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**Department of Biology**

# Study the correlation among some risk factors of heart disease in patients with metabolic syndrome in Kerbala Province

## A Thesis

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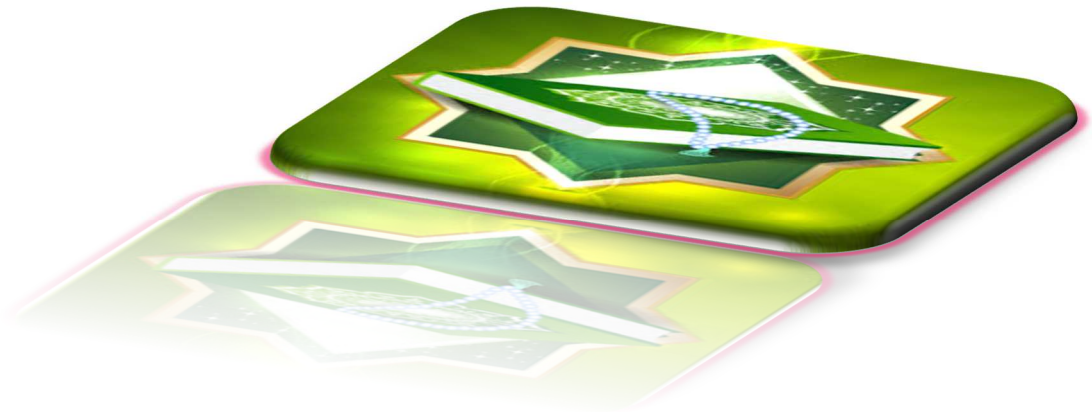
بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ.

صدق الله العلي العظيم

**المجادلة 11**



# *Dedication*

To my husband and my children....

To my brother and sister ....

To my friends.....

*I dedicate this work.*

*Lina*

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**Lina**

## Linguistic certification

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# Summary

Metabolic syndrome is a group of metabolic risk factors that exist in one person and it is a serious health condition. People with it have a higher risk of diseases related to fatty buildups in artery walls, coronary heart disease, which can lead to heart attack, is an example. Stroke and peripheral vascular disease are other examples. People with this syndrome are also more likely to develop T2DM.

The present study include study some of cardiovascular markers in addition to routine biological variables of lipid profile (triglycerides, total cholesterol, high density lipoprotein - cholesterol and low density lipoproteine – cholesterol) and study their correlation with metabolic syndrome and T2DM. Fasting lipid analysis does not give an accurate picture of pre risk of heart disease therefore become necessary to research at more accurate biological variables as predictors of the incident of cardiovascular risk like hs-C-reactive protein, apolipoprotein-A1 and 25-hydroxyvitamin D.

This study was conducted at Al-Hussein Medical City / Kerbala /Iraq and Department of Biochemistry–College of Medicine / University of Kerbala. Samples were randomly selected from the patients attending the diabetic consultation unit at the hospital during Dec,2012 to June, 2013. The patients and control groups were with age ranged between (26 - 65) years .

The study was carried out on 50 patient with metabolic syndrome (MetS), 40 T2DM patients and their history of the disease have at least two years and 30 apparently healthy subjects as control group.

The results of the present study show that 25-hydroxyvitamin D was significantly low in serum of metabolic syndrome patients group and T2DM patients compared with control group ($P < 0.001$) also there was a significant negative correlation with BMI, FBS, TG, hs-CRP and hypertension and it has positive correlation with HDL-C and ApoA1 .

The results show that TG level , Total cholesterol, LDL-C were significant elevated $p<0.001$ in MetS compared with normal control group, while serum HDL-cholesterol level was significantly low in MetS patients group as compared with normal control group($P <0.001$) BMI, systolic and diastolic blood pressure were significantly high in MetS patients at $p<0.001$

The results show significant increase mean value of hs-CRP level in sera of MetS patients compared with normal control group at ($p<0.01$) and with T2DM which undergo uncontrolled or fluctuation in sugar level at ($P < 0.05$)

The results also showed that the mean value of serum apo-A1 was significantly low ($P < 0.05$) in MetS and T2DM patients compared with normal control group.

A non-expected result was showed by serum troponin I mean value which were a non-significant change in T2DM and MetS patients compared with normal control group, it dose not show any significant difference between them ($P > 0.05$).

The results show significant increase in TG level while Total cholesterol, LDL-C show non-significant elevate $p>0.05$ in T2DM patients group compared with normal control group, while serum HDL-C level show non-significant decrease $p>0.05$ compared with normal control group.

It can be concluded that This show significant decrease in serum levels of Apo A1, HDL-cholesterol with a significant elevation in CRP, TG, LDL-C, Total-

Cholesterol, systolic and diastolic blood pressure this factors form seriousness risks are threatening for heart diseases in MetS patients compared with control group.

## Abbreviations

| Abbreviations | Description |
|---|---|
| $1,25\text{-}(OH)_2D$ | 1,25-Dihydroxyvitamin D |
| 25-(OH)D] | 25-Hydroxyvitamin D |
| ATP | Adenosine tri phosphate |
| ADA | American diabetes association |
| ACE | Angiotensin converting enzyme |
| Apo-A-I | Apolipoprotein-A1 |
| ANP | Atrial natriuretic peptide |
| BMI | Body mass index |
| CaBP | Calcium-binding proteins |
| CVD | Cardiovascular disease |
| CHD | Chronic heart disease |
| DBP | Vitamin D3binding protein |
| ER | Endoplasmic reticulum |
| FBS | Fasting blood sugar |
| GDM | Gestational Diabetes Mellitus |
| HDL | High-density lipoprotein |
| hs-CRP | High-sensitivity C-reactive protein |
| HRP | Horse Radish Peroxidase |
| IGT | Impaired glucose tolerance |
| IDDM | Insulin Dependent Diabetes Mellitus |
| IL-1β | Interleukin 1-beta |
| IL-6 | Interleukin-6 |

| | |
|---|---|
| **IDL** | **Intermediate density lipoprotein** |
| **kD** | **Kilo Dalton** |
| **kg/m2** | **Kilograms / square meter** |
| **LADA** | Latent autoimmune diabetes of adults |
| **LCAT** | **Lecithin: cholesterol acyltransferase,** |
| **LPL** | **Lipoprotein lipase** |
| **LDL** | **Low-density lipoprotein** |
| **MRDM** | **Malnutrition-related diabetes mellitus** |
| **MetS** | **Metabolic syndrome** |
| **ng/ml** | **Nanogram / milliliter** |
| **NHANES** | **National Health and Nutrition Examination Survey** |
| **NICE** | **National Institute of Health and Care Excellent** |
| **NIDDM** | **Non-Insulin Dependent Diabetes Mellitus** |
| **NF-κB** | **Nuclear factor-κB** |
| **PTH** | **Parathyroid hormone** |
| **PCOS** | **Polycystic ovarian syndrome** |
| **ROS** | **Reactive oxygen species** |
| **RAS** | **Renin-angiotensin system** |
| **RAAS** | **Renin-angiotensin-aldosterone system** |
| **RXR** | **Retinoic x receptor** |
| **SNS** | **sympathetic nervous system** |
| **TIA** | **transient ischemic attack** |
| **TIA** | **Transient ischemic attack** |
| **TG** | **Triglyceride** |
| **TnT** | **Troponin T** |
| **TNFα** | **Tumor necrosis factor Alfa** |
| **T1DM** | **Type-1 Diabetes Mellitus** |
| **T2DM** | **Type-2 Diabetes Mellitus** |
| **UV** | **Ultraviolet radiation** |
| **VSMC** | **Vascular smooth muscle cells** |
| **VLDL** | **Very-low-density lipoprotein** |
| **Vit-D** | **Vitamin D** |
| **VDR** | **Vitamin D receptor** |
| **WHO** | **World Health Organization** |
| **SNS** | **sympathetic nervous system** |

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## 1.3. Introduction

Metabolic syndrome is the concurrence in an individual of interrelated risk factors for CVD and diabetes. These factors include hyperglycemia, raised blood pressure, elevated triglyceride concentrations, reduced HDL-C, and obesity (particularly central adiposity) [1]. Several reports indicate that the presence of the metabolic syndrome is associated with increased risk for both CVD and type 2 diabetes [2,3]. Persons with the metabolic syndrome have at least a 2-fold increase in risk for CVD, compared with those without. Risk for type 2 diabetes in both men and women is increased about 5-fold [4].

Over the last decade, accumulating evidence has indicated that the concentration of 25-hydroxyvitamin $D_3$in the blood is inversely associated with diabetic, hypertension, and some diseases .

Most of the needed vitamin D3is derived from the synthesis of cholecalciferol (vitamin D3) in the skin derived from the skin 7-dehydrocholesterol through exposure to sunlight[5,6] . The production of vitamin D3 in the skin is depended on the ultraviolet radiation wavelength(290-315nm) and the number of the photons absorbed [7], which affected by many factors including increased skin pigment [8], the sunscreen use, the angle of sunlight reaching the Earth's surface (zenith angle) the time of the day, season and latitude [9-11]. The ultraviolet radiation need to synthesize vitamin D3is blocked by the atmosphere when the sun fails to rise over above the horizon. For this reason, vitamin $D_3$ synthesis is impaired in the morning and evening hours, during the winter months and more so in countries of higher latitude [12-16].

The classical function of is related to calcium absorption and phosphate homeostasis and bone mineralization. some studies suggested that vitamin D3(Vit-D), primarily important for calcium homeostasis and bone metabolism, influences the cardiovascular system through unclear mechanisms. Suboptimal Vit-D status is associated with increased all-cause and cardiovascular disease (CVD) mortality, coronary heart disease, and various cardiovascular risk factors [17,18]. Serum 25-

hydroxyvitamin D3[25-(OH) D] concentration is widely held to be a better indicator of vit. D status than the more functionally active form 1,25-dihydroxyvitamin D3[1,25-$(OH)_2$D or calcitriol. Although there is no consensus on the optimal serum 25(OH)D concentration, most experts recommend an optimal concentration greater than 30 ng/ml in serum and vit.D deficiency is less than 30 ng/ml [19].

Vit.D deficiency is even more prevalent among T2DM patients, endothelial dysfunction predicts cardiovascular events,and represents an underlying event for vascular abnormalities observed in type 2 DM patients[20] . Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with body mass index, family history of diabetes mellitus, smoking and other factors .hs-CRP, a pentameric protein produced by the liver has emerged as the ‘ golden marker for inflammation’.

Inflammation plays a significant role in the pathogenesis of coronary heart diseases [21]. Apparently healthy individuals with increased high-sensitivity c-reactive protein (hs-CRP), a sensitive marker of inflammation, have an increased relative risk of future first cardiac events even after adjustment for age, smoking status, body weight/body mass index (BMI), hyperlipidemia, and hypertension and T2DM [22-24]. In fact, the relative risk of increased hs-CRP is greater than and independent of traditional (cholesterol, HDL, LDL) and novel (lipoprotein (a), apolipoproteins AI and B) markers of cardiovascular risk [25].

## 1.2. Diabetes Mellitus

### 1.2.1. Definition

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, activation or action[26]. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs.

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, diabetic ketoacidosis or a non–ketotic hyperosmolar is stated that may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be presented for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetic complication are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease[27,28].

### 1.2.2. History

Diabetes mellitus has been recognized as a devastating and deadly disease. In the first century A.D. a Greek, Aretaeus, described the destructive nature of the affliction which he named "diabetes" from the Greek word for "siphon"[29]. The term "mellitus" or "from honey" was added by the Britain John Rolle in the late 1700s.

Concerning the sweetness of urine, it was to be noted that the Chinese, Japanese and Korean words for diabetes are based on the same ideographs which mean "sugar urine disease". Matthew confirmed in 1776 that the sweet taste comes from an excess of a kind of sugar in the urine. Many investigations were tried, effective treatment

was not developed until the early part of the 20th century when the Canadians Frederick and Charles first used insulin from bovine pancreas in 1921 and 1922 in treatment · This led to the availability of an effective treatment-insulin injections-and the first patient was treated. For this, Banting and laboratory director MacLeod received the Nobel Prize in Physiology and Medicine in 1923[30].

### 1.2.3. Epidemiology

The highest prevalence of diabetes is in North America where it reached 10.2% of adult population while in Europe the prevalence is 6.9% of population aged 20 to 79 years in 2011. Particularly worrying is constantly increasing in diabetes incidences [31]. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double[32]. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increasing in prevalence is expected to occur in Asia and Africa, where most patients will probably be found by 2030.It is known that diet and way of life increased from Asian developing diabetes as the evolution of the European lifestyle. This led to the increasing in the number of patients with this type of diabetes[32].

### 1.2.4. Classification of Diabetes Mellitus

Most cases of diabetes mellitus fall into three general broad categories: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus (GDM) and another type is a specific type of diabetes.

#### 1.2.4.1. Insulin Dependent Diabetes Mellitus

Insulin dependent diabetes mellitus (IDDM) or type 1 diabetes mellitus (T1DM) occur as a results of auto-immune beta-cell destruction in β-cells of pancreas, characterized by a total absence of insulin production. T1DM is responsible for 5-10% of all cases of diabetes. Associated risk factors include autoimmune, genetic, and

environmental factors. Until the present time, known solutions to prevent diabetes have not been diagnosed[33].

In T1DM, also referred to as juvenile onset is relatively early in life, in childhood or adolescence and usually before the age of thirty. This type of diabetes is a relatively homogeneous disease in which the insulin secretion of β-cells in the pancreas declines and eventually ceases totally[34]. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes . The presence of obesity is not incompatible with the diagnosis, diet and exercise cannot reverse or prevent T1DM [34].

### 1.2.4.2. Gestational Diabetes Mellitus

Gestational diabetes (GDM) could be described as a form of glucose intolerance that affects some women during pregnancy. This kind of diabetes is triggered during pregnancy[35]. Most GDM is resolved naturally after delivery, but 5-10% of women affected during pregnancy are later found to have diabetes, especially T2DM, after pregnancy. Furthermore, women who have had a history of gestational diabetes have 40-60% chance of developing diabetes in the following 10 years. Therefore, changes in lifestyle implemented to normalize blood glucose during pregnancy become essential preventive measures against development of T2DM. Pre-diabetes affect 54 million adults and this places them at risk of developing diabetes later in the nearest future[36].

Untreated gestational diabetes can lead to problems for both the mother and the child. It can lead to fat baby syndrome or microsmatic, in which the baby's body produces extra fat[37].

### 1.2.4.3. Specific Types of Diabetes

Pre-diabetes indicates a state that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of T2DM . This type occurs by many reasons as genetic defects in β-cell function, insulin action, diseases of the exocrine pancreas, drug- or chemical-induced.Many people destined to develop T2DM spend many years in a state of pre-diabetes which has been termed "America's largest healthcare epidemic"[38].

Latent autoimmune diabetes of adults (LADA) is a condition in which T1DM develops in adults. Adults with LADA are often firstly misdiagnosed as having T2DM, based on age rather than etiology. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin, this form is very rare. Genetic mutations (autosomal or mitochondrial) can lead to defects in β-cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes wide damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with extreme secretion of insulin- receptor hormones( plays a key role in the regulation of glucose homeostasis) can cause diabetes (which is usually resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cell [38] .

### 1.2.4.4. Non-Insulin Dependent Diabetes Mellitus

This form of diabetes, accounts for **~90–95%** of diabetic patients, previously referred to as non–insulin dependent diabetes mellitus [NT1DM], or type II diabetes mellitus, T2DM[39]**.** It is a chronic metabolic disorder, in which the body is unable to utilize glucose from food because of the inability of the pancreas to produce sufficient insulin, or the insulin itself is inactive or due to presence of resistance against the action of insulin. The individuals with T2DM don't need insulin treatment to survive. There are probably many different causes of this form of

diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur[40].

Most patients with this type of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another diseases such as infections[41]. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macro-vascular and micro-vascular complications[42]. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated . The higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups[43].It is often associated with a strong genetic predisposition, more than the autoimmune form of T1DM. However, the genetics of this form of diabetes are more complex and not clearly defined[44].

### 1.2.4.4.1. Risk Factors

Risk factors of T2DM include the following[45]:

1. Age ≥ 45 years.
2. Overweight (BMI ≥ 25 kg/m2).
3. Family history of diabetes (i.e., parents or siblings with diabetes).

4. Habitual physical inactivity.
5. Race/ethnicity (e.g., African-Americans, Hispanic-Americans, Native Americans, Asian-Americans, and Pacific Islanders).
6. History of GDM or delivery of a baby weighing > 9 lbs
7. HDL-cholesterol ≤ 35 mg/dl (0.90 mmol/l) and/or a triglyceride level ≥250 mg/dl (2.82 mmol/l).
8. Polycystic ovary syndrome.
9. History of vascular disease.
10. Hypertension (≥ 140/90 mmHg in adults) .

### 1.2.4.4.2. Signs and Symptoms

When symptoms of T2DM occur, they are often ignored because they may not seem serious. Symptoms of T1DM usually come on much more suddenly and are often severe[46].

1. Excessive thirst and appetite.
2. Increased urination (sometimes as often as every hour).
3. Unusual weight loss or gain
4. Fatigue
5. Nausea, perhaps vomiting
6. Blurred vision
7. In women, frequent vaginal infections
8. In men and women, yeast infections
9. Dry mouth
10. Slow-healing sores or cuts
11. Itching skin, especially in the groin or vaginal area

## 1.3. Cardiovascular Diseases

Cardiovascular disease (also called heart disease) is a group of diseases that involve blood vessels of heart (arteries, capillaries, and veins). Cardiovascular disease refers to any disease that affects the cardiovascular system, mainly cardiac disease as (coronary heart disease), vascular diseases of the brain an kidney, and peripheral arterial disease[47]. The causes of cardiovascular disease are various but atherosclerosis and/or hypertension are the majority common. In addition.

There are many complications of Diabetes Mellitus divided into two main sections [48].

1. Macro vascular complications

   It includes coronary artery disease ,Diabetic cardiomyopathy, peripheral vascular disease and stroke. .

2. Micro vascular complications

It includes retinopathy , nephropathy , and neuropathy

Although cardiovascular disease usually affects older adults, the background of cardiovascular disease, particularly atherosclerosis.

Most cardiovascular diseases can be prevented by addressing risk factors such as tobacco uses, unhealthy diet and obesity, physical inactivity, high blood pressure, diabetes and raised lipids[49].

## 1.3.1. Blood Pressure

Blood pressure is the force of blood against the walls of arteries. Blood pressure rises and falls throughout the day. When blood pressure stays elevated over time, it's called high blood pressure[50].

Higher blood pressure, the shorter life expectancy. People with high blood pressure run a higher risk of having a stroke (which damages the brain) or a heart attack. If left untreated for a long time, high blood pressure can lead to kidney failure and even damage the eye. It can also make the heart abnormally large and less efficient (a condition called 'left ventricular hypertrophy'). This can lead to heart failure, which is when the pumping action of the heart becomes less effective. It also known as hypertension –rarely makes people feel ill. It is sometimes called a 'silent threat' because there are usually no symptoms, and it very often goes undiagnosed[51]. Blood pressure is regulated by renin-angiotensin system (RAS).

### 1.3.1.1. Renin-Angiotensin System

Blood pressure and water (fluid) balance are regulated by hormonal system that know renin-angiotensin system (RAS) or the renin-angiotensin-aldosterone system (RAAS). Juxtaglomerular cells in the kidneys secrete renin directly into circulation,when blood volume is low. Plasma renin then carries out the conversion of angiotensinogen released by the liver to angiotensin I [52]. Angiotensin I is subsequently converted to angiotensin II by the enzyme angiotensin converting enzyme (ACE) found in the lungs. Angiotensin II is a potent vaso-active peptide that causes blood vessels to constrict, resulting in increased blood pressure. Angiotensin II also stimulates the secretion of the hormone aldosterone from the adrenal cortex. Aldosterone causes the tubules of the kidneys to increase the reabsorption of sodium and water into the blood which increases the volume of fluid in the body and blood pressure, see figure (1-1)

When the renin-angiotensin-aldosterone system is abnormally active, blood pressure will be too high. There are many drugs that inhibit different steps in this system causing lower blood pressure. These drugs are one of the main ways to control high blood pressure (hypertension), heart failure, kidney failure, and harmful effects of diabetes [53,54].

Angiotensin II is the major bio-active product. Angiotensin II has a variety of effects on the body such as, it is a potent vasoconstrictor of arterioles throughout the body, stimulates $Na^+/H^+$ exchangers located on the apical membranes of proximal tubule cells and thick ascending limb of the loop of Henle in addition to $Na^+$ channels in the collecting ducts. This will ultimately lead to increased sodium reabsorption, it also induce the release of aldosterone. Aldosterone acts on the tubules in the kidneys, causing them to reabsorb more sodium and water from the urine[55]. This increases in blood volume and then increases blood pressure. These effects opposed by atrial natriuretic peptide (ANP) that has exactly the opposite function of the aldosterone [56].

### 1.3.1.2. Diabetes Mellitus and Hypertension

Patients with diabetes have elevated blood sugar compared to patients without diabetes. This excesses sugar has many consequences, including slow but serious damage to sensitive blood vessels or capillaries. Damage to certain capillaries in the kidneys impairs the kidney's blood pressure regulating abilities, leading to higher blood pressure. This increased blood pressure causes small changes in blood flow, which exposes other sensitive capillaries to additional damage. Elevated blood pressure can also affect the delicate insulin secreting areas of the pancreas, leading to higher blood sugar. In this way, the diabetes/high blood pressure combination is a self-reinforcing loop in which both diseases tend to worsen over time [57,58]. In some ways, diabetes and hypertension could be considered as chronic inflammatory diseases and the local renin-angiotensin-aldosterone system (RAAS) plays a very important role in vascular pathophysiology . Also, Ang II down-regulates proinflammatory transcription factors such as nuclear factor-κB (NF-κB), resulting in the generation and secretion of reactive oxygen species (ROS), inflammatory cytokines (eg, interleukin-6 [IL-6]), chemokines, and adhesion molecules. These actions lead to endothelial dysfunction and vascular injury[59,60].

Patient with diabetes mellitus suffered from the increasing of the amount of fluid in the body, when fluid in the body increased, it will lead to increase of the blood pressure [61]. According to the previous research, researcher found that common biological traits of the two diseases are likely to occur together simply because they share a common set of risk factors [62].

