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# Association of serum cathepsin k, interleukin 2 and lipid profile among patients with chronic coronary syndrome in Babylon Governorate

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَالرَّاسِخُونَ فِي الْعِلْمِ يَقُولُونَ

آمَنَّا بِهِ كُلٌّ مِنْ عِنْدِ رَبِّنَا وَمَا يَذَّكَّرُ

إِلَّا أُولُو الْأَلْبَابِ

صَدَقَ اللهُ العَلِيُّ العَظِيم

آل عمران ، الآية ٧

# Dedication

To my lovely father and mother
To my dear husband
To all members of my family
To all friends and lovers
To..... my great country

Alyaa

# ***Supervisor Certification***

We certify that this thesis entitled **Association of serum cathepsin k, interleukin 2 and lipid profile among patients with chronic coronary syndrome in Babylon Governorate**" Was carried under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

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## Summary

Chronic coronary syndrome (CCS) refers to a group of cardiovascular diseases that are caused by the narrowing or blockage of the coronary arteries, which are responsible for supplying blood to the heart muscle. The condition typically develops gradually over time, and is characterized by persistent or recurrent chest pain or discomfort (angina) that occurs during physical activity or emotional stress. Other common symptoms of CCS may include shortness of breath, fatigue, and a sense of tightness or pressure in the chest.

The aim of the study:

This study to show an association between any immune event that starts with tissue destruction and protein lysis which trigger an inflammatory immune response that can involve the possibility of the occurrence of chronic coronary syndrome.

This study includes (100) patients within aged above 40 years, (50) patients includes male (28) and females (22) with a mean ± standard deviation (SD) of age (55.43 ± 5.17) , those patients were to the cardiac center at Imam Al Sadiq Teaching Hospital and diagnosed with chronic coronary syndrome. The second group includes (50) sample of control group, apparently healthy control includes male (28) and females (22) with a mean ± SD of age (53.13 ± 5.09). The control group was matched with patient's groups in sex and age. The samples of participants were collected from first of (September 2022 till January 2023). The part that deals with the practical aspect of the study was accomplished at the laboratory of the department of biochemistry, College of Medicine, University of Babylon. Serum cathepsin k, Interleukin-2 was determined by (ELISA) technique. Lipid profile was determined by spectrophotometer and body mass index were also determined.

The result showed that the levels of cathepsin k are significantly increased in patient group when compared with the control group ($p \leq 0.01$), also the result showed that the levels of Interleukin-2 are significantly increased in patient group when compared with the control group ($p \leq 0.001$). Estimation of total cholesterol (TC) showed that results of the present study showed the mean value of TC was significant in male patients and female patients groups (p-value $\leq$ 0.001) and SD of patients group(4.4 ± 0.80), SD of the control group (3.5 ± 0.55),, estimation of triglyceride (TG) shown that results of the present

study the mean value of TG was no significantly in male patients and female patients groups and (p-value 0.45), estimation of high density lipoprotein cholesterol (HDL-c) shown that results of the present study the mean value of HDL-C was significantly in male patients and female patients groups (p-value 0.001) and SD of patients group (0.90 ± 0.16) SD of control group (1.20 ± 0.23), decreased in the mean of HDL-c level in patients groups compere with control group. In addition estimation of Low density lipoprotein cholesterol (LDL-c) shown that results of the present study the mean value of LDL-c was significantly increase in male patients and female patients groups (p-value 0.001) and SD of patients group (2.66 ± 0.50) SD of control group (1.98 ± 0.67), increased in the mean of LDL-c level in patients groups, estimation of very low density lipoprotein cholesterol (VLDL) shown that results of the present study the mean value of VLDL was no significantly in male patients and female patients groups (p-value 0.71) and SD of patients group (0.63 ± 0.46) SD of control group (0.59 ± 0.36), normal in the mean of VLDL level in patients groups.

There were significant positive correlation between age, cholesterol and cathepsin k (P = 0.01, r =0. 328), (p= 0.003, r= 0.294) respectively. And Interleukin-2 significant positive correlation with age, cholesterol (p=0.00, r= 0.363) (p= 0.034, r= 0.212) respectively, while Interleukin-2 significant negative correlation with HDL (p=0.003, r= 0.298). The conclusion in this study showed that elevated cathepsin K and interleukin-2 are associated with chronic coronary syndrome, as their elevation contributes to tissue destruction and protein lysis, which leads to an inflammatory immune response that may involve the occurrence of chronic coronary syndrome.

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# Lists of Abbreviations

| Symbol | Description |
|---|---|
| ACVD | Atherosclerotic cardiovascular disease |
| ACS | Acute coronary syndrome |
| APOB-100 | Apolipoprotein B-100 |
| ATP | Adenosine triphosphate |
| APOA-I | Apolipoprotein A-I |
| APOE | Apolipoprotein E |
| APOC1 | Apolipoprotein C1 |
| BMI | Body mass index |

| | |
|---|---|
| CCS | Chronic coronary syndrome |
| CAD | Coronary artery disease |
| CSA | Chronic stable angina |
| CHD | Coronary heart disease |
| CVD | Cardiovascular disease |
| CM | Chylomicrons |
| Cat k | Cathepsin k |
| ELISA | Enzyme-linked immunosorbent assay |
| ECG | Electrocardiogram |
| FFA | Free-fatty acids |
| HDL | High density lipoprotein |
| IHD | Ischemic heart disease |
| IL-2 | Interleukin-2 |
| IDL | Intermediate density lipoproteins |
| LDL | Low density lipoprotein |
| LDLR | Low density lipoprotein receptor |
| LPL | Lipoprotein lipase |
| MI | Myocardial infraction |
| NSTEMI | Non–ST- segment elevation myocardial infarction |
| TC | Total cholesterol |
| TG | Triglyceride |
| VLDL | Very low density lipoprotein |

# 1: Introduction& Literature Review

## 1.1: Chronic Coronary Syndrome:

### 1.1.1: Definition:

Ischemic heart disease (IHD) is caused by flow reduction plaque atherosclerosis in the coronary artery causing ischemia in the heart muscle of the plaque. IHD can be divided into two main categories: chronic coronary syndrome and acute coronary syndrome (ACS) [1]. The term chronic coronary syndrome (CCS) refers to is a term that defines coronary artery disease as a chronic progressive course that can be altered, stabilized or improved by lifestyle modifications, pharmacotherapy and coronary revascularization. It has been introduced to replace the previous term ‘stable coronary artery disease [2].

The major difference between chronic coronary syndrome and ACS is the degree of obstruction of the blood flow induced by the coronary plaque. Chronic coronary syndrome is characterized by adequate blood flow in rest [3] .

### 1.1.2: Background:

Chronic coronary syndrome (CCS) is a newly described classification devised by the European Society of Cardiology (ESC) to replace the term “Stable Coronary Artery Disease (CAD).” The main reason for effecting the change is the term is thought to better describe the disease process and encompass a wider spectrum of clinical, pharmacological, and pathophysiological entities. Using this new lexicon, the disease atherosclerosis manifests as CAD is categorized into Acute Coronary Syndrome (ACS) and CCS.” Since this is a relatively new entity, the profile of CCS patients has significantly evolved, challenging clinicians and researchers to have a deeper grasp on the nature of the disease and as a result, develop newer methods of assessing, diagnosing, risk stratifying, and managing patients that fall into the category of CCS [4]

### 1.1.3 Epidemiology of Chronic Coronary Syndrome

Coronary artery disease (CAD) is one of the major cardiovascular diseases affecting the global human population. This disease has been proved to be the major cause of death in both the developed and developing countries. Lifestyle, environmental factors, and genetic factors pose as risk factors for the development of cardiovascular disease [5]. In Iraq, cardiovascular disease is the primary cause of hospitalizations and accounts for 33% of total deaths [6]. With a 13% of death rates being due to CAD, the 2010 Global Burden of Disease Study identified it to be a modern pandemic, the death rate however is escalated to 21% in the MENA region with CAD being the number one cause of death, the prevalence of CAD is slightly higher among men than women in all age groups, however, there is a steep increase in prevalence as the age increases in both genders, the prevalence of CAD in the Kingdom of Saudi Arabia (KSA) was estimated to be 5.5% with slightly higher rates in urban areas when compared to rural areas (6 vs. 4.2%, respectively) [7].

### 1.1.4 Pathophysiology of Chronic Coronary Syndrome:

Coronary artery disease in the presence of flow-limiting lesions. Angina is the result of a mismatch between myocardial perfusion and oxygen demand. In most patients, myocardial ischemia is the result of coronary atherosclerotic stenosis limiting blood flow. A specific pathophysiological view tended to disconnect the hemodynamic effects of severe coronary angina from the risk of subsequent atherothrombotic events [8].

Myocardial cells require O2 and ATP to maintain the contractility and electrical stability needed for normal conduction[9]. As myocardial cells are deprived of oxygen and anaerobic metabolism of glycogen takes over, less ATP is produced, leading to failure of the sodium–potassium and calcium pumps and an accumulation of hydrogen ions and lactate, resulting in acidosis. At this

point, infarction—cell death—will occur unless interventions are begun that limit or reverse the ischemia and injury [10].

Inflammation also participates in the local, myocardial, and systemic complications of atherosclerosis. When the arterial endothelium encounters certain bacterial products or risk factors as diverse as dyslipidemia, vasoconstrictor hormones inculpated in hypertension, the products of glycoxidation associated with hyperglycemia, or proinflammatory cytokines derived from excess adipose tissue, these cells augment the expression of adhesion molecules that promote the sticking of blood leukocytes to the inner surface of the arterial wall. Transmigration of the adherent leukocytes depends in large part on the expression of chemoattractant cytokines regulated by signals associated with traditional and emerging risk factors for atherosclerosis. Once resident in the arterial intima, the blood leukocytes—mainly mononuclear phagocytes and T lymphocytes— communicate with endothelial and smooth muscle cells (SMCs), the endogenous cells of the arterial wall. Recently, much attention has focused on protein mediators of inflammation and immunity, including the cytokines and complement components. Virtually unknown by cardiologists a mere decade ago, the cytokines have joined the mainstream of our specialty. As a major consequence of the inflammatory ferment underway in the early atheroma, SMCs migrate from the tunica media into the intima .These cells proliferate and elaborate a rich and complex extracellular matrix. In concert with endothelial cells and monocytes, they secrete matrix metalloproteinases (MMPs) in response to various oxidative, hemodynamic, inflammatory, and autoimmune signals. MMPs, in balance with their endogenous tissue inhibitors, modulate numerous functions of vascular cells, including activation, proliferation, migration, and cell death, as well as new vessel formation, geometric remodeling, healing, or destruction of extracellular matrix of arteries and the myocardium, Certain constituents of the extracellular matrix (notably proteoglycans) bind lipoproteins, prolong their residence in the

intima, and render them more susceptible to oxidative modification and glycation (nonenzymaticc conjugation with sugars), these products of lipoprotein modification, including oxidized phospholipids and advanced glycation end products, sustain and propagate the inflammatory response, As the lesion progresses, calcification may then occur through mechanisms similar to those in bone formation, In addition to proliferation, cell death (including apoptosis) commonly occurs in the established atherosclerotic lesion, The death of lipid-laden macrophages can lead to extracellular deposition of tissue factor (TF), some in particulate form, The extracellular lipid that accumulates in the intima can coalesce and form the classic, lipid-rich "necrotic" core of the atherosclerotic plaque, these strategies that target arterial stenosis [11].

