ng/ml was increase $p < 0.05$ in T2DM compared to those control group 18.17 ±4.7 ng/ml, while there was strong reduce $p < 0.05$ in Omentin-1 concentration in T2DM 10.57 ± 1.6 ng/ml compared to those control group 12.67 ± 3.9 ng/ml.

The study found that the concentrations of Omentin-1 and YKL-40 in serum were associated with BMI, insulin resistance and oxidative stress index. An increase in the level of YKL-40 and decrease Omentin-1level in the serum in patients with T2DM may lead to antioxidant disturbance and increased production of oxidants that cause the development of complications of type 2 diabetes.

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List of Abbreviations

Abbreviation	Meaning
4-AA	4-amino-antipyrine
AA	Arachidonic acid
Akt	Protein kinase B
AGEs	Advance glycation end products
ATP	Adenosine triphosphate
AMPK	AMP-activated protein kinase
AMP	Adenosine - 5 -monophosphate
AS160	Akt, substrate of 160 kDa
BMI	Body mass index
BAT	Brown adipose tissue
cGMP	Cyclic guanosine monophosphate
Cnos	Constitutive nitric oxide synthase
CoQ_{10}	Co enzyme q 10 (ubiquinol)
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immuno sorbent assay
eNOS	Endothelial nitric oxide synthyase
FFA	Free fatty acid
FR	Free radical
GDM	Gestational diabetes mellitus
GLUT	Glucose transporter

Abbreviation	Meaning
GLUT4	Glucose transporter 4
GOD	Glucose oxidase
GPx	Glutathione peroxidase
GSH	Glutathione
GSSG	Oxidized glutathione
HbA1c	Hemoglobin $A1_C$ (HbA1c)
HDL-Cholesterol	High density lipoprotein-Cholesterol
HONK	Hyperosmolar nonketotic
HO	Heme oxygenase
HRP	Horseradish peroxidase
HSL	Hormone sensitive lipase
HHT	12-l-hydroxy-5,8,10-heptadecatrienoic acid
IFG	Impaired fasting glycemia
IGT	Impaired glucose tolerance
IL-1	Interleukin-1
IL-6	Interleukin-6
IFN-gamma	Interferon Gamma protein
iNOS	Inducible nitric oxide synthase
IRS-1	Insulin receptor substrate -1
IRS-2	Insulin receptor substrate -2
LDL-c	Low density lipoprotein-cholesterol
LCN-2	Lipoclalin-2
Mtor	Mammalian, target, of rapamycin

Abbreviation	Meaning
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
Nnos	Neuronal nitric oxide synthase
NO	Nitric oxide
NFκB	Nuclear factor kappa-protein
NOS	Nitric oxide synthase
$O_2^{\bullet-}$	Superoxide radical
OGTT	Oral glucose tolerance test
$OH^{\bullet}$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
OSI	Oxidative stress index
OPN	Osteopontin
PEG	Polyethylene glycol
PI	Phosphatidylinositol
POD	Peroxidase
PUFA	Polyunsaturated fatty acids
PVAT	Perivascular adipose tissue
PVRFs	Perivascular relaxing factors
PVCFs	Perivascular rcontracting factors
Prxs	Peroxiredoxins
PI3K	Phosphoinositide 3-kinase
PTPN gene	Protein tyrosine phosphatases gene
PPAR-γ	Peroxisome, proliferator activated receptor-γ

Abbreviation	Meaning
R	Correlation-factor
RAGEs	Receptor advance glycation end products
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RPM	Rotation per minute
SD	Standard deviation
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TG	Triglyceride
TMB	Tetra methyl benzidine
TNF-α	Tumour necrosis factor alpha
Type 2 DM	Type 2 Diabetes mellitus
TOS	Total oxidant status
VCAM-1	Vascular cell adhesion molecule-1
VLDL-Cholesterol	Very low density lipoprotein-Cholesterol
WHO	World health organization
YKL-40	A protein with three amino acids at the N-terminus: tyrosine (Y), lysine (K), and leucine (L)
ZAG	Zinc 2-glycoprotein

Chapter One

Introduction

And

Literature review

1. Introduction

1.1- Diabetes Mellitus

1.1.1- Definition

Diabetes mellitus (DM) that affects the body's metabolism a characteristic of chronic rise of glucose in the blood and disordered carbohydrate, lipid, and protein metabolism when not adequately treated or untreated, since insulin isn't produced by the pancreas sufficiency and/or the insulin produced has no effect on the cells [1].

If diabetes hasn't been identified or isn't well controlled, chronic hyperglycemia caused complications, permanent damage to a broad range variety organs and tissues, such as the renal glomeruli (diabetic nephropathy), retina (diabetic retinopathy), and neural tissue (diabetic neuropathy), and blood vessels [2].

Polyuria, polydipsia, blurred eyesight, and polyphagia are all common symptoms with DM, weight loss might occur in its most extreme forms in the absence of active treatment a coma and death can develop [3].

Diabetes mellitus is among the most common chronic disorders in humans. Also, both in the western world and in developing countries the incidence of diabetes continues to increase as changing habits contribute to obesity has increased as a result of decreasing physical exercise.

Projections over the next two decades indicate diabetes is likely to become a pandemic, hitting a rate of 10.5 percent by 2030 (439 million adults worldwide) [4].

1.1.2- Classification

Diabetes mellitus can classified to various types according to pathophysiology as shown in Table (1-1).

Table (1-1): Pathophysiology based classification of DM [5]

Type	Etiology	
T1DM	Infections such as cytomegalovirus (CMV) cause pancreatic beta-cell death, resulting in complete insulin insufficiency.	
T2DM	Insulin resistance and/or insufficiency in insulin secretion	
GDM	Insulin resistance and insulin deficiency occur during pregnancy	
Different forms of DM	Monogenic DM	Gene abnormalities that affect the activity of beta cells
	Diseases	Neoplasm of the pancreatic, Pancreatitis, and pancreatectomy
	Endocrinopathies	Cushing's syndrome
	Drug-Induced DM	Glucocorticoids

1.1.2.1- Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus, T1DM or juvenile-onset diabetes are the names given to this kind of diabetes, which affects only 5-10% of diabetics.

T1DM, which results from the pancreatic degradation of beta cells. It contributes to an absolute lack of insulin total dependency on exogenous insulin to avoid ketoacidosis is the primary characteristic of T1DM [6].

While it can occur at any age in people less than 30 years of age, T1DM is more common. The rate of pancreatic destruction varies but it is normally faster in childhood and slower in adults. Patients typically complain of polyuria, polyphagia, polydipsia and unexplained weight loss, all of which appear to be acute symptoms [7].

1.1.2.2- Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus, T2DM is highly prevalent and one of the major threats to 21st-century human wellbeing. Also, T2DM is associated with a high number of various chronic comorbidities that can undermine the quality of life and the longevity of life a heterogeneous and polygenic metabolic disease. One of the key characteristics of T2DM and cardio metabolic disorders, insulin resistance (IR), is related to different health complications, such as obesity, polycystic ovary syndrome, metabolic disorders, and Atherosclerosis [8, 9].

Most patients with T2DM don't primarily require insulin therapy adults are more likely than children to get T2DM and the chance of developing the disease increases with age especially after the age of 40 [10].

1.1.2.3- Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a condition in which a hormone made by the placenta prevents the body from using insulin

effectively. Glucose builds up in the blood instead of being absorbed by the cells [11]. GDM is most commonly identified during pregnancy's second or third trimester and it affects roughly 4% of all pregnancies. Glucose tolerance normally recovers to normal within six weeks at the end of pregnancy thus the lady should be reclassified at this time. The majority of patients with GDM do progress to DM although a small percentage will move to T2DM [12].

Untreated GDM may impair the fetus or mother's health even if it is just transient. The infant is at risk for macrosomia (birth weight that is excessive), central nervous system disease, and congenital heart. Prenatal death can occur in extreme cases most commonly due to inadequate placental perfusion caused by vascular impairment. Labor induction may be recommended if placental activity is decreased. If the fetus is in significant discomfort or there is a high danger of macrosomia-related damage, it is possible to have a caesarean section [13].

1.1.3- Epidemiology of Type 2 Diabetes Mellitus

In comparison to the other two main forms of diabetes, T2DM is the most prevalent chronic disease, accounting for 90 % of all cases [14]. In recent years, the disease's incidence has been quickly increasing in the western world, because of the traditional western lifestyle of sedentary activity and a high-calorie diet, T2DM has become practically epidemic. In non-Western countries however rates of diabetes are also rising for example, previous study, Africa and Asia are predicted to see the biggest percentage growth in diabetes incidence [15]. According to World Health Organization (WHO) statistics, It is estimated that 180 million people are

affected by T2DM, with 3 million people dying each year as a result of the disease. It is predicted that both estimates will double within the next 25 years [10].

1.1.4- Pathophysiology of Type 2 Diabetes Mellitus

T2DM is characterized by insulin secretion irregularities in the pancreas as well as peripheral IR [16]. Defects in the signaling pathways in the target tissues that process the insulin signal are the cause of IR [2]. Typically, plasma glucose levels known as glucose homeostasis are maintained within a limited and well-regulated range [16].

Pancreatic β-cells, which respond by releasing insulin detect an increase in blood glucose levels following a meal. Increase the amount of glucose absorbed and used by tissues including skeletal muscle and fat cells insulin reduces blood glucose levels. This increase in glucose also prevents the release of glucagon, inhibiting glucose production from other sources, such as the breakdown of glycogen [17].

The liver is the main glucose user and can buffer amounts of glucose. It receives blood rich in glucose directly through the portal vein from the digestive tract. And after a meal, the liver rapidly extracts significant quantities of glucose from the bloodstream [18]. Insulin increases the expression of some lipogenic enzymes. This is due to glucose stored as a lipid within adipocytes. Thus, an increase in fatty acid generation will increase glucose uptake by the cells. Insulin further regulates this process by dephosphorylating and subsequently inhibiting hormone-sensitive lipase, leading to inhibition of lipolysis. Ultimately, insulin decreases serum free fatty acid levels.. In addition, insulin has an anabolic influence on protein

synthesis. It makes it easier for amino acids to enter cells and for proteins to be made from those amino acids [19].

Reduced blood glucose levels (starvation) impede insulin secretion while raising glucagon synthesis. Insulin's actions are blocked by glucagon. Glucagon raises blood glucose levels by causing the liver to mobilize glycogen reserves, resulting in a brief burst of glucose. The glycogen reserves are exhausted in 10-18 hours, and if fasting persists, glucagon enhances glucose development by favoring the hepatic uptake of amino acids, whose carbon skeletons are used to make glucose [20].

T2DM patients have poor insulin secretion and resistance as a result of their diabetes, Hyperglycemia is caused by a disruption in glucose uptake and release by key tissues [16]. IR is thought to represent the start of the disease, accompanied by an increase in pancreatic cell insulin output to keep glucose homeostasis. These cells ultimately experience more damage and apoptosis as a result of the cells' long-term compensation mechanism to keep up with the higher insulin demand. Higher plasma glucose levels occur when the ultimate requirement for insulin release is not met [2].

1.1.5- Etiology of Type 2 Diabetes Mellitus

T2DM is caused by both genetic and environmental factors, similar to other common human complex diseases such as obesity, and hypertension. Furthermore, the relationship of one's genetic history with the multiple environmental exposures experienced during one's life determines the global disease risk. The causes of T2DM are as follows:

1.1.5.1 Environmental factors:

Obesity, inactivity, diet, stress, environmental pollutants, urbanization, all of these variables are hypothesized to play a role in T2DM development [21]. Obesity is another significant risk factor for T2DM. The highest association between increasing BMI, and rise in weight and diabetes risk is found among Asians [22].

Obesity rates have risen in recent decades, coinciding with an increase in T2DM cases among children and adolescents. Over 85% of children with T2DM are overweight or obese at the time of diagnosis, according to research [23]. Dietary factors have an impact on T2DM risk. Excess sugary drinks have been linked to a higher incidence of T2DM [24]. Saturated fats raise the risk of T2DM, hence dietary fats are also crucial. T2DM risk is reduced by polyunsaturated and monounsaturated fats [25]. Eating a lot of white rice tends to be related to a higher risk of T2DM [26]. A lack of exercise is estimated to be the cause of 7% of instances [27].

1.1.5.2 Genetics:

T2DM is a polygenic disease that is caused by a combination of environmental and genetic factors. Researchers employed high throughput candidate gene association approaches to learn more about the genetic components of T2DM etiology [28]. Furthermore, T2DM is multigenic, This suggests that T2DM patients may have numerous different combinations of gene variations, resulting in a clinical pattern that is comparable [29]. Diabetes is caused by a combination of genes in the majority of instances. T2DM is caused by a combination of genes that have been revealed to play a role in the development of the disease. Even when all of these genes are

taken together, they only account for 10% of the disease's overall heredity. A few rare varieties of diabetes (called as monogenic forms of diabetes) are produced by a mutation in a single gene, such as early adult maturity onset diabetes [30].

1.1.5.3 Medical conditions:

A range of medicines and other health issues, such as Cushing's syndrome and some cancers, such as glucagonomas, can put you at risk for diabetes. There are a total of nine concerns that are linked to diabetes [31]. Glucocorticoids are one of these drugs [32]. Women who have had GDM before are more likely to acquire T2DM [33]. T2DM is also linked to a lack of testosterone [34].

1.1.6- Signs and Symptoms of Diabetes Mellitus

The symptoms of both types of diabetes are identical, but the severity of each differs. T1DM symptoms arise more quickly and are more common, but T2DM symptoms develop over time. The majority of cases are discovered by chance or as a result of problems [35, 36]. Some of the indications and symptoms include polydipsia, polyuria, polyphagia, dehydration, unexplained weight loss, poor vision, irritability, fatigue, lethargy or drowsiness, fainting or dizziness, and discomfort in the feet, legs, or hands [37].

1.1.7- Diagnosis of Diabetes Mellitus

For adults and children, there are three conventional methods for diagnosing diabetes, It is based on fasting blood glucose (FBG), oral glucose tolerance test (OGTT), and random blood glucose (RBG) [38]. Patients with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) are now referred to as pre-diabetics, suggesting that they are diabetes has an

increased risk of developing, Glycosylated hemoglobin A1c (HbA1c) testing further eliminates the issue of day-to-day glucose variability by reflecting the average plasma glucose (PG) over the past two months. Table (1-2) lists the diagnostic criteria for DM [15, 39, 40].

Table (1-2): Diagnosis Criteria for DM [39, 40]

Status	Kind of examination	Glucose levels in the blood mmol/l (mg/dl)
Normal	FBG	< 6.1 (< 110)
	OGTT	< 7.8 (< 140)
DM	FBG	≥ 7.0 (≥ 126)
	OGTT	≥ 11.1 (≥ 200)
	RBG	≥ 11.1 (≥ 200)
IFG	FBG	≥ 6.1 (≥ 110) and < 7.0 (< 126)
	OGTT	< 7.8 (< 140)
IGT	FBG	< 7.0 (< 126)
	OGTT	≥ 7.8 (≥ 140) and < 11.1 (< 200)
HbA1c	≥6.5% (in adults)	

1.1.8- Complications of Diabetes Mellitus

Acute and chronic consequences of DM include:

1. Acute Complications:

Some of the outcomes include diabetic ketoacidosis (DKA), hyperosmolar nonketotic (HONK), and hypoglycemia. Diabetic ketoacidosis is far more likely in people with T1DM than in people with T2DM. The liver

uses fat as a source of energy when insulin levels are low. Ketone bodies are intermediate substrates in that metabolic pathway [41]. DKA is caused by high quantities of ketone bodies in the blood, which lowers the blood pH [42].

Hyperosmolarity that is not ketotic is more common. When a person's blood glucose levels are excessively high, in T2DM rather than T1DM, water is osmotically pulled out of cells and into the circulation, and the kidneys finally begin to discharge glucose into the urine. Water is lost and blood osmolality rises as a result of this [43]. Hypoglycemia, or excessively low blood sugar, is an immediate consequence of many diabetic treatments [44].

2. Chronic Complications:

Chronic complications of diabetes affect a variety of organ systems and are the leading cause of morbidity and mortality. There are two types of chronic complications: vascular and nonvascular. The two forms of vascular consequences are microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (cerebrovascular disease, peripheral vascular disease, and coronary artery disease). Infections and skin abnormalities are examples of nonvascular consequences [45, 46].

1.2- Physiological role of adipocytes and adipose tissue

Adipose tissue's main function is to insulate and cushion the body, as well as store and provide fat when it is in excess [47]. Free fatty acids (FFAs) are liberated from lipoproteins during the exogenous and endogenous processes of lipid metabolism (VLDL, chylomicron etc.).

The composition of TGs after hydrolysis by the enzyme lipoprotein lipase (LPL), their (FFAs) subsequent storage as TG in fat depots and remobilization into the periphery by hydrolysis of these, stored TGs by the hormone sensitive lipase (HSL) are all well-known [47,48]. Insulin is a powerful activator and inhibitor of LPL and HSL, respectively, and hence plays a key role in the maintenance of adipocyte fat content [47].

1.2.1- Secretions of adipocytes (adipocytokines)

Adipocytes have recently been classified as endocrine structures due to their wide range of chemical secretions (adipocytokines), which affect a wide range of physiological function and pathological processesin the body, almost all of the body's organs are affected by carbohydrate and lipid metabolism, blood coagulation, blood pressure maintenance, inflammation, and feeding behavior. Increased adipocyte number and adipose-tissue mass has been observed to result in increased plasma adipocytokine levels. Changes in plasma adipokine levels are connected to obesity, T2DM, and metabolic syndrome [49].

Adipose tissue regulates metabolism by secreting hormones, glycerol, and other substances such as leptin, cytokines, adiponectin, and pro-inflammatory chemicals, as well as generating non-esterified fatty acids (NEFAs). In obese people, the secretion of these substances will rise. The most critical element impacting insulin sensitivity is the release of NEFAs [50]. In both T2DM and obesity, increased NEFA release is detected, and it's associated to IR in both situations. Shortly after an acute increase in plasma NEFA levels, patients develop insulin resistance.

1.3- Role of Some Adipokines in Inflammatory Processes with T2DM

Adipose tissue has recently gained a lot of interest due to its crucial role in human metabolic pathways. Adipokines such as leptin, adiponectin, visfatin, TNF-α, and IL-6 are produced by adipose tissue, which functions as an endocrine organ. These adipokines appear to play a role in the etiology of insulin resistance, diabetes, inflammation, atherosclerosis, and vascular endothelial dysfunction, as well as insulin resistance causation [52].

The discovery of a novel adipokine linked to diabetes could open up new possibilities for clinicians in terms of early diagnosis and better management of diabetes and its complications.

1.3.1- Omentin

Omentin (endothelin lectin, HL-1, intelectin, galactofuranose binding lectin, and intestinal lactoferrin receptor) is a peptide with molecular weight of 34 kDa, is an anti-inflammatory and anti-insulin resistance adipokine synthesized abundantly in the visceral adipose tissue. Omentin has two isoforms: omentin-1 and omentin-2, with omentin-1 being the most common in human plasma [53].

T2DM has been connected to Omentin 1 is a 313 amino acid protein with a secretory protein sequence and a fibrinogen related domain. It has a full length of 1269 bp (base pair). generated by a gene on the 1q22-q23 chromosomal region. It may operate as an endocrine agent that affects

muscles, the liver, and the omental adipose depot to improve insulin sensitivity and glucose metabolism on the local level of omental adipose tissue, it affects body fat distribution between visceral and subcutaneous fat depots via increasing insulin signal transduction through activation of protein kinase (Akt/protein kinase B) [54].