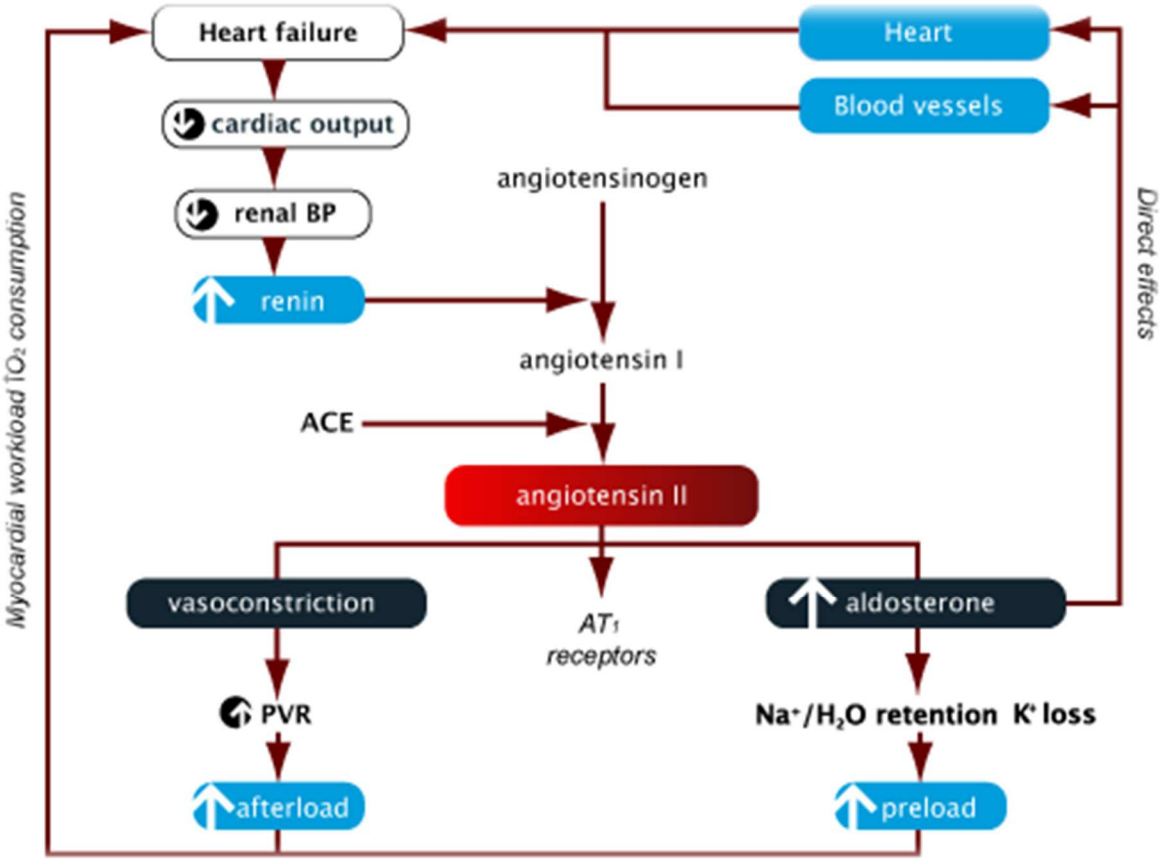


**Figure (1-1)**The renin-angiotensin-aldosterone system (RAAS) and its effects on the cardiovascular system in heart failure [63]

## 1.3.2. Stroke

Stroke is a neurological condition characterized by a disrupted or ceased flow of blood to the brain and can cause permanent neurological damage and death. This is due to ischemia caused by blockage (thrombosis, arterial embolism), or a hemorrhage[64]. It is the second leading cause of death worldwide [65]. Consequently, the affected area of the brain not functioning, which might lead to the loss of a limb movement to one side of the body, inability to understand or formulate speech, or an inability to see one side of the visual field[66]. The risk factors that lead to stroke include old age, high blood pressure, diabetes, hypercholesterolemia, tobacco smoking, previous stroke or transient ischemic attack (TIA) and atrial fibrillation.

High blood pressure is the most important modifiable risk factor of stroke. Strokes can be classified into two major categories [67]. .

1. ischemic stroke (blood supply to part of the brain is decreased, leading to dysfunction of the brain tissue in that area.
2. hemorrhagic stroke (blood inside the skull but outside the brain).

Diabetes mellitus increases the risk of stroke by 2 to 3 times. While intensive control of blood sugar has been shown to reduce micro vascular complications such as nephropathy and retinopathy it has not been shown to reduce macro vascular complications such as stroke[68,69]. High serum cholesterol levels have been associated with (ischemic) stroke[70,71]. Statin drugs have ability to reduce the risk of stroke by about 15% by lowering hyperlipidemia[72]. Hypertension constitutes about 35-50% of stroke risk. It is associated with both ischemic and hemorrhagic strokes[73-75] . .

### 1.3.3. Coronary Heart Disease

Coronary heart disease (CHD), also called coronary artery diseases are the major leading causes of death in the United States and European countries until 2005 for mortality in the present for both men and women. CHD occurs when plaque builds up inside the coronary arteries.Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. Over time, plaque hardens or (atherosclerosis) narrows the arteries, reducing blood flow to the heart muscle causing abnormal artery function[76].

The heart is an aerobic organ that is dependent for its oxygen supply entirely on coronary arteries. Under resting condition, the myocardium extracts the maximum amount of oxygen from the blood it receives there for interruption of coronary blood flow will result in immediate ischemia [77]. The symptoms appear as a chest pain or

angina with physical stress; the pain may spread to the left arm or the neck, back, throat, or jaw.

Research suggests that CHD starts when certain factors damage the inner layers of the coronary arteries. These factors include [78,79].

1. Unhealthy blood cholesterol levels
2. High blood pressure
3. Smoking
4. Insulin resistance
5. Diabetes
6. Overweight or obesity
7. Metabolic syndrome
8. Lack of physical activity
9. Age (older people , the risk for CHD increases)
10. Family history of early heart disease .

### 1.3.4. Peripheral Vascular Disease

It is the obstruction of large arteries as a result of atherosclerosis, inflammatory processes leading to stenosis, an embolism, or thrombus formation. It causes either acute or chronic ischemia (lack of blood supply). Often peripheral vascular disease (PVD) is a term used to refer to atherosclerotic blockages found in the lower extremity. PVD also includes a subset of diseases classified as microvascular diseases resulting from episodal narrowing of the arteries.

Risk factors are diabetes mellitus , dyslipidemia , hypertension , smoking – tobacco and other risk factors as the age over 50, male, obese, heart attack, or stroke which are contributing to PAD [80-82].

## 1.4. Metabolic syndrome

### 1.4.1. Definition

Metabolic syndrome (MetS) is a complex web of metabolic risk factors that are associated with a 5-fold risk of type 2 diabetes (T2DM) and a 2-fold risk of cardiovascular disease (CVD)[83]**.** Although there are different definitions of MetS, The NCEP definition considers the syndrome to be present with at least 3 of the following: elevated fasting glucose, elevated LDL-C, low HDL-C, hypertension and obesity [84].

### 1.4.2. T2DM and Metabolic syndrome

Previous works showed that, in comparison with non-diabetic subjects, circulating the concentrations of commonly recognized acute-phase reactants were increased in T2DM but not T1DM patients who were matched for age, sex, glycemic control [85]. The dyslipidemia common in T2DM (hypertriglyceridemia and low serum levels of HDL-cholesterol) is also a feature of natural and experimental acute-phase reactions as cytokines . Through the action of cytokines on the brain, liver, endothelium, adipose tissue and elsewhere, this process could be a major contributor to the biochemical and clinical features of metabolic syndrome X (glucose intolerance, dyslipidemia, insulin resistance, hypertension, central obesity, accelerated atherosclerosis) but also provides a mechanism for many other abnormalities seen in T2DM, including those in blood clotting, the reproductive system, metal ion metabolism, psychological behavior and capillary permeability [86]. Furthermore, the metabolic syndrome signals /the presence of a proinflammatory state. In fact, increased C reactive protein levels often can be found in patients with the metabolic syndrome, and there is a linear relationship between the number of components of the metabolic syndrome and the degree of inflammation [87]. However, even before levels of blood glucose are high enough for a person to be diagnosed with diabetes, hyperglycaemia and related changes in blood lipids (increase in triglycerides and

decrease in the ‘good’ cholesterol HDL-c) increase a person’s risk of cardiovascular disease[88] .

The elevated levels of glucose and lipids, mainly saturated fatty acids, that are characteristic of insulin resistance synergize at the level of the β-cell to drive parallel increases in FAS expression, activating NK cell ligands, reactive oxygen species, and endoplasmic reticulum (ER) stress, all of which culminate in IL-1β secretion and apoptosis Importantly, IL-1β has been a known mediator of β-cell dysfunction and death for more than 25 years[89-90].

## 1.5. Obesity

### 1.5.1. Definition

Obesity is defined as abnormal condition or excess body fat that has accumulated in adipose tissue, to the extent that health may be impaired and have an adverse effect on health, leading to reduce life expectancy and/or increased health problems [91,92]. However , obese individual differ not only in the amount of fat excess that they store but also in the regional distribution of that fat within the body. The distribution of fat induced by weight gain affects the risks associated with obesity , and the kinds of disease that result [93] . Body fat is not easy to measure directly, and so for epidemiological purposes, measures of relative weight are often used as alternative for body fat[94]. The most widely used indirect measure of obesity that based on relative weight is Body Mass Index (BMI) which is a measurement obtained by dividing a person's weight in kilograms by the square of the person's height in meters. People are considered obese when their body mass index (BMI) exceeds 30 $kg/m^2$[95]. Adults with a BMI of 30-35 $kg/m^2$ have a life expectancy of approximately 2 to 4 years less than those with a BMI of 22.5-25 $kg/m^2$ [96]. The World Health Organization (WHO) developed a classification of BMI for global use, mainly based on its association with mortality in which overweight defined as 25- 29.9 $kg/m^2$, obese as 30-39 $kg/m^2$ and morbidly obese as 40 $kg/m^2$ and above[97]. A major

application of BMI, both in epidemiological and clinical settings, is that height and weight measures can be taken with relative easy and speed. This has led to the widespread use of BMI in a range of settings. in spite of its significant advantages, BMI does have limitations. The relationship between BMI and body fat has been shown to vary with other factors. For example, the relationship between BMI and body fat is age- dependent, as the ratio of fat to lean mass becomes greater when age increases. BMI will also tend to expect too much of body fat in very muscular individuals[98 , 99].

## 1.5.2. Causes of Obesity

A lack of energy balance most often causes overweight and obesity. Energy balance means that the energy input is equals to energy output. An increased intake of energy-dense foods that are high in fat; and a decrease in physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization[100-102]. There are another causes like genes and family history[103]. Health Conditions such as underactive thyroid (hypothyroidism), Cushing's syndrome, and polycystic ovarian syndrome (PCOS), antidepressants, and seizure medicinesage pregnancy especially after a few pregnancies[104,105].

## 1.5.3. Prevalence of Obesity

The prevalence of obesity across the world has escalated in the last three decades and is recognized as a global threat to health[106]. Globally an expected 300 million adults are obese or overweight [107]. This is likely to be a substantial underestimate as the availability and quality of prevalence estimates vary. There are major socio-economic and cultural differences in the prevalence of obesity[108].

In 2004, a medical extensive conducted in the Kingdom of Saudi Arabia conducted with the participation of the Ministry of Health and the King Faisal Specialist Hospital . Preliminary results showed that the rate of obesity and

overweight was 66% among women and 58% among men. World Health Organization for the year 2010 have pointed to the high rate of obesity in Iraq for males reached 8.3%, compared with 19.1% for females[109].

### 1.5.4. Obesity and Clinical Disorders

Overweight and obesity are risk factors for T2DM, heart disease, high blood pressure, and other health problems . An expected 87 million adults in the United States are overweight or obese[110]. These conditions significantly increase the risk of morbidity from hypertension[111], dyslipidemia [112], type 2 diabetes [113-116], coronary artery disease [117], stroke [118], gallbladder disease[119], osteoarthritis[120], and sleep apnea and respiratory problems[121], as well as cancers of the endometrium, breast, prostate, and colon[122. The relationship of obesity to major and emerging risk factors varies, depending on the genetic and acquired characteristics of individuals. The majority of obese persons who develop CVD typically have a clustering of major and emerging risk factors (metabolic syndrome). The constellation of major and emerging risk factors that make up the metabolic syndrome can be called metabolic risk factors [123].

### 1.5.5. Treatment of Obesity

There is no single cause of all overweight and obesity and also no single approach that can help preventing or treating overweight and obesity. Treatment may include a mixture of behavioral treatment, diet by limiting energy intake from total fats and sugars and increasing consumption of fruit and vegetables, exercising and regulation physical activity, and sometimes weight-loss drugs. In some cases of extreme obesity, weight-loss surgery may be an option [124].

## 1.6. Vitamin D3and 25-Hydroxyvitamin D

Vitamin D3is one of the fat-soluble vitamin, it is a pro-hormone derived either from 7-dehydrocholesterol or ergosterol by the action of ultraviolet radiation (290-315 nm) under the skin. The active form of vitamin D3(1,25-dihydroxycholecalciferol or calcitriol) is a hormone and has important effects upon calcium and bone metabolism. In addition to maintain calcium homeostasis, vitamin D3metabolites may also be involved in the functioning of numerous other systems[125].

There is an evidence states that vitamin D3may reduce the risk of cardiovascular disease, hypertension, diabetes mellitus and some cancers.25-hydroxyvitamin D3is the major circulating metabolite of vitamin D. It produced in the liver and it considered as the best indicator of the body's vitamin D3status[126-131].

### 1.6.1. Characteristics

Abbreviated 25-OH Vit D synonym to the 25-hydroxyvitamin D as show in figure (1-2), also known as 25-hydroxycholecalciferol[132].It circulates either as 25-OH Vit $D_2$ or 25-OH Vit $D_3$,this depending on its precursor, vitamin $D_3$ is called (colecalciferol, also known as cholecalciferol) and vitamin $D_2$ (ergocalciferol) [133,134].

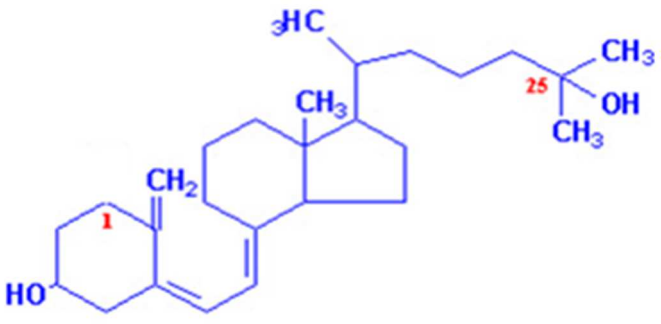


**Figure (1-2) Structure of 25-hydroxyvitamin $D^3$ [133]**

## 1.6.2. Biosynthesis

Human vitamin D3enters the body as crude or inactive vitamin $D_3$ or $D_2$ from the diet (milk,egg yolk, oil fish) or obtained from 7-dehydrocholesterol under the skin by exposure to ultraviolet light (UV) (an intermediate in the biosynthesis of cholesterol that accumulates in the skin) which convert to pre vitamin $D_3$ then none somatically alter to vitamin $D_3$[135]. When vitamin $D_3$ enters the body, it circulates bound to globulin vitamin D3binding protein (DBP) and is rapidly converted to its major circulating form, 25-OH Vit D (Calcifediol) which is a pre-hormone that is produced in the liver by hydroxylation of vitamin $D_3$ (cholecalciferol) by the enzyme cholecalciferol 25-hydroxylase[136,137] as show in figure (1-3). This metabolite is being measured by physicians worldwide to determine a patient's vitamin D3status[138]. Under the influence of parathyroid hormone (PTH), 25(OH) vit D is converted by the 1-alpha-hydroxylase (1α-hydroxylase) in the kidney to the activated form or a hormonal form of vitamin D, 1,25-dihydroxyvitamin D3($1,25(OH)_2$vit. $D_3$). Other tissues in the body have the 1α-OH hydroxylase and can convert 25(OH)D to $1,25(OH)_2D$[139]. However, only the renal 1α-hydroxylase significantly contributes to circulating $1,25(OH)_2D$ level.

A circulating $1,25(OH)_2D$ enters the target cell, either in its free form or facilitated by megalin, and binds to the vitamin D3 receptor (VDR) in the cytoplasm which then translocate to the nucleus and heterodimerizes with the retinoic x receptor (RXR)[140]. $1,25(OH)_2D$-VDR-RXR complex then binds to vitamin D3response elements (VDRE) on DNA to increase transcription of vitamin D3 regulated genes[141].

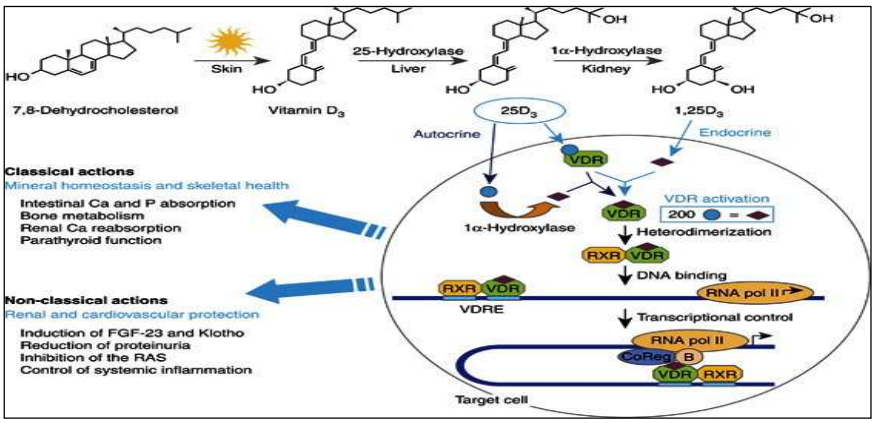


**Figure (1-3) Biosynthesis and role of vitamin D3 in the body[142]**

Vitamin D3status is best determined by a serum 25(OH)D due to several reasons including:

1. Long circulating half-life(~3 weeks versus ~4-6 hours for $1,25(OH)_2D$)[143].
2. The concentration of 25(OH)D is 1000x higher in circulation compared to $1,25(OH)_2D$ (ng/mL vspg/mL).
3. The production of $1,25(OH)_2D$ is mainly under the influence of PTH which tightly regulates calcium levels[144].

Therefore, $1,25(OH)_2D$ levels could be elevated in individuals with severe vitamin D3deficiency in order to maintain normal serum calcium levels[145]. So 25-(OH)Vit D is the best form for detecting the level of vitamin D3in the body.

## 1.6.3. Biological Roles of 25-Hydroxy Vitamin D

At physiological concentrations, 25-(OH)Vit D is biologically inactive. Its principle is to act as the storage form of vitamin D. Vitamin D3receptors (VDR) exist in almost all tissues. They have been identified in bone and kidney cells, skeletal, heart, and smooth muscles, intestinal epithelial cells, stomach, liver, skin keratinocytes and hair follicular cells, breast, pancreatic islets (B cells), thyroid,

parathyroid, adrenal and pituitary glands, immune cells, brain, prostate, ovaries and testes[146,147]. These findings are consistent with a more complex role of vitamin D3in human biology, in addition to mineralization of bone, calcium and phosphate absorption that includes among others also regulation of insulin synthesis and secretion[148], modulation of the inflammatory response, cell maturation, and cell differentiation[149]. Vitamin D3has been shown to have a potential role in cancer prevention, including colon, breast, prostate, and ovarian cancer[150] most probable by its effect on cell maturation and differentiation. The role of vitamin D3in autoimmune and inflammatory conditions is currently aggressively studied and is acting as a negative endocrine regulator of the renin-angiotensin system [151,152].

1,25-$(OH)_2$Vit D is generally acknowledged as a hormone in the classical common sense, while vitamin D3and 25-(OH)Vit D are regarded as pro-hormones. Main classical biologic actions of 1,25-$(OH)_2$Vit D are enhancing absorption of calcium from intestine,mineralization of bone matrix,osteoblast differentiation,inhibition of parathyroid hormone secretion,at high supra physiological concentrations, bone resorption.

## 1.6.4. 25-Hydroxy Vitamin D3Deficiency

### 1.6.4.1. Insulin Resistance

It is a physiological state in which cells fail to reply to the normal actions of insulin hormone. The body produces insulin, but the cells in the body become resistant to insulin (through changes in their surface receptors) and are unable to utilize it as efficiently. Beta-cells in the pancreas increase their production of insulin, extra causative to hyperglycemia.This time and again remains unnoticed and can contribute to a diagnosis of T2DM. Vitamin D3may also decrease insulin resistance and increase insulin secretion in T2DM[153].

Pancreatic islets have both VDR (vitamin D3receptor) and vitamin D-dependent calcium-binding proteins (CaBP), signifying a role for vitamin D3in insulin secretion. Vitamin D3 effects more the β-cells than the α-cells' function. Its effect on the β-cells is by increasing insulin response to glucose stimulation, but it does not affect basal insulin secretion.Vitamin D- deficient rats have been found to have reduced insulin secretion. It was suggested that the effect of the vitamin D3on insulin secretion and synthesis was independent of the effects of calcium levels. Insulin secretion is a process dependent on changes in intracellular calcium concentration. The effects of vitamin D3on β-cells may be by its regulation of extracellular calcium and calcium flux through the β-cell, or through calcium-independent pathways[154-157].

Vitamin D3 or calcium deficiency may alter the balance between intracellular and extracellular calcium in β-cells, interfering with insulin secretion and probably synthesis. Vitamin D3deficiency may also impair insulin secretion through its associated increase in PTH levels. It was planned that vitamin D3deficiency-associated hyperparathyroidism can really cause an illogical increase in intracellular calcium level **[Ca]**. This PTH- induced increase in **[Ca]** causes impair the calcium signal required for glucose-induced insulin secretion[158]. Whether insulin secretion is influenced by the direct action of vitamin D3through its receptor, or through changes in calcium, or PTH, is a subject of continuing studies. It Is also possible that insulin secretion may be influenced by grouping of different mechanisms Systemic inflammation has been found to increase insulin resistance. Obesity and T2DM are conditions of increased inflammatory reaction[159] and therefore vitamin D3may reduce the insulin resistance in these conditions by its immune modulatory and anti-inflammatory effects.

Vitamin D3deficiency is often associated with obesity and T2DM. The deposition of vitamin D3in the fat stores where it becomes less bioavailable is an accepted mechanism explaining this finding as including lack of sunlight exposure from physical inactivity and sequestration of vitamin D3in subcutaneous fat depots. Vitamin D-deficient obese subjects also have elevated PTH levels. Increased PTH

can decrease insulin sensitivity as show in figure (1-4). There is an evidence states that hyperparathyroidism is associated with reduced insulin sensitivity and increased prevalence of impaired glucose tolerance (IGT) and para-thyroidectomy improves fasting and 2 hr post-prandial plasma glugose levels [160-164].