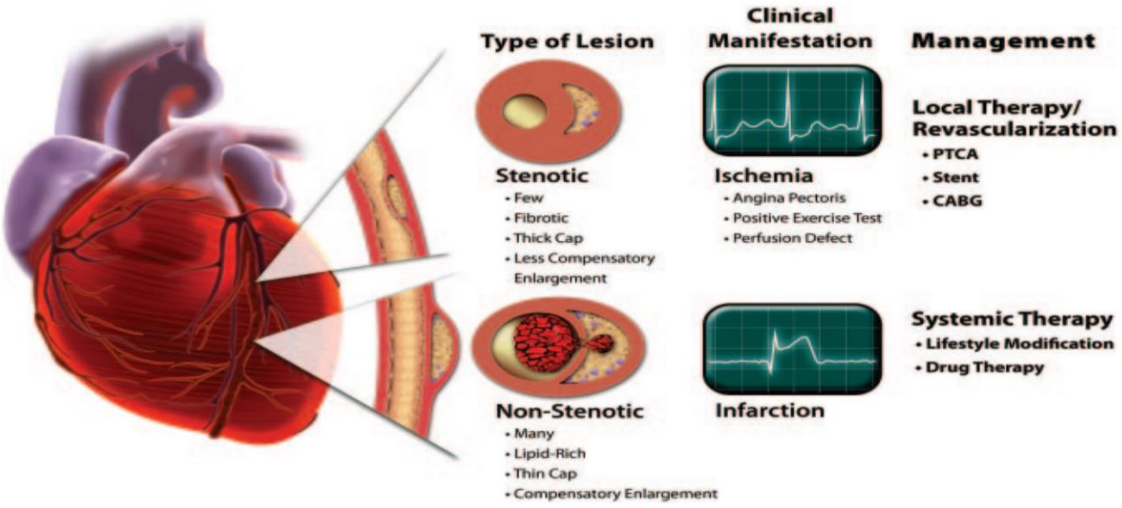


**Figure 1.1 Types lesions in human coronary atherosclerosis** [11].

## 1.1.5 Risk Factors for Chronic Coronary Syndrome:

Ever since the Fr lesions in human coronary atherosclerosis amingham heart study in 1960s identified the important risk factors associated with CAD [12]. There has been a great stress to reduce the burden of CVD by modifying these risk factors [13].

Modifiable risk factors include Diabetes, smoking, hypertension, hyperlipidemia, sedentary life style, obesity, stress and depression [14]. Non-

modifiable factors are advancing age, male gender, family history of premature CAD, while menopause, and personality type being partly modifiable [15].

### 1.1.6 Modifiable Risk Factors:

### 1.1.6.1 Smoking

smoking is an established risk factor for coronary heart disease , as well as a wide range of other conditions, and was estimated to cause 6.4 million deaths globally in 2015 [16].

Cigarette smoking increases morbidity and mortality through the interaction of metabolic and physiologic mechanisms including endothelial dysfunction, oxidative stress, platelet aggregation, and inflammation [17]. Long-term smoking leads to luminal narrowing of the coronary arteries, arterioles, and microvasculature [18] , and is associated with an adverse effect on serum lipids and insulin resistance [19]. A reduced oxygen-carrying capacity affects multiple organ systems and exacerbates existing chronic disease, including metabolic syndrome [20]. Smoking cessation improves the prognosis in patients with chronic coronary syndrome, including a 36% risk reduction in mortality for those who quit [21][2].

### 1.1.6.2 Diabetes mellitus

Diabetes mellitus disease is one of the leading causes of death and disability among patients with cardiovascular [22] .

Studies in stable coronary disease have been more consistent in showing that patients with diabetes have a higher prevalence of silent ischemia compared with those without [23]. In patients with diabetes mellitus, the ability of ischemic tissue to synchronize the molecular and cellular events leading to restoration of tissue perfusion in response to the atherosclerotic occlusion of a patent artery is markedly impaired. As a consequence, adverse tissue remodeling and the extent of ischemic injury are intensified, leading to

increased morbidity and mortality [24]. Diabetes mellitus was found to be stronger predictors of coronary atherosclerosis than hypertension [25].

### 1.1.6.3 Hypertension

Hypertension is the most prevalent cardiovascular risk factor and is closely associated with Chronic coronary syndrome (CCS). BP lowering can significantly reduce major cardiovascular risk, including coronary heart diseases [26][2]. The studies demonstrated that patients with hypertension had more advanced coronary atherosclerosis by coronary CTA and future MACE risk compared with those without hypertension cardiac events, Hypertension has been previously shown to be a predictor of the extent of coronary atherosclerosis as assessed by coronary artery calcium [27].

Insulin resistance is another risk factor hyperinsulinemia is known to contribute to development of both atherosclerosis and hypertension [28].

### 1.1.6.٤ Hyperlipidemia

Dyslipidemia is one of the main risk factors for cardiovascular diseases, in particular for chronic stable angina(CSA) [29]. The risk of death from coronary heart disease increases significantly in patients with a high cholesterol level [30]. These lipoproteins primarily transport triacylglycerol, along with cholesterol, which is mainly synthesized in the liver but accumulates in macrophages in atherosclerotic plaques, especially when combined with defective high-density lipoprotein (HDL)-mediated cholesterol efflux [31].

Even though triacylglycerol is the main component of the particles, the level of low density lipoprotein (LDL) cholesterol is the statistically strongest single risk factor for CAD, which has stimulated interest in the pathways that control its metabolism. However, links between CAD and circulating triacylglycerol have also been noted [32]. Of particular relevance to this review is that LDL is not directly produced by the liver, but is a metabolic product of very low density lipoproteins (VLDL), the primary APO B containing lipoprotein produced by

the liver. Thus, to understand LDL production, one must start with the assembly and secretion of VLDL [33].

### 1.1.6.5 Obesity

The effects of obesity on the cardiovascular system are varied and include increased insulin resistance, elevated blood pressure, systemic inflammation and procoagulant state, dyslipidemia, increased sympathetic activity, heart failure, endothelial dysfunction, coronary artery disease, atrial fibrillation, stroke, and systolic and diastolic dysfunction [34]. The main mechanisms of increased mortality in class III obese individuals (BMI>40 kg/m2) are increased blood volume, increased filling pressures, and increased activation of the sympathetic system [35].

### 1.1.6.6 Age

Risk for (CHD) events increase continuously with age in both men and women, Ageing predisposes patients to a high incidence and prevalence of CAD, in both men and women. Elderly patients (age >75 years) have the greatest mortality and morbidity risk attributable to Chronic coronary syndrome [36] [2] .

The prevalence and incidence of coronary heart diseases (CHD) are significantly increased in the elderly population due to increase of traditional risk factors and prevalence which is associated with aging due to increases of inflammation, endothelial dysfunction and atherosclerosis [37] .

## 1.1.7 Classification

Angina pectoris There are two types of chronic coronary syndrome and Acute coronary syndrome [38] .

Dependent on Troponin blood tests the ACS has classified into unstable angina (UA), non-ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) [39].

The severity of chronic stable angina can be expressed by the Canadian cardiac Society functional classification (CCS) as shown in table (1) below

**Table (1-1): Grading of angina according to CCS classification [40][2].**

| CLASS | Description of stage |
|---|---|
| **Firstly** | Ordinary physical activity does not cause angina "such as walking or climbing stairs. Angina occurs with strenuous rapid or prolonged exertion at work or recreation |
| **Second** | "slight limitation of ordinary activity",angina occurs on walking or climbing stairs rapidly walking uphill, walking or stair climbing after meals,in cold,in wind,or under emotional stress, or only during the few hours after a wakening . |
| **Third** | "marked limitation of ordinary physical activity".angina occurs on walking 1 to 2 blocks on the level and climbing, flight of stairs under normal conditions and a normal pace . |
| **Fourthly** | "inability to carryon any physical activity without discomfort" angina symptoms may be present at rest . |

## 1.1.8 Clinical Presentation in Chronic Coronary Syndrome

Clinical features patients with Chronic Coronary Syndrome can vary depending on the severity of the disease and the underlying cause. Common symptoms include:

**Chest pain**: This is the most common symptom of CCS and is usually described as a tight, heavy, or crushing feeling in the chest. Chest pain may be triggered by physical activity or stress, and it may last for several minutes or be relieved by rest [41].

**Shortness of breath**: This can be caused by a reduction in blood flow to the heart, which can lead to a buildup of fluid in the lungs. Shortness of breath may occur during physical activity or at rest [42] .

**Fatigue**: This can be caused by reduced blood flow to the heart and a buildup of fluid in the body. Fatigue may also be a symptom of heart failure [43] .

The level of angina (and of dyspnea) can be graded on the NYHA or Canadian Cardiovascular Society scales, which both run from I (very mild) to IV (symptoms at rest or on minimal exertion) [44] .

## 1.1.9 Diagnosis

### 1.1.9.1 Physical examination

Physical examination during chest discomfort can provide a high degree of diagnostic certainty when abnormal findings wax and wane with the appearance and disappearance of chest pain [45]. A transient $3 or $4 gallop mitral regurgitation murmur, or paradoxical splitting of the second heart sound indicates that left ventricular function is altered during pain and strongly argues for an ischemic cause of pain [46]. The patient with acute MI often appears anxious and in distress. Vital signs are often normal, but sinus tachycardia is not uncovcxzmmon. The pulse may be rapid or slow if arrhythmias are present either hypotension caused by left or right ventricular dysfunction or arrhythmia or hypertension caused by adrenergic discharge may be present [47]. The murmur of ischemic mitral regurgitation may be present. If a left bundle branch block is present, abnormal splitting of the second heart sound may be heard [48].

### 1.1.9.2 Diagnosis Dependent on ECG Change

The resting ECG is normal in about a quarter of patients with angina. In the remainder, abnormalities include old myocardial infarction (Q

wave), nonspecific ST-T changes, atrioventricular or intraventricular conduction defects, and changes of left ventricular hypertrophy [49]. During 10ngina episode, the characteristic ECG changes is horizontal or down sloping ST segment depression that reverses after the ischemia disappear [50].The management of patients in the chronic coronary syndrome requires the following important information: (I) global and regional contractile function of the heart at rest; (II) valvular function; and (III) local complications, such as mural thrombi, myocardial scars and ventricular septal defects. Information concerning these three points can be provided by basic ECG [51].

### 1.1.9.3 Stress test

A stress test shows how the heart works during physical activity. It also may be called a stress exercise test, exercise makes the heart pump harder and faster,a stress test can show problems with blood flow within the heart .Stress test that involve imaging typically has a superior ability to detect coronary artery disease without an appreciable loss of specificity, the exercise ejection fraction is one of the most important prognostic variables in patients with coronary artery disease.17 Imaging stress tests allow evaluation of left ventricular performance and assessment of the extent of ischemia during stress [52].

### 1.1.9.4 Coronary angiography

Coronary angiography uses X-ray imaging to examine the inside of the heart’s blood vessels, angiographic coronary artery stenosis assessment correlates only modestly with measurements of blood flow restriction in humans [53] .

## 1.2 Cathepsin

Cathepsins are proteases involved in multiple physiological roles ranging from ion channel activity, apoptosis, autophagy, immune regulation and complement system activation. They are divided into several subtypes-serine cathepsins, cysteine cathepsins and aspartic cathepsins[54]. Various pathologies that can be attributed to dysregulation of cathepsins include Ischemia reperfusion (IR) injury is responsible for numerous diseases including myocardial infarction, pancreatitis, acute and chronic kidney disease, arthritis, auto-inflammatory diseases, stroke and IR injury.Lysosomes are responsible for catabolism and recycling of multiple macromolecules and are key degradative compart-ments in the cell. There are two major proteins that mediate lysosomal activity; lysosomal membrane proteins and lyso-somal hydrolases including the cathepsins [55] .