Omentin-1 exclusively increases insulin-mediated glucose transfer; it has no effect on basal glucose transport [53]. However, because it is produced in human blood, it stimulates insulin sensitivity and glucose metabolism at distant locations such as muscles, liver, and subcutaneous fat, allowing it to participate in the storage and breakdown of food [54, 55, 56].

In terms of the direct hypothetical pathways, shown in fig. (1-1 a, b, c), omentin-1 modulates insulin sensitivity and secretion in organs such as adipose, liver, muscle, brain, and other tissues. Omentin-1 stimulates the insulin receptor substrate (IRS) via blocking the rapamycin (mTORp70S6K) pathway, which is triggered by adenosine-5-monophosphate (AMP) activated protein kinase (AMPK) activation, according to several experimental research findings [57]. Furthermore, omentin-1 promotes lipolysis via increasing the expression of the adiponectin gene and mRNA expression of Zinc-alpha-glycoprotein (ZAG), which may be mediated by AMPK. For example, omentin -1 can boost insulin's action by enhancing glucose uptake by visceral fat adipose tissue in vitro via Akt signaling, which is mediated by insulin [58].

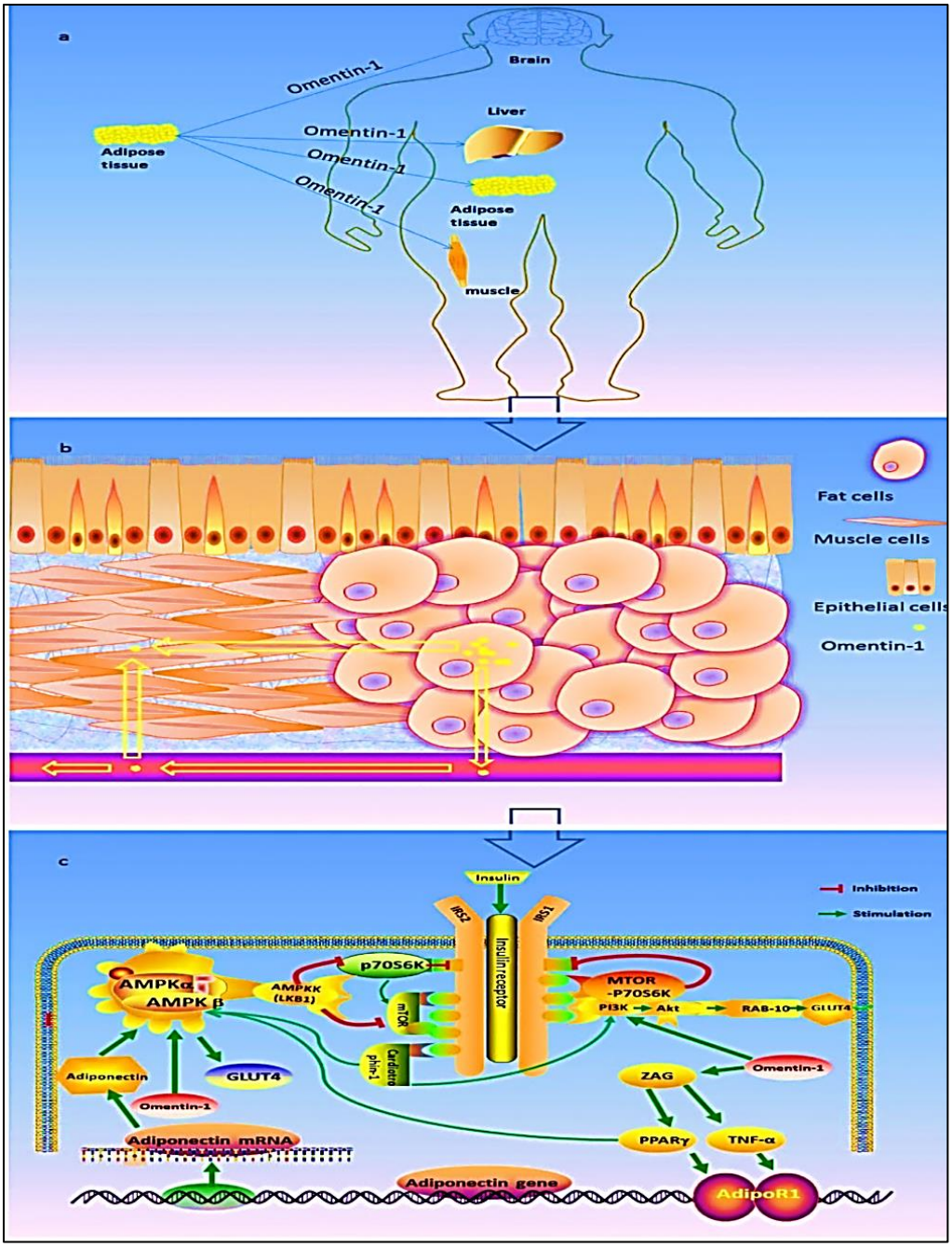


Figure (1-1): The effects of omentin-1 on the immune system a figure depicts the insulin signaling cascade that explains the favorable effects of these adipokines on glycemic management. TNF: Tumor Necrosis Factor; AMPK: AMP-activated protein kinase; ZAG: Zinc 2-glycoprotein; AS160: Akt, substrate of 160 kDa; GLUT: glucose transporter; IRS: insulin receptor substrate; mTOR: mammalian target of rapamycin; PPAR-γ: peroxisome proliferator-γactivated receptor; adipoR1: RAB-10 stands for Ras-related protein RAB-10 [57].

According to earlier studies, plasma omentin levels are inversely related to BMI, fat mass, and fasting plasma insulin, and favorably related to insulin sensitivity, adiponectin, HDL cholesterol, and endothelial function [59, 60]. Some studies suggesting a strong link between omentin level and diabetes [61-64].

Omentin-1 concentrations were found to be lower in those with pre-diabetes in various studies [65]. This phenomena could be explained by the fact that omentin-1 promotes insulin sensitivity, as a result, its reduction could be a factor in pre-diabetic individuals' glucose homeostasis [66]. Omentin-1 affects lipid metabolism, according to experiments in cell models through the activation of AMPK pathways, and energy balance [67]. AMPK is well known for its role as an energy sensor in maintaining energy balance. It increases energy metabolism and produces energy through glucose absorption. It is now commonly acknowledged that AMPK inhibits other energy protein synthesis, gluconeogenesis, and lipogenesis are all metabolic activities that need energy [68]. Furthermore, omentin-1 was reported to boost glucose absorption and increase insulin-induced phosphorylation of Akt/K in isolated adipocytes [69].

However, in clinical studies Omentin-1 levels in plasma and adipose tissue were shown to be lower in obese diabetes people [70].

1.3.2- YKL-40

YKL-40, also known as (chitinase-3-like-1(CHI3L1), human cartilage glycoprotein-39), is a protein with three amino acids at the N-terminus: tyrosine (Y), lysine (K), and leucine (L) [71]. YKL-40 is a heparin and chitin-binding glycoprotein with a size of 40 kDa. Activated macrophages and neutrophils are two cell types linked with the innate immune system[72,73, 74]. YKL-40 is a phylogenetically highly conserved serum protein that belongs to the mammalian chitinase-like proteins family [75]. Vascular smooth muscle and endothelial cells [76], arthritic chondrocytes [77], cancer cells [78], and embryonic and fetal cells all produce YKL-40 [79]. Local inflamed tissues, including adipose tissues in T2DM, produce YKL-40 locally [80]. YKL-40 levels in the blood are higher in T2DM patients and have a favorable correlation with insulin resistance [80]. Furthermore, plasma levels of YKL-40 have been reported to be higher in patients with liver fibrosis [81], human diseases such as atherosclerosis, rheumatoid arthritis, coronary artery disease, Alzheimer's disease, and inflammation [82,83,84]. Various proinflammatory cytokines, such as IL-6, interferon-γ (IFN-γ), IL-1β, and TNF-α, have been demonstrated to regulate YKL-40 expression [85]. As a result, YKL-40 has been linked to disease severity and death in a range of conditions. Furthermore, YKL-40 promotes fibroblast cell proliferation [86]. During the inflammatory response connective tissue development is promoted, while catabolic or degradative processes are inhibited [87], activation of the protein kinase B (AKT) and phosphoinositide-3 kinase (PI3K) signaling pathways and angiogenic function mediate anti-apoptotic activity [88, 89].

Some of the novel proteins generated by adipose tissue that cause inflammatory reactions and metabolic dysfunction include YKL-40, chemerin, (LCN,-2) lipocalin-2, and (OPN) osteopontin [90,91]. These elements have vastly different structures and functions. As a result of aberrant extracellular matrix remodeling, OPN and YKL-40 are associated to increased inflammation and insulin resistance. According to the findings of a recent study, YKL-40 is substantially increased in obesity, regardless of glycaemic status, and is linked to many circulating inflammatory markers [92]. Even in childhood, elevated YKL-40 levels can be seen in obese children and serve as a marker for insulin resistance [93]. The obese prepubertal juvenile population has also been found to have elevatedYKL-40 levels [94], with a strong difference in YKL-40 concentration between insulin resistance and non-insulin resistance participants [93]. Increase YKL-40 concentration have been linked to increased adiposity [95, 96]

YKL-40 levels increased considerably inT2DM patients, according to Rathcke and Vestergaard [97]. Furthermore, they discovered a link between raised YKL-40 levels and dyslipidaemia characteristics in T2DM patients that was out to a two-fold increase. The fact that T2DM patients with insulin resistance exhibited greater plasma YKL-40 levels suggested that insulin resistance rather than decreased insulin production, was associated to YKL-40. T2DM patients have higher amounts of YKL-40 in their plasma than healthy people, according to other studies [98, 99, 100].

1.4- Oxidative Stress

The gap between free radical damage and antioxidant protection in cells is referred to as oxidative stress [101]. It is imposed on cells due to one of three factors: an increase in the generation of oxidants, a reduction

in antioxidant protection, or a mixture of the two (for example an increase in oxidant synthesis and a deficit in antioxidant protection) [102].

A state in which cells produce more oxidants, free radicals (FRs) are released, resulting in cellular destruction [103]. FRs can cause reversible or irreversible damage to a variety of biological substances, including amino acid, nucleic acid, protein, lipid, carbohydrate, and lipoprotein [104].

It's linked to aging and a variety of ailments include diabetes, cancer cardiovascular disease, and diabetic complications [105]. The sufficiency of various antioxidants derived either directly or indirectly from the food is required for defense against all of these activities [106].

1.4.1- Free Radicals

The term free radical (FRs) refers to atoms or molecules that have one or more unpaired electrons, making them highly reactive and unstable [107]. Biological FRs can react with a variety of organic substrates since they have electrons accessible. It reacts with lipids, proteins, and DNA, among other organic substrates. These biomolecules can be damaged by oxidation, which can disrupt normal functions and lead to a range of diseases [108, 109].

1.4.1.1- Types of free radicals

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two forms of FRs species.

A. Reactive Oxygen Species

$O_2^{\bullet-}$ superoxide radical, H_2O_2 hydrogen peroxide , and $OH^{\bullet}$ hydroxyl radical are the 3 basic kinds of ROS [110]. $OH^{\bullet}$ is an efficacious radical capable of altering purines and pyrimidines, strand breakage and DNA

damage occur as a result. It sets off chain reactions that result in the formation of lipid peroxides and organic radicals, as well as immediately contributing to molecules [111].

Although $O_2^{\bullet-}$ is extremely reactive, it has a low lipid solubility and hence cannot diffuse far by interacting non-enzymatically with H_2O_2 in the Haber-Weiss process, it can form the more reactive $OH^{\bullet}$ and hydroperoxy radicals. H_2O_2, on the other hand, is a weak oxidizing agent that is categorized as a ROS due to its ability to create the $OH^{\bullet}$ radical. In the non-enzymatic Fenton reaction, transition metals such as Fe^{2+} or Cu^{+} accelerate the production of $OH^{\bullet}$ from H_2O_2, as seen in fig. (1-2) [112]. During the creation of chemical energy and various other biologic processes in the human body, 98% of the oxygen that is inhaled is decreased. The creation of the three primary forms of ROS is caused by incomplete elimination of the remaining 2%. The concentration of ROS is reduced when oxidative phosphorylation is inhibited, implying that this metabolic cycle produces ROS [113].

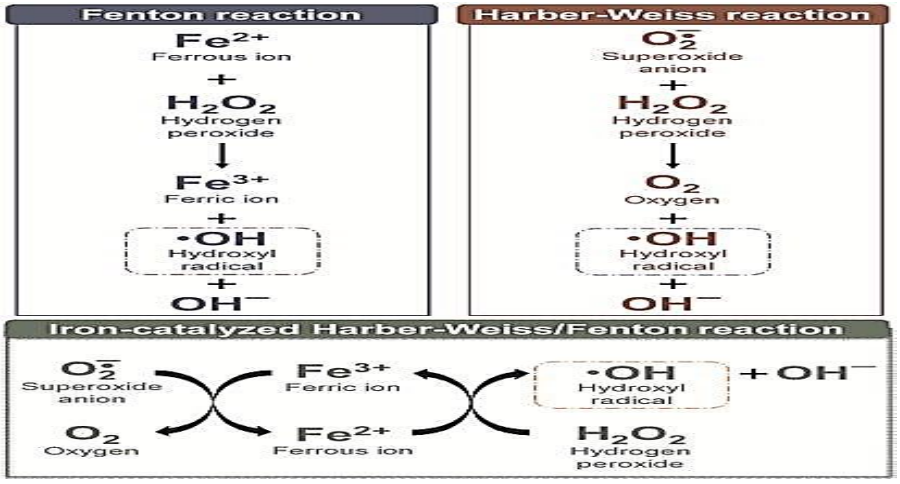


Figure (1-2): The non-enzymatic Haber –Weiss and Fenton Reactions generate OH• [112].

B. Reactive Nitrogen Species

NO nitric oxide and nitrogen dioxide are two examples of RNS [114]. NO regulates a wide range of physiological activities in a number of tissues, but too much of it can be hazardous [115]. NO, on the other hand, has physiological significance as a vasodilator and neurotransmitter. As a result, it can be cytotoxic when used in excess or in combination with oxygen. The result of NO and $O_2^{\bullet-}$ is peroxynitrite ($ONOO^-$). $ONOO^-$ complex can cause energy shortage and cell death by blocking the mitochondrial respiratory chain [116].

1.4.2- Generation of free radicals

ROS can be generated in cells in two ways: enzymatically and non-enzymatically. The respiratory chain and phagocytosis are examples of enzyme processes that produce FRs [117]. Several oxidase systems exist in cells, such as peroxidases, and xanthine oxidase for example, produce $O_2^{\bullet-}$. Once generated, it takes part in a number of processes could generate a variety of ROS such as H_2O_2, $OH^{\bullet}$, and others [118].

Numerous oxidase enzymes are involved in the process like xanthine oxidase, and amino acid oxidase results in non -radical H_2O_2. The last catalyzes both the conversion of hypoxanthine to xanthine and the synthesis of uric acid from xanthine [118].

Free radicals can be produced by ionizing radiation. During oxidative phosphorylation, the non-enzymatic process can also occur in the mitochondria (aerobic respiration) [119, 120]

Oxygen reactive species are formed when oxygen steals electrons from other molecules in the cell, causing damage to cellular constituents. These species play a strong role in the development of illnesses in critically ill patients [121, 122]. Endogenous and exogenous sources both produce reactive oxygen species. Inflammation, immune cell activation, ischemia, severe exercise, cancer, infection, and aging all produce endogenous FRS. Air, cigarette smoke, water pollution, alcohol, and heavy metals (Pb, Cd, Hg, Fe), and radiation all produce exogenous ROS [123].

1.4.3-Nitric oxide (NO)

The nitric oxide synthase family of enzymes produces NO from L-arginine, which is a liposoluble, diffusible radical gas. In tissues, three NOS isoforms are expressed differently [124]. Endothelial cells, which line the inner surface of all blood arteries, produce endothelial nitric oxide synthase (eNOS or NOS-3) isoform in various tissues (heart, pancreas, adipose tissue, skeletal muscle and liver) [125]. Endothelial cells produce endothelial nitric oxide synthase, which modulates smooth muscle vasorelaxation by generating NO [124, 126].

neuronal nitric oxide synthase (nNOS or NOS-1) isoform for the first time, it was detected in the brain, where it's found in abundance in neurons [124, 126]. nNOS is a neurotransmitter that is produced in the CNS. NO is a synaptic signaling neurotransmitter that has been connected to memory, as a result of an increase in intracellular Ca^{2+} concentration that activities of endothelial nitric oxide synthase and nNOS, Calmodulin regulates this process [126,127].

Inducible nitric oxide synthase (iNOS or NOS-2) isoform isn't found in most tissues at rest, unlike eNOS and nNOS, however, it is triggered by acute and chronic inflammatory diseases. Inducible NOS regulation has been found to play a role in macrophage immunity. Calmodulin is required for nitric oxide generation by iNOS and its constitutive equivalents, however, its activation isn't reliant on intracellular Ca^{2+} concentrations because its affinity for calmodulin is independent of Ca^{2+} binding [127].

Because iNOS does not require Ca^{2+} to function, it has the ability to produce large NO levels for longer periods of time than constitutive NOS isoforms, which only produce modest amounts of NO. The principal regulators of iNOS expression are pro-inflammatory cytokines. Due to cytotoxic actions that induce germs to apoptose and/or necrosis, NO production linked to iNOS is thought to be essential for host defense. iNOS-mediated NO production, on the other hand, has been demonstrated to cause harm to a variety of physiological systems, including IR in peripheral tissues [127, 128, 129].

Proteins, polysaccharides, nucleotides, and lipids are all affected by nitric oxide, which is a highly reactive FR. It also plays a role in the demise of cells and tissues, as well as inflammation and adhesion formation [130]. Despite the fact that NO is involved in the control of a range of physiological functions, excessive levels can be dangerous [131]. The level of NO, the cellular redox state, and the amount of metal present in a cell all influence its actions [132].

According to the research, vascular tissues create ROS, such as $O_2^{\bullet-}$, syndromes that play a role in the development of atherosclerosis and IR

[133, 134]. By rapidly interacting with NO to form $ONOO^-$, superoxide promotes vascular dysfunction [135, 136]:

$$O_2^{\bullet -} + NO \longrightarrow ONOO^-$$

(super oxide) (nitric oxide) (peroxynitrite)

Peroxynitrite is a highly reactive agent that can react with DNA bases directly, lipids, antioxidants, thiols, amino acid residues, and other biological targets and cell components, among others [137]. These reactions, however, take a long time to occur. Because of its delayed reaction rate, it has the ability to react more selectively in different parts of the cell. $ONOO^-$ has the ability to cross cell membranes [138].

1.4.4- Chemical and Biological Effects of Oxidative Stress

The severity of oxidative stress's effects is proportionate to the size of the changes, the ability of a cell to overcome minor perturbations and return to its previous state is depending on the magnitude of the changes. More extreme oxidative stress, on the other hand, may cause cell death, and even minor oxidation can lead to apoptosis, while more severe impacts can lead to necrosis [139].

The generation of ROS, which include FRs and peroxides, is a particularly harmful feature of oxidative stress [140]. The majority of these oxygen-derived species are created in small quantities during normal aerobic metabolism, and the harm they cause to cells is regularly corrected. However, when oxidative stress is high enough to cause necrosis,

the damage causes ATP depletion, which prevents regulated apoptosis and forces the cell to simply fall apart [140].