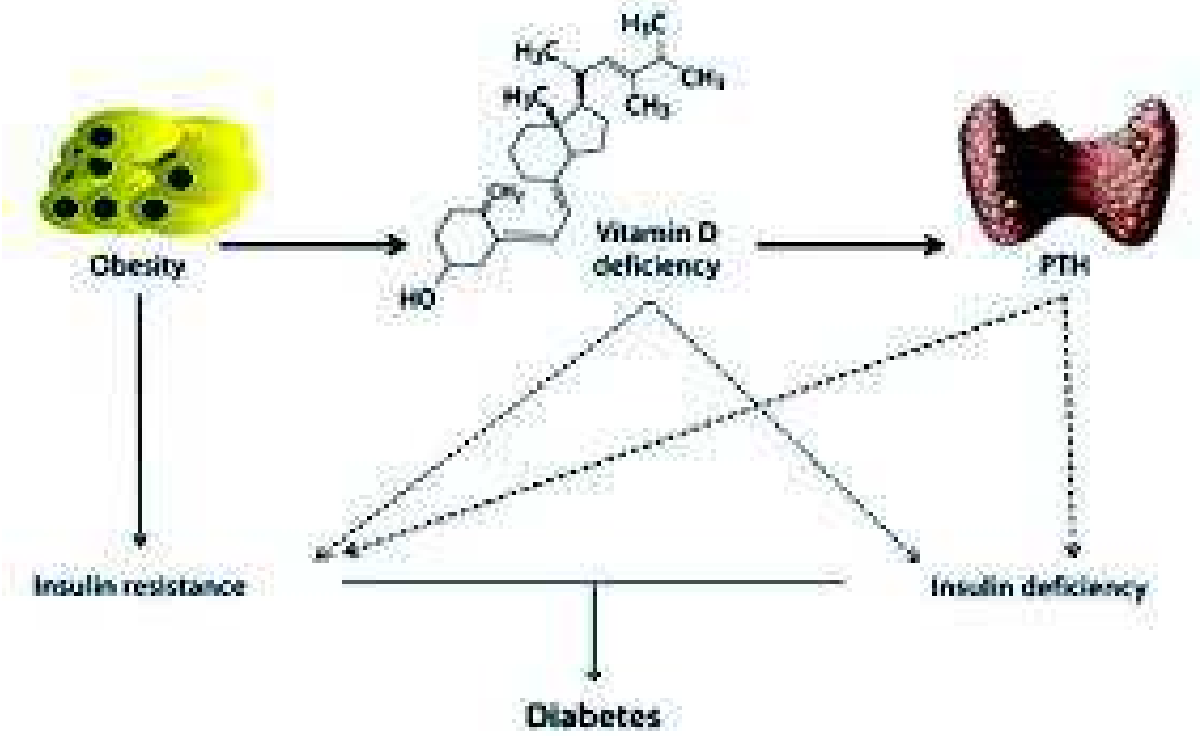


**Fig (1-4) 25-(OH) vitamin D,insulin resistance and obesity correlation[165]**

## 1.6.4.2. 25-Hydroxy Vitamin D3and Cardiovascular Diseases

In addition to the broadly identified effects of vitamin D3on calcium and phosphate homeostasis, other significant effects are implied by the broad distribution of the intracellular vitamin D3receptor in tissues such as leukocytes, vascular smooth muscle cells (VSMC), and juxtaglomerular cells [166--169] and also by the distribution of the 1α-hydroxylase enzyme to a multiplicity of tissues, such as endothelial cells, VSMCs, and different locations in the kidney. Laboratory studies show that **[1,25(OH)2D]** inhibits renin expression in the juxtaglomerular apparatus thereby acting as a negative endocrine regulator of the renin-angiotensin aldosterone

system [170-175] and blocks proliferation of VSMCs, which could influence systemic blood pressure.In humans, skin exposure to ultraviolet radiation, which is the major source of vitamin D, has been associated with lower blood pressure in addition to its another important role[176-182] as show in figure (1-5).

It is consider that vitamin D3 as a protective factor by regulating serum cholesterol levels . Many studies documented that prevalence and mortality rates from coronary heart disease established a physically powerful seasonal pattern with higher rates in the winter, when vitamin D3levels are lowest. Other study reported that blood pressure increased with increasing distance from the equator and suggested that cutaneous synthesized vitamin D3could be playing an important role in the regulation of blood pressure[183,184].

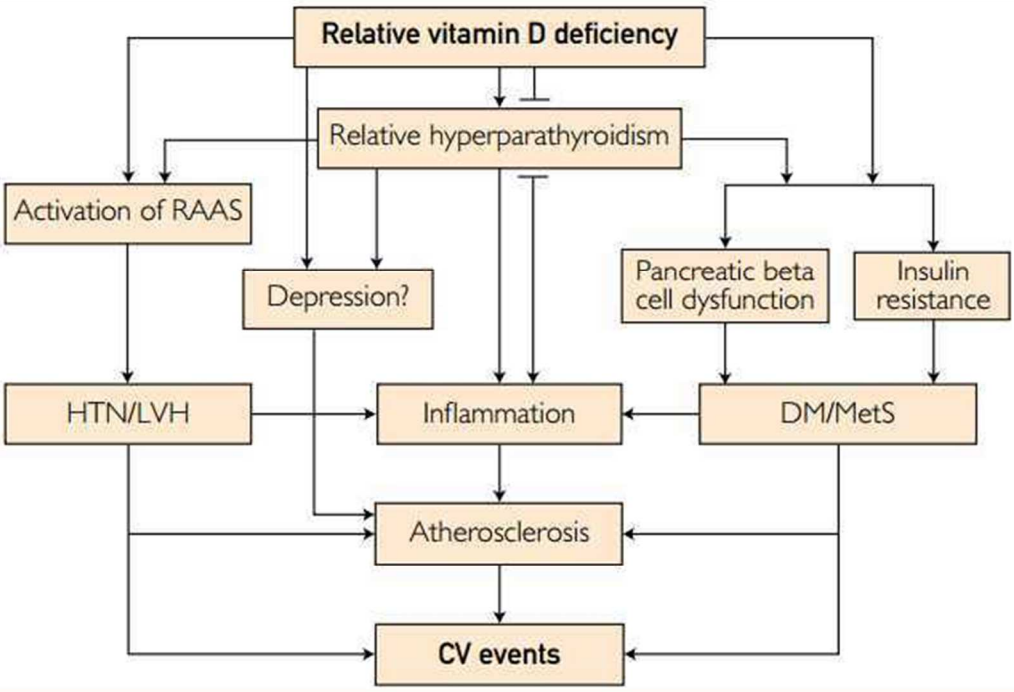


**FIGURE.** Potential mechanisms for CV effects of vitamin D deficiency. CV = cardiovascular; DM = diabetes mellitus; HTN = hypertension; LVH = left ventricular hypertrophy; MetS = metabolic syndrome; RAAS = renin-angiotensin-

**Figure (1-5) Potential mechanisms for CV of vitamin D3 deficiency[185]**

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## 1.7. High Sensitive C-Reactive Protein [186]

C-reactive protein (CRP) is an acute-phase plasma protein, composed of five identical non-glycosylated subunits and a total molecular weight of 105 kDa as show in figure (1-6) . Blood concentration of CRP reflects the presence and intensity of inflammation and . Its physiological role is to bind phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system. CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). It is a member of the pentraxin family of proteins. CRP was diagnosed by Tillett and Francis in 1930. It was initially thought that CRP might be a pathogenic secretion as it was elevated in people with variety of pathological states, including rheumatoid arthritis, tissue trauma, viral or bacterial infection, hepatitis, advanced cancer. and some autoimmune conditions . hs-CRP has also been shown to be a reliable indicator of increased risk of cardiovascular disease and post-operative complications [186] .

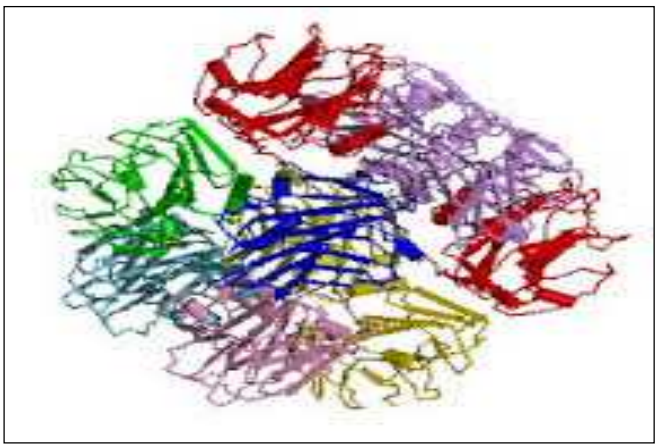


**Fig (1-6).C-Reactive protein (CRP)[187]**

### 1.7.1. Biochemical Roles of hs-C-Reactive Protein

The biochemical role of CRP is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system. CRP binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. Thus, CRP participates in the clearance of apoptotic cells. During the acute phase response, levels of CRP rapidly increase within 2 hours of acute slight, reaching a peak at 48 hours. With resolution of the acute phase response, CRP declines with a relatively short half-life of 18 hours. Measuring CRP level is a monitor for infectious and inflammatory diseases. Marked and speedy increasing of CRP level happen with inflammation, infection, trauma and tissue necrosis, malignancies, and autoimmune disorders [187]. Because there are a large number of different conditions that can increase CRP production, an elevated CRP level does not diagnose a specific disease. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and reached to a peaks at 48 hours. Its half-life is stead, and consequently its level is mainly determined by the rate of production (and therefore the strictness of the precipitating cause). This increasing is due to a ascend in the plasma concentration of IL-6, which is produced predominantly by macrophages in addition to adipocytes[188]. CRP binds to phosphocholine on microbes. It is thought to support in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin mediated phagocytosis), which convey a receptor for CRP. It is also thought to play another essential role in innate immunity, as an early defense system against infections [189].

### 1.7.2. High Sensitivity C-Reactive Protein , Diabetes Mellitus and Coronary Heart Diseases Pathogenesis

Among several markers of inflammation, hs–CRP is found to be significant in people with diabetes. CRP, a pentameric protein produced by the liver has emerged as the ‘ golden marker for inflammation’. It is a non-imuunoglobin protein having five identical subunits. Its protein derives from the fact that it reacts with polysaccharide of streptococcus pneumonia and it is a marker of acute inflammation and is generally used as a measure of inflammatory disease. Recently, CRP increases have been reported in obesity and T2DM. Thus, there is increasing evidence to suggest that insulin resistance is a chronic low-grade inflammatory state. In probable case-control studies, elevated levels of CRP predict the development of T2DM, supporting a possible role for inflammation in diabetogenesis [190-198].

In USA, cardiovascular disease accounts for nearly 40% of all deaths each year.The factors that make up risk score (age, sex, blood pressure, serum total cholesterol or low-density lipoprotein cholesterol level, high-density lipoprotein cholesterol level,cigarette smoking, and diabetes) account for most of the excess risk for incident of coronary heart disease (CHD). However, these factors do not explain all of the excess risk,and approximately 40% of CHD deaths occur in persons with cholesterol levels that are lower than the population average. Several lines of evidence have concerned chronic inflammation in CHD, and inflammatory markers have received much attention as new or emerging risk factors that could account for some of the unexplained variability in CHD risk. Although it is unknown whether CRP is involved in CHD pathogenesis, elevated serum CRP levels are associated with traditional cardiovascular risk factors and obesity [199-204].

## 1.8. Trponin I

### 1.8.1. Definition

**Troponin complex** is a heteromeric protein playing an essential role in the regulation of skeletal and cardiac muscle contraction. The troponin complex is situated on the thin filament of the striated muscle contractile apparatus and consists of troponin T (39 kD), troponin I (26 kD), and troponin C (18 kD), each coded by a separate gene. Each subunit is responsible for a part of troponin complex function as show in figure (1-7) **.** TnT and TnI in cardiac muscle are presented by forms different from those in skeletal muscles**.**TnI inhibits ATP-ase activity of acto-myosin; TnC is a $Ca^{2+}$- binding subunit, playing the main role in $Ca^{2+}$- dependent regulation of muscle contraction[205, 206].

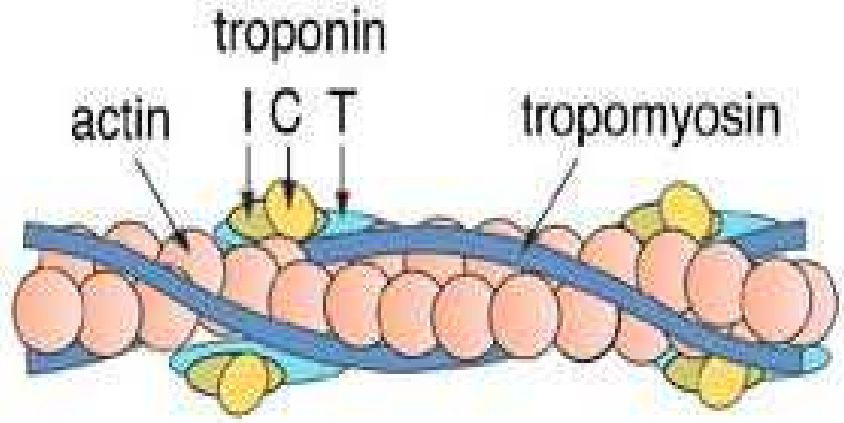



**Fig (1-7) Troponin complex[206]**

### 1.8.2. Characteristics and Functions of Troponins

Human troponin I is presented in cardiac muscle tissue by a single isoform with molecular weight 26 kDa and it consists of 209 amino acid residues. The cTnI molecule contains two serine in the 22 and 23 positions. Both amino acid residues can be phosphorylated in vivo by protein kinase A, so four forms of protein – one dephospho, two monophospho and one bisphospho – can coexist in the cell.

Phosphorylation of cTnI changes the conformation of the protein and modifies its interaction with other troponins as well as the interaction with anti-TnI antibodies**.**

Troponin T structurally binds the troponin complex via troponin C to tropomyosin. Binding of $Ca^{2+}$ to troponin C results in conformational changes and increased affinity of troponin C for troponin I. Troponin I then releases its inhibitory function on actomyosin ATPase, which leads to ATP hydrolysis and muscle contraction. The letter I is given due to its inhibitory character as show in figure (1-8). According to the latest findings significant part of cTnI released into the patient's blood stream is phosphorylated **[207].** In both cardiac and skeletal muscles, muscular force production is primarily controlled by changes in the intracellular calcium concentration. In general, when calcium rises, the muscles contract, and when calcium falls, the muscles relax**.**

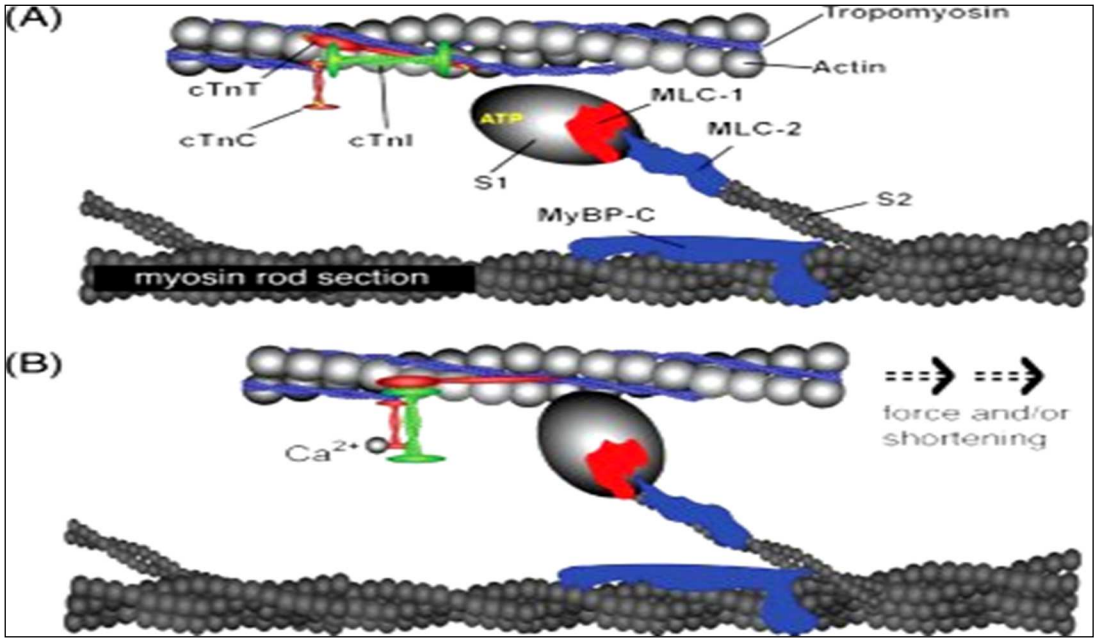



**FIG (1-8) cardiac myofilaments. Myosin (thick filament, contains two head ATPase activity.Thin filament is made up of actin,tropomyosin and troponin (TN). TN-C binds $Ca^{++}$ released by the sarcoplasmic reticulum (S2,S1) TN-I inhibits actin-myosin binding unit $Ca^{++}$ binds to TN-C [207].**

## 1.8.3. Troponin I and Cardiac Diseases

CTnI has been known as a dependable marker of cardiac muscle tissue damage. It is considered to be more sensitive and significantly more specific in diagnosis of the

myocardial infarction than the “golden marker” of last decades – CK-MB isoenzyme, as well as myoglobin and LDH and LDH isoenzymes[208].

Cardiac troponin (I and T) are very sensitive and specific indicators of damage to the heart muscle (myocardium). They are measured in the blood to differentiate between unstable angina and myocardial infarction (heart attack) in patients with chest pain or acute coronary syndrome. A patient who had suffered from a myocardial infarction would have an area of damaged heart muscle and so would have elevated cardiac troponin levels in the blood[209]. This can also occur in patients with coronary vasospasm.

It is important to note that cardiac troponins are a marker of all heart muscle damage, not just myocardial infarction. Other conditions that directly or indirectly lead to heart muscle damage can also increase troponin levels[, such as severe tachycardia (due to supraventricular tachycardia). In severe gastrointestinal bleeding there can also be a mismatch between oxygen require and provide of the myocardium. Troponins are increased in around 40% of patients with critical illnesses such as sepsis. There is an increased risk of mortality and length of stay in the intensive care unit in these patients. Patients with end-stage renal disease can have chronically elevated troponin T levels, which are linked to a poorer prognosis. Troponin I is less likely to be wrongly elevated[210-212].

## 1.9. Plasma Lipid Profile

Total plasma lipid ranged between 400-600 mg/dl[213]. One third is total cholesterol, one third is triacylglycerol and the other includes phospholipids, sphingolipids, free fatty acids and various types of lipoprotein fractions.

Because lipids are hydrophobic and essentially insoluble in aqueous medium of the blood. They are mostly transported in a protein capsule Cholesterol, cholesterol esters, TGs and phospholipids, must be transported through the bloodstream

packaged as lipoproteins. These macromolecules are water-soluble. Therefore, they are complexes with proteins to form lipoprotein, see Figure (1-9) [213] .

The density of the lipids and type of protein determines the destiny of the particle and its effect on metabolism. The concentration of blood lipids depends on intake and excretion from the intestine, and uptake and secretion from cells.The protein part of lipoproteins is called apolipoproteins [214].

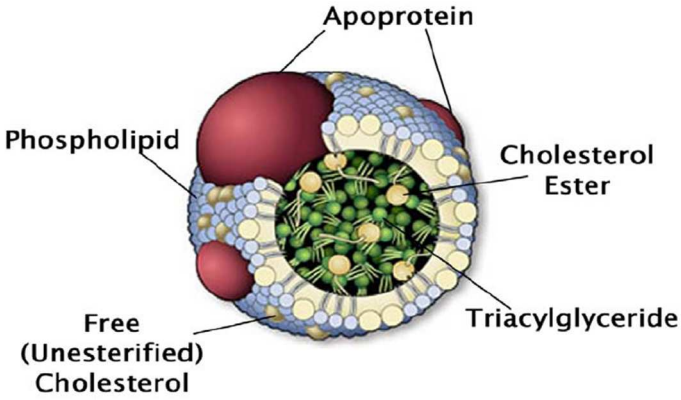



**Fig (1-9) Lipoprotein particle[213]**

## 1.9.1. Cholesterol

Cholesterol is present in tissue and in plasma lipoproteins either as free or combined with a long-chain fatty acid, as cholesteryl ester. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs. Synthesis within the body started with acetyl CoA and finally cholesterol is produced and then eliminated from the body in the bile as cholesterol or converted into bile salts. Essential structural component of animal cell membranes that is required to establish proper membrane permeability and fluidity. In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormone in the body such as corticosteroids, bile acid and vitamin D. Most ingested cholesterol is esterified with the along chain fatty acid on position 3 of hydroxyl group, and esterified cholesterol is poorly absorbed. The body also compensates for any absorption of additional cholesterol by reducing cholesterol synthesis[215,216].

## 1.9.2. Triglyceride T.G

Triacylglycerol or triglycerides (TG) consists of glycerol esterified with three long chain fatty acids molecules. It's present in dietary fat and can be synthesized in the liver and adipose tissue to provide a source of stored energy which can be mobilized when required. For example, during starvation, triacylglycerol containing both saturated and unsaturated fatty acids are catabolized in mitochondria and then act as a source of energy production as ATP , and also as an important components of cell membranes. It constitutes about ninety percent of the lipid in the chylomicron. It is distant from chylomicrons by the action of the enzyme lipoprotein lipase (LPL), located on the luminal surface of the capillary endothelium of adipose tissue, skeletal muscle, cardiac muscle, and lactating breast, so that free fatty acids are delivered to these tissues either to be used as energy sources or after re-esterification to triacylglycerol for energy storage it play an important role in metabolism as energy sources and transporters of dietary fat. This is because fats are less oxidized than are carbohydrates or proteins and hence yield significantly more energy on oxidation. In addition, fats provide about six times the metabolic energy of an equal weight of hydrated glycogen[217]. Triglycerides, is major components of very-low-density lipoprotein (VLDL) and chylomicrons. High levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease and stroke [218].

## 1.9.3. Lipoproteins

Lipoprotein is a biochemical assembly that contains both proteins and lipids, bound to the proteins, which allow fats to move through the bloodstream. Lipids and proteins associate non-covalently to form lipoproteins, which function in the blood plasma as transport vehicles for triacylglycerols and cholesterol because lipids are hydrophobic macromolecules are insoluble in the water of the blood[219] .

## 1.9.3. 1. Structure

Lipids, such as phospholipids, triacylglycerols, and cholesterol, are sparingly soluble in aqueous solution. Hence, they are transported by the circulation as components of lipoproteins, globular micelle like particles that consist of a nonpolar core of triacylglycerols and cholesteryl esters surrounded by an amphipathic coating of protein, phospholipid, and cholesterol. This occurs when the positive charge of the nitrogen atom of the phospholipid (phosphatidylcholine, phosphatidylethanol amine, or phosphatidylserine) forms an ionic bond with the negatively charged hydroxyl ion of the environment. In addition, the shell contains a variety of apoproteins that also increase the water solubility of the lipoprotein. The major carriers of lipids are chylomicrons, VLDL, and HDL. Metabolism of VLDL will lead to IDL and LDL. Metabolism of chylomicrons leads to chylomicron remnant formation[220]. See Table (1-1).

**Table (1-1): The characteristics of the major lipoproteins [219]**

| Lipoprotein class | Density ($g\ mL^{-1}$) | Diameter (nm) | % Protein | % Cholesterol | % Phospholipid | % Triglycerides |
|---|---|---|---|---|---|---|
| HDL | 1.063–1.210 | 5–15 | 33 | 30 | 29 | 8 |
| LDL | 1.019–1.063 | 18–28 | 25 | 50 | 21 | 4 |
| IDL | 1.006–1.019 | 25–50 | 18 | 29 | 22 | 31 |
| VLDL | 0.95–1.006 | 30–80 | 10 | 22 | 18 | 50 |
| Chylomicrons | <0.95 | 100–1000 | <2 | 8 | 7 | 84 |

## 1.9.3.2. Classification

Lipoproteins are particles that contain triacylglycerol, phospholipids and cholesterol and amphipathic proteins called apolipoproteins. Lipoproteins can be differentiated depending upon the basis of their density, but also by the types of apolipoproteins they contain. The percentage and the types of lipid compounds in a lipoprotein affects its density—the lower the density of a lipoprotein, the more lipid it contains relative to protein. The four major types of lipoproteins are chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).In addition to minor classe: IDL (intermediate density lipoproteins, which are intermediate between VLDL and LDL .