In mammals, there are 3 classes of cathepsins based on the amino acid each specific cathepsin breaks down including serine cathepsins, cysteine cathepsins and aspartic cathepsins, of these subtypes, serine and cysteine cathepsins are most abundant [56].

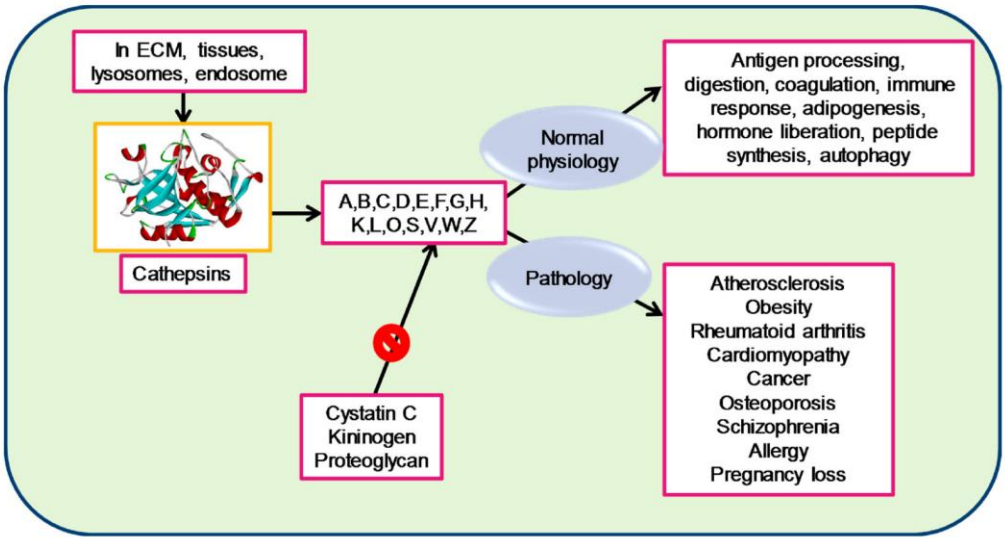


**Figure 1.2 The Dual Role of Cathepsins in Physiological and Pathological Conditions** [54].

**1. Aspartic cathepsins**

Cathepsin **E** is mainly found in dendritic cells, microglia and macrophages and has a role in regulating endo- somal/lysosomal microenvironment and protein sorting in these compartments [57].

Cathepsin **D** is an aspartic protease resident in typical acid vacuolar compartments (i.e., endosomes and lysosomes) ,It is also found in lysosome-related organelles, such as the secretory inflammatory granules of mastocytes, the secretory lytic granules of cytotoxic lymphocytes, the endosome-like MHC compartment of antigen-presenting cells25 and the melanosomes in melanocytes [58].

**2. Serine cathepsins**

Cathepsin **A** (also known as Lysosomal Protective Protein) regulates blood pressure via regulation endothelin-1 (ET-1) a potent vasoconstrictor and forms part of the elastin-binding complex which is responsible of the biogenesis of elastin fibers [59].

Cathepsin **G** (CTSG) is a member of the serine proteases family, which was first found in the azurophilic granules of neutrophil leukocytes and named in 1976 Then, CTSG was detected in other myeloid cells, such as B cells, primary human monocytes, myeloid dendritic cells, plasmacytoid dendritic cells, and murine microglia.3 Recently, studies proved that CTSG also existed in neutrophil traps and human urine exosomes [60].

**3. Cysteine cathepsins**

There are at least 11 cysteine cathepsin subtypes in mouse and human cells (B, C, F, H, K, L, O, S, V, W and X)-all of which have specific roles, but some share similar functions creating a system with certain redundancy [61].

## Cathepsin k

Cathepsin K (CatK) is one of the most potent proteases in lysosomal cysteine proteases family, human CatK is a 329-amino-acid long protein consisting of an N-terminal 15-amino-acid long signal sequence, a 99-amino-acid long propeptide and a 215-amino-acid long catalytic, CatK has the typical three-dimensional structure of a CatL like peptidase [62]. Cysteinyl cathepsins were shown to localize in lysosomes and endosomes and to function there to degrade unwanted intracellular or endocytosed proteins, recent studies have discovered non-traditional roles for Cats in the extracellular space during the development and progression of cardiovascular disease, Among cathepsins, cathepsin K (CatK), which is one of the most potent mammalian collagenases, was first identified in macrophages, Consistent with these biochemical observations, these vascular cells and macrophages can secrete CatK, which degrades type I collagen and elastin, CatK deficiency has been shown to reduce diet-induced atherosclerotic lesion formation [63]. The activity of cathepsin K is optimal at pH 5.5 and degrades many bone matrix proteins including type I collagen, osteopontin, and osteonectin [64] . An increased level of CatK was found in patients with ischemic heart disease. High blood levels of CatK could represent an independent predictor and novel biomarker for the diagnosis of coronary heart disease [65]. The mechanism by which cathepsin K contributes to CAD is not yet fully understood, but it is believed to involve several pathways. One possible pathway is through the activation of matrix metalloproteinases (MMPs), which are enzymes that also play a role in the degradation of ECM proteins. Cathepsin K can cleave and activate pro-MMPs, which can then promote the breakdown of collagen in the arterial wall, leading to the weakening of the vessel wall and the development of atherosclerosis.

Another pathway by which cathepsin K may contribute to CAD is through its effects on immune cells, such as macrophages. Cathepsin K is expressed by macrophages in atherosclerotic plaques, and it has been shown to promote the formation of foam cells, which are a hallmark of atherosclerosis. Foam cells are formed when macrophages take up oxidized low-density lipoprotein (LDL) particles and become loaded with cholesterol, leading to the formation of fatty streaks in the arterial wall [66].

## 1.3 Interleukin

Interleukins are a group of cytokines, which are small signaling proteins produced by various cells in the body that play a crucial role in communication between different types of immune cells. Interleukins specifically help regulate immune and inflammatory responses in the body [67]. There are many types of interleukins, with different functions and roles in the immune system. For example, some interleukins promote inflammation and help attract immune cells to the site of infection or injury, while others help regulate the growth and differentiation of immune cells. Interleukins also play a role in the development and function of certain tissues [68]. One of the primary mechanisms by which interleukins contribute to injury is by promoting inflammation. When tissues are damaged, immune cells are recruited to the site of injury, and they produce a variety of pro-inflammatory cytokines, including interleukins. These cytokines serve to activate other immune cells, enhance vascular permeability, and promote the migration of immune cells to the site of injury. While inflammation is necessary for the repair process, excessive or prolonged inflammation can exacerbate tissue damage and contribute to the development of chronic conditions [69].

## Interleukin-2 (IL-2)

Interleukin-2 (IL-2) is a type of cytokine, a protein signaling molecule that is involved in the regulation of the immune system. It is produced by certain T cells in response to infection, and it helps to stimulate the growth and activation of immune cells, particularly T cells and natural killer cells [70]. T Cell activation and cytokine production: IL-2 is a potent activator of T cells, promoting their proliferation and differentiation into effector T cells. In chronic coronary syndrome, T cells contribute to the local inflammatory response within the atherosclerotic plaques. IL-2 can enhance the production of pro-inflammatory cytokines, such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), by activated T cells. These cytokines can further propagate the inflammatory processes and contribute to plaque destabilization Interleukin-2 (IL-2) is a cytokine that plays a crucial role in regulating immune responses. In coronary artery syndrome, IL-2 has been found to be involved in the progression of atherosclerosis, the main underlying cause of coronary artery disease[71].

Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of lipids and immune cells in the arterial wall, which can lead to the formation of plaques that can rupture and cause heart attacks. IL-2 is known to be involved in the activation and proliferation of T cells, a type of immune cell that plays a key role in atherosclerosis [72].

In chronic coronary syndrome, IL-2 has been shown to promote the activation and proliferation of T cells, leading to the development of atherosclerotic plaques. IL-2 also stimulates the production of other cytokines and chemokines that attract immune cells to the arterial wall, further exacerbating the inflammatory response.

In addition, IL-2 has been implicated in the destabilization of atherosclerotic plaques, which can lead to their rupture and the development of acute coronary

syndromes such as heart attacks. IL-2 promotes the production of matrix metalloproteinase, which can degrade the extracellular matrix that provides stability to atherosclerotic plaques [73].

## 1.4 Plasma Lipid

A class of compounds that are soluble in organic solvents but are nearly insoluble in water and that contain nonpolar carbon-hydrogen bonds. Lipids primarily contain nonpolar carbon-hydrogen (C-H) bonds and often yield fatty acids and/or complex alcohols after hydrolysis [74].

Because of their insolubility in aqueous solutions، body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in white adipocytes, or transported in plasma in association with protein, as in lipoprotein particles, or on albumin [75].

### 1.4.1 Classification of Lipids

1- Simple lipids: They are esters of fatty acids with glycerol or other higher alcohols.
2- Compound lipids: They are fatty acids esterified with alcohol
   a. Phospholipids, containing phosphoric acid.
   b. Non-phosphorylated lipids.
3- Derived lipids: They are compounds, which are derived from lipids or precursors of lipids, e.g. fatty acids, steroids.
4- Lipids complexed to other compounds[76].

### 1.4.2 Cholesterol

Cholesterol can be synthesized by all cells in the body, and the enzyme catalyzing the rate-limiting step is 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. HMG-CoA reductase is inactivated when bound to statins and is transcriptionally reduced when intracellular cholesterol levels are high.

Cholesterol is an important component in cell membranes and is a substrate for the synthesis of steroid hormones and bile acids [77].

Due to its hydrophobicity, cholesterol in blood plasma is transported in lipoproteins [78].

HDL cholesterol has an inverse relationship to coronary heart disease [79],[80], although it has never been shown that raising HDL lowers the risk for cardiovascular disease in humans [81].

### 1.4.3. Triglyceride

Triglyceride (TG) consists of a group of glycerol and three fatty acids listed in the carbon hydroxyl groups of glycerol. Every TG may contain a combination of various fatty acids or contain three of the same fatty acids [82].

Triglycerides are transported from the liver and intestines by very-low-density lipoprotein (VLDL) and chylomicrons, respectively, and transferred to peripheral tissues to provide energy that needs [83].

Because triglycerides do not accumulate in foam cells, the association of plasma triglycerides and Atherosclerotic Cardiovascular Disease (ASCVD) these may be due to the remaining lipoproteins. The residues can accumulating in the arterials lining, where it can be ingested by macrophages, promoting the formation of foam cells, and ultimately, the formation of a fatty line that leads to the development of plaque [84][85].

Hypertriglyceridemia is also associated with higher concentrations of small dense LDL particles (which may be more atherogenic than other LDL particles), reduced HDL particle and APOA-I concentrations, and greater concentrations of APOC3–containing particles [86].

Lipoprotein lipase at the endothelial cell surface and within the subendothelial space hydrolyzes remnant triglycerides and generates pro-inflammatory mediators, including free fatty acids [87][88].

## 1.4.4. Classification of Lipoproteins

Since fat is less dense than water, the density of a lipoprotein decreases as the proportion of lipid to protein increases . Four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis.