1.4.4.1- Oxidative Damage to Lipids (Lipid Peroxidation)

Lipid peroxidation is a chain reaction involving PUFA and ROS or reactive nitrogen species. Lipid peroxides and hydrocarbon polymers are produced, both of which are extremely toxic to cells [141]. FRs are created when peroxide is produced from FAs with methylene-interrupted double bonds, such as those contained in PUFA found in nature, as seen in the diagram, are assumed to be the cause of the negative repercussions fig. (1-3) [142].

Because active bis-allylic methylene groups are present, on these active methylene units, the bond dissociation energy of the carbon hydrogen bond is reduced, allowing their hydrogen atom to be extracted more easily by radical reaction. As the number of unsaturated sites in the lipid chain grows, so does PUFA peroxidation [143].

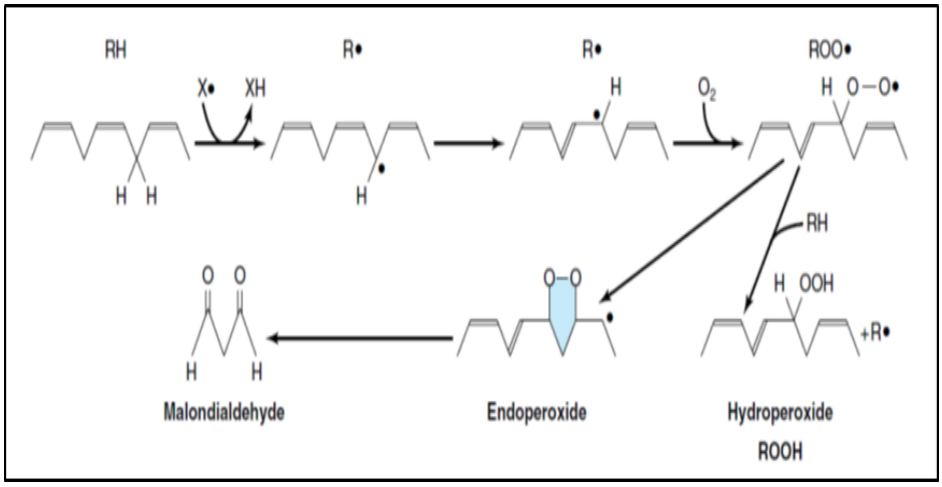


Figure (1-3): Peroxidation of Lipids. ROOH: Lipid peroxide, RH: Polyunsaturated lipid, X•: Free radical, R•: Lipid radical, ROO•: Lipid peroxy radical [142].

Lipid peroxidation is a chain reaction that generates FRs in a continuous stream, allowing for further peroxidation to occur. The following stages will walk you through the entire process [142]:

- First step (Initiation):

$$ROOH + Metal^{(n)+} \longrightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$$

$$X^{\bullet} + R\,H \longrightarrow R^{\bullet} + X\,H$$

- Second step (Propagation):

$$R^{\bullet} + O_2 \longrightarrow R\,OO^{\bullet}$$

$$RO\,O^{\bullet} + R\,H \longrightarrow RO\,OH + R^{\bullet}, \text{ e tc}$$

- Third step (Termination):

$$RO\,O^{\bullet} + ROO^{\bullet} \longrightarrow R\,OOR + O_2$$

$$R\,OO^{\bullet} + R^{\bullet} \longrightarrow R\,OOR$$

$$R^{\bullet} + R^{\bullet} \longrightarrow R\,R$$

The structure of lipid molecules is always changed or damaged when they are peroxidized. The aldehydes formed during membrane lipid peroxidation can crosslink proteins, in addition to being self-destructive. The cohesive lipid bilayer arrangement and stable structural order are compromised when damaged lipids are constituents of biologic membranes. Further FRs synthesis could be triggered if mitochondrial membrane integrity is disrupted [109]. Peroxidation of membrane lipids by FRs causes an increase in membrane fluidity and permeability, as well as a loss of membrane integrity, which causes cell injury [144].

1.4.4.1.1- Malondialdehyde (MDA)

Malondialdehyde (MDA) is a byproduct of PUFA and related esters peroxidation . MDA is a biological marker of oxidative stress-induced lipid peroxidation [145]. MDA is extremely reactive, interacting with amine groups in amino acids like lysine and arginine [146]. It also combines with other ketones or aldehydes, such as linked sugars or glycation products, to produce ketones or aldehydes [147].

MDA can be found in two forms in tissues and blood: free and attached to proteins with-SH and/or -NH_2 groups, nucleic acids, and lipoproteins [148]. The active ingredient form of Free MDA is used as a marker for recent damage [149]. The binding fraction discharged in urine [150] is symptomatic of a previous injury.

1.4.5- Free radicals Advantageous Actions

FRs are required for the maturation of cellular structures at low or moderate concentrations and can be used as weapons by the host defense system. Indeed, disease releases FRs to destroy invading pathogenic microorganisms as part of the body's struggle against phagocytes (neutrophils and macrophages) [151].

Free radicals promote both the generation of ATP from ADP in the mitochondria (oxidative phosphorylation) and the detoxification of xenobiotics by cytochrome P-450 (oxidizing enzymes).The physiological involvement of FRs in the function of a number of cellular signaling systems are also advantageous [152]. Non-phagocytic cells also produce NADPH oxidase isoforms, in a variety of non-phagocytic cells, which are

vital in the regulation of intracellular signaling cascades, including VSMC, endothelial cells, and cardiac myocytes [153].

1.4.6- Antioxidants

Antioxidants are chemicals or substances that scavenge FRs. Endogenous and exogenous antioxidants are the two forms of antioxidants. Most antioxidants are electron donors, and when they come into contact with FRs, they produce harmless end products, such as water. These antioxidants bind to FRs and render them inactive. As a result, antioxidants protect cells from oxidative stress and damage [105].

1.4.6.1-Endogenous Antioxidants:

Endogenous defense mechanisms have evolved in biological systems to help protect cells from FRs-induced cell damage. Endogenous antioxidants include both enzyme-based and non-enzymatic antioxidants. Primary and secondary enzymes are among the enzymatic antioxidants. Superoxide dismutase (SOD), catalase, and glutathione peroxidase are the main enzymes involved in the direct removal of ROS [154, 155].

Glutathione reductase and glucose -6- phosphate dehydrogenase are examples of secondary enzymes, and cytosolic glutathione -S- transferase, aid in ROS detoxification by reducing peroxide levels or maintaining a continuous supply of metabolic intermediates such as glutathione and NADPH, which are required for the proper operation of the key antioxidant enzymes [154, 155].

Endogenous antioxidants such as glutathione, sulfhydryl groups, NADPH, thioredoxin, uric acid, bilirubin, ceruloplasmin, myoglobin, albumin, and melatonin are non-enzymatic compounds [156].

A. Reduced Glutathione (GSH):

It's a glutamine, cysteine, and glycine linear tripeptide. The cysteinyl part of the glutathione molecule has a sulfhydryl (SH) group, which accounts for its strong electron donating properties. The molecule becomes oxidized when electrons are lost, and two such molecules are connected (dimerized) by a disulfide bridge to produce glutathione disulfide or oxidized glutathione (GSSG). This connection is reversible when glutathione reductase is used to re-reduce it [157]. Glutamine is an essential cellular protector. It acts as a major shield against radiation damage to the skin, lens, cornea, and retina [158].

B. Glutathione Peroxidase:

Glutathione peroxidase is a tetrameric glycoprotein containing selenium, meaning it has four selenocysteine amino acid residues. Because glutathione peroxidase is critical for the integrity of cellular and subcellular membranes, The antioxidative defense system of glutathione peroxidase requires selenium [159]. The function of glutathione peroxidase is to convert lipid hydro peroxides to alcohols and free H_2O_2 to water. An example of a reaction catalyzed by glutathione peroxidase is shown below:

$$2GSH + H_2O_2 \xrightarrow{\text{glutathione peroxidase}} GSSG + 2H_2O$$

C. Glutathione Reductase:

Glutathione disulphide (GSSG) is reduced to the sulfhydryl form GSH by this enzyme. One mole of NADPH is required for every mole of

GSSG. The pentose phosphate pathway is used to produce NADPH (glucose -6- phosphate dehydrogenase) [160].

$$GSSG + NADPH + H^{+} \xrightarrow{\text{Glutathione reductase}} 2\,GSH + NADP^{+}$$

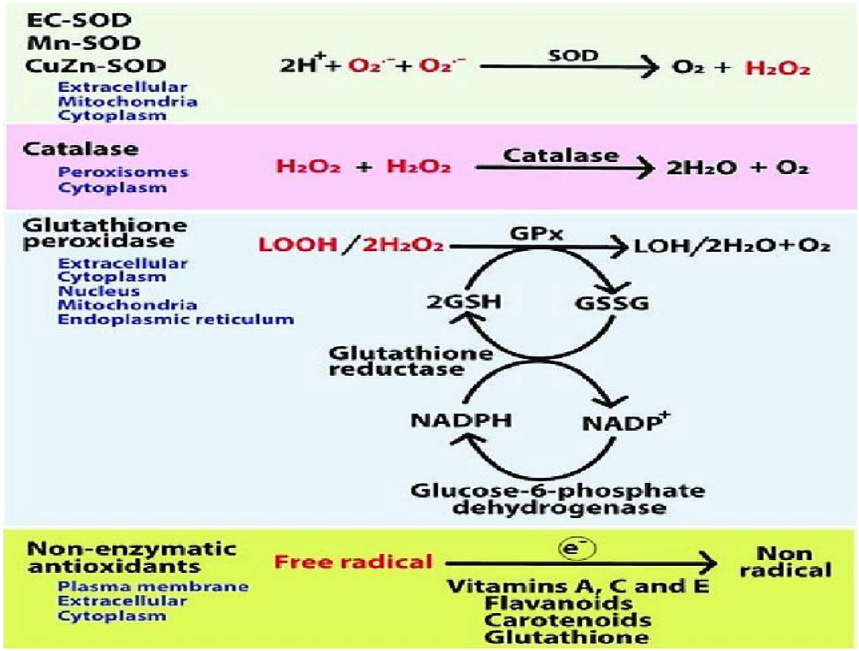


Figure (1-4): In cells, the antioxidant system . Antioxidants (enzymatic and non-enzymatic) catalyze processes that donate electrons to neutralize FRs. Enzymatic antioxidants catalyze reactions that neutralize specific FRs, such as SOD, which converts superoxide to H_2O_2, hydrogen peroxide is converted to water by catalase and glutathione peroxidase (GPx) [160].

D. Coenzyme Q_{10} (CoQ_{10}) :

Ubiquinone, commonl known as CoQ, is a lipophilic molecule that can be totally oxidized, partially reduced (ubisemiquinone), or fully reduced (ubiquinol) [161].

It is found in the plasma membrane as well as various internal membranes, including mitochondrial membranes, it is used in the

production of energy and the formation of ROS. CoQ, in its oxidized state, may have antioxidant properties. In tests, biquinone has been reported to protect against protein carbonylation and oxidative DNA damage [162, 163]. Furthermore, ubiquinone has been found to protect membrane phospholipids against peroxidation [164] and regenerate other potent antioxidants like alpha tocopherol and ascorbate by recycling them back to their reduced active forms, hence enhancing cellular antioxidant resources [165]. Because of these qualities, ubiquinone is a good food supplement for improving cellular bioenergetics and preventing age-related illnesses.

CoQ_{10} has been found to decrease the peroxidation of cell membrane lipids and diminish the oxidation of circulating lipids in their reduced form. It prevents the oxidation of low-density lipoprotein more effectively in vitro than other antioxidant compounds like α–tocopherol or β–carotene [166].

T2DM is the most severe result of insulin resistance. When compared to healthy people, patients with T2DM have a shortage of CoQ_{10} plasma levels [167,168].

1.4.6.2-Exogenous Antioxidants

They primarily serve as free radical scavengers, neutralizing free radicals, repairing oxidized membranes, and reducing the generation of reactive oxygen species. Vitamins (A, C, E, and K), enzyme cofactors Ubiquinone, nitrogen molecules (uric acid), minerals (Zn and Se), among the exogenous antioxidants are polyphenols (flavonoids, and phenolic acid). Manganese, zinc, copper, iron, and selenium are metals that improve the

catalytic activity of antioxidant enzymes. It has been proposed that a diet deficient in specific trace elements may diminish the effectiveness of antioxidant defense mechanisms [169,170].

1.5-Relevance of Inflammatory with Diabetes Mellitus and oxidative stress

When the production of reactive oxygen species (ROS) outnumbers the antioxidant defense, oxidative stress occurs. Obesity increases oxidative stress levels in the body. In adipose tissue, NADPH oxidase, xanthine oxidase, and the mitochondrial oxidative phosphorylation system can all produce ROS. On the one hand, ROS production in the adipose tissue of obese patients promotes IR, aberrant adipokine release, inflammation, and increased protein carbonylation.

In brown adipose tissue (BAT), on the other hand, ROS may promote adipocyte growth and thermogenesis. SOD, catalase, GPx, HO, and Prxs are just a few of the enzymes that can reduce ROS burden and act as antioxidant defense in adipose tissue.

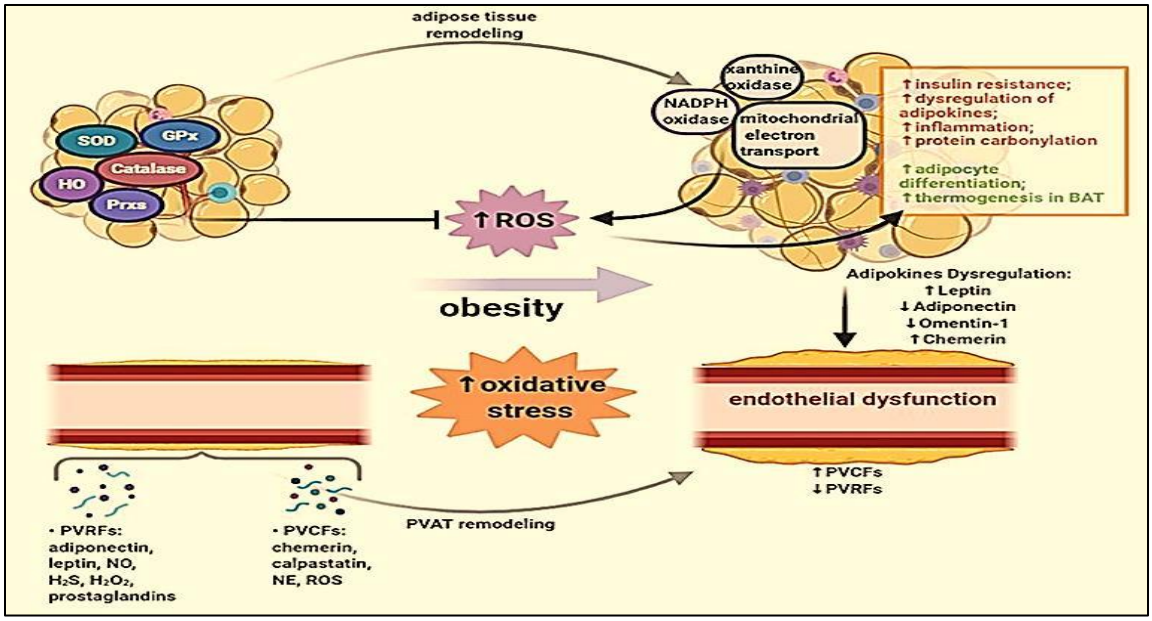


Figure (1-5): Relevance of inflammatory with Diabetes Mellitus and oxidative stress [170].

By releasing a number of bioactive molecules, including circulating adipokines, adipose tissue directly impacts the circulatory system. Perivascular adipose tissue PVAT is an essential adipose tissue that influences vascular function and remodeling due to its close proximity. The release of perivascular adipose tissue releasing factors PVRFs and perivascular adipose tissue contracting factors PVCFs regulates vascular contractility increased oxidative stress, inflammation, and eNOS dysfunction in the PVAT in obese patients may alter the balance of PVRFs and PVCFs. PVAT dysfunction caused by obesity results in a decrease in PVRFs and a rise in PVCFs, leading in greater vasocontraction. Chronic changes in the adipokine profile might potentially cause aberrant vascular remodeling, raising the risk of CVDs even more [171].

1.8- Aim of Study

1. Evaluation both of serum Omentin-1 and YKL-40 in male T2DM patients.
2. Evaluation of serum oxidant parameters: Total Oxidant Status (TOS), Nitric Oxide (NO), and Malondialdehyde (MDA). And antioxidant parameters: Total Antioxidant Capacity (TAC), Glutathione (GSH), and Co enzyme 10 (CoQ_{10}) in male T2DM. And finding the correlation between each other and between all studied parameters.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1- Materials

2.1.1- Subjects

There are two categories in this study, control and patients group. All samples were collected from the Anbar governorate. The study's practical component took place at the laboratories of Fallujah maternity and children hospital.

2.1.1.1- Control Group

The control group consists of 30 men who appear to be in good health. This group's age ranged from 32 to 55 years old. They were gathered from medical personnel and families. They were free of any illness symptoms and indicators.

2.1.1.2- Patient Group

A total of 60 T2DM patients were enrolled in the trial men. The patients' ages ranged from 31 to 55 years old. Physicians diagnosed all of the samples in this group. They were gathered in Anbar governorate.

Patients use metformin as treatment. Any subject (control or patient group) who had any of the following issues was not included in the current study:

- Patient with renal dysfunction or cardiovascular diseases.
- Infections

- ❖ Patients with malignancies
- ❖ Antioxidants and other drugs that alter oxidative stress.

2.1.2- Blood Collection

In the sitting position, venous blood samples were taken from control and patient volunteers using a disposable syringe (5mL). Fasting subjects (8-12) hours will be asked to come in for blood sample. Each individual had five milliliters of blood drawn from their veins and slowly put into plain disposable tubes. Venous blood samples were taken and separated into two tubes: a gel tube and an EDTA coated tube. Samples in gel tubes were allowed to coagulate for 10-15 minutes at 37°C before being centrifuged at 3000 xg for 10-15 minutes to obtain serum samples, then split the serum into five parts and kept at -20°C until they are analyzed serum (YKL-40, Omntin-1, Co enzyme q $_{10}$ (CoQ_{10}), glutathione (GSH), total antioxidant capacity (TAC), insulin, nitric oxide (NO), total oxidant status (TOS), maloinaldyhyde (MDA), total cholesterol (CHO), high density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) concentration will be measured. Glycated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) were determined using samples packed in EDTA coated tubes.

2.1.3- Instruments and Materials

The instruments and materials used in this study are shown in Table(2-1).

Table (2-1): Instruments and Equipment

No.	Instruments and Equipment	Origin
1	Balance	Sartorius (Germany)
2	Centrifuge EBA 20	Hettich (Germany)
3	Deep freezer	Medicald jet (Syria)
4	differents micropipettes	Watson Nexty (Japan)
5	Distillatory	Bibby science (England)
6	ELISA system, micro plate reader	Bio-tech instrument(USA)
7	E170 cobas e411	Roche (USA) analyzer
8	FUJI DRI-CHEM (FDC)	Jaban
9	I-Chroma instrument	Korea
10	Incubator	Fisher scientific (USA)
11	Spectrophotometer UV-VIS	Cecil, CE10N (England)
12	Vortex (Electronic)	Bionex (Korea)
13	Water bath	Grant (England)

2.1.4- Chemicals

Specific chemicals were employed to measure some parameters in this investigation, whereas standard kits were utilized to measure others, as shown in Table (2-2).