## 1.9. 3.2.1. Chylomicrons

Chylomicrons are act to transport exogenous dietary lipids including triacylglycerols and cholesterol from the intestines to the tissues. Chylomicrons are the lowest density of lipoproteins and largest in size and contain the highest percentage of lipid and the smallest percentage of protein. Chylomicrons are assembled in the intestinal mucosa as a means to transport dietary cholesterol and TGs to the rest of the body. The predominant lipids of chylomicrons are TGs. Nascent chylomicrons formed in the intestinal mucosa are secreted into the lymphatic system and enter the circulation at the left subclavian vein through the thoracic duct[221]. In the bloodstream, chylomicrons acquire apoC-II and apoE from plasma HDLs. In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the TG by the action of lipoprotein lipase (LPL), which is found on the surface of the endothelial cells of the capillaries. The apoC-II in the chylomicrons activates LPL in the presence of phospholipids and returns to HDL[222]. The main sites for removal of chylomicrons are the muscle and liver, lipoprotein lipase, an enzyme bound to the capillary endothelium of extra hepatic tissues, hydrolyzes triacylglycerols in chylomicrons, and VLDL into free fatty acids and glycerol. After

entering adipose tissue or muscle, these compounds are esterified and stored. The smaller remanant particles contain mainly cholesterol and pass to the liver where they are metabolized further [222] as show in figure (1-10).

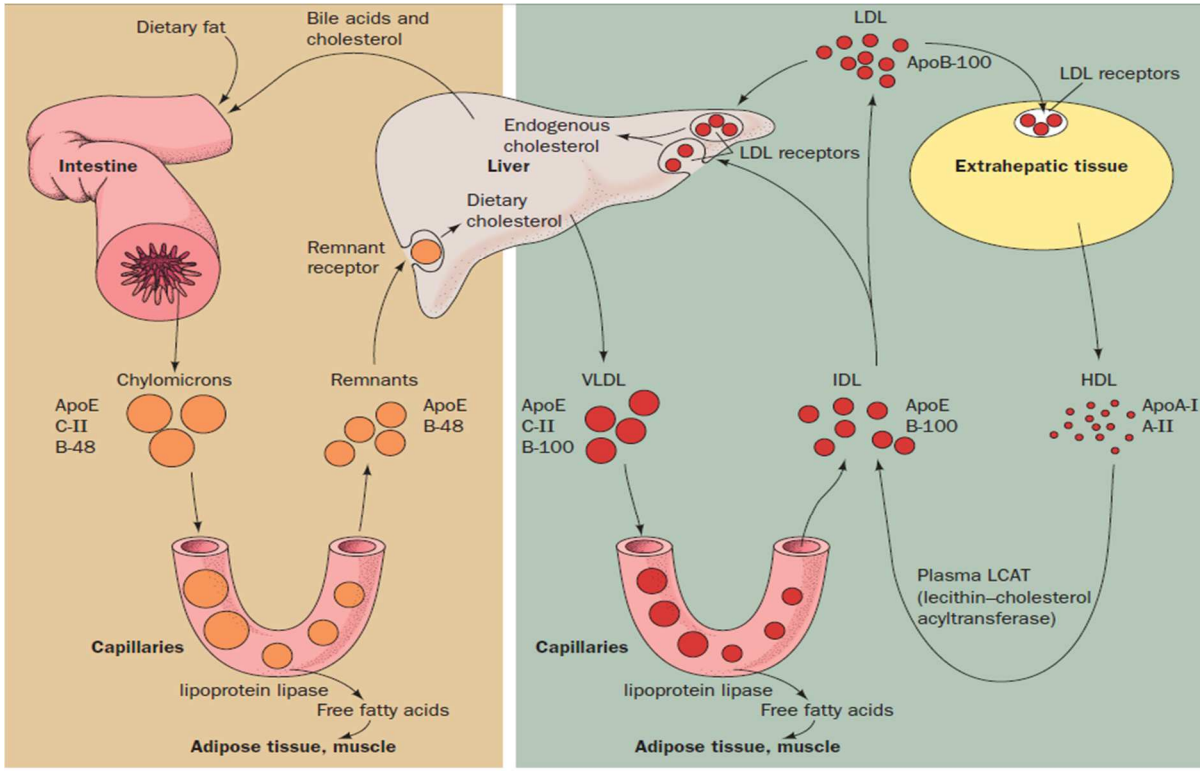



**Figure (1-10) Metabolism of lipoprotein [223]**

## 1.9.3.2.2. Very Low Density Lipoprotein

The excess dietary intake of fat and carbohydrate leads to their conversion into TGs in the liver. These TGs are packaged into VLDLs and released into the circulation for delivery to the various tissues (primarily muscle and adipose tissue) for storage or production of energy through oxidation. VLDLs are, therefore, the molecules formed by liver to transport endogenously derived TGs to extra-hepatic tissues. In addition to TGs, VLDLs contain some cholesterol and cholesteryl esters and the apoproteins, apo-B-100, apo-C-I, apo-C-II, apo-C-III and apo-E. Like nascent chylomicrons, newly released VLDLs acquire apo-Cs and apo-E from circulating HDLs[224] .

The fatty acid portion of VLDLs is released to adipose tissue and muscle in the same way as for chylomicrons, through the action of lipoprotein lipase; glycerol is also released as mentioned above. The action of lipoprotein lipase coupled to a loss of certain apoproteins (the apo-Cs) converts VLDLs to intermediate density lipoproteins (IDLs), also termed VLDL remnants. The apo-Cs are transferred to HDLs. The predominant remaining proteins are apo-B-100 and apo-E. Further loss of TGs converts IDLs to LDL particles[224] as show in figure (1-10).

### 1.9.3.2.3. Low Density Lipoprotein

IDL is converted to low-density lipoprotein particles (LDL), largely by the liver, by removal of additional TGs. In addition to its formation from VLDL, some LDL is produced and released by the liver. LDL is a major transport form of cholesterol and cholesteryl esters. The relative rates of VLDL and LDL release by the liver depend on the availability of cholesterol. If the regulatory pathways signal the liver to increase its cholesterol output, then the liver increases its LDL production. LDL has specific cell surface receptors[225] as show in figure (1-10). It is internalized by receptor-mediated endocytosis. The receptor-LDL complex is transported to lysosomes, for degradation of the particle, while most of the LDL receptors are recycled to the cell surface. The amount of LDL receptor is regulated by the cellular requirement for lipids, with the primary regulatory lipid being cholesterol[226]. High levels of LDL cholesterol are associated with elevated risk of heart disease. LDL cholesterol is the “bad cholesterol” of the popular literature.

The cellular requirement for cholesterol as a membrane component is satisfied in one of two ways: either is synthesized de novo within the cell, or it is supplied from extra-cellular sources, namely, chylomicrons and LDLs. As indicated above, the dietary cholesterol that goes into chylomicrons is supplied to the liver by the interaction of chylomicron remnants with the remnant receptor. In addition, cholesterol synthesized by the liver can be transported to extra-hepatic tissues if packaged in VLDLs. In the circulation VLDLs are converted to LDLs through the

action of lipoprotein lipase. LDLs are the primary plasma carriers of cholesterol for delivery to all tissues[226].

### 1.9.3.2.4. High Density Lipoprotein

High density lipoprotein (HDL) particles are synthesized de novo in the liver and small intestine, as primarily protein-rich disc-shaped particles. These newly formed HDLs are nearly devoid of any cholesterol and cholesteryl esters. The primary apoproteins of HDLs are apo-A-I, apo-C-I, apo-C-II and apo-E. In fact, a major function of HDLs is to act as circulating stores of apo-C-I, apo-C-II and apo-E as show in figure (1-10). HDLs are converted into spherical lipoprotein particles through the accumulation of cholesteryl esters [227]. This accumulation converts nascent HDLs to $HDL_2$ and $HDL_3$ fractions.

Any free cholesterol present in chylomicron remnants and VLDL remnants (IDLs) can be esterified through the action of the HDL-associated enzyme, lecithin: cholesterol acyltransferase, (LCAT). LCAT is synthesized in the liver and so named because it transfers a fatty acid from the C-2 position of lecithin to the C-3-OH of cholesterol, generating a cholesteryl ester and lysolecithin. The activity of LCAT requires interaction with apo-A-I, which is found on the surface of HDLs. HDL is the main transport form of cholesterol from peripheral tissue to liver, which is later excreted through bile, HDL contains the highest protein concentration and it was known to be protective against heart attacks[227].

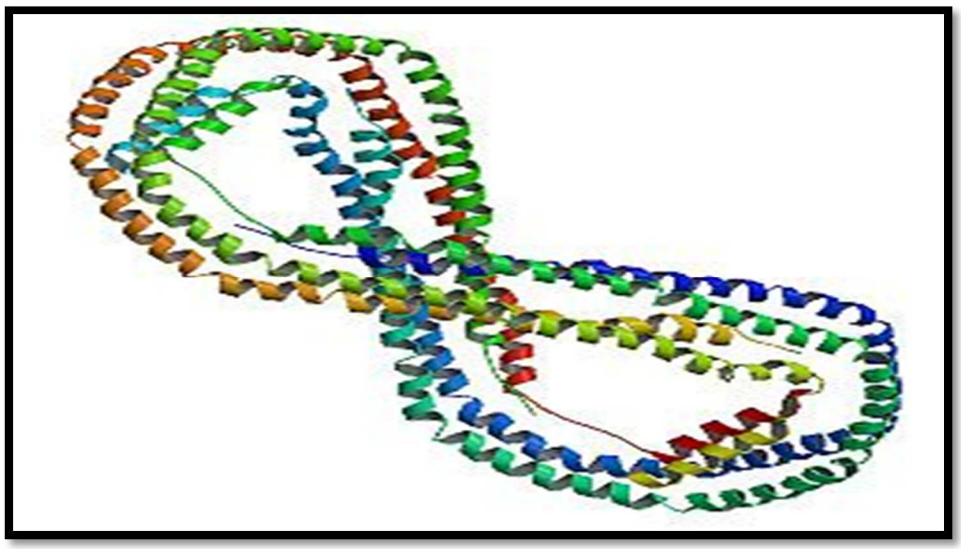


**Fig (1-11) Structure of Apo A-1 protein[228]**

## 1.10. Apolipoprotein - A1

Apolipoprotein A1 (Apo-A1) is primarily found in high density lipoprotein (HDL) particle. It serves the function of preventing the accumulation of cholesterol loaded macrophages which deposit on the arterial wall as foam cells. This is the prominent early feature of atherosclerotic lesion formation ultimately resulting in atherosclerosis. Apo-A1 is a single polypeptide , 243-amino acid with a molecular weight of 28 K**D** [228] as show in figure (1-11). Its primary function is to activate LCAT within the HDL complex, which catalyzes the esterification of cholesterol. This results in a more soluble HDLcholesterol complex which increases the cholesterol transport capacity of the HDL particle for subsequent removal by the liver . Apo A1 is therefore a convenient marker for assessing the cholesterol clearing capacity of the blood, and studies have clearly indicated that it is a better discriminator of angiographically documented coronary artery disease than HDL cholesterol[229] .

Series analysis indicates that apoA-I consists mainly of repeated amphipathic helices of 11 or 22 residues that provide the protein's lipid-binding regions. These assumed helices, as well as similar helices that occur in most other apolipoproteins, have their hydrophobic and hydrophilic residues on opposite sides of the helical cylinders

Furthermore, the polar helix face has a zwitterionic character in that its negatively charged residues project from the center of this face, whereas its positively charged residues are located at its edges[230]. Evidently, the structural role of apoA-I, and probably most other apolipoproteins, is fulfilled by its helical segments rather than by any organized tertiary structure. This suggests that lipoprotein helices float on phospholipid surfaces,much like logs on water. The phospholipids are presumably arrayed with their charged groups bound to oppositely charged residues on the polar face of the helix and with the first few methylene groups of their fatty acid residues in hydrophobic association with the nonpolar face of the helix[230].

Apo A-1 appears to have effects on the atherosclerosis inhibition, revers cholesterol transport and anti-inflammation. Oxidation of specific amino acid residues in Apo A-1 may contribute to atherogenesis by impairing cholesterol efflux from macrophages[231].

## 1.10.1. Apolipoprotein-A1 and Heart Diseases

Atherosclerosis is a progressive disease that begins as intracellular lipid deposits in the smooth muscle cells of the inner arterial wall. These lesions eventually become fibrous, calcified plaques that narrow and even block the arteries. The resultant roughening of the arterial wall promotes the formation of blood clots, which may also occlude the artery. A blood flow stoppage, known as an infraction, causes the death of the deprived tissues. Although atheromas can occur in many different arteries, they are most common in the coronary arteries, the arteries supplying the heart. This results in myocardial infarctions or “heart attacks,” the most common cause of death in Western industrialized countries[231].

As a major component of the high-density lipoprotein complex (protective "fat removal" particles), apo A-I helps to clear fats, including cholesterol, from white blood cells within artery walls, making the WBCs less likely to become fat overloaded, transform into foam cells, die and contribute to progressive atheroma.

Five of nine men found to carry a mutation (E164X) who were at least 35 years of age had developed premature coronary artery disease. One of four mutants of apo A-I is present in roughly 0.3% of the Japanese population, but is found 6% of those with low HDL cholesterol levels[232].

## 1.11.Aim of the study

The aims of the presented study are to investigate the levels of some parameters in sera of T2DM and MetS patients and compared with control group. Then the correlations between these biomarkers will determine to see the effects of different diabetic complication and MetS on the levels of these parameters .

These parameters include:

1. BMI
2. Blood presure
3. Fasting blood sugar
4. 25-(OH)D
5. APO A1 lipoprotein
6. CRP protein
7. Troponin I
8. Lipid profile

## 2.1. Subjects

This study was conducted at Al-Hussein Teaching Hospital / Kerbala- Iraq and in the department of Biochemistry–College of Medicine - Kerbala University . All samples were randomly selected from the patients attending the Diabetic Consultation Unit at the hospital during the period from Dec, 2012 till June, 2013. While control group was selected from healthy hospital staff . It is worth mentioning that patients and healthy controls were free from inflammatory disease or any injury ,trauma or acute inflammations.

### 2.1.1. Patients

The study was carried out on 90 patients attending the diabetic consultation unit at Al-Hussein Teaching Hospital which were divided into two group depending on some criteria .

1. T2DM patients N=40 individuals, equally male and female were suffering from diabetes since at least two years.

2. Patients with metabolic syndrome . N =50 equally male and female.

According to American Heart Association guidelines (2013), any three of the following traits in the same person meet the criteria for the metabolic syndrome:- obesity (BMI $\geq$ 30 kg/m$^2$) or Abdominal obesity( a waist circumference over 102 cm (40 inches) in men and over 88 cm (35 inches) in women) , HDL-C less than 40 mg/dl for men and less than 50 mg/dl for women , fasting blood triglycerides are 150 mg/dL or more, elevated blood pressure of 130/85 mm Hg or higher or taking medicine for high blood pressure , fasting blood glucose of ≥126 mg/dl or above and abnormal cholesterol .

Patients suffered from the following cases were excluded from the current study :-

1. Patients on corticosteroid or thyroxin treatment.
2. Patients complain of chronic liver disease.
3. Patients with thyroid trouble (hyper or hypothyroidism).

4. Patients kidney failure .

The mean±SD of all parameters in all groups were determined to compare among diabetic patients , MetS patients and control group in order to find out the effect of metabolic syndrome and diabetes on the biochemical parameters mean under this study. In the second the correlation among these parameters were determined .

## 2.1.2. Control Group

A group of 30 apparently healthy individuals were ranged from **(26** to **65)** years from males and females . They were collected from those individuals who were free from signs and symptoms of any chronic diseases like diabetes mellitus , hypertension , inflammations and other disease. Non-diabetic (control group) subjects were have BMI $\leq 25$ kg/m$^2$ ; N=30. Show Table- 2-1.

**Table- 2-1.-Details of subjects included in this study**

| Subjects | | Number | Total Number |
|---|---|---|---|
| Type – 2 Diabetes Mellitus | With MetS | 50 | 90 |
| | Without MetS | 40 | |
| Control (Non-Diabetic Subjects) | BMI ≤ 25 kg/m$^2$ | | 30 |

Serum fasting blood sugar, 25-hydroxyvitamin D [25-(OH)D], apolipoprotein-A1 (Apo-A1), hs- CRP (C-reactive protein), troponin I and lipid profile. BMI, systolic

and diastolic blood pressure have been determined in all control and patients included samples in the study.

The correlation coefficient r is used to describe the association between the different studied parameters; $p < 0.05$ was considered statistically significant.

### 2.1.3. Blood Collection

After an overnight fasting (14 hours), about five milliliters of venous blood was aspirated using disposable syringes. The blood was allowed to clot in plain tubes for 30-45 minutes at room temperature and serum was separated after centrifugation at 3000 x g for 10 minutes and transferred into plain plastic tubes and kept frozen at -20 $^{o}$C until the time of assays or analysis **.**

### 2.1.4. Body Mass Index

Body Mass Index (BMI) was calculated by weight (in kilograms) divided by the square of height (in meters); weight and height are measured by the same scale for the all sample subjects[233].

BMI = Weight (kg)/Square Height ($m^2$).The units of BMI is kg/ $m^2$. Body weight was measured with a digital scale to the nearest 0.1 kg

### 2.1.5. Measurement of Blood Pressure

Three measurements were taken and then averaged. If the range of values exceeded 0.5 cm, a fourth measurement was taken and the outlying value discarded. Blood pressure was obtained after the subject had been seated for at least 5 minutes. Systolic and diastolic pressures were measured twice, with value averaged, by the use of an automated blood pressure measurement device.

## 2.2. Materials

### 2.2.1. Instruments

All the instruments and tools which are used in this study are listed in the table - (2.2.) :

**Table - (2-2): Instruments and tools with their suppliers**

| Instruments | Suppliers |
|---|---|
| Centrifuge | H-19F{ KOKUSAN}- Japan |
| UV-VIS Spectrophotometer | (303 )APEL – Japan |
| Water bath | { Memmert }- Germany |
| ELISA Washer | ELX800 – USA |
| ELISA Reader | ELX800 – USA |
| Printer | Epson |
| Freezer | Express cool-Austria |
| Sphygmomanometer | Germany |
| Mini Vidus instrument | BioMérieux - France |
| I-Chroma Reader | SYCOmed-France |
| Vortex –mixture | (Karlkole)Germany |
| Micropipette and Multichannel Pipette | (Watson) Japan |
| | |

### 2.2.2. Chemicals

The chemicals and kits that were used in this study are listed in the Table - (2.3.) with their suppliers:

**Table - (2.3.): Chemicals and kits with their suppliers**

| Chemicals | Source |
|---|---|
| 25-OH Vitamin D ELISA Kit | Eagle Biosciences USA |
| Apo-lipoprotein A1 (Apo-A1) ELISA Kit | Eagle Biosciences USA |
| I-CHROMA HS-CRP Test | BioMrieux®sa. France |
| Vidas Troponin I Ultra (TNIU) Assay kit | BioMrieux®sa. France |
| Glucose kit | Plasmatic , France |
| Total cholesterol MR kit | Linear, Spain |
| Triglyceride kit | BioMrieux®sa. France |
| HDL-cholesterol kit | Linear, Spain |

## 2.3. Methods

## 2.3.1. Determination of Blood Glucose Concentration

### 2.3.1.1. Principle

Glucose is oxidized by glucose oxidase to gluconate and hydrogen peroxide according to the following equation [234]:

$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} H_2O_2 + \text{D-gluconate}$$

$$2H_2O_2 + \text{phenol} + 4\ \text{Amino-antipyrine} \xrightarrow{\text{Peroxidase}} 4H_2O + \text{Quinonimine}$$

### 2.3.1.2.Reagents

| | | |
|---|---|---|
| **Reagent 1 Buffer solution** | Tris buffer PH 7 | 100 mmol/l<br>0.3 mmol/l |
| **Reagent 2** | Glucose oxidase<br>Peroxidase<br>4-Amino-antipyrine | 10000 U/l<br>1000 U/l<br>6.2 mmol/l |
| **Reagent 3 Standard** | Standard glucose | 100 mg/dl<br>5.56 mmol/l |

### 2.3.1.3. Procedure

| | **Reagent Blank** | **Standard** | **Sample** |
|---|---|---|---|
| Working Solution | 1 ml | 1 ml | 1 ml |
| Standard | - | 10 µl | - |
| Sample or Unknown | - | - | 10 µl |

Tubes were mixed and incubated for 10 minutes at 37 ºC or 30 minutes at 25ºC then they were measured at wavelength about (505 nm) at room temperature.

### 1.3.1.5. Calculation

$$\text{Glucose concentration (mmol/l)} = \frac{A_{sample}}{A_{standard}} \times \text{Conc. of standard glucose}$$

Concentration of standard glucose solution = 100mg/dl

## 2.3.2. Determination of Serum 25-OH Vitamin D concentration

### 2.3.2.1. Principle

The new 25-OH Vitamin D ELISA assay test kit is designed for the determination of 25-OH Vitamin D in human serum or plasma samples. In the first analysis step, the calibrators and patient samples are diluted with biotin-labelled 25-OH vitamin D and added to microplate wells coated with monoclonal anti-25-OH vitamin D antibodies. During the incubation an unknown amount of 25-OH vitamin D in the patient sample and a known amount of biotin-labelled 25-OH vitamin D compete for the antibody binding sites in the microplate wells plate. Unbound 25-OH vitamin D is removed by washing. For the detection of bound biotin-labelled 25-OH vitamin D, a second incubation is performed using peroxidase-labelled streptavidin. In a third incubation using the peroxidase substrate tetramethylbenzidine (TMB) the bound peroxidase promotes a color reaction. The color intensity is inversely proportional to the 25-OH vitamin D concentration[235] .

Table (2-4) Description state of 25-OHD3[235]

| Description state of 25-OHD3 | Concentration ng/ml |
|---|---|
| Very severe Vitamin D deficiency | <5 |
| Severe Vitamin D deficiency | 5-10 |
| Vitamin D deficiency | 10-20 |
| Suboptimal Vitamin D provision | 20-30 |
| Optimal Vitamin D level | 30-50 |
| Upper norm | 50-70 |

**2.3.2.2. Reagents**

1. Micro titer wells, 12 × 8 strips, 96 wells coated with monoclonal anti- antibody (vitamin D2 and D3).
2. Standards 1 – 6 (CAL 1 – CAL 6) 6 vial , 1ml each, colored red –brown ready for use with concentration as:

| Standard | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|
| ng/ml | 120 | 60 | 25 | 10 | 4 | 0 |

3. Control 1 + 2 (Conc. 1 Conc. 2 ) 2 vial , 1 ml each, colored red-brown ,to be diluted 1:26 in working strength biotin values for the control are given on the vial label.
4. Biotin , 100 x concentrated; colored blue.
5. Sample buffer , colored yellow, ready for use .
6. Enzyme conjugate , colored blue, ready for use
7. Substrate from TMB / $H_2O_2$, ready for use.
8. Wash Buffer (WASH) 10 x concentrated
9. Stop Solution (STOP) ready for use 0.5 M sulphuric acid.

## 2.3.2.3. Preparation of Reagents

**A. Coated wells** Ready for use.

B. **Calibrators and controls:** The reagents was mixed thoroughly before use.

**C. Biotin:** The biotin is a 100 x concentrate, was mixed thoroughly before diluting. The required volume was removed with a clean pipette tip and were diluted in sample buffer (1 part biotin + 99 parts sample buffer). Example: 1 ml biotin concentrate plus 99 ml sample buffer.

**D. Sample buffer:** It was used for sample dilution after adding the biotin concentrate.

**E. Enzyme conjugate:** Ready for use. The enzyme conjugate was mixed

thoroughly before use.

**F. Wash buffer:** The wash buffer is a 10x concentrate. The quantity required was removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).