(1) chylomicrons، derived from intestinal absorption of triacylglycerol and other lipids

(2) VLDL, derived from the liver for the export of triacylglycerol

(3) low density lipoproteins (LDL), representing a final stage in the catabolism of VLDL

(4) high-density lipoproteins, (HDL), Involved in transport of cholesterols as well as in VLDL and metabolism of chylomicron [89].

### 1.4.4.1 Chylomicrons (CM)

Chylomicrons are present in chyle formed just via the lymphatic system that drains the intestine and responsible for transporting all dietary lipids to the blood circulation [89].

Chylomicrons surface is a layer of phospholipids, where the head groups face the aqueous phase. Triacylglycerol's sequestered in the interior (yellow) make up more than 80% of the mass. Many apolipoproteins that emerge from the surface (C-2, B-48, C-3) work as signals in the absorption and metabolism of chylomicron [90].

Newly released or "nascent" chylomicrons contains a small worth of apolipoproteins E and C, and the complete supplementation of HDL is obtained in the circulation [91] .

### 1.4.4.2 Very Low-Density Lipoprotein (VLDL)

Very low density lipoprotein particles which transport TGs from the liver to peripheral tissues. VLDL is synthesized and secreted from the liver in a process based on APOB-100 [92].

Packages require hepatic TGs with APOB-100, cholesterol esters, PLs, and vitamin E to form a nascent VLDL induced enzyme microsomal triglyceride transfer protein (MTP) [93]. IDL TGs are in turn degraded by hepatic lipase (HL) to produce low-density lipoprotein (LDL) or removed by the interaction of apolipoprotein E with LDLR on the liver surface, Figure (1-3) [94].

### 1.4.4.3 Low-Density Lipoprotein (LDL)

Low density lipoproteins (LDL): These particles are derived from VLDL and IDL particles and are further enriched in cholesterol. LDL carries the majority of cholesterol found in the circulatory system. The dominant apolipoprotein is B-100 and every LDL particle contains one APO B-100 molecule, Figure (1-3) [95].

Low density lipoproteins consists of a spectrum of particles that vary in size and density. An abundance of small dense LDL particles are seen in association with hypertriglyceridemia, low HDL levels, obesity, type 2 diabetes and infectious and inflammatory states. These small dense LDL particles are considered to be more pro-atherogenic than large LDL particles for several reasons [96].

Small dense LDL particles have low affinity for LDL receptors, resulting in a long retention period in the circulation. In addition, they easily enter the arterial wall and are more passionately attached to proteoglycans within the arteries, which trap them in the arterial wall. Finally, small dense LDL particles are more susceptible to oxidation, which can lead to increased uptake by macrophages [97].

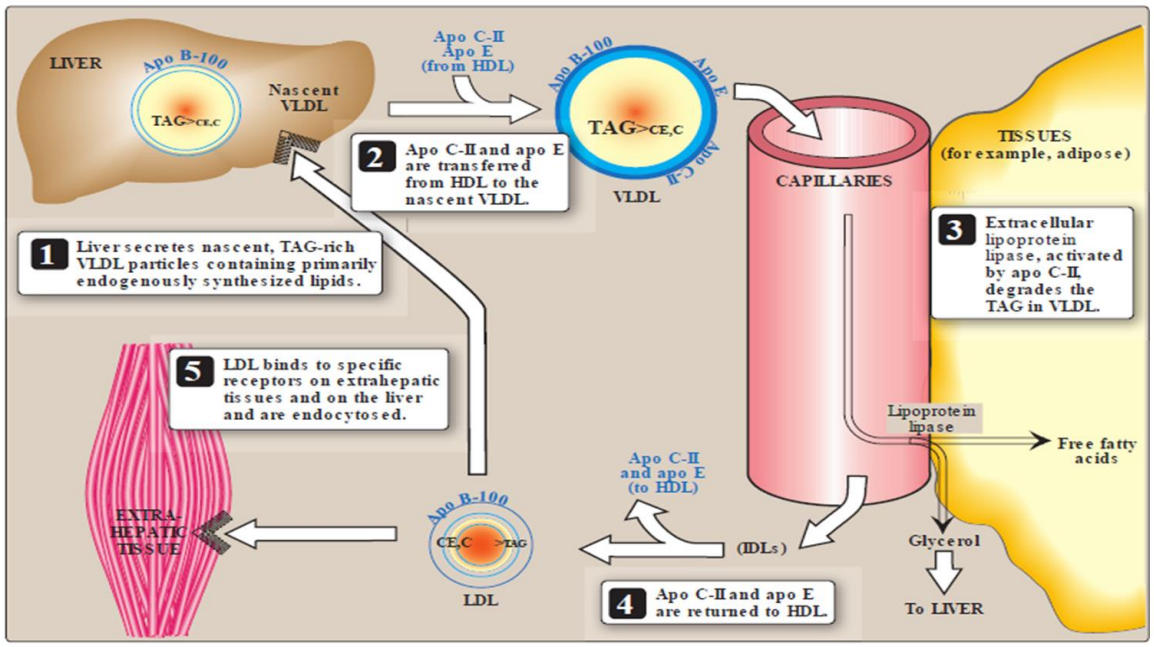


**Figure 1.3 Metabolism of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). APO B-100, APO C-2, and APO E are apolipoproteins found as specific components of plasma lipoprotein particles** [91].

## 1.4.4.4. High Density Lipoprotein (HDL)

High density lipoprotein cholesterol is known to be beneficial cholesterol and is inversely related to the risk of coronary artery disease (CAD) in humans [98].

High density Lipoprotein has been shown to protect against atherosclerosis and other vascular diseases based on anti-atherosclerotic effects such as reverse cholesterol transport and lipid homeostasis [99][100].

High density lipoprotein particles are manufactured by the intestines and liver [101]. The main protein on HDL is APOA1 that lends structural stability to the particle and stimulates efflux of cholesterol from cells to HDL, enlarging the particles [102].

High density lipoprotein promotes reverse cholesterol transport, mediating eclectic cholesteryl ester uptake from lipoproteins into the liver and steroidogenic tissues, as well as cholesterol efflux from macrophages. Besides

its turn, in regulating cholesterol metabolism, HDL has been shown to have anti-inflammatory and antioxidant effects in blood vessels, Figure (1-4) [103].

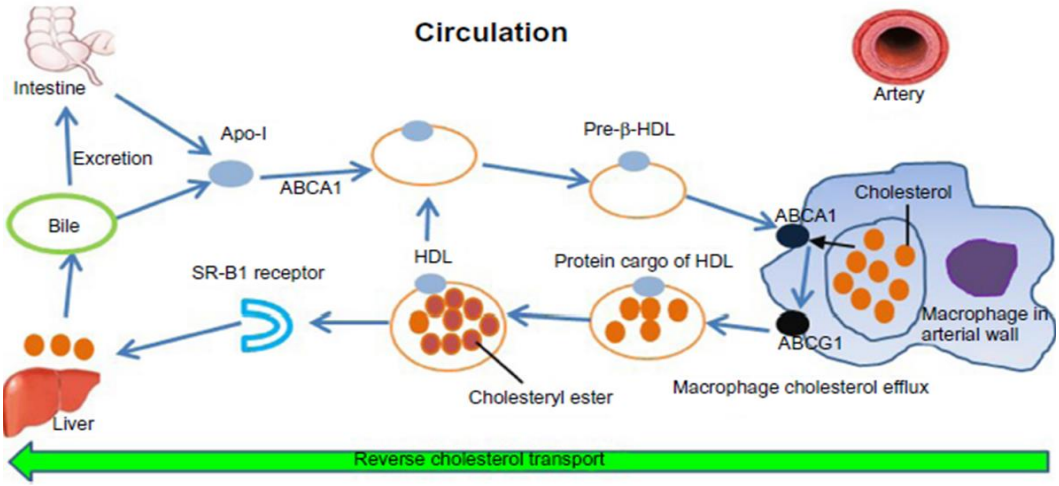


**Figure 1.4 Life cycle of HDL ABCA, ATP-binding cassette transporter family A; ABCG, ATP-binding cassette transporter family G; Apo, apolipoprotein; HDL, SR-B1, scavenger receptor class B1 ؛high-density lipoprotein** [103].

## Aim of the Study

This study to show an association between any immune events that starts with tissue destruction and protein lysis which trigger an inflammatory immune response that can involve the possibility of the occurrence of chronic coronary syndrome.

# 2.1 Materials and Methods

## 2.1.1Chemicals

The standard kits and the chemicals used in this study were shown in the following table.

**Table (2-1) Chemicals and kits used in this study**

| No. | CHEMICALS | COMPANY AND COUNTRY |
|---|---|---|
| 1 | Cathepsin K ELISA Kit | Elabscience (China) |
| 2 | Interleukin 2 ELISA Kit | Elabscience (China) |
| 3 | Triglyceride kit | Biolabo SAS France |
| 4 | Total Cholesterol | Biolabo SAS France |
| 5 | HDL-Cholesterol | Biolabo SAS France |

## 2.1.2 Instruments and Equipment

The table below showings the instruments used in this study

**Table (2-2) Instruments and equipment's used**

| No. | Instruments and equipment | Company, Origin |
|---|---|---|
| **1** | Centrifuge | Hettich, Germany |
| **2** | Spectrophotometer | C-cell, England |
| **3** | Deep freeze | GFL, Germany |
| **4** | Disposable syringe (5 mL) | Alrawabi, Jordan |
| **5** | Disposable test tube (10 mL) | Meheco, China |
| **6** | Distiller | GFL, Germany |
| **7** | Elisa reader and washer | Biotek, USA |
| **8** | Eppendorf tube (1.5 μL) | BC, China |
| **9** | Incubator | Memmert, Germany |

| | | |
|---|---|---|
| **10** | Micropipettes (5-50 µL), (2-20 µL), (20-200 µL), (100-1000 µL) | Slamed, Germany |
| **11** | pipette tips 0.2 mL | BC, China |
| **12** | pipette tips 1 mL | BC, China |
| **13** | Test tube with separation gel | AFCO, Jordan |
| **14** | Water bath | Memmert, Germany |

## ٢.2 Subjects

In this case-control study, there are two groups: the first includes patients chronic coronary syndrome, and the second includes control group people. The sample size was determined according to the Fisher formula for calculate sample size[104].This formula is :

$$n= \frac{Z^2 P(1-P)}{d^2}$$

Where n= sample size

Z= Z statistic for the level of confidence interval 95% which = 1.96.

P= Prevalence of coronary artery disease (CAD) which is 5.5 % in the Kingdom of Saudi Arabia [105].

D= precision (in proportion of one; if 5%, d = 0.05).

This research was carried out at the laboratory of the College of Medicine at the University of Babylon. The collecting of samples carried out from Septmber 2022 to January 2023. Questionnaires were created to collect data from the control and patients group.

### 2.2.1 Patients Group

The patients group that consisted of 50 patients with chronic coronary syndrome. This group was separated into two subgroups:

- 28 males.

- 22 females.

They were obtained from the Cardiac Catheterization Center at Imam Al-Sadiq Hospital. All patients were previously diagnosed by physicians at the Cardiac Catheterization Center

### 2.2.2 Control Group

The control group consists of 50 individuals and were separated into two subgroups:

- 28 males.

- 22 females.

### 2.2.3. Exclusion Criteria

patient with acute coronary syndrome (ACS).

### 2.3. Ethical Approval and Consent

All participants in this study were informed before to collecting samples, and verbal agreement was obtained from each of them.