Table (2-2): Chemical substances and kits are used in this study

No.	Chemical substance	Origin
1	Acetone	VWR, Chemicals BDH
2	Ammonium Iron(II) Sulphate-6-hydrate	Sigma-Aldrich
3	DTNB reagent	Sigma-Aldrich
4	Glucose kit	Glucose kit, linear chemical, Spain)
5	Glycerol	Sigma-Aldrich
6	HDL-C	HDL-c Fuji film
7	HumanChitinase-3-like-protin1 (YKL-40,CHI3L1) ELISA Kit CAT.#: EKHU-2211	Melsin Medical Co., Limited (China)
8	Human Coenzyme Q10(CoQ10) ELISA Kit CAT.#: EKHU-2007	Melsin Medical Co., Limited (China)
9	Human Omentin 1 ELISA Kit CAT.#: EKHU-1593	Melsin Medical Co., Limited (China)
10	Hydrochloric acid	Sigma-Aldrich
11	Hydrogen peroxide	Sigma-Aldrich
12	I-Chroma-HbA1c kit	Boditech HbA1c Calibrator (Korea)
13	Insulin kit ECLIA	Modular analytics E170(UAS)
14	Nitric oxide ELISA Kit	Melsin Medical Co., Limited (China)
15	o-dianisidine	Sigma-Aldrich

No.	Chemical substance	Origin
16	Potassium chloride	Sigma-Aldrich
17	Sodium chloride	Sigma-Aldrich
18	Sulfuric acid	Sigma-Aldrich
19	Trichloro acetic acid	Hokin and Williams (England)
20	Triglyceride kit	Triglyceride kit, linear chemical, Spain)
21	Total cholesterol kit	cholesterol kit, linear chemical, Spain)
22	Vitamin C	Sigma-Aldrich
23	Xylenol orange	Sigma-Aldrich

2.2- Methods

2.2.1- Measurement of Body Mass Index (BMI)

The BMI calculation preformed in a mathematical equation [172].

$$\text{BMI= Weight (kg)/ (Height m)}^2$$

2.2.2- Determination of Fasting plasma glucose concentration

Principle:

In the Trinder reaction, GOD is a glucose oxidase which converted glucose to gluconate, releasing in the process H_2O_2. As shown in the following reactions, a mixture of phenol and 4-AA was oxidized by H_2O_2 in the presence of peroxidase (POD) to produce a red quinoneimine dye proportionate to the amount of glucose in the sample [173]:

Reagents:

Table (2-3): Colorimetric method manual insert in chemicals reagents of fasting plasma glucose concentration test.

Reagents	Composition	
Monoreageant	Phosphate buffer	100 mmol/L pH 7.5
	Glucose oxidase	> 10 KU/L
	Peroxidase	> 2 KU/L
	4-aminoantipyrine	0.5 mmol/L
	Phenol	5 mmol/L
Standard	Glucose 100 mg/dL or	5.56 mmol/L

Procedure:

Table (2-4): Colorimetric method manual of fasting plasma glucose test.

Reagents	Blank	Standard	Sample
W.R	1 Ml	1 mL	1 mL
Standard	-	10µL	-
Sample	-	-	10 µL

Allow for 10 minutes at room temperature or 5 minutes at 37 °C after thoroughly mixing. Compare the absorbance (A) of the samples, the standard, and the reagent blank at 500 nm. The color lasts around 2 hours when protected from light.

Calculation:

$$\textbf{Glucose (mg/L)} = \frac{A.of\ sample}{A.of\ standard} \times 100$$

2.2.3-Determination of HbA1c percentage

Principle:

The test uses a sandwich immune-detection technique, in which the detector antibody in the buffer binds to the antigen in the sample, forming antigen-antibody complexes that migrate onto the nitrocellulose matrix and are caught by the other immobilized antibody on the test strip. The more antigen in the sample, the more antigen-antibody complexes are generated, resulting in a stronger fluorescence signal on the detector. On an i-chroma testing apparatus, the concentration of glycated hemoglobin in blood is represented as a percentage of total hemoglobin in blood.

Reagent:

1- The cartridge includes a test strip with anti-human HbA1c on the test line and rabbit IgG on the control line, as well as a membrane. Each cartridge is packed in an aluminum foil pouch with a desiccant inside.

2- The detection buffer contains anti-human HbA1c fluorescent conjugate, anti-rabbit IgG fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer, and sodium azide in phosphate buffered saline (PBS) as a preservative.

3- In PBS, there is a non-ionic detergent and sodium azide as a preservative in the hemolysis buffer.

Procedure:

1) A volume of 100 µL of hemolysis buffer were drawn and put to the detection buffer tube.

2) by using a 10 µL micropipette, draw 5 µL of whole blood from the EDTA tube and was place it in the detection buffer tube.

3) The cover was Closed of the detection buffer tube and the tube was shook it 15 times to fully mix the sample.

4) Half of the cartridge was removed from the i-Chamber slot.

5) a volume of 75 µL of the sample mixture was pipetted out and put into a sample well in the test cartridge.

6) The flow of the sample mixture was waited till it appeared in the windows. (Approximately 10 seconds).

7) The cartridge was Inserted into the i-Chamber slot.

8) The cartridge was leave in i-Chamber for 12 min. before removing it.

9) The sample-loaded cartridge was scanned immediately when the incubation time is over. If not, it will cause inexact test results.

10) Inserted the sample-loaded cartridge into the instrument's cartridge holder for i-chroma tests to scan it. Before inserting the cartridge all the way into the cartridge holder, double-check its orientation. On the cartridge, an arrow has been carefully marked for this reason.

11) To begin the scanning procedure, press the 'Select' button on the device for i-chroma testing.

12) The instrument for i-chroma tests will immediately begin scanning the sample-loaded cartridge.

13) For i-chroma tests, On the instrument's display screen, read the test result.

2.2.4-Determination of serum fasting insulin concentration

Serum insulin level was determined full automated using modular analytics E170 cobas e411 instrument Roche (USA).

2.2.5-calculation of insulin resistance by hemostasis model assessment of insulin resistance (HOMA-IR)

The hemostasis model assessment of insulin resistant was calculated using the HOMA-TR calculator- Insulin Resistance [174].

2.2.6- Determination of Total Cholesterol concentration

Principle:

The concentration of CHO was determined enzymatically using Allain C. et al. technique's [175]. as evidenced by the reactions that follow:

$$\text{CHO esters} + H_2O \xrightarrow{\text{CHO esterase}} \text{CHO} + \text{FAs}$$

$$\text{CHO} + O_2 \xrightarrow{\text{CHO oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AA} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4\ H_2O$$

Reagents:

Table (2-5): Colorimetric method manual insert in chemicals reagents of CHO test.

Reagents	Composition	
Monoreagent	PIPES	200 mmol/L pH 7.0
	Sodium cholate	1 mmol/L
	Cholesterol esterase	> 250 U/L
	Cholesterol oxidase	> 250 U/L
	Peroxidase	> 1 KU/L
	4-aminoantipyrine	0.33 mmol/L
	Phenol	4 mmol/L
	Non-ionic tension actives	2 g / L (w / v)
Reagent (Standard)	CHO 200 mg/dL or 5.17 mmol/L	

Procedure:

Table (2-6): The procedure of CHO

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
D.W	10µl	-	-
Standard	-	10 µL	
Sample	-	-	10 µL

Allow for 10 minutes at room temperature or 5 minutes at 37 °C after thoroughly mixing. Compare the absorbance (A) of the samples, the standard, and the reagent blank at 500 nm. The color lasts about 30 minutes when protected from light.

Calculation:

$$\textbf{CHO (mg/L)} = \frac{A.of\ sample}{A.of\ standard} \times 200$$

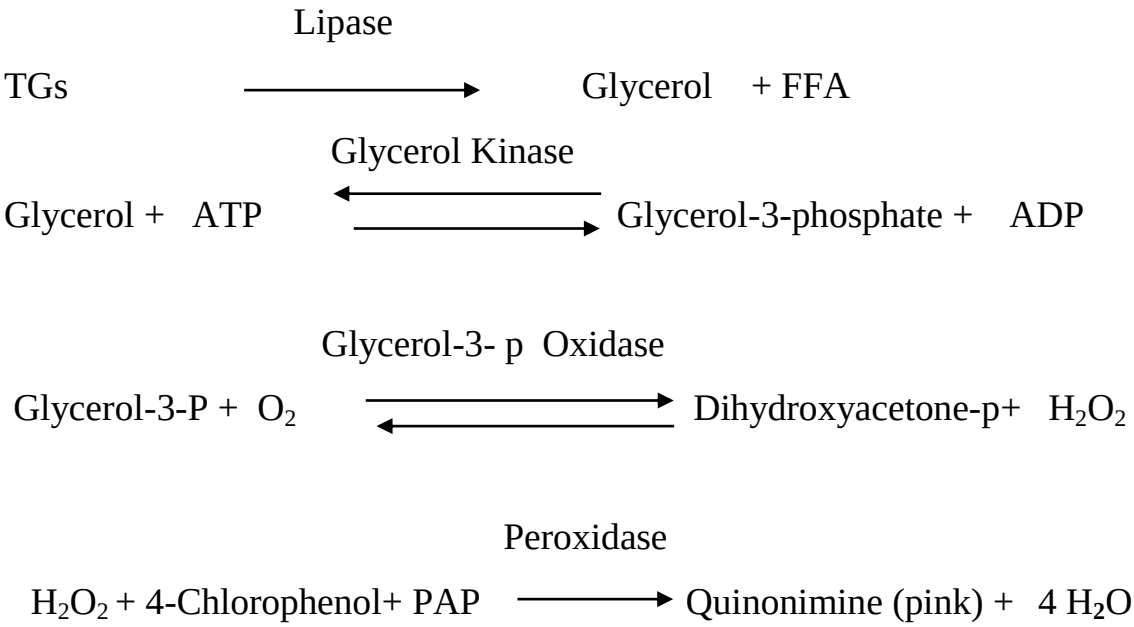
2.2.7- Determination of High Density Lipoprotein concentration

Serum of HDL-C levels was determined full automated using FUJI DRI-CHEM (FDC) system [176].

2.2.8- Determination of Triglycerides concentration

Principle:

The concentration of TGs was determined using an enzymatic approach developed by Allain F. and the Prencipe technique linked to the Trinder reaction [177,178], as illustrated in the two reactions below:



The absorbance (quinonimine) of the colored complex is proportional to the amount of TGs in the specimen.

Reagents:

Table (2-7): Colorimetric method manual insert in chemicals reagents of TG test

Reagents	Composition	
Monoreageant	PIPES buffer	50 mmol/L pH 6.8
	LPL	≥12KU/L
	GK	≥ 1 KU/L
	GPO	≥ 10 KU/L
	ATP	2.0 mmol/L
	Mg^{2+}	40 mmol/L
	POD	≥ 2.5 KU/L
	4-AA	0.5 mmol/L
	Phenol	3 mmol/L
	Non-ionic tension actives	2g/L(w/v)
Standard	TGs 200 mg/dL or	2.26 mmol/L

Procedure:

Table (2-8): The procedure of TG

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
D.W	10Ml	-	-
Standard	-	10 µL	
Sample	-	-	10 µL

Allow for 15 minutes at room temperature or 5 minutes at (16-25) °C after thoroughly mixing. Compare the absorbance (A) of the samples and the standard to that of the reagent blank at 500 nm. The color lasts around 1 hour when protected from light.

Calculation:

$$\textbf{TGs (mg/L)} = \frac{A.of\ sample}{A.of\ standard} \times 200$$

2.2.9- Calculation of Very Low Density Lipoprotein Concentration

VLDL-C levels was calculated by dividing TGs value obtained in section (2.2.8) by 5 [179].

$$\textbf{VLDL-C (mg/dL)} = \frac{TGs}{5}$$

2.2.10- Calculation of Low Density Lipoprotein Concentration

LDL-C level was calculated by using Friedewald equation [180, 181]. This equation is only valid at TGs less than 400 mg/dL.

LDL-C (mg/dl) = Total CHO - [HDL-C + VLDL-C]

2.2.11-Determination of Serum Omentin-1 Concentration

Principle:

The quantitative enzyme immunoassay approach (double-antibody sandwich) is used to assay Omentin-1 in human serum in order to determine serum Omentin-1 levels. The wells of the micro-Liter plate are coated with an anti- Omentin-1 antibody. Pipette Omentin-1 samples and standards into the wells to see if the coated antibody would bind to them.

After a washing phase to remove unbound compounds, an enzyme-linked antibody specific for Omentin-1 is injected to the wells. After a wash, Chromogen Solution A and B are added to the wells to remove any unattached antibody enzyme reagent. The color of the liquid will shift to blue in proportion to the amount of Omentin-1 bound in the first stage. Due to the acid's effect, the color ultimately turns yellow. The color change is detected using spectrophotometry at a wavelength of 450 nm. the concentration of Omentin-1 in the samples is determined by comparing the O.D. of the samples to the standard curve.

Reagents:

The reaction contained the following reagent**:**

- Omentin-1 micro-plate: 96 well polystyrene micro-plate (12 strips of 8wells) covered with a polyclonal anti-Omentin-1 polyclonal antibody.
- Standard (1 set): 0.3 mL, 6 vials

- ❖ Wash solution (20×): 25 mL, 1 vial
- ❖ HRP- Conjugate reagent: 10.0 mL, 1 vial
- ❖ Sample diluents: 0.6 mL, 1 vial
- ❖ Chromogen Solution A: 0.6 mL
- ❖ Chromogen Solution B: 0.6 mL
- ❖ Stop Solution: 0.6 mL
- ❖ Standard concentration was followed by 64, 32, 16, 8, 4, 0 ng/mL.

Reconstitution of Reagents:

Wash solution : The 20 × wash solution concentrate was diluted into deionized water 1:20.

Procedure:

- A volume of 50 micro liter of each standard (64, 32, 16, 8, 4, 0 ng/mL), was added per well and 10 micro liter of sample , was added per well then testing sample well received the sample diluent 40 micro liter; the blank well received nothing.
- 100 µL of HRP-conjugated reagent was added to each well, which was then covered with an adhesive strip and incubated for 1hours at 37 °C.
- Each well was cleaned and aspirated. The washes were completed by utilizing an auto washer to filled each well with 400 mL of wash solution. To achieve good results, each step must be completely liquid-free. For a total of three washes, the process was repeated twice more. Any residual 400 mL wash solution was eliminated by aspirating or decanting following the last wash invert the plate and wipe it clean with paper towels.

- Each well was filled with 50 microliters of Chromogen A and 50 microliters of Chromogen B solutions, which were gently mixed and incubated at 37 °C for 15 minutes.
- A stop solution of 50 microliters was poured into each well. The color was modified from blue to yellow. If the color in the well is green or the color change isn't uniform, gently tap the plate to ensure complete mixing.
- Using a microplate reader set to 450 nm, optical density of each well was calculated in 15 minutes.

Calculation:

The absorbance of the standards was plotted against their concentration, and the best curve was created. absorbance value was drawn it on the y-axis and stretched a horizontal line to the standard curve to get the Omentin-1 concentration of each sample, then extended a vertical line to the x-axis at the intersection, as shown in fig. (2-1), and read the associated sample concentration.

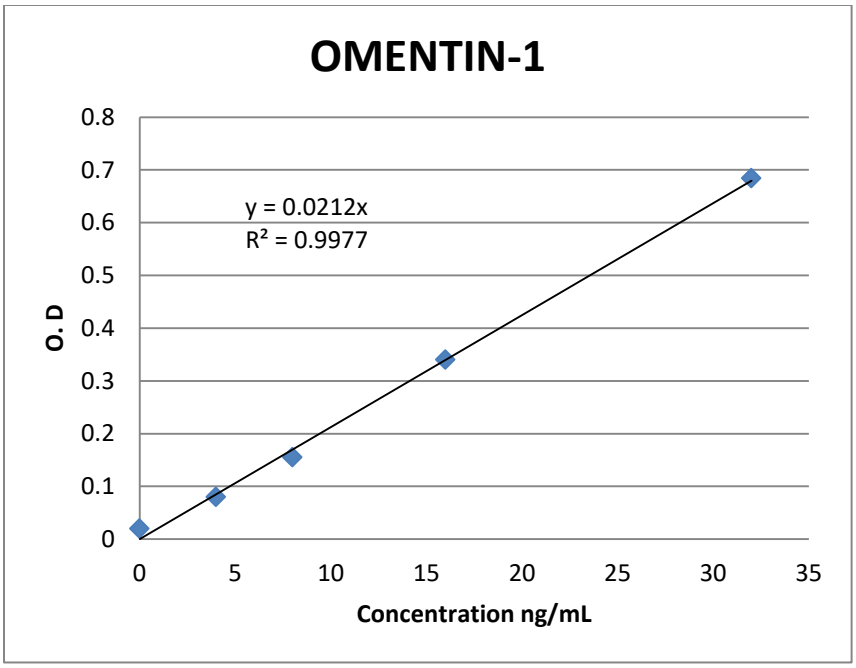


Figure (2-1): Standard Curve for Determination of Omentin-1Concentration.

2.2.12- Determination of Serum HumanYKL-40, serum NO and serum CoQ_{10} Concentrations as mention in (2.2.11)

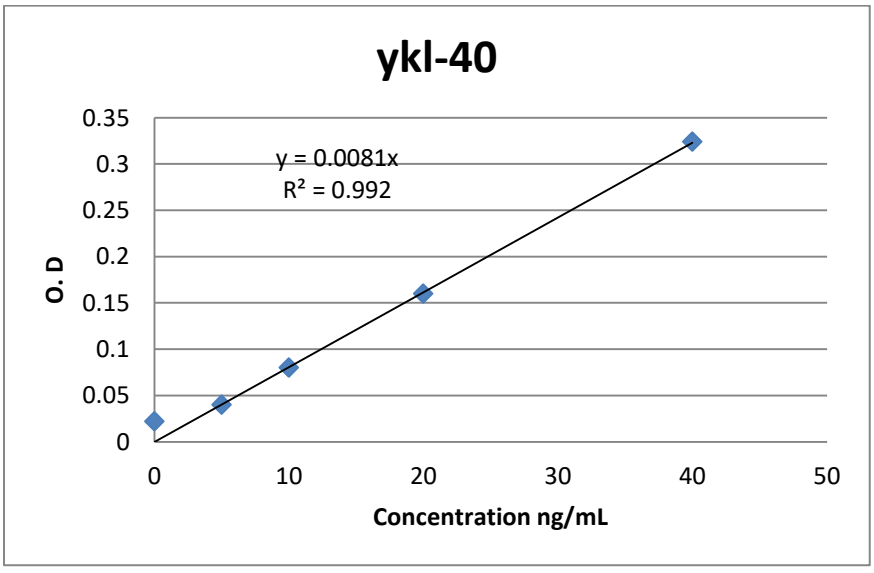


Figure (2-2): Standard Curve for Determination of Human YKL-40 Concentration.

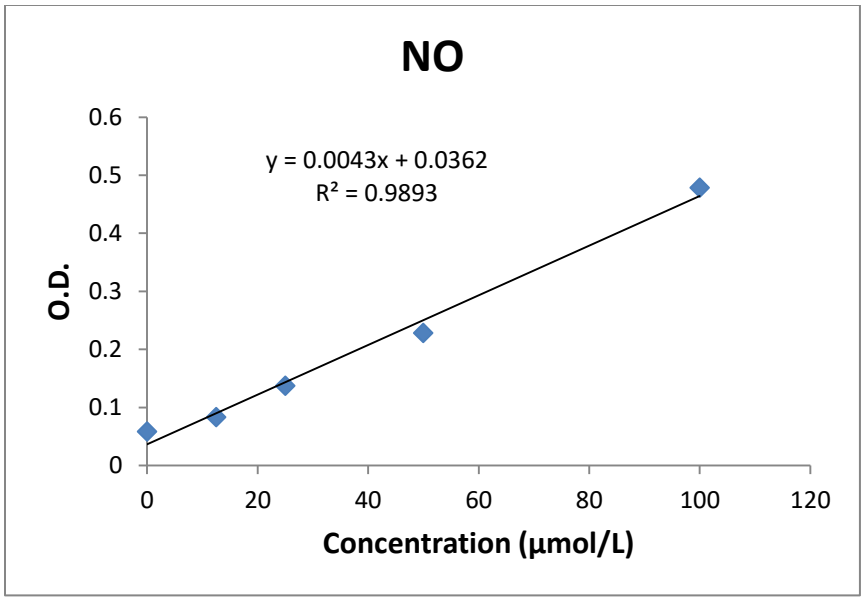


Figure (2-3): Standard Curve for Determination of NO concentration.