**G. Chromogen/substrate solution:** Ready for use.

**H. Stop solution:** Ready for use.

**2.3.2.4. Procedure**

1. 200 µl of each pre-diluted standards 1 - 6, pre-diluted control 1 and control 2 were dispensed into the appropriate wells.
2. 200 µl of each were dispensed patient sample diluted in biotin/sample buffer into each well to be used.
3. The wells were incubated at room temperature (+18 °C to +25 °C) for 2 hours.
4. After the 2 hour incubation, the samples were aspirated or discarded from the wells and were added 300 µl of wash buffer and were aspirated or discarded again. Washing were repeated with each 300 µl wash buffer two more times for a total of three washings. The inverted wells gently were tapped on a clean dry absorbent surface to remove any droplets of wash buffer.
5. 100 µl of Enzyme Conjugate were dispensed into each well and incubated for 30 min at room temperature (+18 °C to +25 °C).
6. After the 30 minute incubation, the reagent were aspirated or discarded from the wells, were added 300 µl of Wash Buffer were added and aspirated or discarded again. washing was repeated with each 300 µl Wash Buffer two more times for a total of three washings. Tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of wash buffer.
7. 100 µl of chromogen / substrate solution were dispensed into each well and were incubated for 15 minutes at room temperature without shaking.
8. The substrate reaction was stopped by addition of 100 µl of Stop Solution to each well (this caused the blue color to turn yellow).

9. Photometric measurement of the color intensity were made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution**.**

### 2.3.2.5. Calculation

The absorbance for each set references standard , control and sample was measured. Standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper. With absorbance on vertical (y) axis and concentration on the horizontal (x) axis. The corresponding concentration of 25-(OH)D (ng/ml) from the standard curve was determined.

### 2.3.2.6. Standard curve of 25-(OH)D determination

Result of the typical standard run with absorbance reading at 450 nm shown on the y axis against 25-(OH)D concentration shown on the x axis.

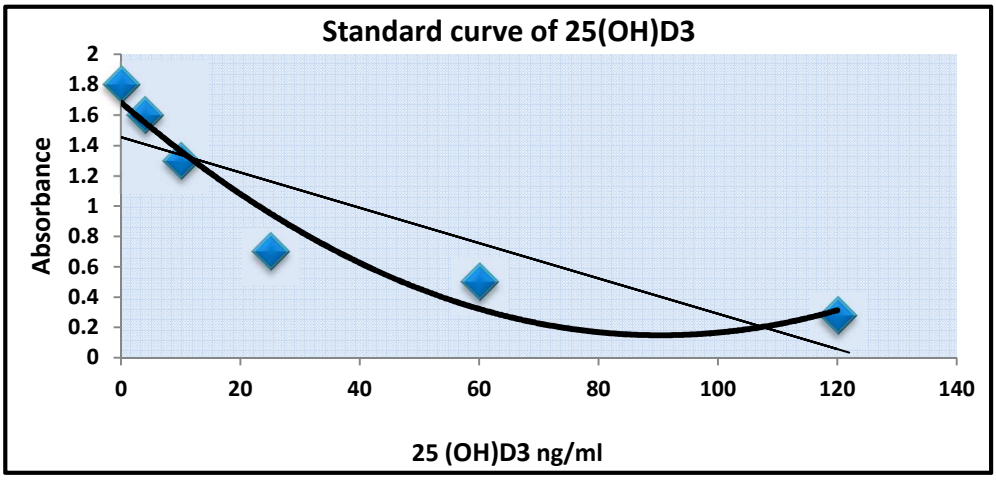



Fig. (2-1) : **Standard curve of human 25-(OH)D obtained by ELISA reader at wave length 450nm.**

## 2.3.3. Determination of Serum Apolipoprotein-A1 concentration

### 2.3.3.1. Principle

The apolipoprotein-A1 (Apo-A1) ELISA assay kit determines human apolipoprotein-A1 according to the "sandwich" principle. Apolipoprotein-A1 in samples and standards binds to antibodies which are coated to the micro-titer plate[236]

.

After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a micro-titer plate reader. The apolipoprotein-A1 concentration can be calculated from the standard curve.

### 2.3.3.2. Reagents

1. Anti-human apo-A1 coated micro well strips – 12x8 with plastic frame
2. HRP conjugated affinity purified goat anti-apo-A1 – 12 ml
3. Apo-A1 standard (pre-diluted 1:10,000) – 1 ml (store at -20 oC)
4. TMB/Peroxide Substrate color developer III – 12 ml
5. Stop solution: Sulfuric acid termination reagent (0.5 N) – 12 ml
6. 15X wash buffer concentrate – 2 x 60 ml

#### 2.3.3.2.1. Reagent and Sample Preparation:

1. The wash buffer provided in the apolipoprotein-A1 (Apo-A1) ELISA assay kit was diluted 15 X , 1:15 using one part wash buffer concentrate and 14 parts reagent grade water.
2. Each serum or plasma specimen was diluted to be tested 1:10,000 with diluted wash buffer.

3. Serial two-fold dilutions of the human apo-A1 standard *were prepared* (neat, 1:2, 1:4, 1:8, 1:16, 1:32. 1:64) with the diluted wash buffer. The diluted wash buffer was used alone in the blank control well.

### 2.3.3.3. Procedure

1. 100 µl of diluted specimen or standard were added to each micro well.
2. The wells were incubated at room temperature for 2 hours.
3. Each micro well were washed four times with diluted wash buffer (dilute wash buffer 1:15 with reagent grade water).
4. 100 µl HRP conjugated goat apo-A1 was added into each well.
5. The wells were incubated at room temperature for 2 hours
6. Washing as in step 3.
7. 100 µl TMB/peroxide substrate was added and incubated at room temperature for 30 minutes.
8. The reaction was terminated with 100 µl of Stop Solution 0.5N sulfuric acid.
9. The absorbance or OD was read at 450 nm after using the blank control well for zero the micro-well reader.

### 2.3.3.4. Calculation

1. The absorbance for each set references standard ,control and sample was calculated
2. Standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in µg/dL on graph paper. With absorbance on vertical (y) axis and concentration on the horizontal (x) axis .
3. The corresponding concentration of Apo A-1 (µg/dL) from the standard curve was determined .

### 2.3.3.5. Standard curve of Apo A-1 determination

The result of the typical standard runs with absorbance reading at 450 nm shown on the y axis against Apo A-1 concentration shown on the x axis.

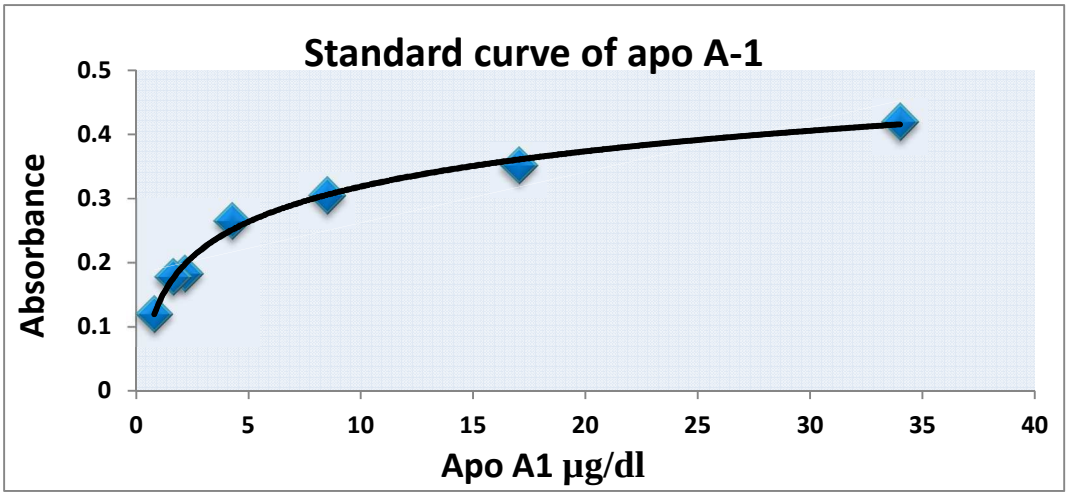



**Fig. -(2-13) :Standard curve of human apo A-1 obtained by ELISA reader at wave length 450nm.**

## 1.3.4. Determination of Serum HS-CRP Concentration

### 2.3.4.1. Principle

The **I-CHROMA HS-CRP Test** is based on fluorescence immunoassay technology[237]. The **I-CHROMA HS-CRP** Test uses a sandwich immune-detection method, such that by mixing detector buffer with blood specimen in test vial, the fluorescence-labeled detector anti-HS-CRP antibody in buffer binds to HS-CRP antigen in blood specimen. As the sample mixture is loaded onto the sample well of the test device and migrates the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and HS-CRP are captured to anti-HS-CRP sandwich pair antibody that has been immobilized on test strip. Thus the more HS-CRP antigen is in blood specimen, the more complexes are accumulated on test strip.

Signal intensity of fluorescence of detector antibody reflects amount of HS-CRP captured and is micro-processed from I-CHROMATM Reader to show HS-CRP concentration in blood specimen. The default result unit of **I-CHROMA HS-CRP Test** is displayed as an mg/L from I- CHROMATM Reader.

### 2.3.4.2. Procedure

1. ID Chip was checked and inserted onto the instrument. So that the Test Device lot matches with ID Chip.
2. A tube was took out containing Detector Buffer from refrigerator and was left it at room temperature for a couple of minutes.
3. A puncture was made on the top of the detector tube by inserting an empty blood collection capillary.
4. A prick was made on a finger with a lancet. whole blood were drew with a blood collection capillary.
5. The capillary and the tube were collected into one.
6. The assembled tube was shook gently 5 times by inversion to take the blood out of capillary.
7. The cap was removed off the top of tube. Two drops of reagent were removed onto the paper towel before applying to the cartridge.
8. Two drops only were applied onto the sample well of a cartridge
9. Test Device was inserted onto the holder of **I-CHROMA** Reader. Instrument will automatically scan the Test Device after 3 min.
10. The results were examined on the display screen of I-CHROMA Reader.

### 1.3.4.3. Calculates:

The HS-CRP concentration of samples were read by screen of I-CHROMA Reader in mg/dl .

## 2.3.5. Determination of Serum Troponin I concentration

### 2.3.5.1. Principle

The VIDAS Troponin I Ultra (TNIU) assay is one-step, sandwich enzyme-linked fluorescent immunoassay (ELFA) performed with an automated VIDAS or a Minividas instrument [238]. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR), serves as the solid phase as well as a pipettor for the assay. Reagents for the assay are pre-dispensed in the sealed TNIU reagent strips [238] . All assay steps and assay temperatures are controlled by the instrument. The sample is transferred into the wells containing the conjugate (alkaline phosphatase-labeled mouse monoclonal anti-cardiac troponin I antibody). The sample and conjugate mixture is cycled in and out of the SPR several times. Unbound sample is removed from the SPR during the wash step. During the detection step, the fluorescent substrate (4-methyl-umberlliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme remaining in the SPR catalyzes the hydrolysis of the substrate into a fluorescent product 4-methylumbellferone. Fluorescence is measured at 450 nm wavelength by the optical scanner in the instrument. The intensity of the fluorescence is directly proportional to the concentration of analyte present in the sample.

### 2.3.5.2. Reagents

Each VIDAS Troponin I Ultra kit contains 60 tests.

1. The kit is comprised of: 60 TNIU reagent strips. The TNIU reagent strips consist of 10 wells. Five of the wells contain either conjugate (alkaline phosphatase-labeled mouse monoclonal anti-cardiac troponin I antibody and preservative), wash buffer. One well is designated for the sample and the remaining wells are empty.
2. 60 Solid Phase Receptacles (SPR), The interior of the (SPR) are coated with mouse monoclonal anti-cardiac troponin I antibody.

3. TNIU controls (C1 and C2), are supplied with the kit as four, 2 ml vials of lyophilized human serum, troponin I, and preservative; 2 vials of C1 and 2 vials of C2.
4. TNIU calibrators (S1 and S2), are supplied with the kit as four, 2 ml vials of lyophilized human serum, troponin I, and preservative; 2 vials of C1 and 2 vials of C2.
5. TNIU Diluent, is ready-to-use. It is supplied as one 2 ml vial and contains human serum with preservatives.
6. One Master Lot Entry (MLE) Card.

### 2.3.5.3. Procedure

1. The required reagents were removed from the refrigerator .
2. One TNIU strip and one TNIU SPR were used for each sample, control or calibrator to be tested.
3. 200 µl for each of calibrator ,controls and samples were mixed by using a vortex type mixer.
4. The TNIU SPRS and "TNIU" strips were inserted into the instrument ,by this step the assay immediately is initiated and all the assay steps are performed automatically by the instrument.
5. The results were analyzed automatically by the computer once the assay completed. Fluorescence was measured twice in the reagent strips reading cuvette for each sample tested.

### 2.3.5.3. Calculation

Concentration of samples were read from computer once the assay completed in ng/ml

### 2.3.6. Measurements of Serum Lipid Profiles

#### 2.3.6.1. Total Serum Cholesterol

##### 2.3.6.1.1. Principle

Total serum cholesterol (TC) was measured according to the method described by Allain, *et al* (1974), as shown in the following Cholesterol concentration was determined enzymatically reactions [239].

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-amino-antipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4H2O$$

##### 2.3.6.1.2. Reagents

| Reagents | Composition | |
|---|---|---|
| Reagent 1 (Buffer) | Phosphate buffer | 100 mmol/l |
| | Chloro-4-phenol | 5.0 mmol/l |
| | Sodium chloride | 2.3 mmol/l |
| | Triton x 100 | 1.5 mmol/l |
| | Preservative | |
| Reagent 2 (Enzymes) | Cholesterol oxidase | 100 IU/l |
| | Cholesterol esterase | 170 IU/l |
| | peroxidase | 1200 IU/l |
| | 4-amino-antipyrine | 0.25mmol/l |
| | PEG 6000 | 167μmol/l |
| Reagent 3 (Standard) | Cholesterol 200 mg/dl (5.17 mmol/l) | |

### 2.3.6.1.3. Procedure

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (buffer), mix gently until complete dissolution (approximately 2 minutes) to prepare work reagent. The procedure was carried out as in the following:

| Reagents | Blank | Standard | Sample |
|---|---|---|---|
| Reagent | 1 ml | 1 ml | 1 ml |
| Demineralized water | 10 µl | - | - |
| Standard | - | 10 µl | |
| Sample | - | - | 10 µl |

The tubes were mixed and then let stands for 5 minutes at 37˚C. Record absorbance at 500 nm (480-520) against blank. The color is stable for 1 hour.

### 2.3.6.1.4. Calculation

$$\text{Cholesterol (mmol/l)} = \frac{A_{Sample}}{A_{Standard}} \times \text{Conc. of standard cholesterol}$$

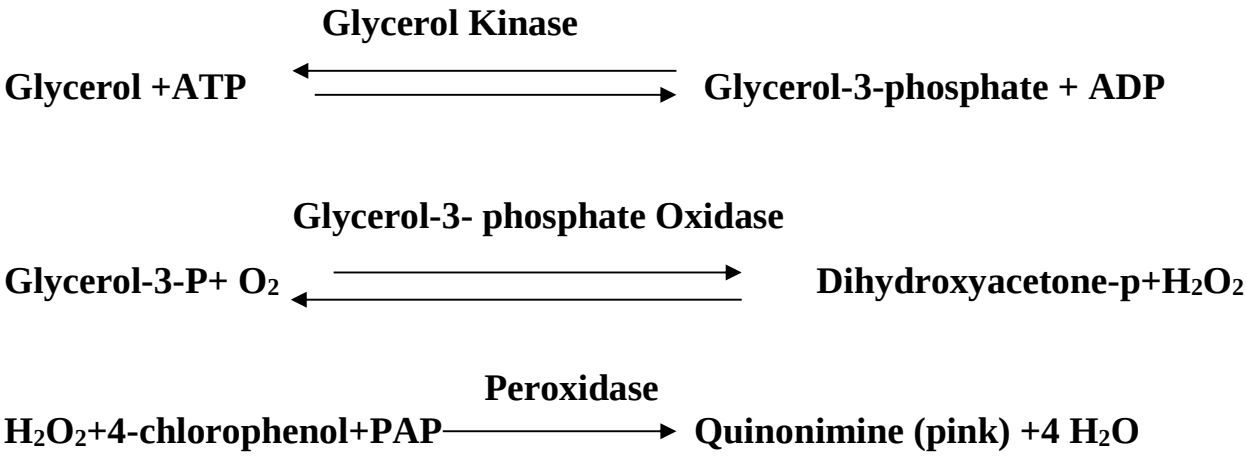
Standard Cholesterol Solution = (5.17mmo/l)

## 2.3.6.2. Serum Triglyceride

### 2.3.6.2.1. Principle

Serum Triglyceride concentration was determined enzymatically according to the method described by Allain (1982) and principle method associated with Trinder reaction, as shown in the following reactions [240].

$$\textbf{Triglycerides} \xrightarrow{\textbf{Lipase}} \textbf{Glycerol + free fatty acids}$$




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

**2.3.6.2.2. Reagents**

| Reagents | Composition |
|---|---|
| Reagent 1 (Buffer) | PIPES 100 mmol/l<br>$MgCl_2$ 9.8 mmol/l<br>Chloro-4-phenol 3.5 mmol/l<br>Preservative |
| Reagent 2 (Enzymes) | Lipase 1000 IU/l<br>Peroxidase 1700 IU/l<br>Glycerol-3-p-oxidase 3000 IU/l<br>Glycerol kinase 660 IU/l<br>PAP 0.5 mmol/l<br>ATP 1.3 mmol/l |
| Reagent 3 (Standard) | Glycerol equivalent to triglycerides 200 mg/dl or (2.28 mmol/l) |

#### 2.3.6.2.3. Procedure

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

| Reagents | Blank | Standard | Sample |
|---|---|---|---|
| Reagent | 1 ml | 1 ml | 1 ml |
| Demineralized water | 10 µl | - | - |
| Standard | - | 10 µl | |
| Sample | - | - | 10 µl |

The tubes were mixed, and then let stands for 5 minutes at 37˚C or 10 minutes at room temperature. Record absorbance at 500 nm (480-520) against blank. The colored complex is stable for 1 hour.

#### 2.3.6.2.4. Calculation

$$\text{Triglyceride Concentration (mmol/l)} = \frac{A_{Sample}}{A_{Standard}} \times 2.28$$

Standard triglyceride solution = 200mg/dl

### 2.3.6.3. Serum High Density Lipoprotein-Cholesterol

#### 2.3.6.3.1. Principle

LDL, VLDL and chylomicron from specimens were precipitated by phospho-tungstic acid and magnesium chloride. HDL-cholesterol obtained in supernatant after centrifugation is then measured with total cholesterol reagent [241].

### 2.3.6.3.2. Reagents

| Reagents | Composition | |
|---|---|---|
| Reagent 1 (precipitant) | Phosphotungstic acid | 13.9 mmol/l |
| | Magnesium chloride pH 6.2 | 490 mmol/l |

### 2.3.6.3.3. Procedure

The procedure was carried out as indicated in the following table:

| Reagents | Volume |
|---|---|
| Serum | 0.5 ml |
| Precipitant (reagent 1) | 50 µl |

The tubes were mixed vigorously, then let stand for 10 minutes at room temperature. Centrifuge 15 minutes at 1400-1800 x g. Then apply next procedure which includes measurement of cholesterol in supernatant:

| Reagents | Blank | Standard | Sample |
|---|---|---|---|
| Reagent | 1 ml | 1 ml | 1 ml |
| Distilled water | 25 µl | - | - |
| Standard 2.58mmol/l | - | 25 µl | |
| Supernatant | - | - | 25 µl |

The tubes were mixed, and then let to stands for 5 minutes at 37˚C. The absorbance were measured at 500 nm (480-520) against blank. The colored complex solution is stable for 1 hour.

### 2.3.6.3.4. Calculation

$$\text{HDL-C Concentration (mmol/l)} = \frac{A_{Sample}}{A_{Standard}} \times 2.58$$

Standard cholesterol solution = 2.58 mmol / l

## 2.3.6.4. Measurement of serum Low Density Lipoprotein-Cholesterol

Low density lipoprotein-cholesterol (LDL-C) concentration was calculated indirectly by using Friedewald and colleagues equation (2006) , which become the more common method in routine clinical labs[242].

**LDL-C = [Total-chol.] – [HDL-C] –[TG/5 ]**

Where all concentrations are given in mg/100 ml (TG / 2.22 is used when LDL-cholesterol is expressed in mg/100 ml). The factor [TG / 5] is an estimate of VLDL-cholesterol concentration, and is based on the average ratio of triglyceride to cholesterol in VLDL fraction[243].

In practice , the Friedewald calculation is reasonably accurate, but there are several well-known circumstances under which the Friedewald equation cannot be used. First, calculation is precluded in samples that have TG concentration above 400 mg/100 ml or in those that contain great amount of chylomicrons (non-fasting specimens). At high TG concentration, , the factor [TG/5] as an estimate of VLDL-cholesterol concentration is not appropriate because such samples can also contain chylomicrons, chylomicron remnants, or VLDL remnants, all of which have higher TG/cholesterol ratios. Under these circumstances , the use of the factor [TG/5] would overestimate VLDL-cholesterol and therefore underestimate LDL-cholesterol.

The Friedewald equation has been found to be more accurate in samples with TG levels below 200 mg/100 ml, but the error becomes unacceptably large (i.e. , <10%) at TG concentration greater than 400 mg/100 ml.

## 2.4. Statistical Analysis

Statistical analysis was done by SPSS statistical software (SPSS 20 for Windows, standard version). The results were presented as mean ± standard deviation (SD).Continuous variables were tested for normality according to the Kolmogorov-Smirnov test. Independent sample T-test and Mann-Whitney U-tests were performed to compare the results. The correlation analysis was done using the Pearson test, a P value of <0.05 was considered statistically significant.

## 3.1. Comparison between T2DM and control group

### 3.1.1 .Fasting blood sugar (FBS)

The results obtained show that serum FBS was significantly high in T2DM patients group compared with normal control group ($P < 0.001$), Table (3-1).

The present study showed that serum FBS correlates negatively with serum 25-(OH)$D_3$ , HDL- cholesterol, ($r = -0.560$, $p=0.0001$), ($r =-0.397$, $p=0.0001$) respectively show Figure (3-1,3-2) and also FBS correlates non-significant with serum Apo-A1($r= -0.085$, $p>0.05$). While it has positive correlation with serum hs-CRP and BMI ($r= 0.305$,$p=0.003$), ($r=0.281$, $p=0.006$) respectively. Also it correlates positively but non-significant with total cholesterol, LDL-C, VLDL-C, TG, Total-cholesterol/HDL-C ratio, Troponin I, systolic and diastolic blood pressure ($r = 0.011$), ($r=0.017$), ($r= 0.048$), ($r= 0.051$), ($r = 0.212$), ($r = 0.082$), ($r = 0.190$), ($r =0.163$) respectively at $p >0.05$.

The present study showed a relationship between diabetes and increase appetite, which leads to increase bad cholesterol and decrease HDL-C this finding consist with previous reported about correlation between diabetes and decrease HDL-C levels [244]. Results of the present study are in good agreement with previous studies regarding the role of 25(OH)$D_3$ in diabetes which suggested that overweight or obesity are booked vitamin D in the subcutaneous fat, because the 25-(OH)$D_3$ inhibits rennin enzyme also RAAS is controlled by sympathetic nervous system SNS ,which is activated by many factors associated with T2DM , including hyperinsulinemia, excess weight , and an unhealthy diet [245,246].