## 2.4. Methodologies

### 2.4.1. Collection of Samples

Using a disposable syringe (5 mL), venous blood samples were obtained from control and patients.All samples were collected in fasting status at 8:00 am. Subjects were asked to come in for a blood sample. Five milliliters of blood were extracted through vein puncture and progressively pumped into disposable tubes containing separating gel. The blood in the gel-containing tubes was allowed to clot for 10 minutes at room temperature before being centrifuged for 10 minutes at 2000 xg, then separated into small volumes and kept in a deep freezer (-20$^{o}$ C) to carry out the assay. The blood samples obtained from the

groups were used to estimate serum Cathepsin k, Interleukin 2 and lipid profile.

### 2.4.2. Body Mass Index (BMI)

BMI: is widely used in the screening, diagnosis, and classification of overweight and obesity. BMI is an anthropometric measurement that inter-relates height and weight (kg/m2) and does not provide a direct measure of adipose tissue mass. Furthermore, BMI does not indicate the degree to which excess adiposity can lead to adiposopathy, or adversely affects the health of individual patients [106].

**Table (2-3) Body mass index established by WHO** [107]

| BMI | Classification |
|---|---|
| < 18.5 | underweight |
| 18.5- 24.9 | Normal weight |
| 25.0- 29.9 | Overweight |
| 30.0-34.9 | Class 1 obesity |
| 35.0- 39.9 | Class 11 obesity |
| _> 40.0 | Class 111 obesi |

## 2.4.3 Plasma Lipids Measurements

Plasma levels of TC, TG and HDL were measured by enzymatic colorimetric methods, LDLC level was measured by Friedewald formula .

### 2.4.3.1 Determination of Serum Total Cholesterol [108].

Total cholesterol concentration in serum were measured by enzymatic colorimetric method, and the reaction of the chemicals was as the following:

Cholesterol esters $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + free fatty acids

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

$2H_2O_2$ +phenol + PAP $\xrightarrow{\text{peroxidase}}$ Quinoneimine (pink) + $4H_2O$

**Procedure:**

The content of vial reagent 2 which contains the enzymes was added to the content of vial reagent 1 which contain the buffer and mix gently until the dissolution was completed in order to prepare the working reagent. Then, the procedure was carried as in the following:

**Table (2-4) procedure for determination serum total cholesterol**

| Reagents | Blank | Standard | Assay |
|---|---|---|---|
| Working reagent | 1 ml | 1ml | 1ml |
| Deionized water | 10µl | - | - |
| Standard | - | 10µl | - |
| Sample | - | - | 10µl |

The tubes were mixed then after mixing they let stand for about 5 minutes at 37℃ or 10 minutes at 25℃( room temperature), the absorbance was recorded at 500nm against reagent blank. Colour was stable for 1hour.

**Calculation**

The outcome was determined as follows:

$$\text{cholesterol} = \frac{\text{Absorbance of the assay}}{\text{Absorbance of the standard}} \text{X Concentration of standard}$$

## 2.4.3.2 Serum Triglyceride Determination: [109]

Triglyceride was measured by Fossati and Prencipe method related with Trinder reaction. As shown in the following scheme:

Triglyceride $\xrightarrow{\text{lipase}}$ Glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3-phosphate +ADP

Glycerol-3-phosphate+ $O_2$ $\xrightarrow{\text{Glycerol-3-phosphate oxidase}}$ Dihydroxyacetonphosphate +$H_2O_2$

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

The absorbance of quinonimine which is the colored complex, was proportional to amount of the sample.

**Procedure:**

The content of vial reagent 2 which contain the enzymes was added into the content of vial reagent 1 which contain the buffer, then mix gently and wait until complete dissolution (for about 2 minutes) before using the working reagent.

The procedure was continued as in the following:

**Table (2-5) procedure used for determination serum triglyceride**

| Reagents | Blank | Standard | Assay |
|---|---|---|---|
| Working reagent | 1ml | 1ml | 1m |
| Deionized water | 10µl | - | - |
| Standard | - | 10µl | - |
| Sample | - | - | 10µl |

The tubes were mixed and then let stands for about 5 minutes at 37℃ or 10 minutes at room temperature, then absorbance at 500 nm was measured against reagent blank. The reaction was stable for 1hour.

**Calculation:**

The result was calculated as follows:

$$\text{Triglyceride} = \frac{\text{Absorbance of the assay}}{\text{Absorbance of the standard}} \text{ X Standard concentration}$$

### 2.4.3.3 Determination of Serum HDL-Cholesterol: [110]

Magnesium chloride and Phosphotungestic acid (PTA) act on precipitating low density lipoprotein (HDL), very low density lipoprotein. HDL-cholesterol which obtained in the supernatant after centrifugation was measured with total cholesterol reagent.

**Procedure:**

In a clean plain tube 0.5 ml of sample (serum) was added, then the precipitant (50µl) was added. The tubes were mixed in a vigorous manner and let stand for 10 minutes at 25 $^0$C (room temperature). Then centrifuge for 15 minutes at 03,000 x g. The following procedure was applied for the measurement of HDL-cholesterol in the supernatant:

**Table (2-6) procedures used for determination serum HDL**

| Reagents | Blank | Standard | Assay |
|---|---|---|---|
| Working reagent | 1ml | 1ml | 1ml |
| Distilled water | 25µl | - | - |
| Standard | - | 25µl | - |
| Supernatant | - | - | 25µl |

The tubes were mixed and let stands for 5 minutes at 37℃ or 10 minutes at room temperature. Absorbance was recorded at 500nm against blank. The color was stable for 1 hour.

**Calculation:**

$$\text{HDL} = \frac{\text{Absorbance of the assay}}{\text{Absorbance of the standard}} \text{ x concentration of standard}$$

## 2.4.3.4 Determination of Serum Low Density Lipoprotein: [111]

LDL-cholesterol was measured by using Friedewald equation as follows:

$$LDL_{cholesterol}\ (mmol/L) = T._{cholesterol} - HDL_{cholesterol} - VLDL_{cholesterol}\left(\frac{Triglyceride}{2.22}\right)$$

## 2.4.4 Determination of Cathepsin k

### Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA), it is type sandwich ELISA. The plate has been pre-coated with Human CTSK antibody. CTSK present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human CTSK Antibody is added and binds to CTSK in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CTSK antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human CTSK. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm[112].

### Reagent Preparation

1- All reagents should be brought to room temperature before use.

2- Standard Reconstitute the 120µl of the standard (48pmol/L) with 120µl of standard diluent to generate a 24pmol/L standard stock solution. Allow the standard to sit for 15 mines with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (24pmol/L) 1:2 with standard diluent to produce12pmol/L, 6pmol/L, 3pmol/L and1.5pmol/L solutions. Standard diluent serves as the zero standard (0 pmol/L). Any remaining solution should be frozen at -20°C and used within one month.

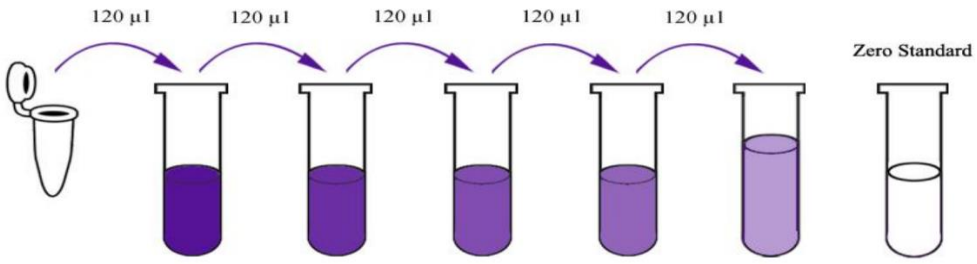


**Figure 2.1 Concentration of standards of Cathepsin k**

3- Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

**Assay procedure**

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. A standard solution of 50µl was added to standard well. Note: Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

4. A volume of 40 µL from sample was added to each sample wells and then added 10µl anti-CTSK antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well ). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Each well was received 50 µL of Substrate A and then added 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7 The reaction was stopped by adding 50 µL of stop solution to each well., the blue color will change into yellow immediately.

8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

**Calculation of Results**

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

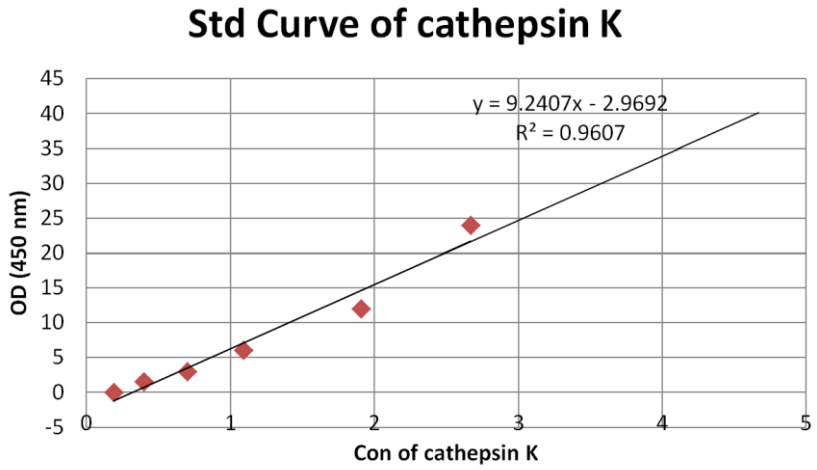


**Figure 2.2 -Standard curve of Cathepsin k**

## 2.4.5 Determination of Interleukin-2, IL-2

### Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA), it is type sandwich ELISA. The plate has been pre-coated with Human IL2 antibody. IL2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL2 Antibody is added and binds to IL2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm[113].

### Reagent Preparation

- All reagents should be brought to room temperature before use.

- Standard Reconstitute the 120ul of the standard (2400ng/L) with 120ul of standard diluent to generate a 1200ng/Lstandard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1200ng/L) 1:2 with standard diluent to produce 600ng/L, 300ng/L, 150ng/L and 75ng/L solutions. Any remaining solution should be frozen at -20°C and used within one month.

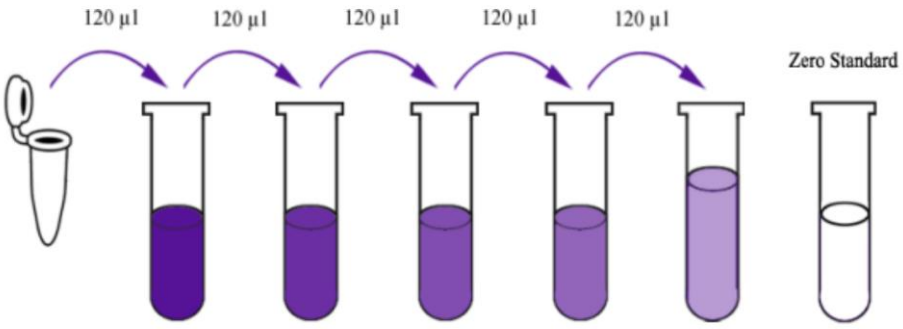


**Figure 2.3 Concentration of standards of Interleukin-2, IL-2**

• Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

**Assay procedure**

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. A standard solution of 50µl was added to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4. A volume of 40 µL from sample was added to each sample wells and then added 10ul Human IL2 antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For

automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Each well was received 50 µL of Substrate A Reagent. and then added 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. The reaction was stopped by adding 50 µL of stop solution to each well; the blue color will change into yellow immediately.