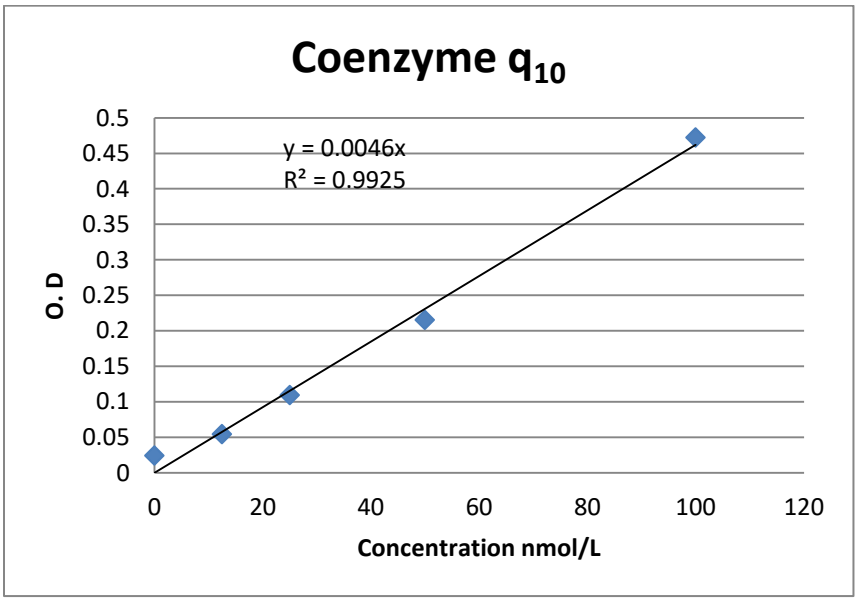


Figure (2-4): Standard Curve for Determination of Human CoQ_{10} Concentration.

2.2.13- Determination of Serum Malondialdehyde concentration

The concentration of MDA is measured using a modified technique outlined by (Buege and Aust) [182].

Principle:

The method relies on MDA's reaction with thiobarbituric acid (TBA), MDA-TBA2 product is formed, which absorbs substantially at 532 nm.

Preparation of Reagents :

A 0.188 g of TBA (0.013 N) and 7.5 g tri-chloroacetic acid (TCA) reagent (0.46 N) diluted in an appropriate volume of HCL (0.25N), The mixture was agitated and heated at 70°C until complete solubility was achieved, and then the volume was increased to 100 mL of HCL (0.25 N).

Procedure:

In a 0.5 mL serum sample, 1 mL of the reagent was applied. Vortexing was used to thoroughly mix the tube, which was then heated for 20 minutes at 70 °C. The mixture was centrifuged for 10 minutes at (6000 Xg) using a microfuge after cooling. The clear supernatant was compared to a blank containing 1 mL of D. W and 2 mL of reagent at 535 nm.

Calculation:

$$\textbf{MDA (µmol/L)} = \frac{\textbf{Abs} \times \textbf{D.f}}{\varepsilon \times \textbf{d}}$$

d **:** Light path (1cm)

Abs: Absorbance.

Ɛ**:** Extinction coefficient (1.56×10^5 M^{-1} cm^{-1})

D. *f* **:** Dilution factor

2.2.14- Determination of Serum Glutathione concentration

The concentration of thiol in the serum was determined using the Ellman method [183]:

Reagents:

(a) By dissolving (0.2 g) in (100 mL) D.W, H_2NaPO_4 (0.2 M) was prepared.

(b) Dissolving (0.2 g) in (100 mL) D.W yielded HNa_2PO_4 (0.2 M).

(1) Reagent A: (Phosphate buffer 0.2 M, pH=7) was made by mixing 41 mL of (b) with 9 mL of (a). D.W. finished the volume to 100 mL and corrected the pH.

(2) Reagent B: (Phosphate buffer 0.2 M, pH=8) was made by combining 5 mL of (a) with 45 mL of (b), adding D.W to bring the volume to 100 mL, and adjusting the pH before and after adding D.W.

(3) Reagent C: (DTNB reagent): This solution was made by dissolving (39.6 mg) DTNB in 10 mL reagent A with a trace of Na_2CO_3 added.

Procedure:

1) In a test tube, 20 µL of serum was added to 1000 µL of D.W.

2) After that, 1000 µL of reagent B was added and thoroughly mixed.

3) a volume of 1500 µL of the aforesaid mixture was drained, and 20 µL of reagent C was added.

The solution was thoroughly mixed and incubated for 60 minutes at 37 degrees Celsius.

4) Blank was made in the same way as (1, 2) and (3), except that in step 1 the same volume of D.W. was used instead of serum.

5) At 420 nm, the absorbance was read.

Calculations:

The level of GSH is calculated according to the equation:

$$\text{GSH (µmol/L)} = \frac{(T-B)\times d.f}{\varepsilon \times 10^6}$$

T: Test absorbance

Ɛ: Extinction coefficient = $13600 M^{-1} cm^{-1}$

B: Blank absorbance

d. *f*: Dilution factor =102.

2.2.15- Determination of Total Antioxidant Capacity concentration

Erel [184] described a method for determining TAC.

Principle

From a standardized solution of Fe^{2+} o-dianisidine complex reacting with a standardized solution of H_2O_2, a Fenton-type reaction yields $OH^{\cdot}$ These potent ROS convert colourless o-dianisidine molecules to yellow-brown coloured dianisidyl radicals at low pH. New oxidation reactions emerge as the oxidation processes between dianisidyl radicals advance. As the number of oxidation reactions grows, so does the colour creation. Antioxidants in the sample prevent oxidation processes and colour formation. This reaction can be monitored using spectrophotometry.

Reagents:

Table (2-9): colorimetric method manual insert in Chemical reagents of TAC test.

Reagents	Composition
Solution A: Clark and Lubs (75 mM ; pH 1.8)	a. A weight of 5.591g of KCl was dissolved in 1L of D.W. reagent grade HCl36.5% 6.41mL was diluted to 1L with D.W. the prepared KCl solution 800 mL was mixed with 200 mL of conc. HCl solution to obtain Clark and Lubs solution. b. A weight of 2.44g of O-dianisidine was dissolved in small amount of acetone, then 0.01764 g of Fe $(NH_4)_2(SO_4)_2$.6 H2O was added (final conc. 45 µM) and complete the volume to 1L with Clark and Lubs solution. (Stable for at least6months at 4 ^oC)
Reagents	**Composition**
Solution B: (7.5 mM; H_2O_2)	The solution of Clark and Lubs was used to dilute a volume of 0.5 mL of 15% H_2O_2 solution to 1L. The presence of H_2O_2 was confirmed by absorbance at 240 nm using a spectrophotometer. At 4 ^oC, this solution is stable for at least one month.
Solution C: (2.0 mM standard vitamin C solution)	This solution was prepared by dissolving 0.174 g at vitamin C in 1L of D.W.

Procedure

A. Standard curve of TAC

1. Different standard concentrations of vitamin C (0, 0.4, 0.8, 1.2, 1.6, and 2.0) mM/L were prepared from 2.0 mM vitamin C solution.

2. The first absorbance A_1 was read at a wavelength of 444 nm after a volume of 1000 µL of reagent A was introduced to 25 µL of each standard L-ascorbic acid tubes. (The sample blank was represented by this value).

3. After adding 50 µl of Reagent B to each tube, the second absorbance A_2 was measured after four minutes.

4. The standard curve of TAC (Figure 2-5) was obtained by plotting the absorbance of the standard vitamin C solutions ($\Delta A=A_2- A_1$) against their corresponding concentrations using the zero concentration of vitamin C as a blank.

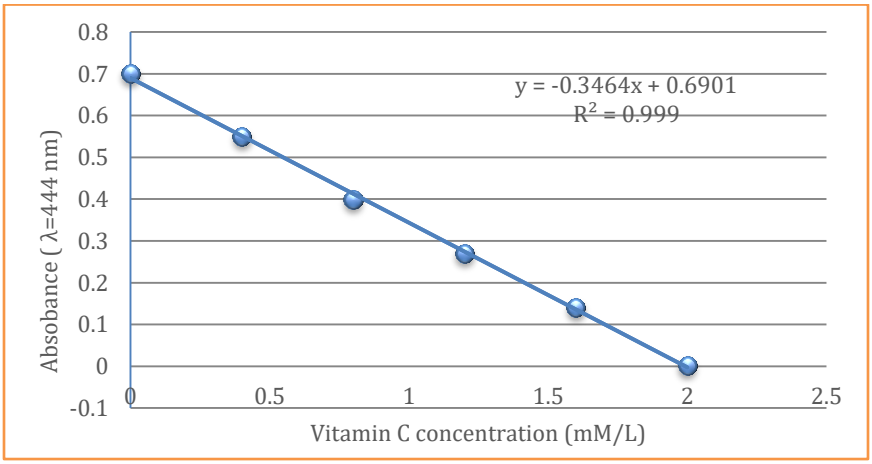


Figure (2-5): Standard Curve of TAC.

B. Serum TAC determination

A volume of 25 µL of serum was used instead of a standard vitamin C, and then treated with the same steps as in section A (steps 2-4). The obtained equation of the straight line from this standard curve was used to determine the unknown total antioxidant concentration as shown in figure (2-5).

2.2.16-Determination of Total Oxidant Status concentration

The approach suggested by Erel [185] was used to determine TOS.

Principle:

The ferrous ion o-dianisidine complex is oxidized to ferric ion by oxidants in the sample. The presence of a large number of glycerol molecules in the reaction fluid speeds up the oxidation process. In acidic circumstances, the ferric ion forms a vivid complex with xylenol orange. The total number of oxidant molecules present in the sample is related to the color intensity, which can be quantified using spectrophotometry. The assay is calibrated using hydrogen peroxide (µmol H_2O_2 Equiv./L), and the findings are presented in micro molar hydrogen peroxide equivalent per litter.

Reagents:

Table (2-10): Colorimetric method manual insert in chemicals reagents of TOS test.

Reagents	Composition
Solution A: (150 µM xylenol orange), (140 mM, NaCl), (1.35 M glycerol pH 1.75)	In 900 mL of 25 mM H_2SO_4, 114 mg of xylenol orange and 8.18 g of NaCl were dissolved, then 100 mL of glycerol was added to the solution. (This reagent is stable for at least 6 months at 4^oC.)
Solution B: (5 mM ferrous ammonium sulfate), (10 mM o-dianisidine)	In 1L of 25mM H_2SO_4,1.96 g of ferrous ammonium sulfate and 2.44 g of o-dianisidine were dissolved. (This reagent is stable for at least 6 months at 4^oC.) Because o-dianisidine is a poisonous and carcinogenic drug, gloves and a face mask must be worn when working with it.
Solution C: (standard solution H_2O_2) (200 µM)	This solution was prepared by diluting (13 µM) of (15%) commercial H_2O_2 (485×10^4 µM) up to (1 L) with D.W

Procedure

A. Standard curve of TOS

1. Different volumes (0, 13, 26, 53, 79,105 μL) of 200 μM hydrogen peroxide were pipetted into a set of test tubes. Then the volumes were made up to 105μL with deionized water to give a final concentration of (0, 25, 50,100, 150, 200 μmol/L) of hydrogen peroxide.

2. A volume of 625 μL of reagent A was added to 105 μL of each standard hydrogen peroxide tube, Then the first absorbance A_1 was read at a wavelength λ=560 nm. (This value represented the sample Blank).

3. A volume of 33 μL of reagent B was added to each tube, then the second absorbance A_2 was read after four minutes.

4. The standard curve of TOS was obtained by plotting the absorbance of the standard H_2O_2 solutions ($\Delta A=A_2-A_1$) against their corresponding concentrations using the zero concentration of H_2O_2 as a blank. The straight line equation obtained from this standard curve was used to determine the unknown TOS concentration.

B. Serum TOS determination

A volume of 105 μL of serum was used instead of standard H_2O_2, and then treated following the same steps as in section A (steps 2-4).

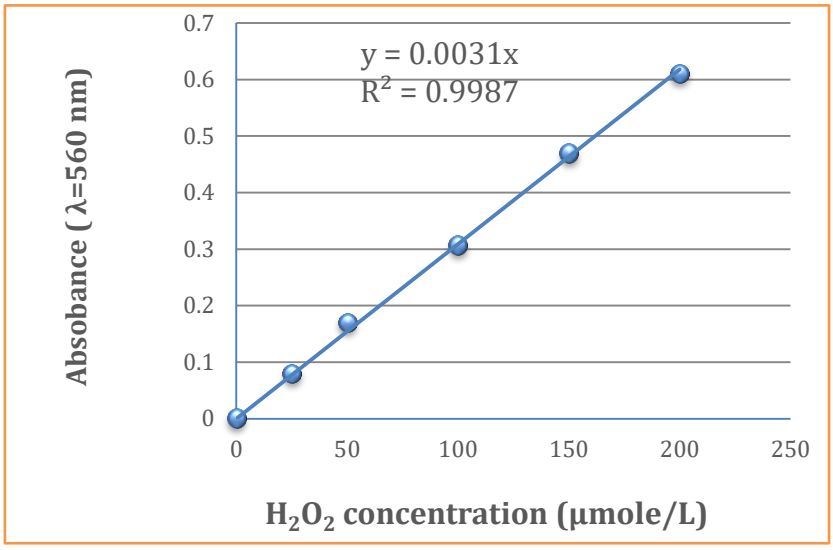


Figure (2-6): Standard Curve of TOS.

2.2.17- Calculation of Oxidative Stress Index (OSI):

The oxidative stress index (OSI), which is a measure of the degree of oxidative stress [186], was derived using the following equation:

OSI (arbitrary unit) = TOS (µmol H_2O_2 Eq. /L)/ TAC (µmol Vit C. Eq. /L)

2.3- Statistical Analysis

The data was evaluated using linear regression analysis and the findings were expressed as mean ± SD. SPSS version 23.0 was used to conduct statistical analysis. Statistical significance was defined as a $p < 0.05$.

Chapter Three

Results and Discussion

3.Results and discussion

3.1-Demographic measurements:

Table (3-1) contains the age and BMI, in the form of Mean ± SD, the study included a 60 male patients and 30 male as a healthy control to get a homogeneous group.

Table (3-1): Demographic Characteristics of T2DM Patient and control subject

Parameters	Control, N=30 Mean ± SD	T2DM, N=60 Mean ± SD	*p*-value
Age (year)	48.4 ± 5.04	48.36 ± 6.04	0.450
BMI ($kg.m^{-2}$)	23.7 ± 2.9	29.9 ±4.8	0.03

*SD: standard deviation. The values is significant at $p < 0.05$

The finding show significant increase $p < 0.05$ in BMI of T2DM patients than the control, while there are no significant differences $p< 0.05$ in age T2DM patients when compared to those of the control group.

Obesity could be determined using the body mass index (BMI). Obesity is associated with a low quality of life and an increased risk of chronic diseases and health outcomes. For instance, obesity, especially abdominal obesity, might be associated with T2DM by developing insulin resistance. Obesity might also be related to several cardiometabolic risk factors, such as increased blood pressure, dyslipidemia, inflammation, and endothelial dysfunction. Collectively, insulin resistance and other cardiometabolic risk factors might be associated with an increased risk of CVD among obese individuals [187].

3.2- Glycemic factors

Hyperglycemia occurs when blood glucose is not used by tissue due to insulin resistance in type 2 diabetic mellitus, therefore insulin will be increase. When glucose rise in the blood, it glycates to hemoglobin. The more glucose in the blood, means more it glycates. And it can stay there for around three months, or about how long the average red blood cell lives.

The current study shown increase in FPG, HbA1c, fasting insulin and insulin resistance as shown in table (3-2)

Table (3-2): FPG, HbA1c, HOMA-IR and fasting insulin.

Clinical parameters (unit)	Control Mean ± SD (Range)	T2DM Mean ± SD (Range)	*p*- value
FPG (mg/dL)	93± 17.1(70–122)	169± 50.8(95-270)	< 0.01
HbA1c (%)	5.2 ± 0.9(4.0–6.42)	9.8± 2.6(7.5–17.27)	<0.01
Insulin (µIU/mL)	12.5±4.7(3.7-22.3)	22.7±12.0(6.5-50.0)	<0.05
HOMA-IR*	1.8± 0.29(0.5–3.1)	3.2 ± 1.7(0.87–7.25)	<0.05

*HOMA-IR: homeostatic model assessment-insulin resistance Hemoglobin A1c (HbA1c)

3.3- Lipid profile levels in T2DM Patients and Control subject

lipid profiles concentrations in T2DM patients and a control subjects were measured, the finding of the present study shown a strong rise $p < 0.01$ in CHO, TGs, VLDL-C, and LDL-C (169.6±41.5, 164.8±66.6, 31.3±12.9, 122.7±42.1) mg/dL respectively when compared to the control

subject (109.8±27.1, 83.3±25.1, 16.6±5.0, 99.2±25.4) mg/dL respectively. While the level of HDL concentration in T2DM patients was found to be substantially decrease $p < 0.05$ (35.8±8.7) mg/dL than in the control subject (53.5±18.2) mg/dL, as evidenced by Table (3-3).

Table (3-3): Mean ± SD of CHO, HDL, TGs, VLDL and LDL levels in T2DM patients and control subject.

Parameter (mg/dL)	Subjects	Mean ± SD	Range	*p*-value
CHO	Control	109.8±27.1	65-175	< 0.01
	Patient	169.6±41.5	70-240	
HDL-C	Control	53.5±18.2	22-90	< 0.05
	Patient	35.8±8.7	25-56	
TGs	Control	83.3±25.1	44-130	< 0.01
	Patient	164.8±66.6	45-300	
VLDL-C	Control	16.6±5.0	8.8-26	< 0.01
	Patient	31.3±12.9	9-60	
LDL-C	Control	99.2±25.4	44-147.0	< 0.05
	Patient	122.7±42.1	44.9-206.88	

*The values is significant at $p < 0.05$

The explanation for the shift in lipid levels could be for a variety of reasons.

- TG and VLDL metabolism VLDL metabolism is influenced by IR, which has been shown to promote hepatic VLDL triglyceride TG synthesis. Increased hepatic apo B-100 production is therefore connected to increased VLDL synthesis. Hypertriglyceridemia, fluctuating increases in particle number represented by VLDL apo B-100, and reduced HDL-C concentrations result as a result of this. Increases in hepatic triglyceride lipase (HTGL) are also linked to IR, which could lead to faster HDL-C clearance and lower HDL-C levels. In addition, HTGL activity has recently been suggested as a key regulator of insulin clearance. An accelerated rate of lipolysis

of stored TG-derived free fatty acids (FFA) from adipose tissue, with resulting increases in FFA flow to the liver, is a crucial role in the process of both IR and enhanced VLDL-C synthesis. Furthermore, despite the fact that insulin is an important stimulator of adipose lipoprotein lipase (LPL), a pathway that reflects the provision of TG-rich lipoprotein (VLDL, chylomicron)-derived FFA for adipose tissue uptake and storage, insulin (ATLPL) dose response curves shift to the right in IR states. As a result, IR may inhibit VLDL-C breakdown, leading to a rise in hypertriglyceridemia. [188].