### 3.1.2 . 25-hydroxy Vitamin D3

The results obtained show that serum 25-(OH)D was significantly low in T2DM patients group compared with normal control group ($P < 0.001$) as show in Table (3-1).

Serum 25-(OH)D correlates negatively with Total-cholesterol/HDL-C ratio, systolic and diastolic blood pressure, BMI and hs-CRP (r= ─ 0.540, p=0.0001), (r= ─ 0.419, p=0.0001), (r= ─ 0.408, p=0.0001), (r= ─ 0.266, p=0.012), (r= ─ 0.295, p=0.004) respectively as show in Figures (3-3,3-4,3-5), and it correlates positively with serum HDL-C and Apo-A1(r=0.540,p=0.0001), (r =0.472,p=0.0001) respectively. While it correlates negatively but non-significant with serum total cholesterol, LDL-C , TG and Troponin I (r = ─ 0.206), (r = ─ 0.218), (r = ─ 0.227), (r= ─ 0.04) respectively at p> 0.05.

The results of the present study showed that diabetic subjects were older with mean age of 51 years than the normal subjects. They also had higher systolic and diastolic blood pressure ( P<0.05 ), FBS ( P<0.001 ) especially with smokers of diabetic patients.

Also this study showed that vitamin D deficiency or insufficiency status was associated with an increase risk of T2DM. Vitamin D deficiency or insufficiency was present in 68% of the samples under the study and the women undergo this deficiency more than the men , and serum 25-(OH)D levels were lower in patients who were over weight/obese or associated with hypertension and diabetic when compared with individuals of non-diabetic ,healthy weight(16.8 ± 9.8 ng/ml vs. 36.0 ± 9.4) respectively .

More recently, animal and human studies have suggested that vitamin D is a potential modifier of diabetes risk [247-249]. Vitamin D has been show to play an important role in the disorders of glucose and insulin metabolism[250,251].

The results from the present study are in good agreement with previous studies regarding the role of 25-(OH)D in T2DM, although some authors have reported on conflicting results [252,253] or the relationship did not retain statistical significance in multivariate models of diabetics[254].

Vitamin D deficiency is associated with major effects on the innate immune system. This could potentially influence the risk of diabetes and that clarifies the role of vitamin D in both insulin secretion ,insulin resistance and reducing risk of infection of islet cells where vitamin D may directly induce insulin secretion by binding the active form 1,25-$(OH)_2D_3$ to receptors are present on pancreatic β-cells and skeletal muscle, and the activating enzyme, 25-(OH)D-1α-hydroxylase, is expressed in pancreatic β-cells[255]. The indirect effects of vitamin D may be mediated via its important role in regulating extracellular calcium and calcium flux through the beta cell That's where vitamin D-induced stimulation of osteocalcin , which may improve insulin sensitivity [456]. Importantly, this study confirms the findings of previous studies which have suggested an association between deficiency of Vitamin D3 status and cardiovascular risk factors (eg, hypertension) [257,258].

### 3.1.3. HS-C-reactive protein

The results obtained showed that serum hs-CRP was significantly high in T2DM group compared with normal control group ($P < 0.05$) Table (3-1).

Serum hs-CRP correlates positively with BMI ($r= 0.253, P=0.013$). It also has a positive non-significant correlation with, LDL-C , TG ,Troponin I , systolic and diastolic blood pressure ($r= 0.076$), ($r = 0.010$) , $r = (0.057)$, ($r= 0.116$) , ($r = 0.113$) respectively at $p>0.05$ .While it has a negative non-significant correlation with serum HDL-C and Apo-A1at $p>0.05$.

The present study showed that hs-CRP protein was increasing especially with patients who suffer from fluctuation in sugar level and have these obtained results in consistent with previous report which showed the positive relationship between diabetes and elevated hs-CRP [ 259] and it agree with many researchers have studied the effect of blood glucose fluctuation on the vascular complications of T2DM [260,261]. These studies confirmed that the blood vessel endothelium are damaged greater by blood glucose fluctuation than by chronic constant hyperglycemia as a result of increasing oxidative stress T2DM patients[262] therefore hs-CRP remained a significant predictor of diabetes risk even after adjusting with body mass index, family history of diabetes, smoking and other factors [263,264]. Consequently inflammation could also promote hepatic insulin resistance to a greater extent in those who are genetically prone to diabetes, as hepatic insulin resistance exacerbates fasting glycemia and hypertriglyceridaemia then production of proinflammatory cytokines and other inflammatory pathways in vascular endothelial cells, the diabetic state promotes oxidative stress mediated by reactive oxygen species and carbonyl groups[265].

### 3.1.4. Apolipoprotein- A1

The results obtained showed that serum Apo-A1was decrease in T2DM group compared with normal control group as show in Table (3-1) at $p<0.05$.

The results obtained show that serum Apo-A1correlates positively with HDL-C ($r= 0.625$, $p=0.0001$) show Figure (3-6). It is also has a negative significant correlation with Total-cholesterol / HDL-C, systolic blood pressure , TG ,VLDL-C ($r = - 0.467$, $p=0.0001$) , ($r = - 0.485$, $p=0.0001$) , ($r = - 0.279$, $p=0.007$) , ($r = - 0.280$, $p=0.007$) show Figure (3-7) .While it correlates negatively but non-significantly with diastolic blood pressure, BMI and LDL-C ($r = - 0.222$), ($r = - 0.136$) , ($r = - 0.187$) at $p>0.05$.

The results obtained in this study in consistent with previous reports which showed the positive relationship between HDL-C and Apo-A1level [266]. This is

because that (Apo-A1), the major protein moiety of the high density lipoprotein (HDL) particle thus one of the hallmarks of insulin resistance or the metabolic syndrome is reduced plasma concentration of HDL-C and low plasma levels of its major apoprotein, namely Apo-A1 [267].

It seems from previous studies that the diabetes-related reduction in Apo-A1is not only related to increased plasma clearance of the protein but also is the result of down regulation of Apo-A1expression at a transcriptional level. Although the accurate nature for this change is not known, it is likely that increased cytokine production may contribute significantly to the inhibition of Apo-A1gene transcription [267a,267b] .

### 3.1.5. Lipid Profile.

The results obtained showed that serum TG, Total cholesterol, LDL-C show non-significant elevate $p>0.05$ in T2DM patients group compared with normal control group, while serum HDL-C level show significant decrease $p<0.05$ compared with normal control group as show in Table (3-1)

Firstly, serum total cholesterol shows a positive correlation with LDL-C, TG and Total-cholesterol/HDL-C ratio ($r = 0.684$, $p=0.0001$), ($r = 0.447$, $p=0.0001$),( $r=0.797$, $p=0.0001$) respectively, it also show a non-significant negative correlation with HDL-C ($r = 0.108$) at $p>0.05$ and a non-significant positive correlation with BMI , systolic and diastolic blood pressure.

Secondly, TG shows a significant negative correlation with HDL-C ($r= ─ 0.309$, $p=0.003$) as show in Figure (3-8) and a significant positive correlation with Total-cholesterol/HDL-C ($r= 0.575$, $p=0.0001$) and a non-significant positive correlation with LDL-C, BMI, systolic and diastolic blood pressure at $p>0.05$ .

Thirdly, LDL-C has a significant positive correlation with Total-cholesterol/HDL-C ( $r = 0.745$, $p=0.0001$) and has a non-significant positive correlation with BMI, systolic and diastolic blood pressure and a non-significant negative correlation with HDL-C.

Finally, HDL-C shows a significant negative correlation with BMI, systolic and diastolic blood pressure (r = ─ 0.297, p=0.004 ), (r = ─ 0.463, p=0.0001), (r = ─ 0.340, p=0.001) respectively.

These results are in a good agreement with the previous reports [268]. The lipid profile abnormalities associated with insulin resistance affect all lipid fractions. They are characterized by elevated fasting triglyceride levels, elevated postprandial triglyceride rich remnant lipoproteins, low HDL-C, and small density LDL-C particles. This pattern correlates strongly with cardiovascular risk, and treatment decreases this risk. The positive correlation between serum 25-(OH)D level and HDL-C in this study results [r = 0.540, p=0.0001], is consistent with other authors [269].

### 3.1.6. Troponin I

The results obtained show that serum Troponin I was non-significant change in T2DM group in comparison with normal control group, as show in Table (3-1).

This finding consist with previous study which reported Patients with diabetes show normal troponin-I levels [270] .

Troponin I showed non-significant correlation FBS and serum 25-(OH)D, Apo-A1, hs-CRP, lipid profile, BMI , systolic and diastolic blood pressure .

The reasons for the normal results of Troponin I that analysis must be conducted from patients who presented to the emergency department within 2-4 h of the onset of chest pain and this finding consist with previous studies which show that Troponin I is a contractile protein that normally is not found in serum. It is released only when myocardial necrosis occurs [271]. Serum levels increase within 3-12 hours from the onset of the chest pain , peak at 24-48 hours [272].

**Table (3-1): Comparison between diabetic and control group in the measured parameters**

| Parameters | T2DM patients (N=40) | Control group (N=30) | P value |
|---|---|---|---|
| BMI , kg/ $m^2$ | 28.09 ± 2.92 | 23.65 ± 1.26 | P<0.01 |
| Systolic B.P. mmHg | 133.22 ± 3.36 | 119.00 ± 2.52 | NS |
| Diastolic B.P. mmHg | 85.60 ± 2.51 | 78.50 ± 2.87 | P<0.05 |
| Fasting blood sugar , (mg/dl) | 240.63 ±101.91 | 100.20 ± 7.79 | P<0.001 |
| Cholesterol , (mg/dl) | 201.68 ± 45.46 | 167.80 ± 26.22 | NS |
| TG , (mg/dl) | 167.10 ± 23.74 | 87.70 ± 9.85 | P<0.05 |
| HDL-C , (mg/dl) | 36.82 ± 6.36 | 45.60 ± 8.10 | P<0.05 |
| LDL-C , (mg/dl) | 120.8 ± 21.42 | 104.7 ± 27.61 | NS |
| Total/ HDL-C Ratio | 5.50 ± 2.36 | 3.78 ± 0.94 | P<0.05 |
| VLDL-C , (mg/dl) | 33.47 ± 16.39 | 17.70 ± 1.949 | P<0.001 |
| 25-(OH)$D_3$ , (ng/ml) | 17.00 ± 9.78 | 36.45 ± 5.042 | P<0.001 |
| HS-CRP , (mg/L) | 3.74 ± 1.96 | 1.30 ± 0.470 | P<0.05 |
| Troponin I , ng/ml | 0.59 ± 1.01 | 0.50 ± 0.513 | NS |
| Apo-A1, µg/dl | 1.90 ± 0.99 | 5.65 ± 2.796 | P<0.05 |

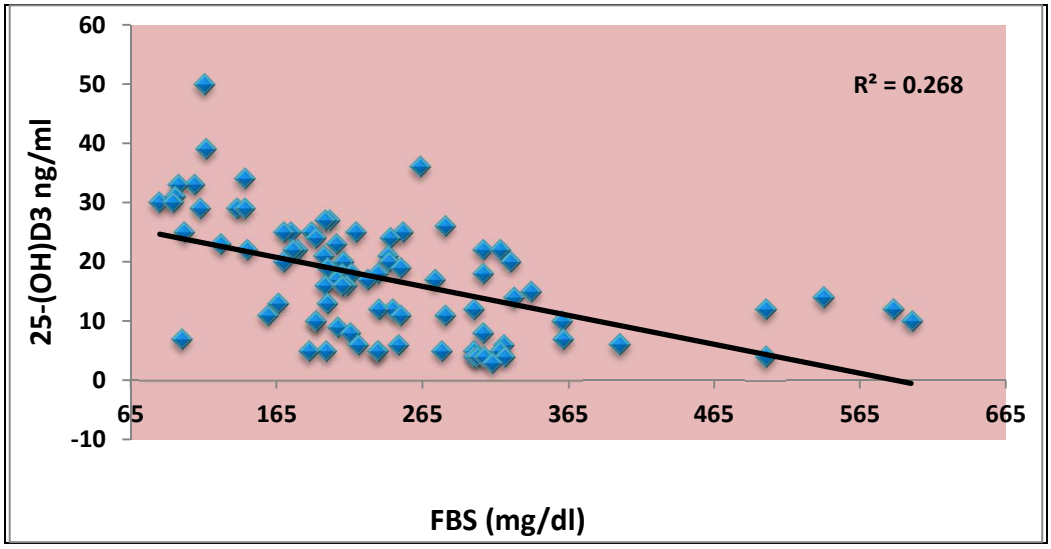



**Figure (3-1): Correlation between serum 25-(OH)D3 and fasting blood sugar in T2DM**

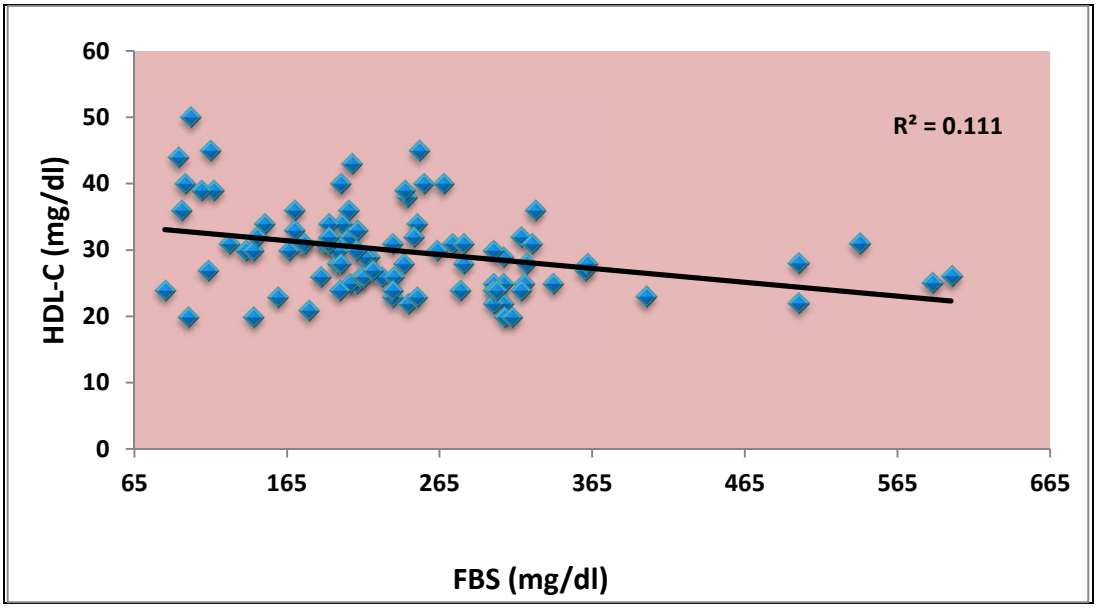



**Figure (3-2): Correlation between HDL-C and fasting blood sugar in T2DM.**

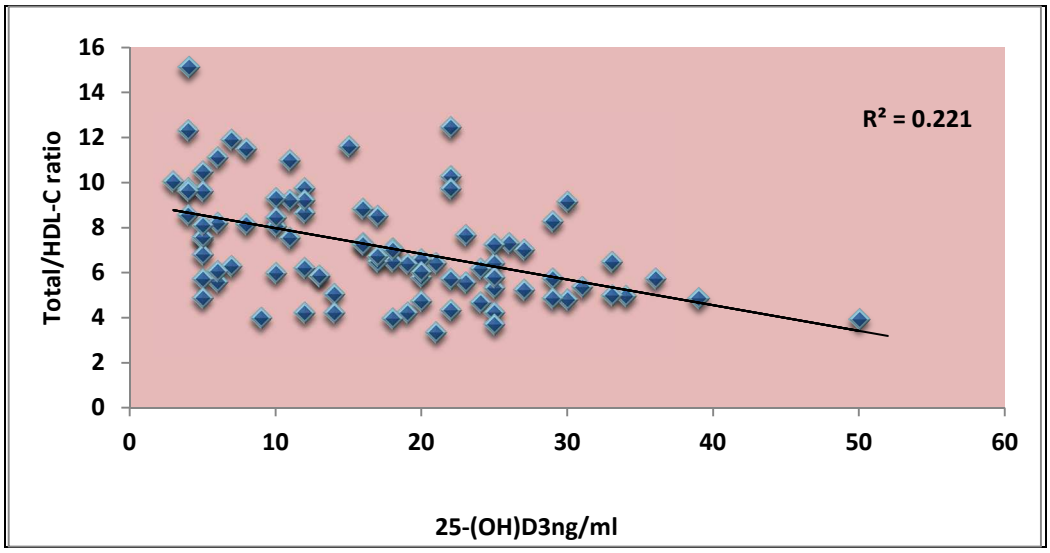



**Figure (3-3): Correlation between serum 25-(OH)D3 and Total/ HDL-C ratio in T2DM**

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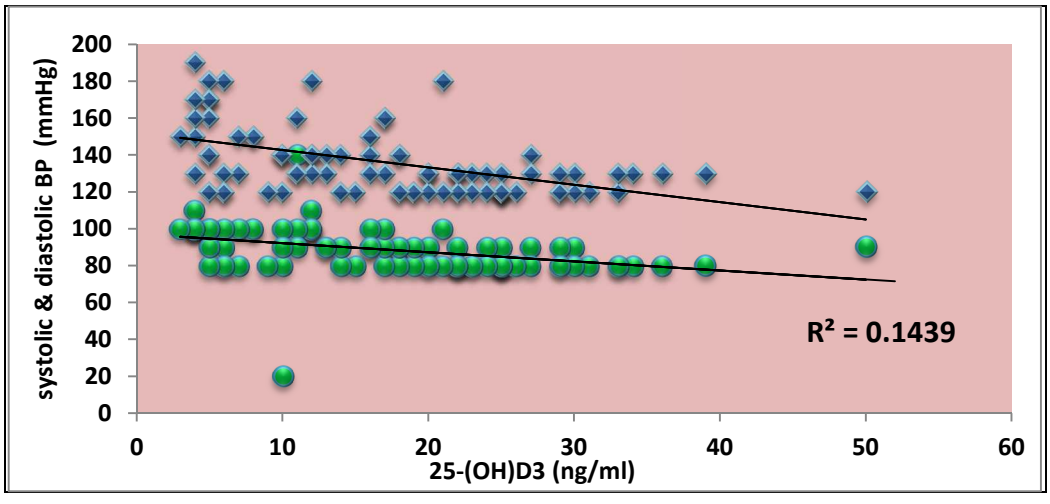


**Figure (3-4): Correlation between serum 25-(OH)D and systolic & diastolic BP in T2DM**

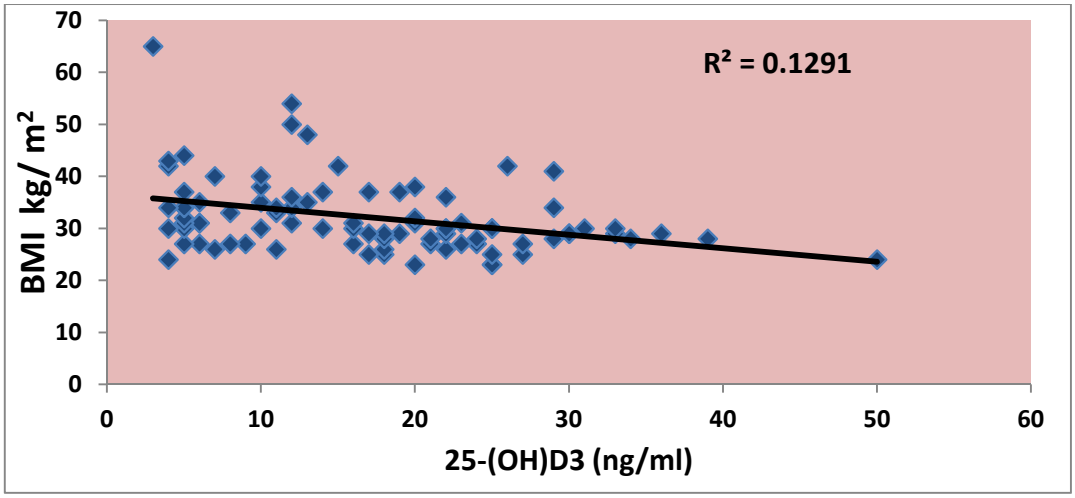


**Figure (3-5): Correlation between serum 25-(OH)D and BMI in T2DM**

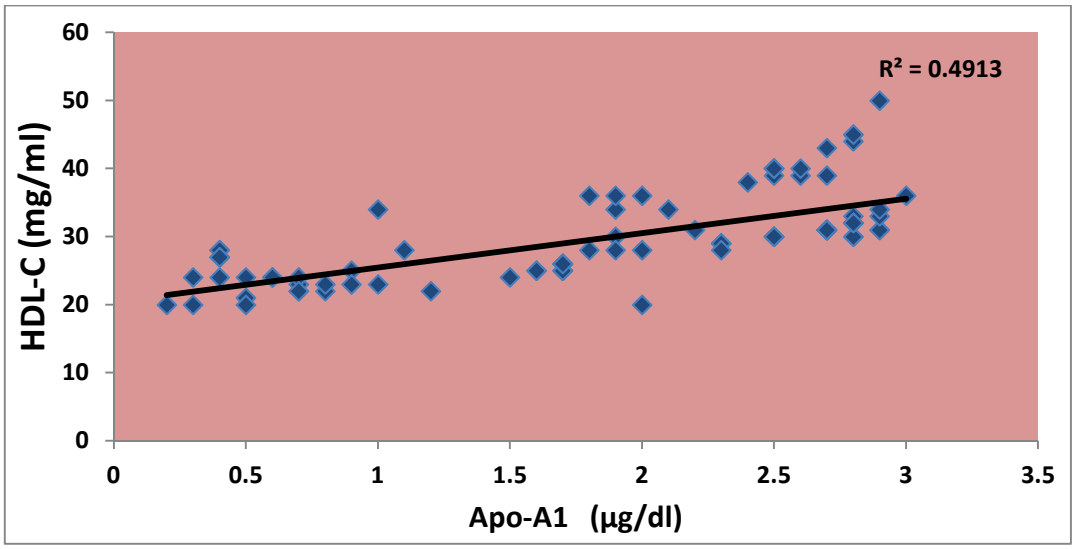


**Figure (3-6): Correlation between serum Apo-A1and HDL-C in T2DM.**

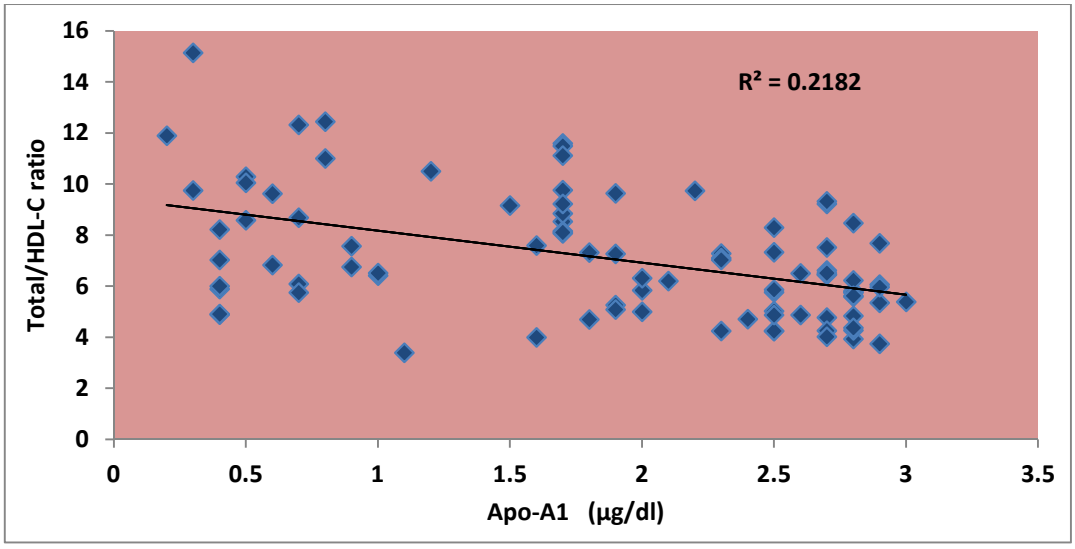


**Figure (3-7): Correlation between serum Apo-A1and Total-cholesterol/HDL-C ratio in T2DM.**

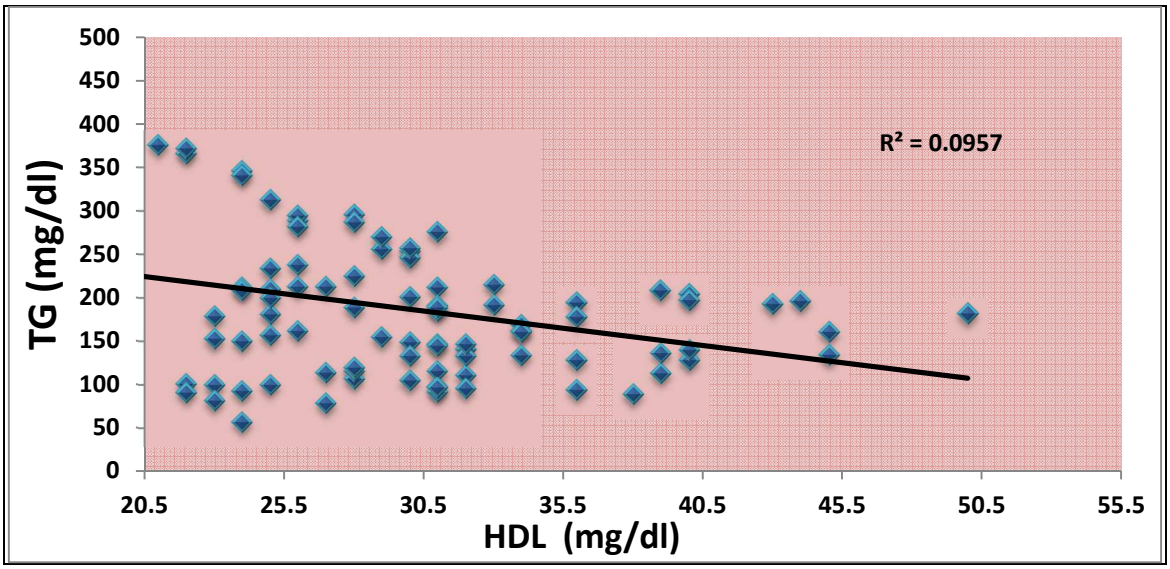


**Figure (3-8): Correlation between serum HDL-C and TG in T2DM.**

## 3.2. Comparison between metabolic syndrome and control group

### 3.2.1. Fasting Blood Sugar

The results obtained show that serum fasting blood glucose was significantly high in MetS patients group compared with normal control group. ($p< 0.001$) as show in Table (3-2).