8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

**Calculation of Results**

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

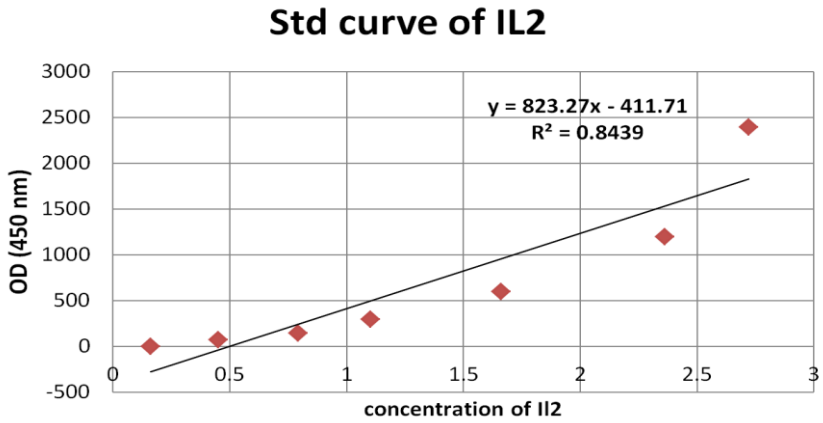


**Figure 2.4 - Standard curve of Interleukin-2, IL-2**

### 2.4.6 Statistical Analysis

Statistical analysis was carried out using SPSS version 25. Categorical variables were presented. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. Paired t-test was used to compare means for two paired readings. A *p*-value of $\leq 0.05$ was considered as significant. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of CCS.

# 3. Results and Discussion

## 3.1. Demographic Characteristics in Patients and Control.

### 3.1.1. Age and BMI

The patients and control that were included in this study were aged above 40 and BMI (20-29.9) Kg/$m^2$ as shown in table (3-1)

**Table (3-1) The general characteristics of patients and controls according to study**

| **Study variables** | | **Patients (N=50)** Mean ± SD | **Controls (N=50)** Mean ± SD | **P value** |
|---|---|---|---|---|
| **Age (years)** | | 55.43 ± 5.17 | 53.13 ± 5.09 | 0.56 |
| **BMI (kg/$m^2$)** | | (28.9 ± 3.0) | (28.0 ± 3.8) | 0.09 |
| **Gender** | **Male(n)** | 34 | 28 | 0.22 |
| | **Female(n)** | 16 | 22 | |

Age is a significant factor in the development and prevalence of chronic coronary syndrome (CCS), the most indicator of cardiovascular hazard in any hazard condition is age [114], With nearly three times risk with each decade as reported Jani B *etal* [115]. As shown below in Figure (3.1).

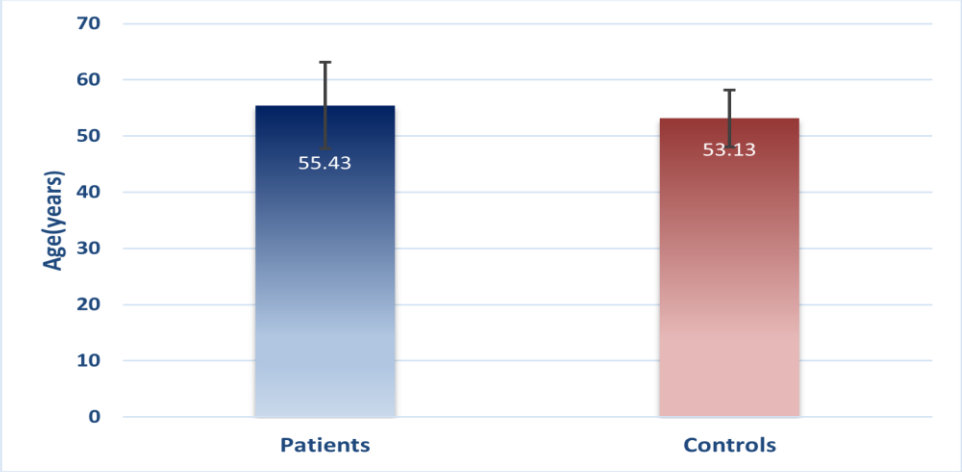


**Figure 3.1 The mean differences of age according to study group (P<0.001*)**

In this study, both groups were overweight. This match takes out contrasts in parameter results. Where the free fatty acids (FFA) released from abdominal fat, enter the portal vein, thus, direct access to the liver.

This is agree with Elisa Fabbrini *etal* study that concluded the Obesity linked with multiple metabolic risk factors for cardiovascular disease, including diabetes, insulin resistance, and dyslipidemia and the study showed that insulin resistance in muscle, adipose tissue and liver changes in free fatty acid metabolism [116].

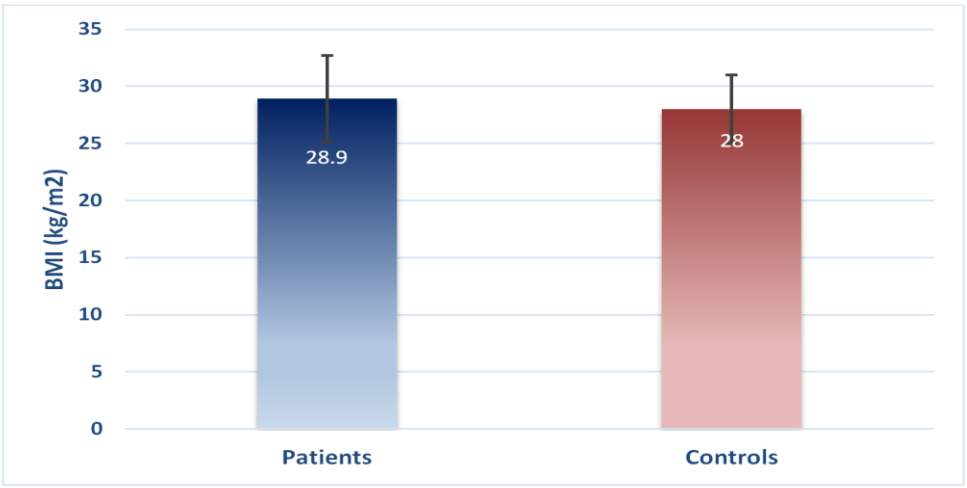


**Figure 3.2 The mean differences of BMI according to study groups (P<0.001*)**

### 3.1.2. Gender Distribution in patients and control groups.

Amongst fifty patients with chronic coronary syndrome who contributed to this study, there were 28 males and 22 females, and this represents 56% and 44% of patients respectively, as shown in figure (3.3).

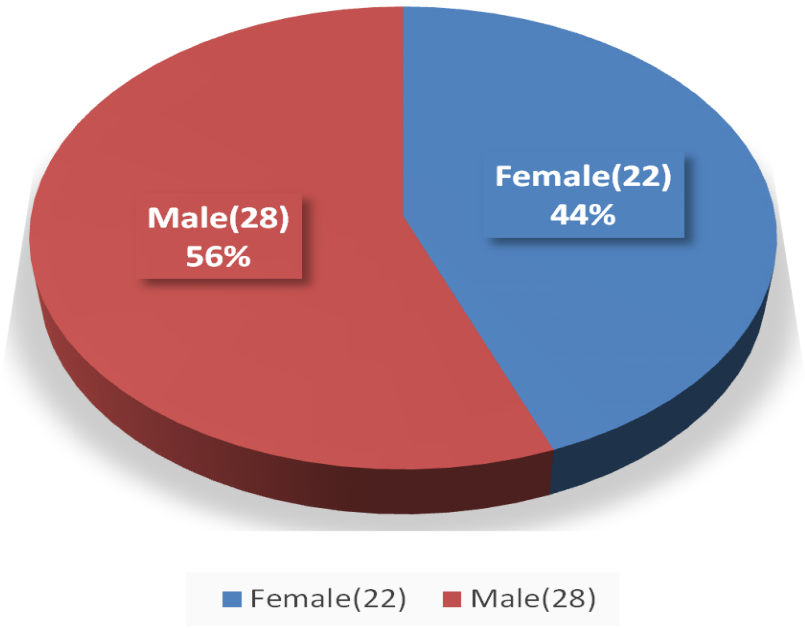


**Figure 3.3 The distribution of gender in study group.**

## 3.2. Measurements of Cholesterol, Triglyceride, Very low density lipoprotein, Low density lipoprotein and High density lipoprotein Concentration in Patient and Control Group

The result exhibit that the levels of total cholesterol P value (≤0.001), TG P value (≤0.45), VLDL P value(≤0.71) and LDL P value(≤0.001) are significant increased except TG and VLDL are no significant normal and HDL is significant decrease in patients group when compared with the control group as shown in table(3-2).

**Table (3-2): The mean differences of lipid profile according to study group including (CCS patients and control group).**

| Study variables | Study group | N | Mean ± SD | P-value | Normal value |
|---|---|---|---|---|---|
| Total Cholesterol (mmol/L) | CCS patients | 50 | 4.4 ± 0.80 | < 0.001* | 3.9 – 6.2 |
| | Control group | 50 | 3.5 ± 0.55 | | |
| TG (mmol/L) | CCS patients | 50 | 1.21 ± 0.36 | 0.45 | 0.4 – 1.4 |
| | Control group | 50 | 1.31 ± 0.80 | | |
| HDL (mmol/L) | CCS patients | 50 | 0.90 ± 0.16 | 0.001* | 0.9 – 1.4 |
| | Control group | 50 | 1.20 ± 0.23 | | |
| LDL (mmol/L) | CCS patients | 50 | 2.66 ± 0.50 | 0.001* | 1.8 – 4.3 |
| | Control group | 50 | 1.98 ± 0.67 | | |
| VLDL (mmol/L) | CCS patients | 50 | 0.63 ± 0.46 | 0.71 | < 0.53 |
| | Control group | 50 | 0.59 ± 0.36 | | |

*P value ≤ 0.05 was significant

The results revealed that there was no significant differences in the level of CCS between males patients and females patients groups (p-value >0.05) for TG and VLDL, while significant differences between males patients and females patients groups (p-value ≤ 0.05) for total cholesterol ,LDL and HDL, the means, standard deviation, and statistical parameters are listed in the Table (3.2).

Estimation of Total cholesterol (TC) Table (3-2) showed that results of the present study the mean value of TC was significantly in patients groups (p-value ≤ 0.001) and SD of patients group(4.4 ± 0.80),SD of control group(3.5 ± 0.55).

The cholesterol may quickly be linked with chronic coronary syndrome. The elevated level of cholesterol in serum lead to the increase of the progressing

macro vascular complications, such as MI [117]. High cholesterol concentration participates to build-up of plaque in the arteries, blood vessels that carry oxygen-rich blood to body organs and various portions of the body. Additionally, a plaque solidifies and narrows the arteries, subsequently forming of atherosclerosis. Atherosclerosis restrictions the movement of oxygen-rich blood and can influence in anybody arteries, including arteries in the heart [118]. Other study denote a gradual rise in CVD risk as the serum TC exceeds 5.0 mmol/L [119].

Estimation of Low density lipoprotein cholesterol (LDL-C) Table (3-2) showed that results of the present study the mean value of LDL-C was significantly in male patients and female patients groups (p-value 0.001) and SD of patients group (2.66 ± 0.50) SD of control group (1.98 ± 0.67), increased in the mean of LDL-c level in patients groups.