- High density lipoprotein oxidation Insulin has a significant impact on HDL metabolism, and low HDL-C levels are prevalent in IR patients. Increased exchange of TGs from chylomicrons and VLDL for cholesterol esters from HDL-C particles, resulting in a reduction in HDL-C, is associated with IR, a process mediated by cholesteryl ester transfer protein (CETP). Reduced LPL activity reduces TGs hydrolysis from chylomicrons and VLDL-C, potentially limiting the input of TG-rich lipoprotein-derived HDL particles. In IR conditions, greater HTGL activity is linked to improved HDL-C clearance and thus reduced HDL-C concentrations. Reduced apo A-I production and secretion from the liver and gut may potentially contribute to low HDL-C levels [189].
- Low density lipoprotein oxidation insulin resistance appears to have a smaller impact on LDL-C metabolism than it does on VLDL metabolism. Insulin is known to up regulate LDL-C receptor function [190].
- Lipoprotein lipase hydrolyzes chylomicrons in the vasculature, releasing their fatty acids to peripheral cells. The hydrolysis of chylomicron TGs is influenced by the insulin resistance-induced

decrease in LPL activity. This is especially true if excessive hepatic very low density lipoprotein saturates all of the endothelium's accessible LPL binding sites [191].

3.4- Omentin-1 level

Patients with T2DM had a substantially big decline in serum Omentin-1 amounts $p < 0.05$, 10.57 ± 1.6 ng/mL compared to controls 12.67 ± 3.9 ng/mL fig. (3-8).

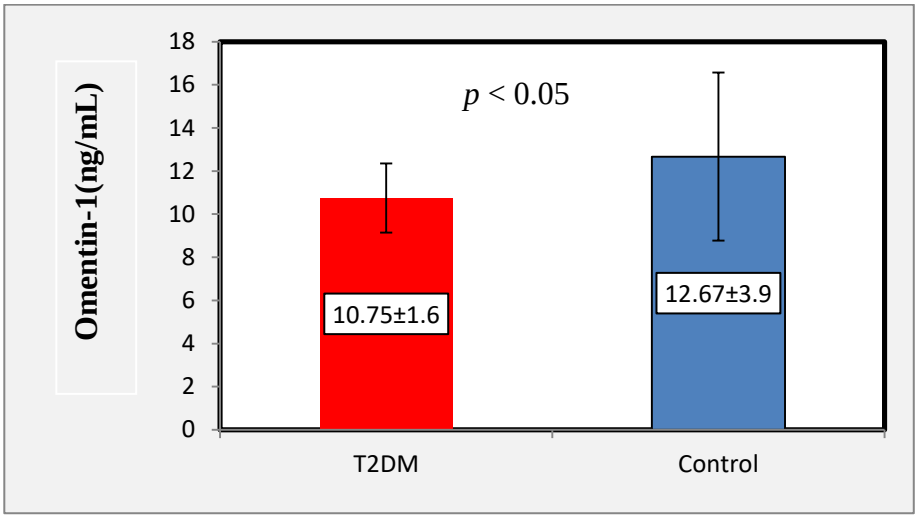


Figure (3-1): Comparison Mean ± SD of serum level of Omentin-1 between T2DM and control.

Because omentin-1 is largely expressed in the human visceral omental tissue, obese people have lower levels. T2DM has also been linked to lower omentin-1 levels [192], Omentin-1 has negative connections with insulin resistance and obesity characteristics, indicating that it could be used as a biomarker for a number of metabolic illnesses.

T2DM patients, those with poor glucose tolerance, and those who are obese had decreased levels of Omentin-1[192, 193]. These investigations support the findings of the current study, which found a substantially big decline reduction in average Omentin-1 concentrations in T2DM subject compared to controls. Also concur with another study which observed that

diabetic patients had significantly lower omentin-1 levels before and after BMI modification than control with or without ischemic heart disease [194].

3.5- YKL-40 level

There is substantially big decline increase $p< 0.05$ in YKL-40 concentrations in T2DM 25.16 ± 8.7 ng/mL compared to controls 18.17 ±4.7 ng/mL fig. (3-9).

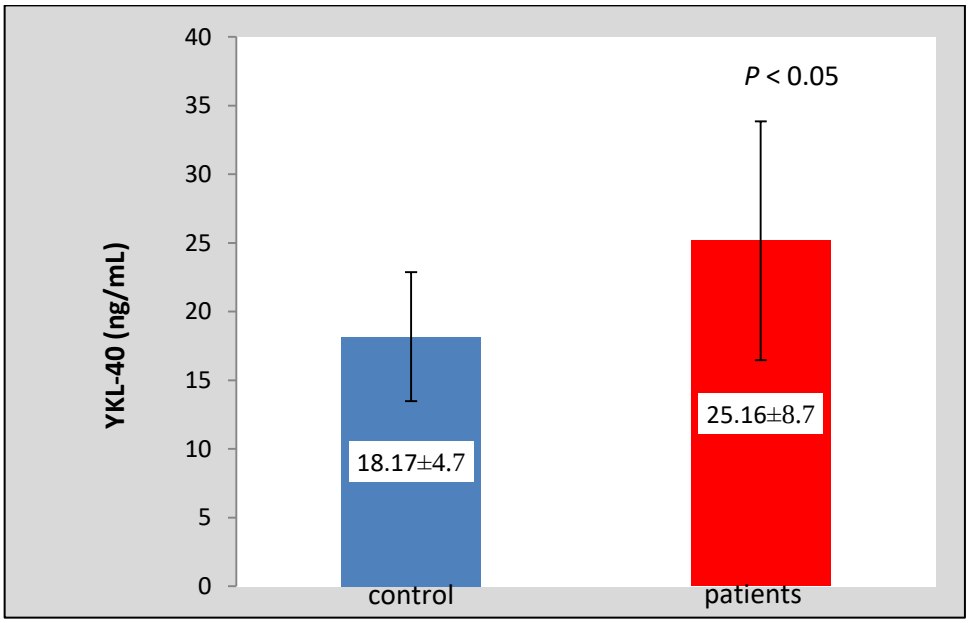


Figure (3-2): Comparison Mean ± SD of serum level of YKL-40 between T2DM and control.

Secretion of YKL-40 are dependent on inflammation mediators such oxidize low density lipoprotein. Obesity is related to increased macrophage infiltration of adipose tissue and plays an important role in the development of insulin resistance. YKL-40 is possibly with relation to the insulin resistance based on the macrophage infiltration and adipose tissue [195].

Type 2 DM patients had higher levels of concentration of YKL-40 than control subjects in our study, There was a positively association between YKL-40 and fasting insulin in our study, which was similar to Maha H. and others [196]. Previous studies discovered that YKL-40 levels are linked to HbA1c and FPG, which corroborated the findings of the current study. Endothelial dysfunction has been linked to a greater level of concentration of YKL-40 in several investigations [197, 198].

3.6- Oxidative Stress

3.6.1- Nitric Oxide level

The level of NO has increased significantly $p < 0.05$ in T2DM 59.91 ± 8.9 µmol/L fig. (3-3) compared to healthy control 48.20 ± 6.7 µmol/L. The increase of NO level in T2DM patients of the present study might be attributed to the activation of the iNOS.

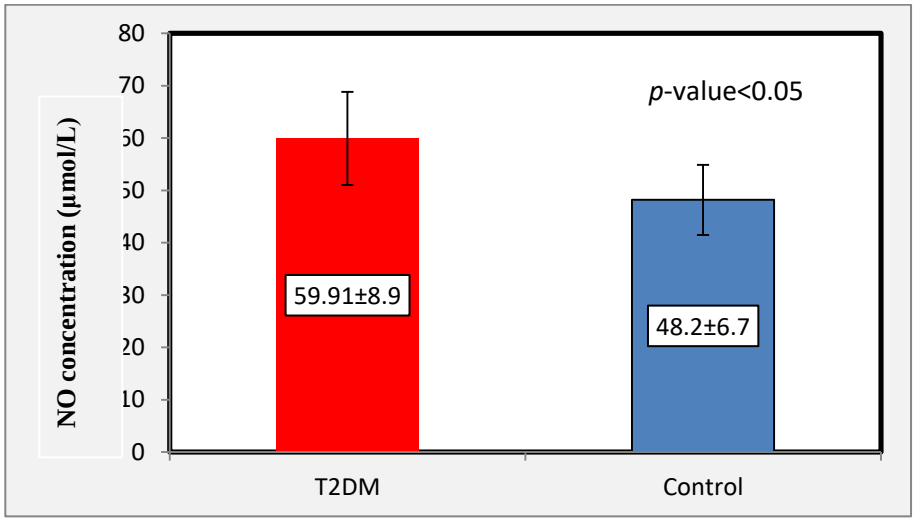


Figure (3-3): comparison Mean ± SD of serum level of NO between T2DM and control.

There is a high free radical (FR) load in T2DM, which is linked to hyperglycemia. The increased glucose binds to proteins and glycates them, resulting in the development of advanced glycation end-products

(AGEs) and a large number of FRs [199]. Tissue damage and aging have been linked to AGEs [200]. Glycation of proteins raises the rate of FR synthesis by roughly fifty times when compared to control levels in diabetes [201].

Once generated, AGEs bind to their RAGE cell surface receptors in endothelial cells and macrophages, triggering postreceptor signaling, the production of intracellular ROS, and the stimulation of gene expression. Endothelial cells exposed to AGEs produce more tumor necrosis factor alpha (TNF-α), enhance iNOS activity and increase adhesion molecule synthesis (including vascular cell adhesion molecule-1) [202].

Nitric oxide can be converted into $ONOO^-$ by reacting with another free radical, such as $O_2^{\bullet-}$.

Lipids and proteins can be nitrated by peroxynitrite. Protein dysfunction can be caused by nitration, and various proteins that seem nitrated in vivo have been found, one of which is nitrotyrosine, which is a hallmark of ONOO synthesis [201, 202].

The latest findings are in line with previous findings of Sarita A. and others [203], they found a substantial rise in NO concentration in T2DM patients than the control group.

3.6.2- Serum Malondialdehyde level

Malondialdehyde (MDA) concentration was measured control and type tow DM patients group. The finding of present study show strong increase *p* less than 0.01 in MDA level T2DM patients 2.58 ± 0.6 μmol/L than the control group 0.78 ± 0.1 μmol/L fig (3-4).

Type 2DM subject had a statistically strong rise in serum MDA levels in this study. The following are the most likely causes of an elevated MDA level in T2DM: [204, 205, 206]:

1- Abnormally high levels of lipid, lipoprotein, and MDA in plasma in type 2DM patients may be attributed to aberrant lipid metabolism

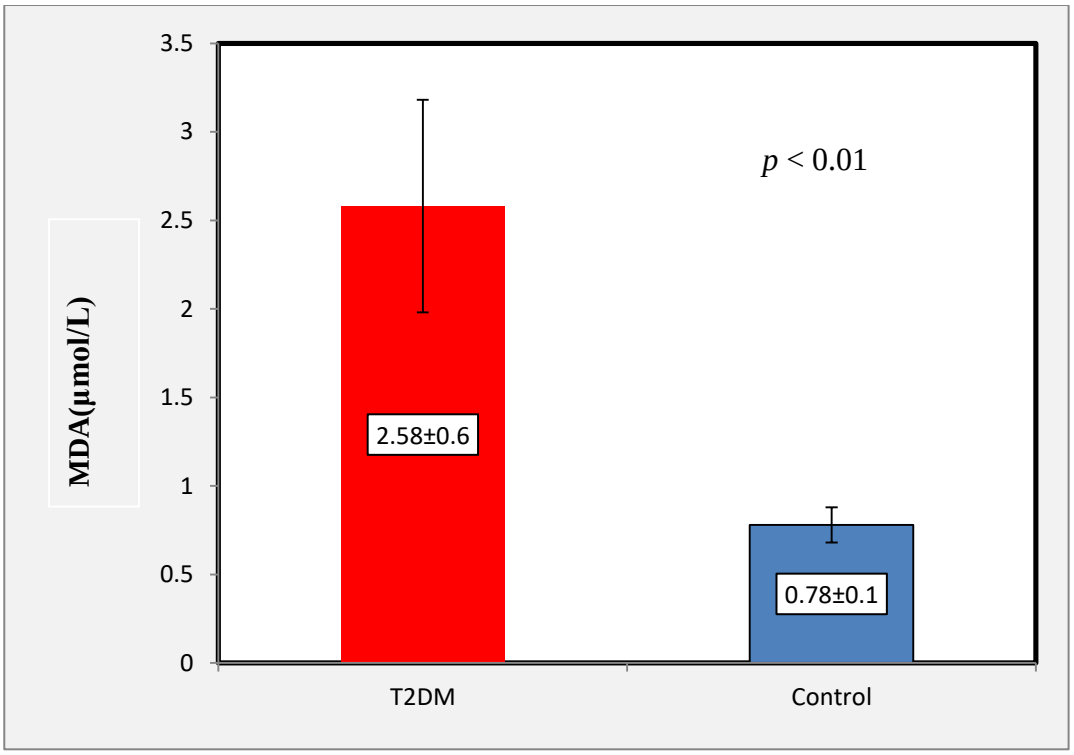


Figure (3-4): Comparison Mean ± SD of serum level of MDA between T2DM and control.

Increased MDA levels in T2DM may be attributed to a change in the function of the erythrocyte membrane. This suppresses the function of the define system, resulting in a buildup of $O_2^{\bullet-}$, which causes the most MDA and tissue damage in diabetes.

2- Increased MDA could be related to increased protein glycation in T2DM. The glycated protein could be a source of FRs in and of itself. There is a definite link between MDA and blood glucose

levels, which could explain why MDA levels are higher in T2DM patients.

3- A lack of antioxidant activity has been linked to a greater MDA content. Due to the lack of an antioxidant system, there may be an imbalance between the formation and scavenging of FR.
4- Mitochondria and microsomal membrane phospholipids contain a high proportion of poly unsaturated fatty acids. FA with 2, 4, 5, and 6 double bonds are among them. Because they have three or more double bonds, they are more vulnerable to FR assault, resulting in increased lipid peroxidation. As a result, the rate of peroxidation may be significant, resulting in increased MDA and FRs concentrations in T2DM patients [204, 205, 206].

The current findings are consistent with those of Madhikarmi N., et al. [207], who found a substantial rise in MDA concentration in T2DM patients when compared to a control group.

3.6.3-Reduced Glutathione level

A strong significant $p < 0.01$ reduced of GSH concentration type 2 diabetes mellitus 810.2 ± 155.8 μmol/L compared to healthy control 1311.1± 33.1 μmol/L fig.(3-5).

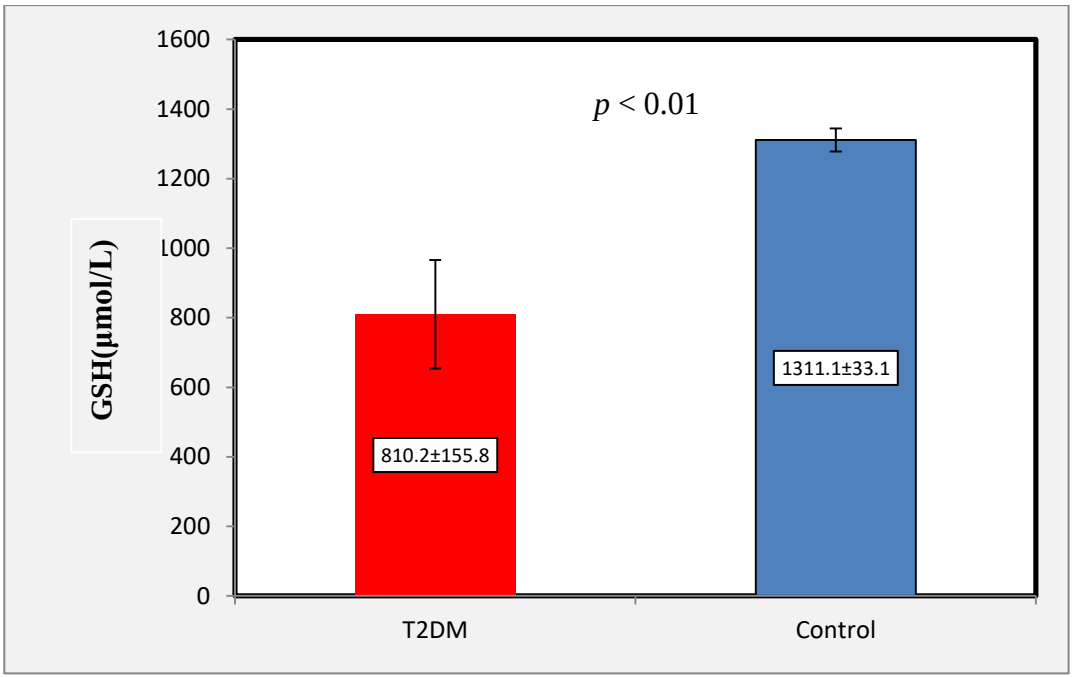


Figure (3-5): Comparison Mean ± SD of serum level of GSH between T2DM and control.

The cause of decrease reduced glutathione was to convert lipid hydroperoxides to alcohols and to convert free H_2O_2 to water in the present of Glutathione peroxidase's [159].

The mean levels of GSH in participants with T2DM were considerably lower than in normal people, according to Hakki K.I. and others. They got to the conclusion at the end of their research that GSH depletion plays a significant role in the pathophysiology of DM [208].

3.6.4- Coenzyme Q_{10} level

There is statistically strong decrease $p < 0.05$ in serum CoQ10 levels in T2DM 28.4 ± 9.7 nmol/L compared to controls 33.04 ±7.5 nmol/L fig. (3-6).

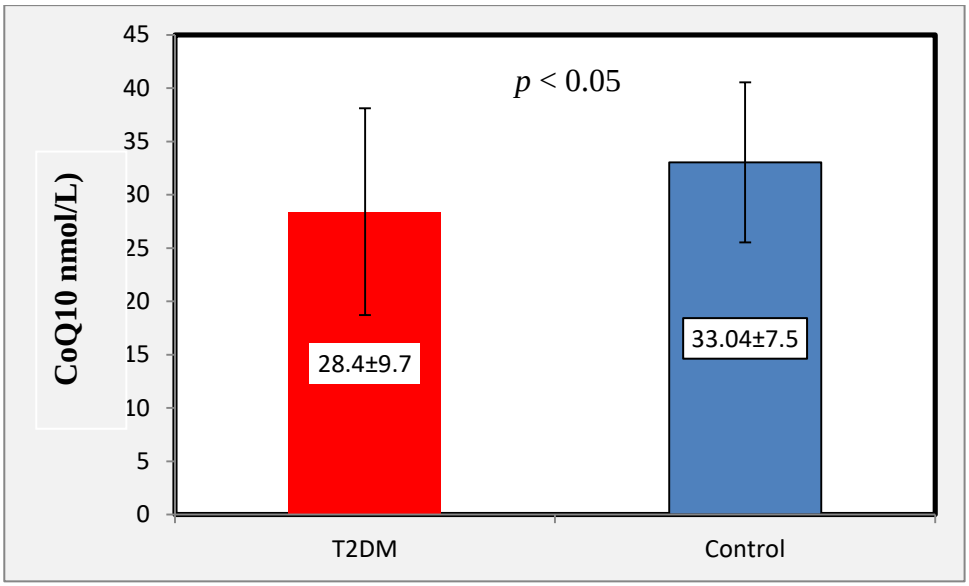


Figure (3-6): Comparison Mean ± SD of serum level of CoQ_{10} between T2DM and control.