These results showed that serum FBS correlates positively with BMI , Triglyceride , systolic and diastolic blood pressure and hs-CRP ($r=0.820$, $p=0.0001$), ($r=0.614$, $p=0.0001$), ($r=0.560$, $p=0.013$), ($r =0.554$, $p=0.014$), ($r=0.317$, $p=0.04$) respectively show Figure (3-9). While it correlates positively but non-significantly with serum cholesterol, LDL-C , VLDL-C, Total-cholesterol/ HDL-C ratio ,Troponin I, at $p > 0.05$ level of significant. Also correlates negatively with serum 25-(OH)$D_3$, HDL-C, ($r = -0.487$, $p=0.0001$), ($r= ─ 0.344$, $p=0.00$) respectively Figure (3-10).

These results showed a rise in average fasting blood glucose level in MetS group compared with the control group this consistent with previous report which showed that obesity elevate glucose concentration and it is associated with an increase risk of MetS **[273]** .

Also the present study showed the association between 25-(OH)D obesity and disorder of FBS this finding in consistent with previous recently, animal and human studies have suggested that vitamin D is a potential modifier of diabetes risk **[274-277]**. Vitamin D has an important role in the disorders of glucose and insulin metabolism **[278,279]**. It has been observed that vitamin D with calcium supplementation produces a significant decrease in fasting glucose and insulin resistance in patients with MetS **[280].**

### 3.2.2. 25-Hydroxy vitamin D

The results obtained show that serum 25-(OH)D was significantly lower in MetS patients group compared with normal control group ($p < 0.0001$) as show in Table (3-2).

Serum 25-(OH)D correlates negatively with serum hs-CRP, BMI ,Total-cholesterol /HDL-C ratio, systolic and diastolic blood pressure respectively , ($r=-0.336$, $p=0.006$), ($r= -0.409$, $p=0.001$), ($r= -0.449$, $p=0.0001$), ($r= -0.565$, $p=0.0001$), ($r=-0.441$, $p=0.0001$) respectively show Figures (3-11 to 3-13) and positively with serum HDL-C , Apo-A1($r = 0.615$, $p=0.0001$), ($r = 0.412$, $p=0.001$) respectively show Figure (3-14). While it correlates negatively but non-significantly with serum total cholesterol, LDL-C, TG and Troponin I ($r = -0.229$), ($r = -0.054$), ($r = -0.228$), ($r= -0.058$) respectively at $p> 0.05$ level of significant.

The present study showed increasing in systolic and diastolic blood pressure levels correlates with lower concentration of 25-(OH)D this accepted with previous studies which confirm that patients with vitamin D levels below the recommended 75 nmol/l (30 ng/ml) have higher systolic and diastolic blood pressure levels [281,282] .

The present study showed that vitamin D deficiency or insufficiency status was associated with an increased risk of metabolic syndrome these results are in good agreement with previous studies regarding the role of 25-(OH)D in MetS [283].

Potential mechanisms for the effects of vitamin D on glucose homeostasis have been suggested based on the findings from animal and in vitro studies. It appears that vitamin D may play a role in both insulin secretion and insulin resistance. The present study also showed that vitamin D receptors are present on pancreatic b-cells and skeletal muscle and the activating enzyme, 25-(OH)D-1α-hydroxylase, is expressed in pancreatic β-cells[284] . Vitamin D may directly induce insulin secretion by binding

to vitamin D receptors on b-cells, or it may indirectly affect b-cell function by regulating extracellular calcium level and calcium flux through b-cells. Vitamin D stimulates the expression of insulin receptor and enhances insulin responsiveness for glucose transport in cells. It also indirectly affects insulin sensitivity in tissues such as skeletal muscle and fat by regulating extracellular calcium level and ensuring adequate intracellular cytosolic calcium pool, which is essential for insulin-mediated intracellular processes [285] .

### 3.2.3. Hs-C-Reactive Protein

The results obtained show that serum hs-CRP was significantly high in MetS patients group compared with normal control group ($P<0.01$) as show in Table (3-2).

Serum hs-CRP correlates positively with BMI, Cholesterol, systolic blood pressure ($r =0.351$, $p=0.006$), ($r = 0.479$, $p=0.001$), ($r=0.267$, $p=0.002$) respectively . It also has a positive non-significant correlation with, LDL-C , TG ,Troponin I, Total-cholesterol/HDL-C ratio at $p>0.05$ , and it has negatively significant correlation with serum HDL-C and Apo-A1($r= - 0.457$, $p =0.001$), ($r = - 0.448$, $p=0.001$) respectively.

The results obtained in this study are in consistent with previous report which showed the positive relationship between MetS and elevated hs-CRP [259]. Hs-CRP became a significant predictor of MetS risk even after adjusting with body mass index, family history of diabetes mellitus, smoking and other factors [263,264]. HS-CRP concentrations have also been observed to predict the development of hypertension[264a]. By the results of this study showed a negative correlation between hs-crp and 25-(OH)D this mean that increase inflammation correlate with vitamin D deficiency as mentioned in the previous study[286].

Vitamin D performs different functions besides its well-known role in calcium-phosphorus metabolism, as indicated by the presence of vitamin D receptors (VDRs) and CYP271B (enzyme responsible for 25-(OH)D synthesis) in different tissues. An

important regulatory role for vitamin D in the immune system is suggested by the presence of VDRs on activated T lymphocytes and inflammatory might decrease 25(OH)D levels[287]. The suppressive inhibiting effect of 1,25-dihydroxyvitamin D in different autoimmune diseases, and in vitro and in vivo findings of vitamin D induced changes in immune functions[288].

### 3.2.4. Apolipoprotein-A1

The results obtained show that serum Apo A1 was decreased in MetS patients group compared with normal non-obese control group as show in Table (3-2).

Serum Apo-A1has a negative significant correlation with Total-cholesterol/ HDL-C ratio, BMI, TG, systolic blood pressure (r=─ 0.408, p=0.0001), (r =─0.263, p=0.028), (r= -0.303, p=0.011), (r=─ 0.467p=0.0001) respectively Figure (3-15). While it has a negative insignificant correlation with diastolic blood pressure, Total cholesterol , LDL-C and troponin I (r = 0.216), (r = 0.030) , (r = 0.034) , (r = 0.013) respectively at p>0.05 . The results also show a highly significant positive correlation with HDL- C (r =0.653, p=0.0001) Figure (3-16) .

As was mentioned above from obtained results a negative correlation between Apo-A1level and obesity which consistent with previous reports [289 ,289a].This results also showed reduced levels of HDL-C and Apo-A1levels in MetS patients which consistent with previous study [290]. Apo-A1is the major protein component of HDL, then reduced concentrations of HDL and apolipoprotein-A1 promote the accumulation of cholesterol in the vessel wall and lead to atherosclerosis.

Inflammation and dyslipidemia may also explain the link between obesity and endothelial dysfunction. Adipose tissue, especially visceral fat, is known to produce several pro-inflammatory molecules, such as adipocytokines and acute-phase reactants. Concentrations of high-sensitive C-reactive protein (hs-CRP) and soluble adhesion molecules have been found to be higher in obese than in lean subjects[291].

There is great interest in determining the mechanisms responsible for reduced serum HDL-C and Apo-A1concentrations, particularly in insulin resistant states. Accepted cardio-protective functions of HDL particles include a direct inhibition of pro-atherogenic processes at the arterial wall, including inhibition of LDL oxidation, prevention of monocyte adhesion and chemotaxis, reduction in macrophage formation, and inhibition of endothelial dysfunction and apoptosis [292].

### 3.2.5. Lipid Profile

The results obtained showed that serum Total cholesterol, Total-cholesterol/HDL-C ratio, TG , LDL-C ,VLDL-C were significantly high in MetS patients group compared with control group . while serum HDL-C level was significantly lower in MetS patients group compared with normal control group at( $p< 0.001$), as show in Table (3-2).

BMI and systolic and diastolic blood pressure were significantly high in MetS patients group compared with control group ($P < 0.001$) , Table (3-2).

Firstly, serum total cholesterol showed a positive correlation with TG, LDL-C, VLDL-C and Total-cholesterol/ HDL-C ($r=0.376$, $p=0.001$), ($r=0.942$, $p=0.0001$), ($r = 0.377$, $p=0.001$), ($r = 0.820$, $p=0.0001$) respectively as show in Figure (3-17) , it also show a significant negative correlation with HDL-C ($r=-0.252$, $p=0.036$) and a non-significant positive correlation with BMI , troponin, systolic and diastolic blood pressure at $p>0.05$ .

Secondly, TG shows a significant negative correlation with HDL-C ($r = -0.407$, $p=0.0001$) and a significant positive correlation with BMI, Total-cholesterol/ HDL-C and systolic blood pressure ($r = 0.251$, $p=0.007$), ($r =0.550$, $p=0.0001$), ($r = 0.900$, $p=0.0001$), ($r =0.330$, $p=0.011$) respectively and positive correlation with diastolic blood pressure ($r=0.407$, $p=0.0001$) and a non-significant positive correlation with LDL-C and troponin I .

Thirdly, LDL-C has a significant positive correlation with Total- cholesterol / HDL-C (r= 0.747, p=0.0001) and a non-significant positive correlation with BMI, troponin I ,systolic and diastolic blood pressure and a non-significant negative correlation with HDL-C.

Finally, HDL-C shows a significant negative correlation with Total-cholesterol/ HDL-C , VLDL-C , BMI , systolic and diastolic blood pressure (r = ─ 0.721, p=0.0001), (r = ─ 0.405, p=0.001) , (r= ─ 0.354, p=0.003), (r = ─ 0.509, p=0.0001), (r =─ 0.404, p=0.001).

As mentioned above the results are in a good agreement with the previous epidemiological studies, which found that the prevalence of dyslipidemia was higher in high BMI subjects than in normal weight or BMI [303-304].

These results indicate that serum (VLDL-C) levels are directly and independently related to obesity. The well-known inverse association between obesity and serum HDL-C is not independent, but secondary to the elevated VLDL-C or TG levels associated with obesity. While associations of obesity and lipoprotein cholesterol are found, far fewer occur with apolipoproteins, especially apo-A1.

These results agreement with the previous epidemiological studies, which found that the prevalence of dyslipidemia in the obesity may be associated with MetS and insulin resistance[293,295]. Insulin is a lipid synthetic hormone, thus alteration in a gene regulating insulin gene transcription may alter lipid metabolism as well and contribute to dyslipidemia. The liver is the main target organ of the insulin effect. Insulin resistance can descend the repression of insulin on the concentrations of plasma free fatty acids, increase the plasma levels of free fatty acids, promote free fatty acids into the liver, and stimulate the synthesis and release of very low density lipoprotein (VLDL) in the liver. At the same time, insulin resistances can also decline the activity of lipoprotein lipase (LPL), reduce the metabolism of VLDL, and increase the levels of serum VLDL[296].

### 3.2.6. Troponin I

Results obtained show that serum Troponin I concentrations were a non-significant change in MetS patients compared with control group. Troponin I level was 0.62 ±1.16 in MetS patients group vs. 0.50 ± 0.51 in control group as show in Table (3-2).

The presented data also showed a non-significant positive correlation with FBS, hs-CRP, BMI, systolic and diastolic blood pressure , and non-significant positive correlation with 25-(OH)D, Apo-A1as indicated above .

These results may be due to a previous disorders such as the patient had suffered from a myocardial infarction would have an area of damaged heart muscle and so would have elevated cardiac troponin levels in the blood[297] . This can also occur in patients with coronary vasospasm a type of myocardial infarction involving severe constriction of the cardiac blood vessels.

It is important to note that cardiac troponins are a marker of all heart muscle damage, not just myocardial infarction, which is the most severe form of heart disorder. However, diagnostic criteria for raised Troponin indicating Myocardial Infarction is currently set by the WHO at a threshold of 2 or higher. Critical levels of other cardiac biomarkers are also relevant, such as creatine kinase isoenzymes. Other conditions that directly or indirectly lead to heart muscle damage and death can also increase troponin levels, such as renal failure[298].

\

**Table (3-2): Comparison between metabolic syndrome and control group in the measured parameters**

| Parameters | Control group (N=30) | MetS Patients (N=50) | P-Value |
|---|---|---|---|
| BMI , kg/ $m^2$ | 23.65 ± 1.26 | 34.13 ± 6.722 | P<0.001 |
| Systolic B.P. , mmHg | 119.00 ± 5.52 | 140.01 ± 6.26 | P<0.001 |
| Diastolic B.P. , mmHg | 78.50 ± 5.87 | 89.00 ± 5.74 | P<0.001 |
| Fasting blood sugar , (mg/dl) | 100.20 ± 7.79 | 247.16 ± 109.65 | P<0.001 |
| Total Cholesterol , (mg/dl) | 167.80 ± 26.22 | 206.44 ± 46.34 | P<0.001 |
| TG , (mg/dl) | 87.70 ± 9.85 | 191.09 ± 30.72 | P<0.001 |
| HDL-C , (mg/dl) | 45.60 ± 8.10 | 29.61 ± 5.71 | P<0.01 |
| LDL-C , (mg/dl) | 104.7 ± 27.61 | 139.03 ± 24.07 | P<0.05 |
| Total/ HDL-C Ratio | 3.78 ± 0.94 | 7.31 | P<0.001 |
| VLDL-C , (mg/dl) | 17.70 ±1.949 | 38.32 ± 16.40 | P<0.001 |
| 25-(OH)$D_3$ , (ng/ml) | 36.45 ± 5.042 | 16.48 ± 9.59 | P<0.0001 |
| HS-CRP , (mg/L) | 1.30 ± 0.470 | 4.13 ±2.02 | P<0.01 |
| Troponin I , ng/ml | 0.50 ± 0.513 | 0.62 ± 1.16 | NS |
| Apo-A1 , µg/dl | 5.65 ± 2.796 | 1.89 ± 1.01 | P 0.05 |