Hyperlipidemia, especially high serum levels of low-density lipoprotein (LDL) cholesterol (LDL-c), is a major risk factor for endothelial dysfunction and CAD in the general population [120]. Increased circulating LDL-c levels may be related with obesity, systemic inflammation and CAD [121]. The rise of LDL cholesterol may does not reflect impaired LDL catabolism but rather implies increased production of LDL-c [122]. The LDL-c act as a transporter of lipids, cholesterol in the body. The oxidation of LDL-C to become oxidized (ox-LDL) leading to becoming atherogenic in the arterial wall, ox-LDL, identified as a small dense LDL (sd-LDL), activates the cascade of local inflammation [123]. ox-LDL, alternatively, has been linked with formation of plaque and increased risk of stroke and heart attack when build up in the endothelium [124]. Moreover to its inducing endothelial dysfunction, ox-LDL exhibits proinflammatory actions, including chemotactic effects expression of macrophage colony-stimulating factors, and adhesive molecules. Finally, oxidized LDL is trapped by the monocyte/macrophage scavenging receptors, leading in turn to monocyte activation [125].

Estimation of High density lipoprotein cholesterol (HDL-c) Table (3-2) showed that results of the present study the mean value of HDL-c was significantly in patients groups (p-value 0.001) and SD of patients group (0.90 ± 0.16) SD of control group (1.20 ± 0.23), decreased in the mean of HDL-c level in patients groups compere with control group.

HDL-c characterizes an important cardioprotective factor assumed the role in reverse cholesterol transfer, its effects on endothelial cells, and it has a role antioxidant effect [126]. A possible reduction in HDL is associated with obesity and also a risk factor for coronary artery disease. which is agrees the recent studies (Krishnan et al 2016) [127]. (Ahn N, et al 2016) [128], (Chen Q, et al 2016) [129]. Showed the decrease of HDL-c concentration in the blood stimulates cardiac disease and that rising concentration of HDL-c may be beneficial, especially in low level of HDL-c patients, many arguments suggestions a protective role of HDL against LDL oxidave modification [130]. however experimental studies advocate a direct role for HDL-c in stimulating efflux of cholesterol from foam cells in the atherosclerotic plaque depots in blood vessels to the liver for cholesterol excretion. HDL-c also reveals potent anti-inflammatory properties that inhibit the atherogenic process [131]. The protectivety of HDL-c against cardiovascular disease predominantly the function in reverse cholesterol transport, more than 40% of MI patients have Low level of HDL-c in blood [132].

Estimation of Triglyceride (TG) Table (3-2) showed that results of the present study the mean value of TG was no significantly in patients groups and (p-value 0.45)

Triglycerides are a type of fat found in your blood, and high levels of triglycerides are associated with an increased risk of cardiovascular disease, including chronic coronary syndrome. However, it is possible that in some cases, triglyceride levels may not be significantly elevated in individuals with chronic coronary syndrome.

Other risk factors may be more significant: While high triglyceride levels are a risk factor for cardiovascular disease, other factors such as high blood pressure, smoking, and high levels of LDL (bad) cholesterol may be more significant in some individuals [133].

Estimation of Very Low density lipoprotein cholesterol (VLDL) Table (3-2) showed that results of the present study the mean value of VLDL was no significantly in patients groups (p-value 0.71) and SD of patients group (0.63 ± 0.46) SD of control group (0.59 ± 0.36), normal in the mean of VLDL level in patients groups.

VLDL is the precursor of LDL, that have low content of cholesterol, but also contains some triglycerides, and these are also a risk factor for CVD [134]. High levels of VLDL cholesterol can be associated with an increased risk of coronary artery disease, but in some cases, VLDL levels may remain normal even in the presence of chronic coronary syndrome. One possible explanation for this is that in chronic coronary syndrome, the focus is more on the buildup of LDL cholesterol in the coronary arteries, which can lead to the formation of plaques that can obstruct blood flow to the heart. In contrast, VLDL cholesterol may play a lesser role in the development of plaques in the coronary arteries [135].

## 3.3 Measurements of Cathepsin K in Patient and Control Group

The result showed that the levels of cathepsin k are significantly increased in patient group when compared with the control group (p 0.01), as shown in table (3-3).

**Table (3-3) Mean ± standard deviation and p value of Cathepsin k concentration in patients group and control group**

| Study variables | Study group | N | Mean ± SD | P-value |
|---|---|---|---|---|
| Cathepsin K (pmol/L) | CCS patients | 50 | 9.29 ± 4.22 | 0.01* |
| | Control group | 50 | 6.65 ± 2.40 | |

Recent studies have shown that cysteinyl cathepsin K is the most abundant and significant protease synthesized by the cardiovascular cells and inflammatory cells, and that it is relevant to atherosclerosis-related cardiovascular disease and its implications. The protein level of cathepsin K was increased in atherosclerotic plaques and injury-related lesions in animals and humans [136].

Studies have shown that patients with CAD tend to have higher levels of CatK compared to individuals without the disease. CatK is primarily known for its role in bone remodeling, but emerging research suggests its involvement in cardiovascular diseases as well. In the context of CAD, CatK has been found to promote the migration and proliferation of smooth muscle cells. [137].

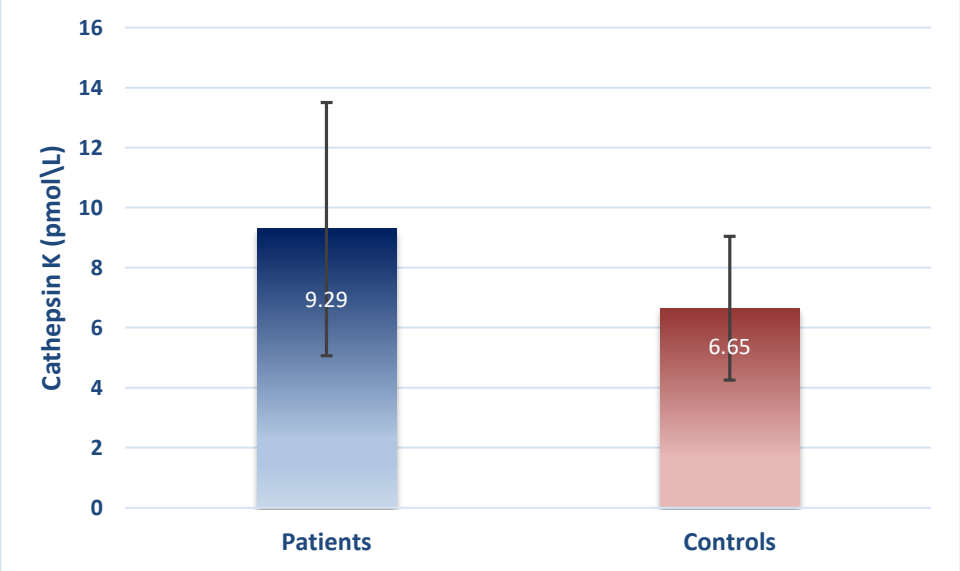


**Figure 3.4 The mean differences of Cathepsin K (P= 0.01*)**

## 3.4 Measurements of Interleukin-2 (IL-2) in Patient and Control Group

The result showed that the levels of Interleukin-2 are significantly increased in patient group when compared with the control group (p 0.001), as shown in table (3-4).

**Table (3-4) Mean ± standard deviation and p value of Interleukin-2 concentration in patients group and control group**

| Study variables | Study group | N | Mean ± SD | P-value |
|---|---|---|---|---|
| IL2 (ng/L) | CCS patients | 50 | 1.41 ± 0.59 | 0.001* |
| | Control group | 50 | 0.97 ± 0.32 | |

In cardiovascular disease, chronic inflammation plays a key role in the development and progression of atherosclerosis, which is a narrowing of the arteries due to the buildup of fatty deposits (plaques) on the artery walls. This chronic inflammation leads to the activation of immune cells, including T cells and macrophages, which release pro-inflammatory cytokines, including IL-2 [138].

IL-2, in turn, promotes the activation and proliferation of immune cells, including T cells and natural killer (NK) cells, which can contribute to the development and progression of atherosclerosis. Additionally, IL-2 has been shown to promote the production of other pro-inflammatory cytokines, which can also contribute to the inflammatory process in cardiovascular disease [139]. Studies have shown that IL-2 can stimulate the proliferation and migration of vascular smooth muscle cells, which are key processes involved in the formation of atherosclerotic plaques. In vitro studies have demonstrated that IL-

2 can directly stimulate the proliferation and migration of smooth muscle cells, while in vivo studies have shown that IL-2 can increase the thickness of the intima layer of the arterial wall, which is a hallmark of atherosclerosis [140].

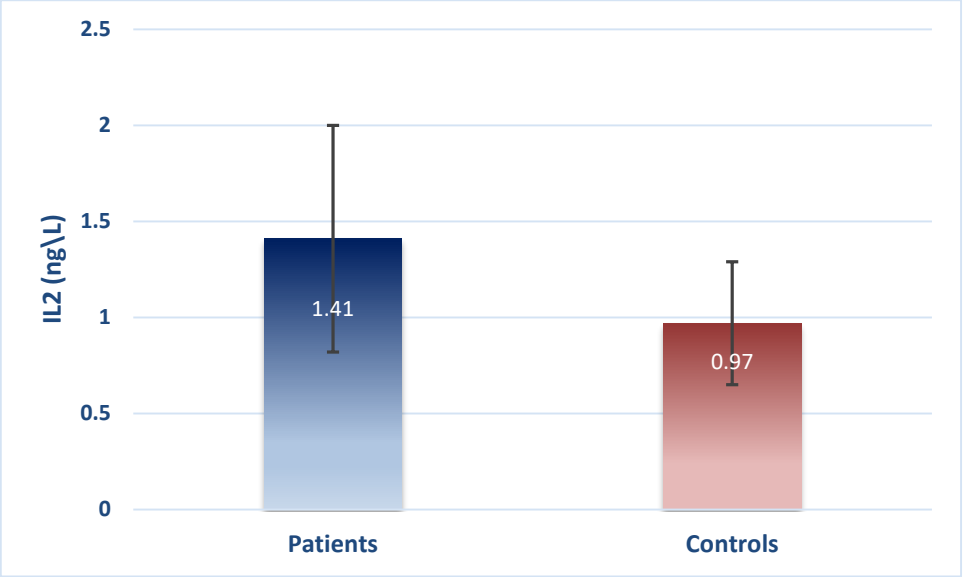


**Figure 3.5 The mean differences of IL2 (P=0.001*)**

# 3.5- Correlation between age, cholesterol and HDL in patients of CCS and control

**Table: 3.5-Correlation between biochemical parameter**

| | | Cathepsin K | IL2 |
|---|---|---|---|
| **Age** | r | 0.328 | 0.363 |
| | Sig | **0.001** | **0.00** |
| | N | 50 | 50 |
| **Cholesterol** | r | 0.294 | 0.212 |
| | Sig | **0.003** | **0.034** |
| | N | 50 | 50 |
| **T.G** | r | -0.099 | -0.014 |
| | Sig | 0.325 | 0.890 |
| | N | 50 | 50 |
| **HDL** | r | -0.171 | -0.298 |
| | Sig | 0.088 | **0.003** |
| | N | 50 | 50 |
| **VLDL** | r | -0.096 | -0.058 |
| | Sig | 0.343 | 0.569 |
| | N | 50 | 50 |

r: correlation coefficient

In this study there is a positive correlation between age with cathepsin k p-value ≤0.001, as seen in figure (3.5)

there is evidence to suggest that increased cathepsin K activity with age may contribute to the development of CAD [141].