It is reduces lipid peroxidation by acting as an electron carrier during oxidative phosphorylation and diffusing to the cell membrane's phospholipid layer via a unique chain structure, and thus it is level decrease in the present study.

Several studies have found that T2DM individuals had considerably lower blood CoQ_{10} levels, which correlate with higher of glucose concentration, oxidative stress indicators, and HbA1c [209].

Earlier clinical trials examining the efficacy of CoQ_{10} supplementation on T2DM glycaemic management yielded conflicting results, as a result CoQ_{10} supplementation considerably improved long-term glycaemic management, according to Hodgson et al [210].

Zhang SY et al [211] showed that after take CoQ_{10} supplement to T2DM and compared them with the control group, coenzyme Q_{10} decrease the HbA1c and FBG and this improve T2DM health. These studies mentioned above its opposite this study which shown decrease in

CoQ_{10} level in T2DM without treatment with CoQ_{10} supplement and this may agree with the studies above when T2DM treat with CoQ_{10} supplement that will lower FBG and HbA1c and improve their health.

3.6.5- Total Antioxidant Capacity level

Level of TAC has decreased significantly $p < 0.01$ T2DM 0.65± 0.087 µmol Vit C. Eq. /L compared to healthy control 1.51 ± 0.084 µmol Vit C. Eq. /L fig (3-7).

Opara EC et al [212] has discovered that, in comparison to a control subject, patients' overall TAC was severely depleted. Furthermore TAC depletion is related to a higher risk of diabetes complications, according to the researchers.

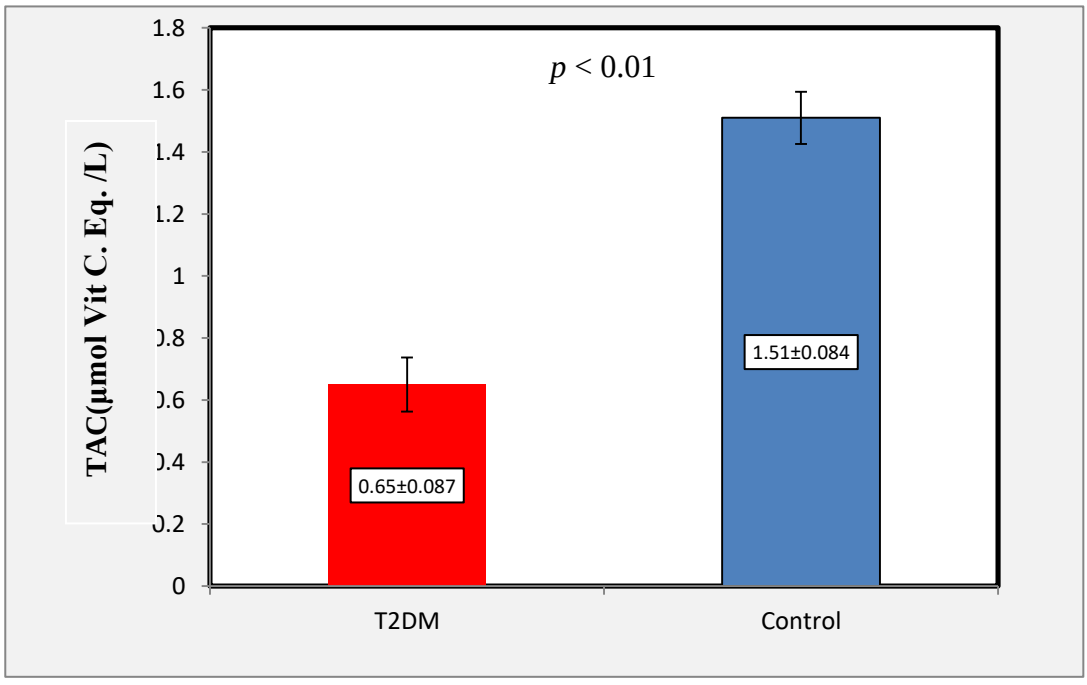


Figure (3-7): Comparison Mean ± SD of serum level of TAC between T2DM and control.

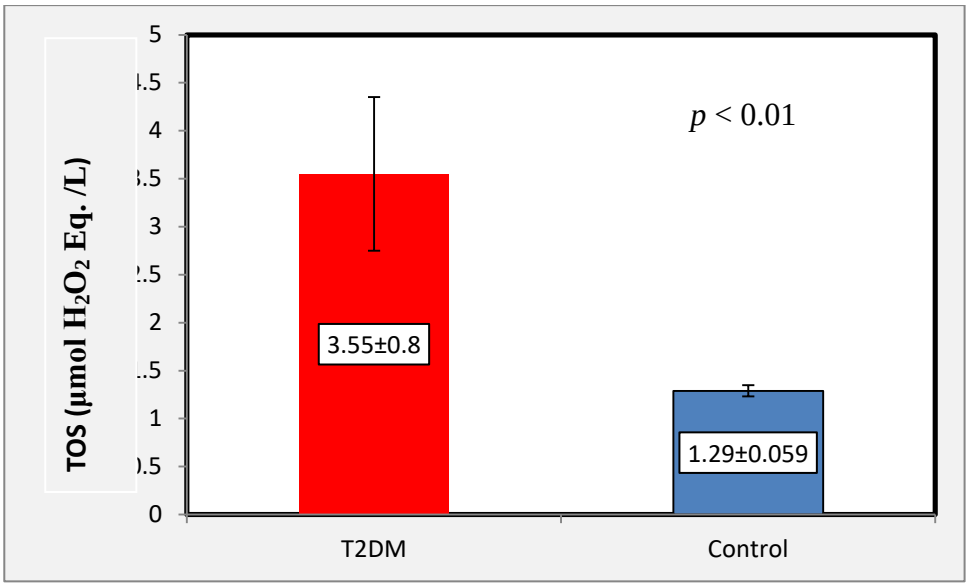
Also the study of Çapaş M and others [213] revealed that increasing niacin and antioxidant intake during eating can help decrease HbA1c and fasting plasma glucose levels.

Pieme and others mentioned A subset of diabetic individuals with problems had statistically higher levels of MDA, NO, and TOS, and they also suggested that the levels of reduced GSH and TAC potentially offer data on the likelihood of acquiring diabetic complications given that the modification of these biomarkers levels was correlated to oxidative stress [214].

The findings of this study support those of Ganjifrockwala and others, they found T2DM patients had a substantial increase in HbA1c, triglycerides, MDA, and LDL, in addition to a significant reduction in TAC and HDL-C when compared to controls [215].

3.6.6- Total Oxidant Status level

Total Oxidant Status TOS has elevated significantly $p < 0.01$ in type 2 diabetes mellitus 3.55 ± 0.8 μmol H_2O_2 Eq. /L compared to healthy control 1.29 ± 0.059 μmol H_2O_2 Eq. /L fig (3-8).



Figure(3-8): Comparison Mean ± SD of serum level of TOS between T2DM and control.

Mehmet A. et al [216] shown that OSI, and TOS were higher in patients with diabetic compared to healthy control subject.

Aslan M. et al. [217] found that the patient group's TOS and OSI were considerably greater than the control groups. Furthermore, they claimed that insulin resistance is linked to an increase in oxidative stress.

Akin et al [218] found that patients' TOS levels were much higher than healthy controls. Söğüt et al [219] They have observed significant increase of TOS level in the patients. the previous studies measured the level of oxidants individually. Existing methods allow for the detection of blood each oxidant/antioxidant level independently, but They take a long time and need a lot of effort, and For two reasons, they may not be correct: (1) Unknown oxidants/antioxidants it's possible that it's still present in the serum, and (2) multiple types of oxidants/antioxidants in the same system may combine and provide an additive or synergistic effect. To demonstrate a definitive relationship between oxidative stress and T2DM, measuring merely one or a few particular

oxidants/antioxidants in the blood is insufficient. As a result, TOS is commonly used to evaluate the body's total oxidation state [220].

3.6.7- Oxidative Stress Index level

There is highly significant $p < 0.01$ increase of oxidative stress index in T2DM 5.24 ± 1.17 compared to healthy control 0.85 ± 0.06 fig. (3-9).

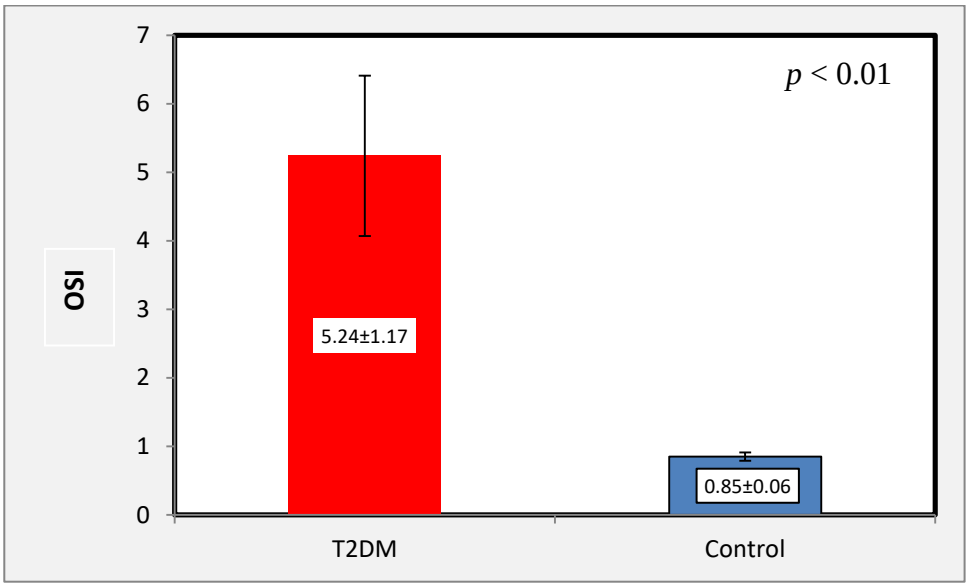


Figure (3-9): Comparison Mean ± SD of serum level of OSI between T2DM and control.

Ihsan Boyac [221] discovered that the OSI was significantly decrease in those of control group than T2DM, and he also indicated that insulin resistance is the primary pathophysiologic mechanism in T2DM. Insulin resistance causes hyperinsulinemia as a secondary effect. Hyperinsulinemia causes oxidative stress, which results in cell death and insulin shortage. According to these findings, breaking and preventing insulin resistance in T2DM should be an aim of our treatment techniques.

3.7-Correlation Analysis of Nitric Oxide level with Insulin Resistance and Total Antioxidant Capacity levels in T2DM patients

The findings of linear regression analysis demonstrate that there is a strong positive association $p < 0.05$, r = 0.56 of HOMA-IR level with NO and a strong negative association $p < 0.05$, r = - 0.50 of serum TAC with NO in T2DM patients group. This correlations shown in fig. (3-10) and Table (3-4).

The present study agree with Alessandra Magenta et al [222] showed that NO activity and superoxide generation are elevated in pathological circumstances such as diabetes mellitus as a result ROS increase and total antioxidant decrease [223].

Table (3-4): Correlation analysis between Nitric Oxide and Insulin Resistance and Total Antioxidant Capacity in T2DM patients

Parameters	Correlation coefficient r	*p*-value
HOMA-IR	0.560	0.001
TAC(µmol Vit.C.Eq./L)	- 0.50	0.001

Significant *p*: < 0.05

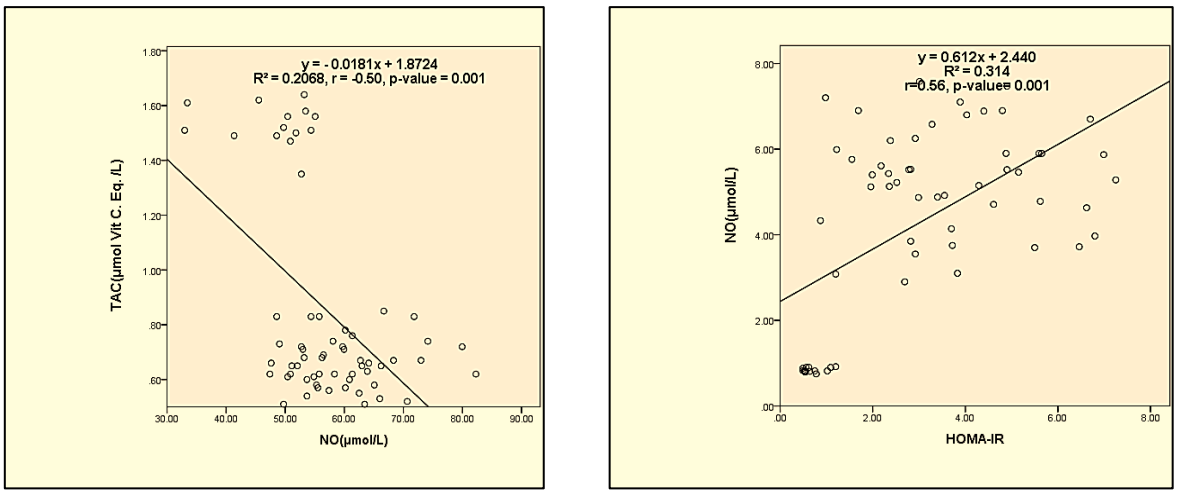


Figure (3-10): Correlation Analysis between Nitric Oxide and Insulin Resistance and Total Antioxidant Capacity in T2DM patients

3.8-Correlation Analysis between Omentin-1 level and HbA1c percentage level in T2DM patients

The findings of linear regression analysis demonstrate that there is a significant strong correlation p < 0.05 r = - 0.62 HbA1c level with Omentin-1 in T2DM patients group. This correlations shown in fig. (3-11)

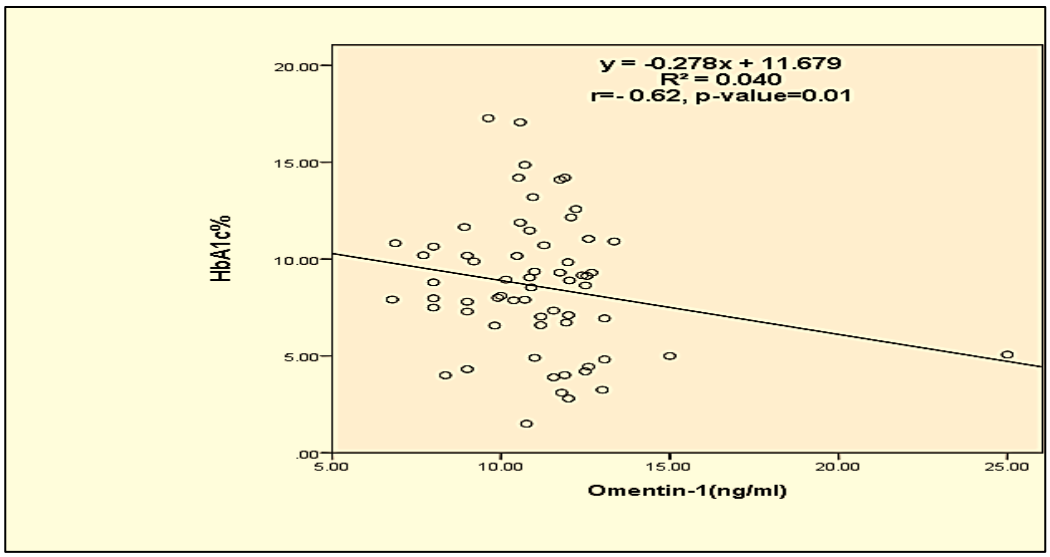


Figure (3-11): Correlation Analysis between Omentin-1 level and HbA1c percentage level in T2DM patients

3.9-Correlation Analysis of Total Antioxidant Capacity level and different parameters levels in T2DM patients

Significant positive correlations are found in the findings of linear regression analysis. $p < 0.05$, r = 0.81, 0.451 of serum GSH, and CoQ10 concentration with TAC respectively and significant strong correlation $p < 0.05$, r = - 0.737, - 0.897, - 0.762, - 0.627 of serum TOS, OSI, MDA, HOMA-IR and HbA1c concentration with TAC in T2DM patients group. This correlations shown in fig. (3-12) and Table (3-5).

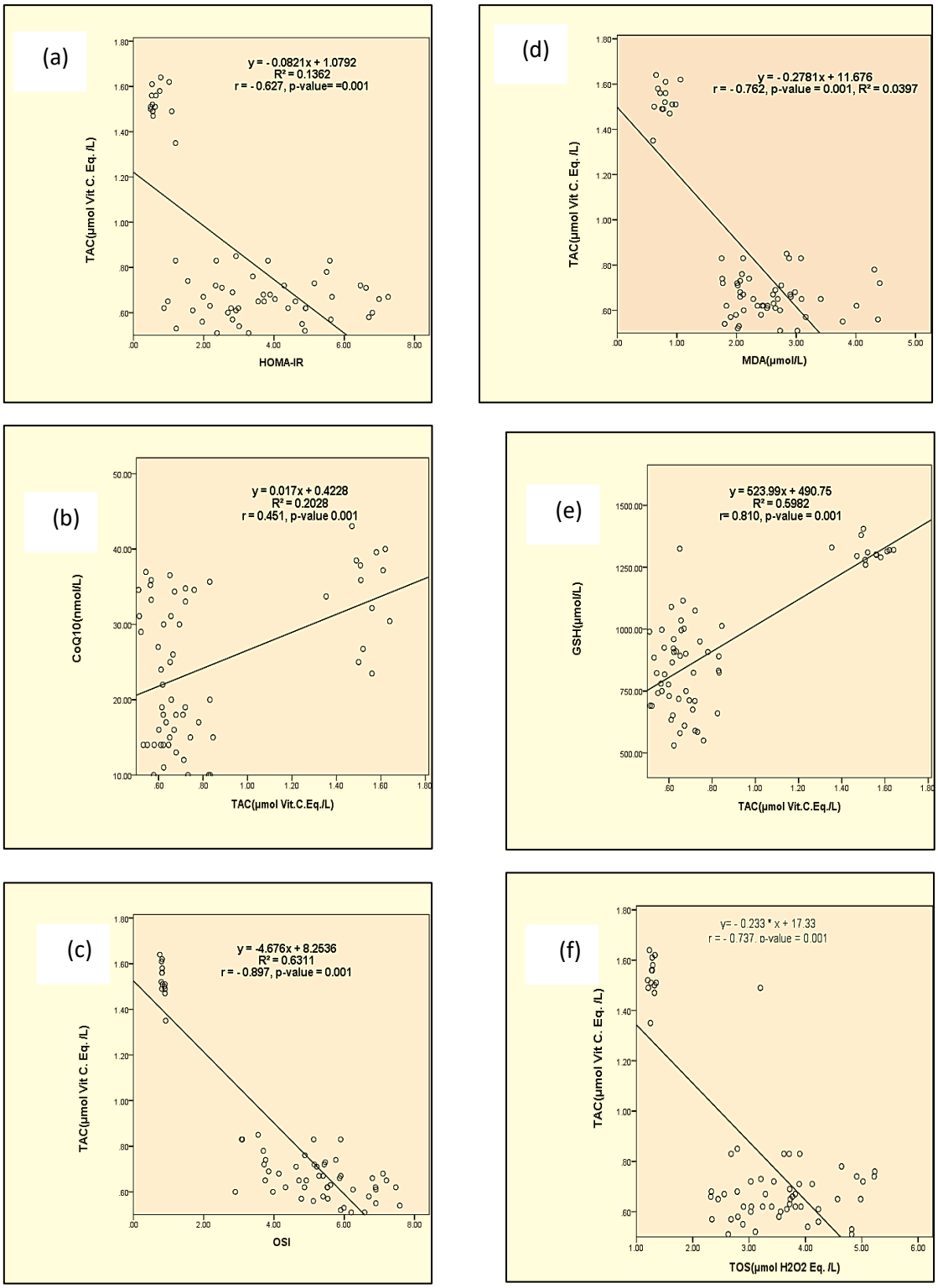


Figure (3-12): Correlation Analysis between Total Antioxidant Capacity level and different parameters levels in T2DM patients

Table (3-5): Correlation Analysis between Total Antioxidant Capacity level and different parameters levels in T2DM patients

Parameters	Correlation coefficient r	*p*-value
HbA1c%	- 0.726	0.001
HOMA-IR	- 0.627	0.001
GSH (µmol/L)	0.810	0.001
TOS (µmol H2O2 Eq./L)	- 0.737	0.001
OSI	- 0.897	0.001
MDA (µmol/L)	- 0.762	0.001
CoQ10 (nmol/L)	0.451	0.001

3.10- Correlation Analysis between Total Oxidant Status level and different parameters levels in T2DM patients

Significant positive correlations are found in the findings of linear regression analysis. $p < 0.05$, r = 0.638, 0.639, 0.517 of serum OSI, MDA and HbA1c concentration with TOS respectively and a major negative relationship $p < 0.05$, r = - 0.603 of serum GSH concentration with TOS in T2DM patients group. This correlations shown in fig. (3-13) and Table (3-6).