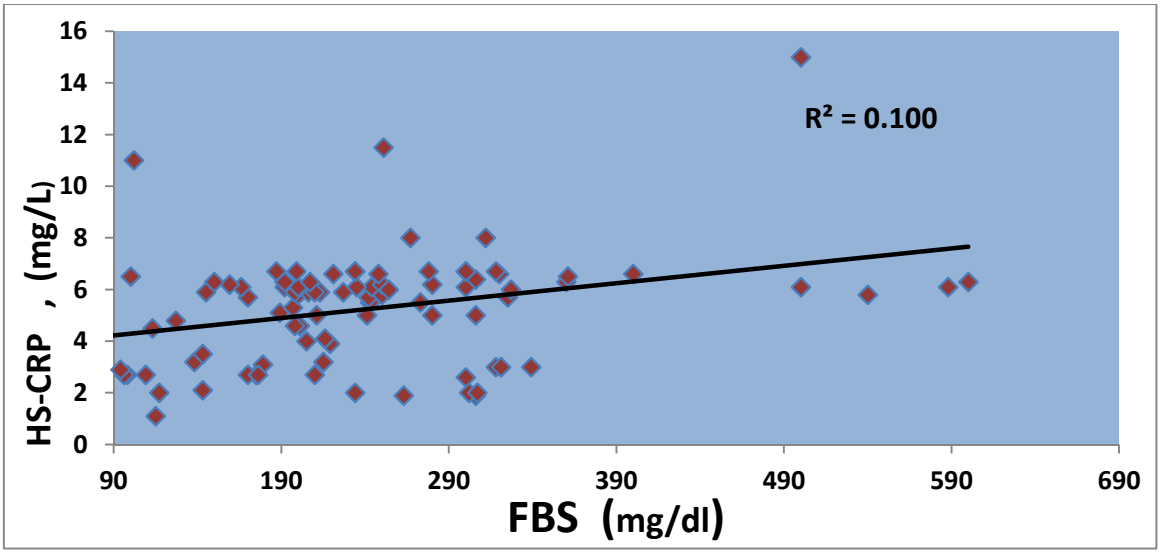


**Figure (3-9):Correlation between serum Blood glucose and hs-CRP in MetS patients**

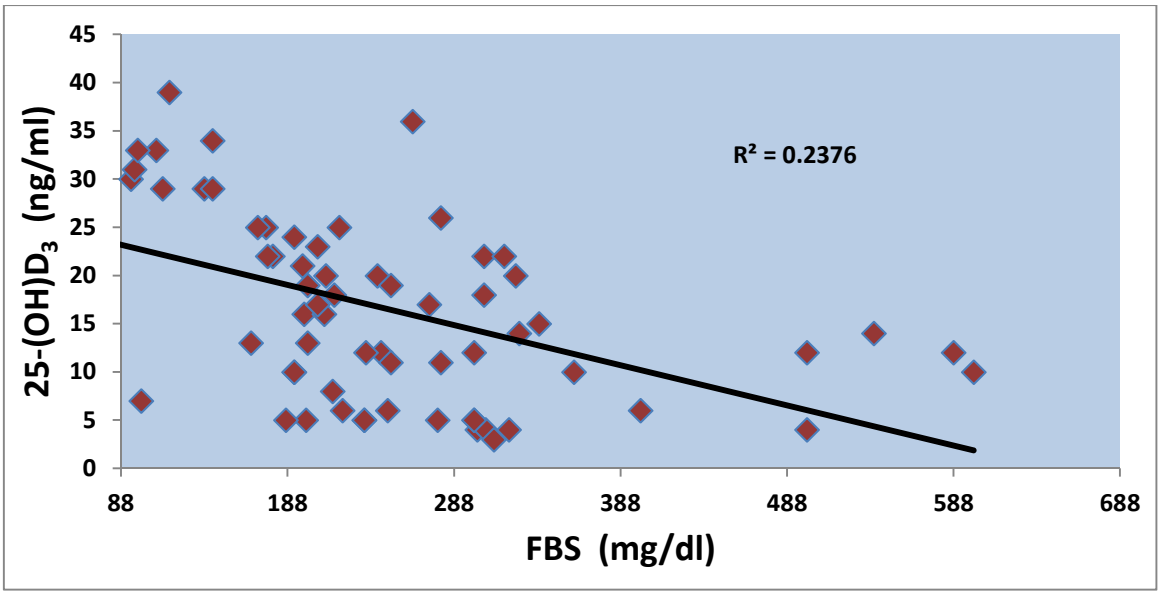


**Figure (3-10): Correlation between FBS and 25-(OH)D in MetS patients**

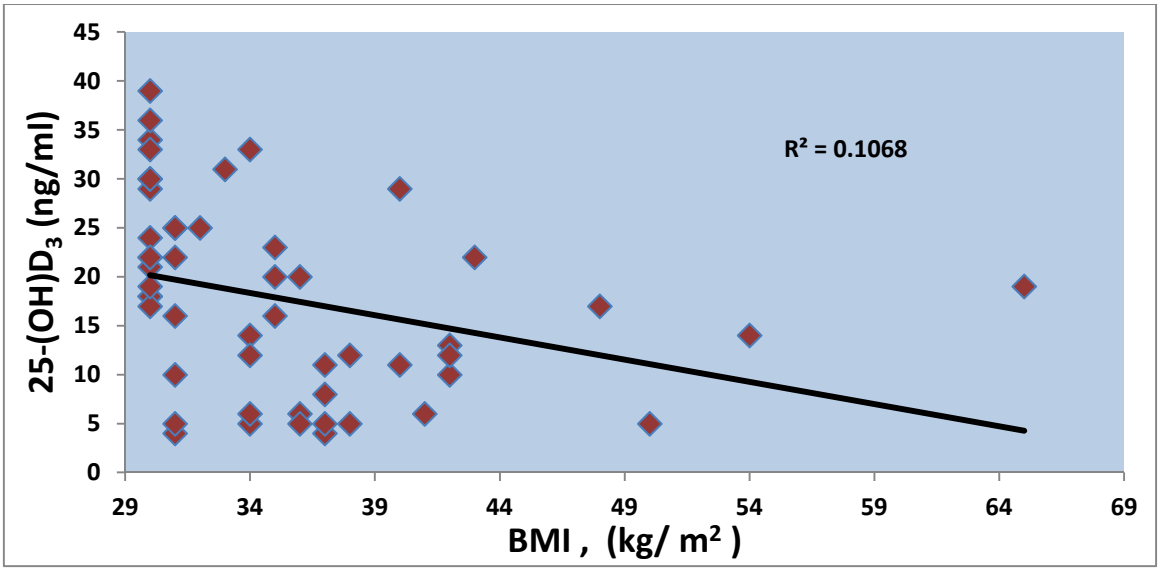


**Figure (3-11): Correlation between serum 25-(OH)D and BMI in MetS patients**

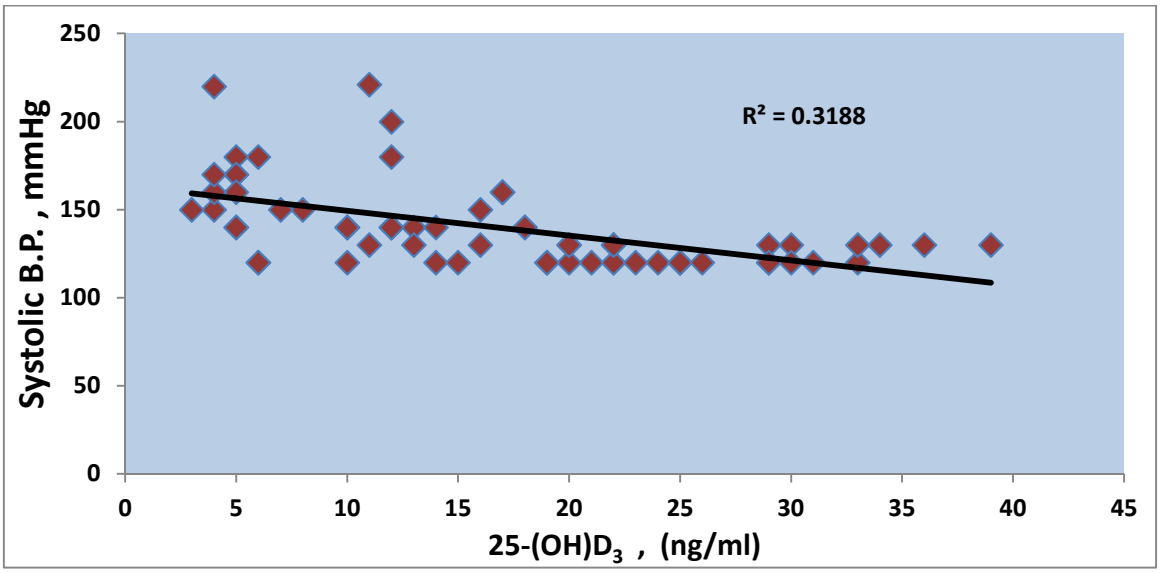


**Figure (3-12): Correlation between serum 25-(OH)D and systolic BP in MetS patients**

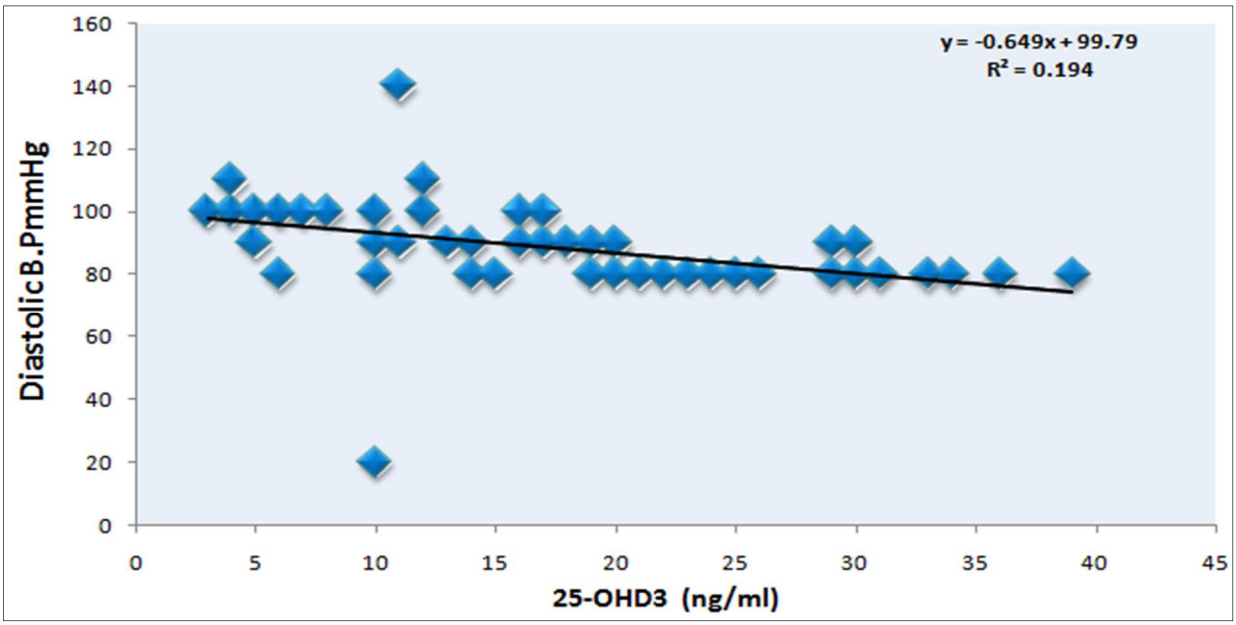


**Figure (3-13): Correlation between serum 25-(OH)D and Diastolic BP in MetS patients**

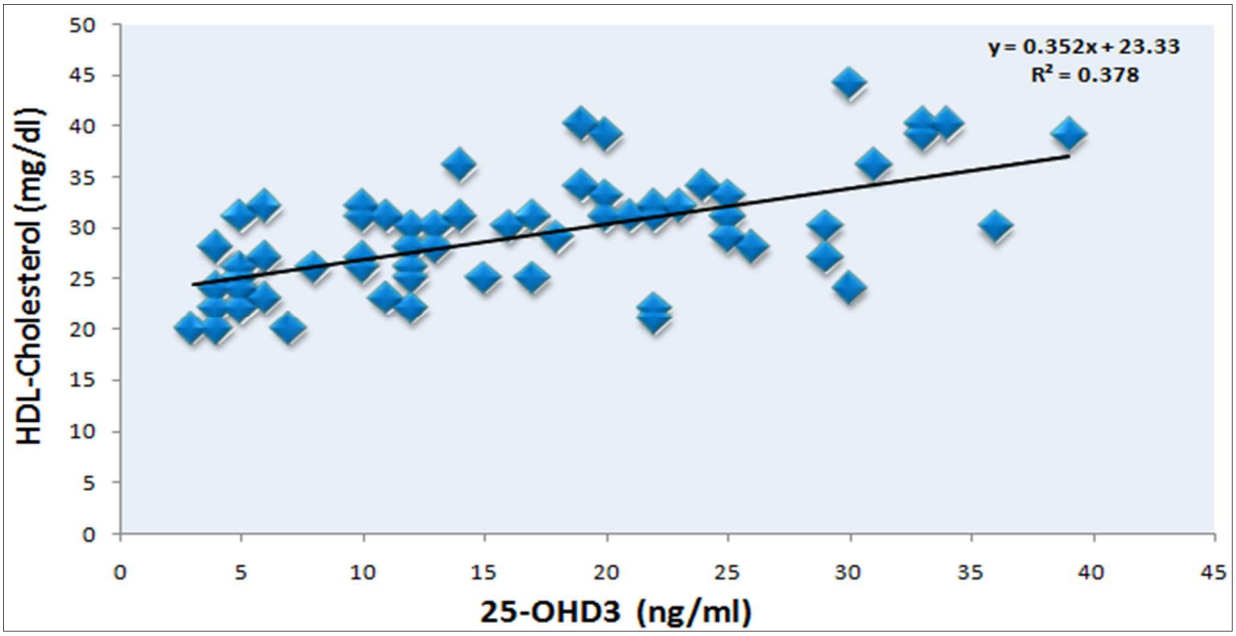


**Figure (3-14): Correlation between serum 25-(OH)D and HDL-C in MetS patients**

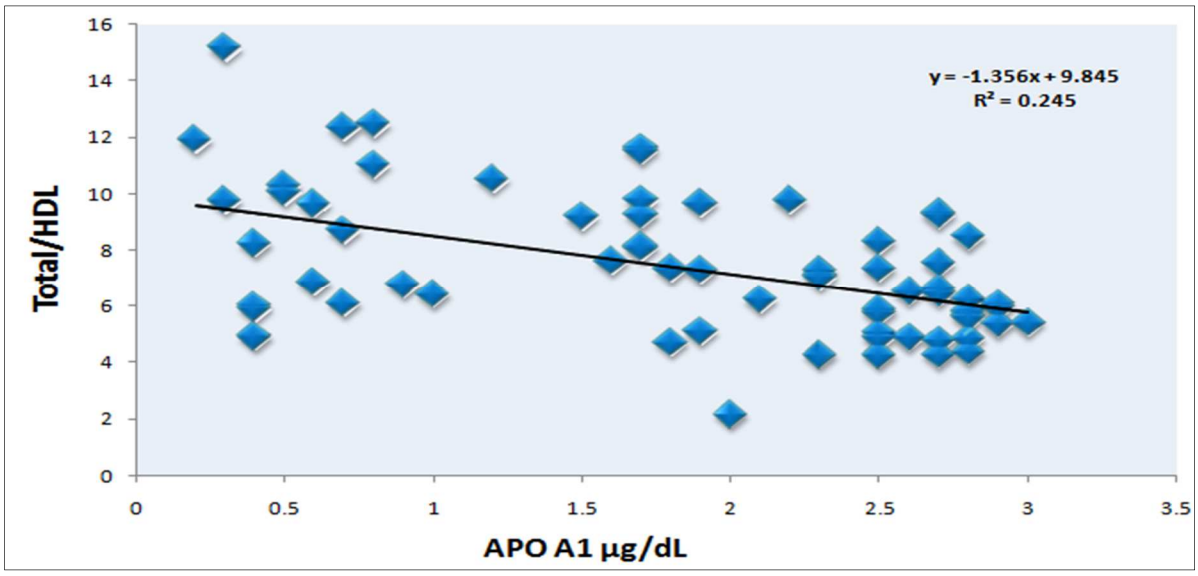


**Figure (3-15): Correlation between serum Apo-A1and Total-cholesterol/ HDL-C ratio in MetS patients**

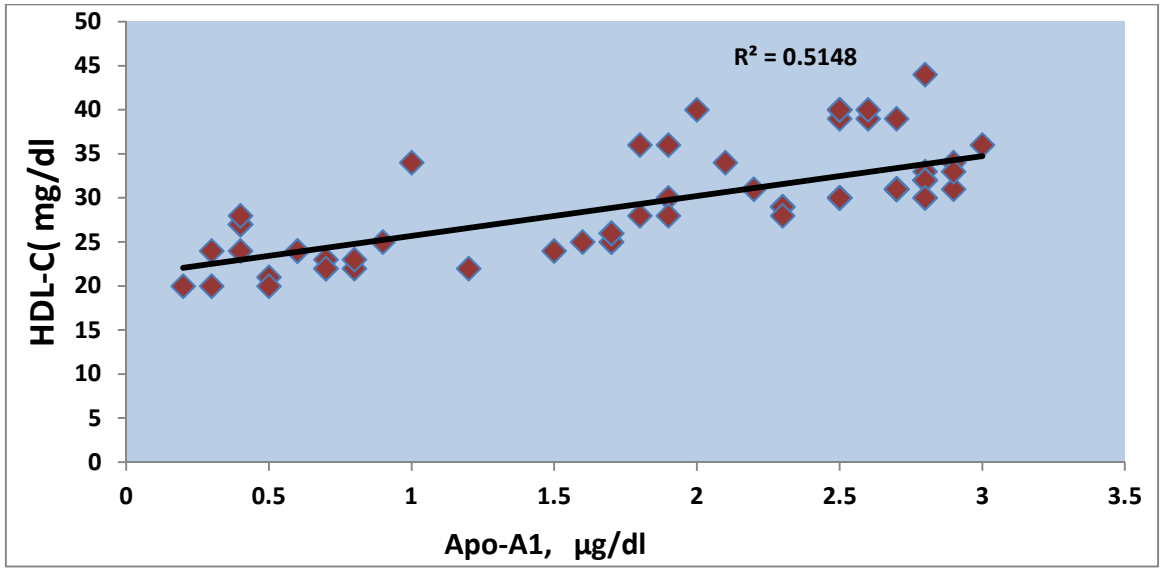


**Figure (3-16): Correlation between serum Apo-A1and HDL-C in MetS patients**

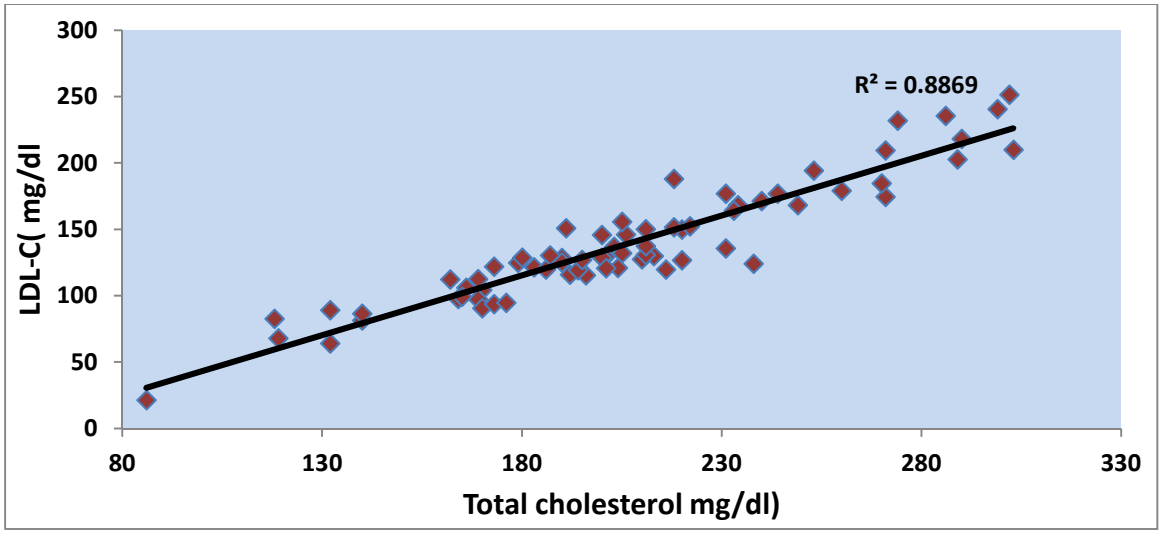


**Figure (3-17): Correlation between serum Total cholesterol and LDL-C in MetS patients**

# Conclusions

1. The present study show significant decrease in serum levels of Apo A1,HDL-C with a significant elevation in CRP, TG, LDL-C, Total-Cholesterol, systolic and diastolic blood pressure in MetS patients compared with control group.

2. These risk factors are threatening for heart diseases in MetS patients as compared with control group.

3. The present study revealed a deficiency in vitamin D in over weight/obese women with MetS and T2DM patients and this deficiency has inverse correlation with FBS, CRP, TG, systolic and diastolic blood pressure while it correlates positively with ApoA1 and HDL-C.
4. ApoA1 was correlated negatively with Total-cholesterol/HDL-C ratio, BMI, TG and systolic blood pressure.

5. There is no significant difference between males and females in patients with metabolic syndrome.

6. There are significant increase in hs-CRP level in sera of T2DM which undergo uncontrolled or fluctuation in sugar level.

7. The increase Total/HDL ratio and decrease 25(OH)D, HDL-C and Apo A1 lipoprotein seems to be a very effective way of characterized predicted risk of cardiovascular in MetS irrespective of their lipoprotein abnormality. Patients with diabetes or metabolic syndrome can have normal LDL-C level but possess aspects of atherogenic lipid profile, and these individual often have a high ratio of Total-cholesterol/HDL and less Apo A1 concentration which is correlates with cardiovascular risk.

8. The results showed insignificant difference in the level of troponin I, which its presence in the serum mean presence damage in the heart muscle. Therefore it is not considered as a predictive risk factor for heart diseases.

# Recommendations

1. Study of the correlation between 25-(OH)$D_3$ and development of metabolic syndrome.

2. Physicians should focus at tests: apo-A1, Total/HDL ratio and inflammatory marker hs-CRP more than triglycerides and cholesterol tests in diagnoses the pathogenic case to the heart because these parameters more accuracy.

3. Study the correlation between elevated 25-(OH)D level and dyslipidemia with the development of vascular complications in MetS patients.

4. Study the correlations between hs-CRP and ApoA1 in MetS patients.

5. Study the correlations between serum ApoA1 and atherogenic agents in MetS patients.

6. Study the correlations between 25-(OH)D with various antioxidant status in MetS patients.

7. Study the correlation between 25-(OH)D and BMI in MetS patients .

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# الخلاصة

متلازمة الأيض هي مجموعة من عوامل الخطر الأيضية التي توجد في شخص واحد، وهي حالة مرضية خطيرة. الأشخاص الذين يعانون من تلك المتلازمة هم الأكثر خطرا للإصابة بالأمراض المرتبطة بترسب الدهون على جدران الشرايين، وأمراض القلب التاجية، والتي يمكن أن تؤدي إلى أزمة قلبية، و مثال على ذلك. السكتة الدماغية وأمراض الأوعية الدموية المحيطية وغيرها . أيضا فأن الأشخاص الذين يعانون من هذه المتلازمة هم أكثر عرضة للإصابة بداء السكري النوع الثاني. وقد شملت الدراسة الحالية بعض العلامات القلبية بالإضافة إلى المتغيرات البيولوجية الروتينية لصورة الدهون مثل الدهون الثلاثية والكولسترول الكلي والكولسترول النافع والضار وعلاقتها مع مرض المتلازمة الايضية والسكري .إن التحليل الصيامي للدهون لا يعطي الصورة الدقيقة للمعرفة المسبقة للمخاطر القلبية لذا أصبح من الضروري البحث عن متغايرات بيولوجية استباقية وأكثر دقة للمخاطر القلبية مثل البروتين النشط عالي الحساسية ،صورة النظير غير الفعال لفيتامين د أو ما يسمى كولكالسيفيرول،وكذلك البروتين الدهني(A1)الناقل للدهون الضارة.

أجريت تلك الدراسة في مستشفى الحسين التعليمي - مدينة الحسين الطبية / مديرية صحة كربلاء - كربلاء - العراق في قسم الكيمياء السريرية وفي مختبرات فرع الكيمياء الحيوية - كلية الطب – جامعة كربلاء وقد تم جمع العينات بصورة عشوائية من المرضى الذين يراجعون وحدة استشارة السكر من الفترة كانون الأول 2012 ولغاية حزيران 2013 إضافة لجمع عينات مجاميع السيطرة وقد تراوحت أعمار المجموعتين من المرضى والأصحاء بين (25-65) وأجريت الدراسة على 90مريضا وتم تقسيمهم إلى مجموعتين 50 مريضا يعانون من متلازمة الايض 40 من مرضى السكري النوع الثاني الذين اصيبوا بالمرض لفترة لاتقل عن السنتين و30 شخصا من الاصحاء مع الاخذ بعين الاعتبار خلو جميع العينات من أي مرض التهابي يؤدي الى خطأ في النتائج .

أظهرت نتائج الدراسة الحالية ان مستوى كولكالسيفيرول كان منخفضا بصورة معنوية ($P < 0.001$)في مصل دم مرضى متلازمة الايض و مرضى السكري النوع الثاني بالمقارنة مع مجموعة السيطرة وقد تبين انه يرتبط بعلاقة عكسية مع سكر الدم ومؤشر كتلة الجسم و البروتين النشط عالي الحساسية ومع ضغط الدم في ارتباط عالي المعنوية ($P < 0.001$) وكانت نسبة انخفاض فيتامين د لدى النساء أكثر من الرجال .

كما أظهرت نتائج الدراسة إن معدل قيم تركيز البروتين الفعال –ج- عالي الحساسية مرتفعا بصورة معنوية ($P < 0.05$)في مصل مرضى السكري النوع الثاني وتحديدا مع المرضى اللذين يعانون من تقلب مستوى السكر لديهم أي من غير المسيطر على نسبة السكر في دمائهم ومرضى متلازمة الايض بالمقارنة مع مجموعة السيطرة وقد بينت النتائج انه يرتبط بعلاقة طردية مع معدل سكر الدم ومؤشر كتلة الجسم بينما هو يرتبط بصورة عكسية مع الكولكالسيفيرول والبروتين الدهني (A1) ($P< 0.01$) .

كما بينت نتائج الدراسة الحالية ان مستوى البروتين الدهني (A1) كان منخفضا بصورة معنوية ($P < 0.05$) في مصل الدم مرضى السكري النوع الثاني مرضى متلازمة الايض بالمقارنة مع مجموعة السيطرة ($P < 0.05$) وقد اظهر علاقة طردية مع الكولسترول النافع (HDL) وعلاقة عكسية مع ضغط الدم .

وأوضحت النتائج للدراسة الحالية إن مستوى الدهون الثلاثية مرتفعا بصورة معنوية (P < 0.01)في مصل دم مرضى السكري النوع الثاني بالمقارنة مع مجموعة السيطرة بينما مستوى الكولستيرول النافع (HDL-C) كان منخفضا بصورة غير معنوية (P >0.05) في مصل دم مرضى السكري النوع الثاني بالمقارنة مع مجموعة السيطرة كما لم يبدي الكولستيرول الكلي والكولسترول الضار فرقا معنويا .

وقد أظهرت النتائج ايظا إن مستوى الكولسترول الكلي والكولسترول الضار (LDL-C) والدهون الثلاثية مرتفعا بصورة معنوية (P < 0.01)في مصل دم ومرضى متلازمة الايض مقارنة مع مجموعة السيطرة بينما مستوى الكولستيرول النافع (HDL-C) كان منخفضا بصورة معنوية (P < 0.01) .

ايظا اظهر ضغط الدم الانقباضي والانبساطي ارتفاعا معنويا لدى مرضى المتلازمة الايضية مع مؤشر كتلة الجسم وبالتالي تشكل تلك الدوال خطرا يهدد للإصابة بإمراض القلب والأوعية الدموية

حسب نتائج الدراسة لم تظهر مستوى التروبونين Iأي فرق معنويا في مصل دم كل من مرضى السكري النوع الثاني ومرضى متلازمة الايض بالمقارنة مع مجموعة السيطرة وبالتالي لم يظهر أي علاقة معنوية مع بقية الدوال .

يمكن أن نخلص إلى أن هذا الدراسة أظهرت انخفاضا كبير في مستويات مستوى البروتين الدهني (A1) ، الكولسترول النافع (HDL-C) مع ارتفاع كبير في CRP البروتين النشط عالي الحساسية ، TG، LDL-C، إجمالي-الكولسترول، ضغط الدم الانقباضي والانبساطي هذه العوامل تشكل مخاطر جدية تهدد للاصابة بإمراض القلب في مرضى متلازمة الايض مقارنة مع مجموعة السيطرة.

جمهورية العراق

# وزارة التعليم العالي والبحث العلمي

## جامعة كربلاء- كلية العلوم

## قسم علوم الحياة

# دراسة العلاقة بين بعض مؤشرات الخطر للإصابة بإمراض القلب عند مرضى المتلازمة الايضية في محافظة كربلاء

رسالة

مقدمة إلى عمادة كلية العلوم في جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل


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