Table (3-6): Correlation Analysis between Total Oxidant Status level and different parameters levels in T2DM patients

Parameters	Correlation coefficient r	*p*-value
HbA1c%	0.517	0.001
GSH (µmol/L)	- 0.603	0.001
OSI	0.638	0.001
MDA(µmol/L)	0.639	0.001

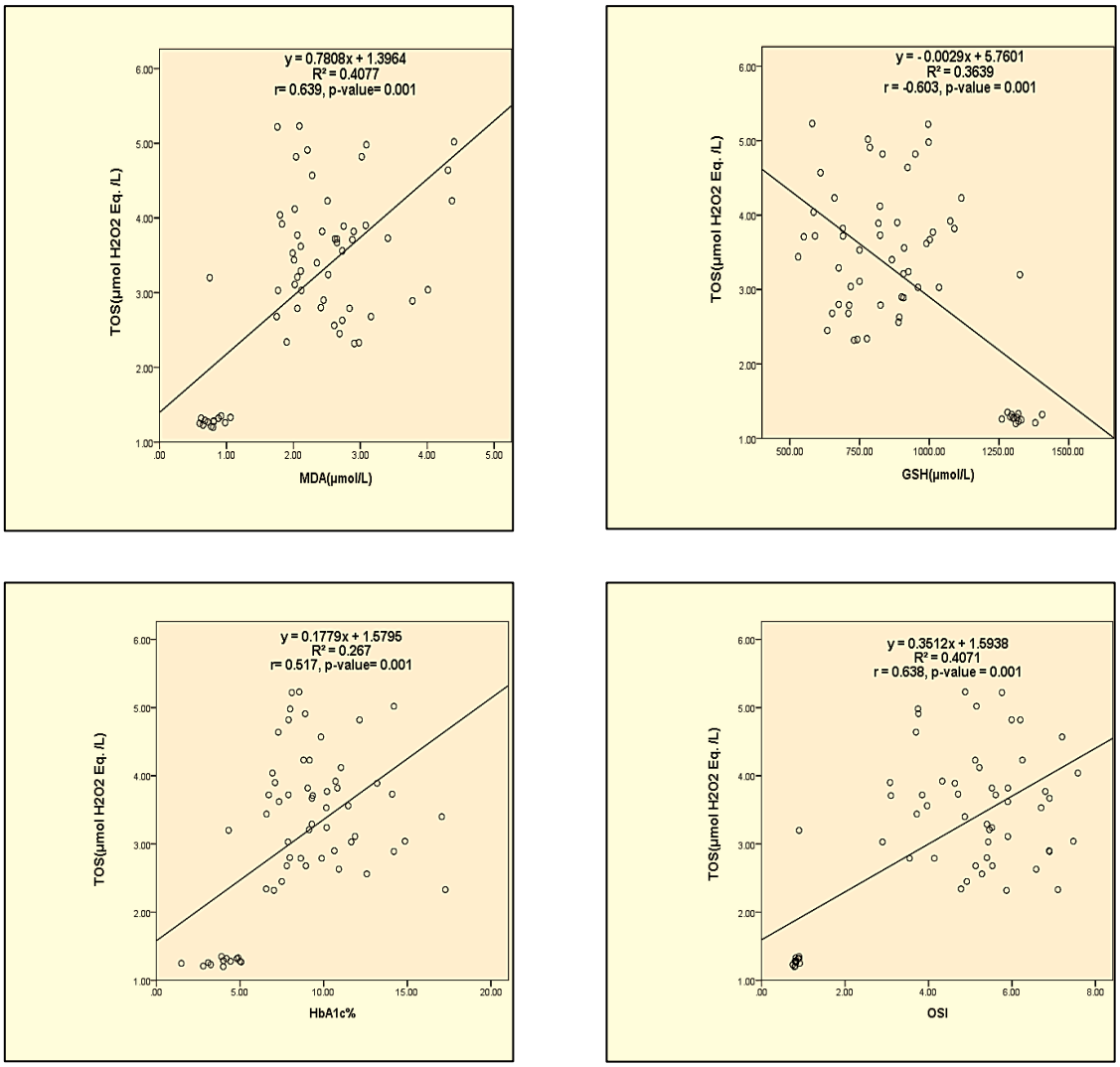


Figure (3-13): Correlation Analysis between Total Oxidant Status level and different parameters levels in T2DM patients

3.11- Correlation analysis of Malondialdehyde level with Insulin Resistance and Glutathione levels in T2DM patients

Significant positive correlations are found in the findings of linear regression analysis. $p < 0.05$, r = 0.58of HOMA-IR level with MDA respectively and a major negative relationship $p < 0.05$, r = - 0.686 of serum GSH concentration with MDA in T2DM patients group. This correlations shown in fig. (3-14) and Table (3-7).

Table (3-7): Correlation Analysis of Malondialdehyde level with Insulin Resistance and Glutathione levels in T2DM patients

Parameters	Correlation coefficient r	*P*-value
HOMA-IR	0.580	0.001
GSH	- 0.686	0.001

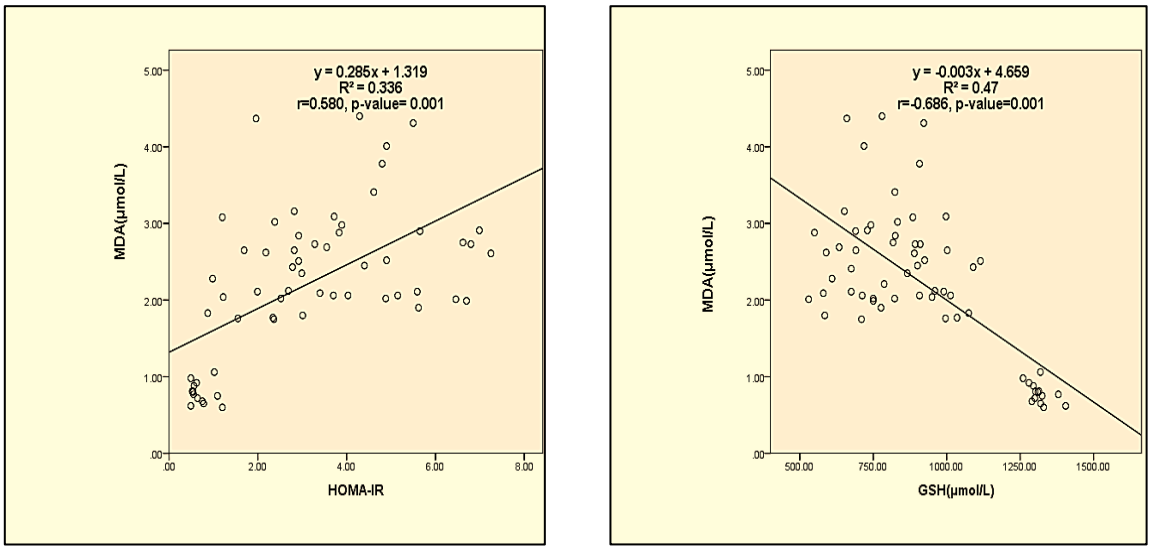


Figure (3-14): Correlation analysis of Malonialdehyde level with Insulin Resistance and Glutathione levels in T2DM patients

3.12-Correlation analysis of Glutathione level with Insulin Resistance and Oxidative Stress Index levels in T2DM patients

A major negative relationship $p < 0.05$, r = (-0.584, -0.721, -0.54) of insulin resistance, OSI and HbA1c level with GSH level in T2DM patients group. This correlations shown in fig. (3-15) and Table (3-8).

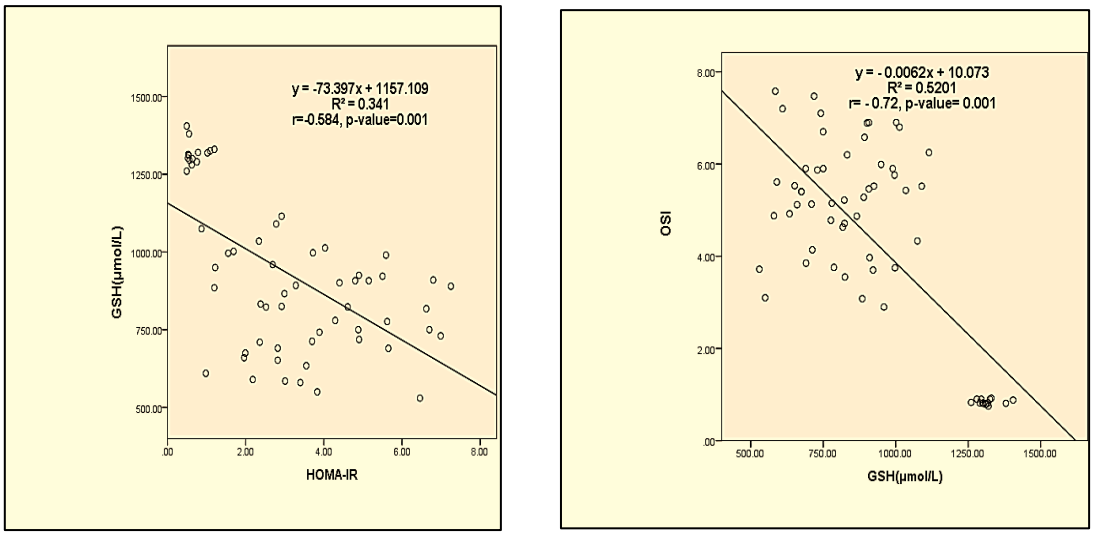


Figure (3-15): Correlation analysis of Glutathione level with Insulin Resistance and Oxidative Stress Index levels in T2DM patients

Table (3-8): Correlation Analysis of Glutathione level with Insulin Resistance and Oxidative Stress Index levels in T2DM patients

Parameters	Correlation coefficient r	*p*-value
HOMA-IR	- 0.584	0.001
OSI	- 0.721	0.001

Reactive oxygen metabolites and their products are increased in hyperglycemia [224]. GSH depletion causes higher in oxidative stress because it is a key antioxidant. Powell and others [225] Hyperglycemia caused oxidative stress and a decrease in GSH levels in the vascular straight muscles, according to the findings. HbA1c and GSH levels were shown to have a strong negative connection $p < 0.05$ in this study. And This deal with the study results of linear regression analysis show strong positive association $p < 0.05$, of serum GSH, and CoQ10 concentration with TAC and strong negative association $p < 0.05$ of serum TOS, OSI, MDA,HOMA-IR, HbA1c, NO, and insulin concentration with TAC in T2DM patients group.

Our findings support those of Seghrouchni et al [226], who found a negative connection between GSH and HbA1c levels in diabetes patients. A study by Giugliano et al. [227] yielded a similar outcome.

3.13- Correlation analysis of OSI with Insulin Resistance and HbA1c percentage levels in T2DM patients

Significant positive correlations are found in the findings of linear regression analysis. $p < 0.05$, r = 0.56, 0.735 of HOMA-IR and HbA1c level with OSI respectively. This correlations shown in fig. (3-16) and Table (3-9).

Table (3-9): Correlation analysis of OSI with Insulin Resistance and HbA1c percentage levels in T2DM patients

Parameters	Correlation coefficient r	*p*-value
HbA1c%	0.735	0.001
HOMA-IR	0.56	0.04

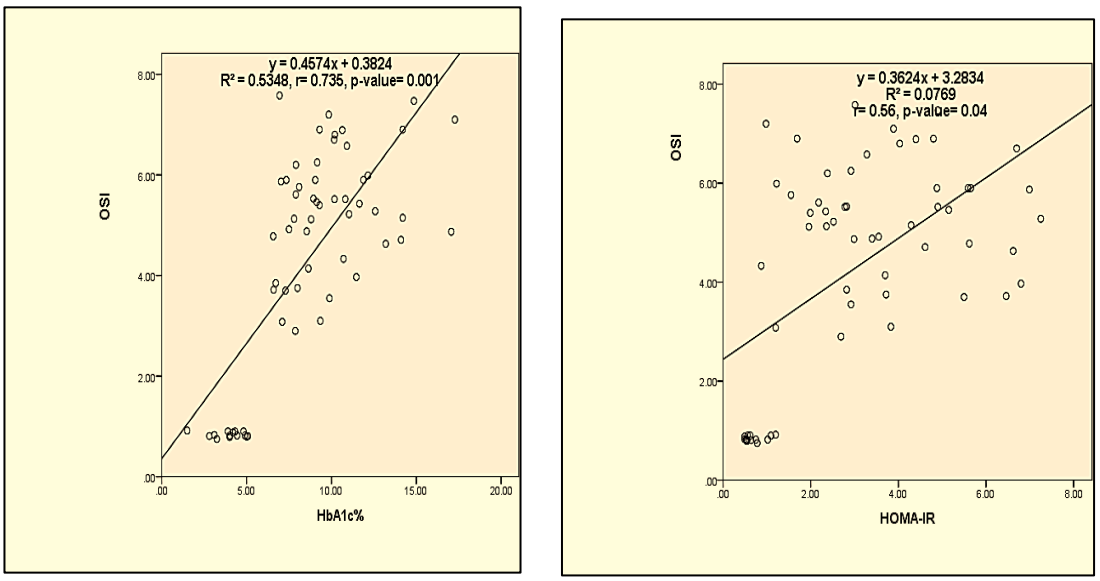


Figure (3-16): Correlation analysis of OSI with Insulin Resistance and HbA1c percentage levels in T2DM patients.

Conclusions

Serum Omentin-1level was found to be significantly lower in T2DM as compared to control group. We also found that serum omentin-1 is inversely related to overweight. A statistically significant negative correlation analysis between insulin resistance and oxidants levels with serum omentin-1 level in T2DM.

Our study showed that YKL-40 level are increase in T2DM. We also found that serum YKL-40 level is positively related to overweight. A statistically significant positive correlation analysis between insulin resistance and oxidants levels with serum YKL-40 level.

Increase in the level of YKL-40 and decrease Omentin-1level in the serum in patients with type 2 diabetes may lead to antioxidant disturbance and increased production of oxidants that cause the development of complications of type 2 diabetes.

Recommendations and future work

1. Molecular analysis of YKL-40 gene expression in T2DM patients.
2. Molecular analysis of Omentin-1 gene expression in T2DM patients
3. Gene expression examination of enzymatic antioxidants for patients with T2DM.
4. Monitoring the levels of omentin1 and ykl-40 in people with diabetes because they give an indication of the high oxidative stress that ends with a rise in complications of diabetes, and these levels can be adopted in the diagnosis of type 2 diabetes.
5. It is important for people with diabetes to take antioxidants to reduce the effects of free radicals and oxidative stress.

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الخلاصـــــــــة

داء السكري من النوع الثاني هو مرض يُصاب به المرء عندما لا يستطيع الجسم إنتاج ما يكفي من الأنسولين أو استخدامه بفعالية.

هدفت هذه الدراسة إلى تقييم المتغير Omentin-1 كمضاد للالتهابات والمتغير YKL-40 كعامل التهابي وعلاقتهما ببعض المتغيرات الكيموحيوية في المرضى الذكور الذين يعانون من مرض السكري النوع الثاني. حيث تضمنت الدراسة ٦٠مريضاً تتراوح أعمارهم بين ٣١-٥٥ سنة و ٣٠ شخص صحياً تتراوح أعمارهم بين ٣٢-٥٥ عامًا.

حيث اشتملت هذه الدراسة على قياس مؤشر كتلة الجسم، وتحديد عوامل نسبة السكر في الدم ، ودراسة خصائص الدهون ، والإجهاد التأكسدي.

كشفت النتائج عن زيادة معنوية في مؤشر كتلة الجسم و عوامل نسبة السكر في الدم بالإضافة الى ارتفاع في نسبة الكوليسترول والدهون الثلاثية وانخفاض في نسبة الكوليسترول الجيد لمرضى سكري النوع الثاني مقارنة بالأشخاص الاصحاء.

أظهرت الدراسة الحالية زيادة كبيرة في مستوى المؤكسدات وانخفاض مستويات مضادات الأكسدة لمرضى سكري النوع الثاني مقارنة بالأشخاص الاصحاء، كما تزداد أيضًا مستويات مؤشر الإجهاد التأكسدي في المرضى.

زاد تركيز YKL-40 ١٦ ,٢٥ ± ٧,٨ نانوغرام / مل وكانت القيمة الاحتمالية اقل من ٠٥,٠ لمرضى سكري النوع الثاني مقارنة بالأشخاص الاصحاء ٧,٤ ± ١٧, ١٨ نانوغرام / مل ، بينما كان هناك انخفاض في تركيز Omentin-1 لمرضى سكري النوع الثاني٥٧,١٠ ± ٦,١ نانوغرام / مل مقارنة بالأشخاص الاصحاء ٦٧ ,١٢ ± ٩,٣ نانوغرام / مل.

في مصل الدم كانت مرتبطة بمؤشر YKL-40 و Omentin-1وجدت الدراسة أن تركيز كل من YKL-40كتلة الجسم ومقاومة الأنسولين ومؤشر الإجهاد التأكسدي. قد تؤدي زيادة مستوى في المصل لمرضى سكري النوع الثاني إلى اضطراب مضادات Omentin-1وانخفاض مستوى الأكسدة وزيادة إنتاج المؤكسدات التي تسبب تطور مضاعفات مرض السكري من النوع الثاني.

جمهوريـــــــــــة العـــــــــراق
وزارة التعليم العالي والبحث العلمي
جامعـــــــــة الأنبـــــــــار
كليـــــــــة العلـــــــــوم

علاقة اومنتين-١و YKL-40

مع بعض المتغيرات الكيموحيوية لدى الرجال المصابين بمرض السكري من النوع الثاني في محافظة الانبار

أطروحة مقدمة إلى مجلس كلية العلوم في جامعة الأنبار كجزء من متطلبات نيل شهادة دكتوراه الفلسفة في علوم الكيمياء

اعداد الطالبة

سراب طارق محمود حمزه المحمدي

بكالوريوس علوم كيمياء/ جامعة الأنبار(٢٠١٢- ٢٠١٣)

ماجستير علوم كيمياء/ جامعة الأنبار(٢٠١٦- ٢٠١٧)

بأشراف

الأستاذ الدكتور خالد فاروق الراوي **الأستاذ الدكتور مصطفى طه محمد**

١٤٤٤ هـ ٢٠٢٢ م