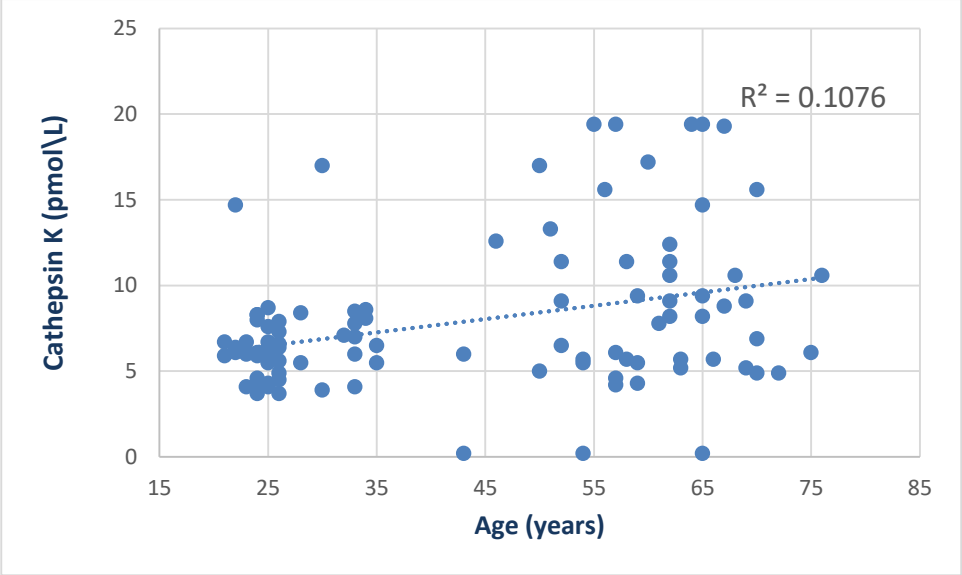


**Figure 3.6 the correlation between age and Cathepsin K**

Our data indicate there is a positive correlation between age with Interleukin-2 p-value=0.00, as seen in figure (3.7)

The correlation between age and Interleukin-2 in CAD suggests that as people get older, their immune system may become more activated, leading to an increase in Interleukin-2 production. This may contribute to the chronic inflammation that is seen in CAD[142].

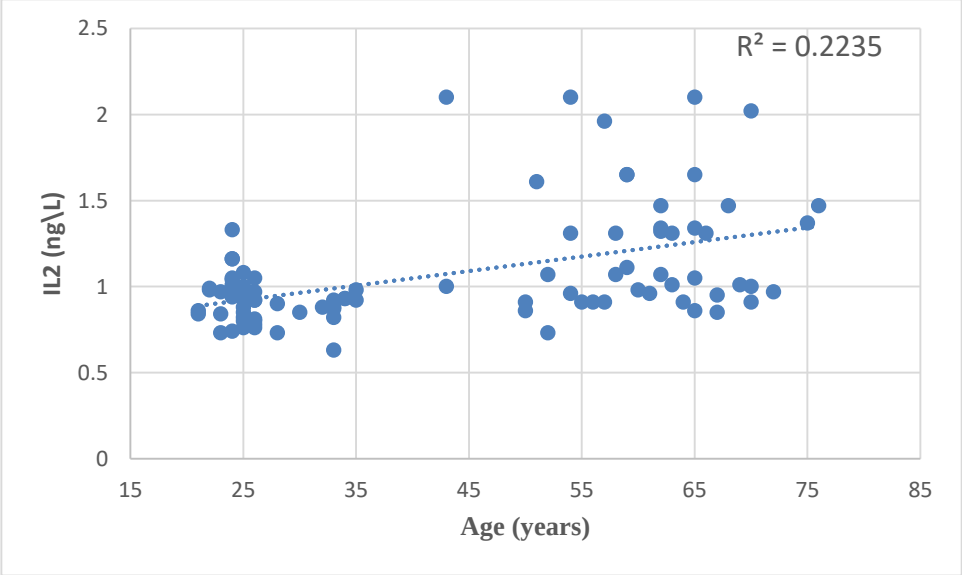


**Figure 3.7 The correlation between age and IL2 .**

In this study significant positive correlation between cholesterol with cathepsin k p-value= 0.003, as seen in figure (3.8)

It has been demonstrated that adipose tissue expresses cathepsin K. This is critical because atherogenesis—the process by which fatty deposits build up in the walls of the arteries and contribute to the onset of cardiovascular disease—has been linked to these proteases.

Larger adipose tissue, especially visceral adipose tissue, has been linked to a higher risk of cardiovascular disease, according to the available research. The increased expression of cathepsins in adipose tissue may help to promote the growth and development of atherosclerosis and, as a result, raise the risk of cardiovascular events [143].

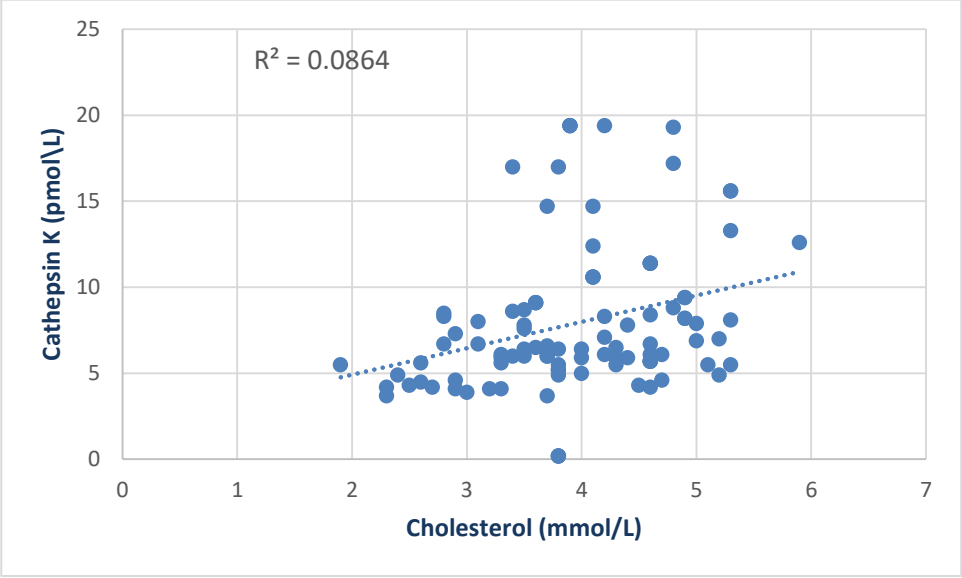


**Figure 3.8 The correlation between cholesterol and Cathepsin K.**

The present study showed that significant positive correlation between cholesterol with Interlrukin-2 p-value=0.034, as seen in figure (3.9)

it is that high levels of cholesterol in the blood can cause inflammation in the walls of the arteries. This inflammation can trigger the release of cytokines such as interleukin-2 by immune cells in the area. These cytokines can then

contribute to the development of atherosclerosis, a condition in which plaque builds up inside the arteries and can lead to heart disease [144].

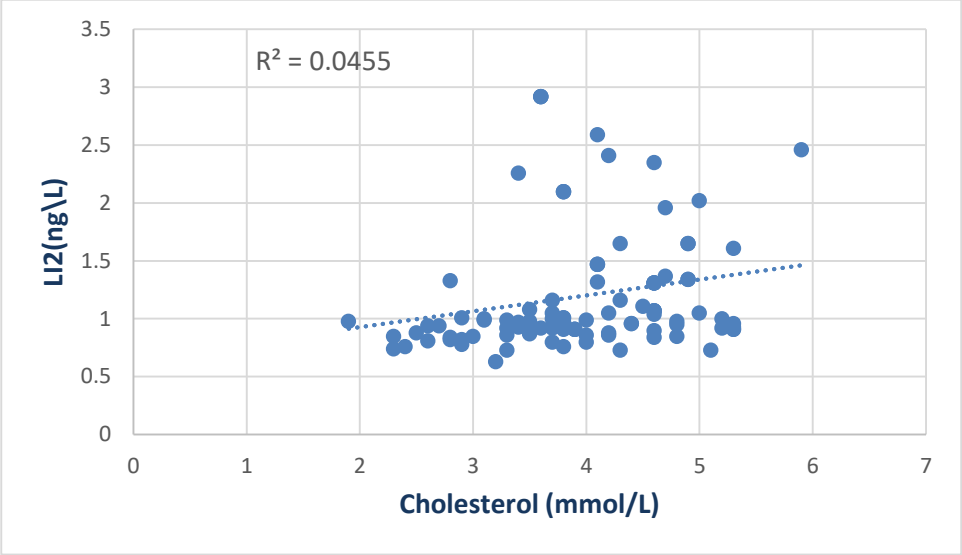


**Figure 3.9 The correlation between cholesterol and IL2.**

Our data indicate there is a negative correlation between HDL with Interleukin-2 p-value=0.003, as seen in figure (3.10)

One study published in the Journal of Internal Medicine found that there was a negative correlation between HDL levels and IL-2 levels in patients with stable coronary artery disease (CAD). The study showed that patients with lower levels of HDL also had higher levels of IL-2, suggesting a link between these factors in the development of CAD [145].

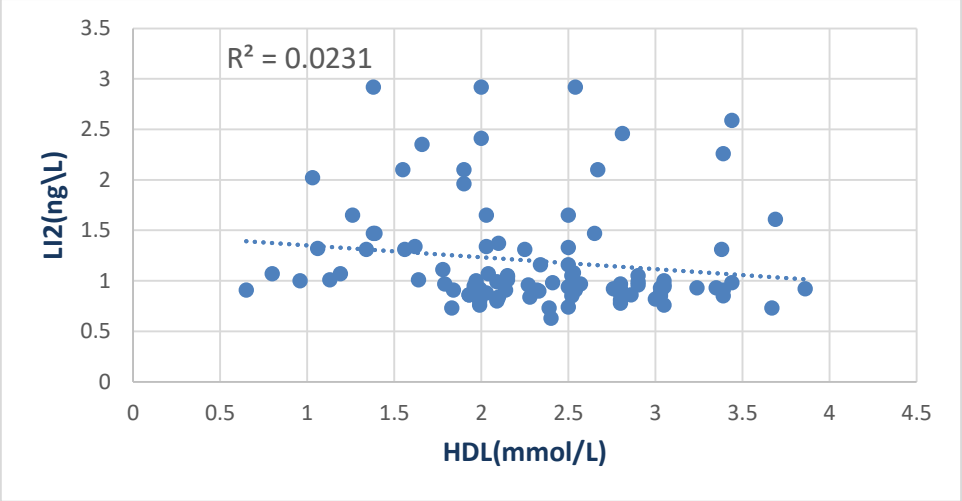


**Figure 3.10 The correlation between HDL and IL2.**

# 3.6. ROC curve of biochemical parameters

**Table 3.6 ROC curve of Cathepsin K**

| Parameter | Cathepsin K |
|---|---|
| **Cut-off point** | **8.6** |
| **AUC** | **0.66** |
| **sensitivity** | **88.0 %** |
| **specificity** | **54.0 %** |
| **P value** | **0.005** |

## 3.6.1 ROC curve for Cathepsin K

ROC curve for the sensitivity and specificity of cathepsin k (pmol/L) for diagnosis of chronic coronary syndrome (CCS), (Cut-off point was 8.6

**(**pmol/L), AUC=0.66, P 0.005, the sensitivity and the specificity was 88.0 %, 54.0 % respectively, as shown in figure (3-11).

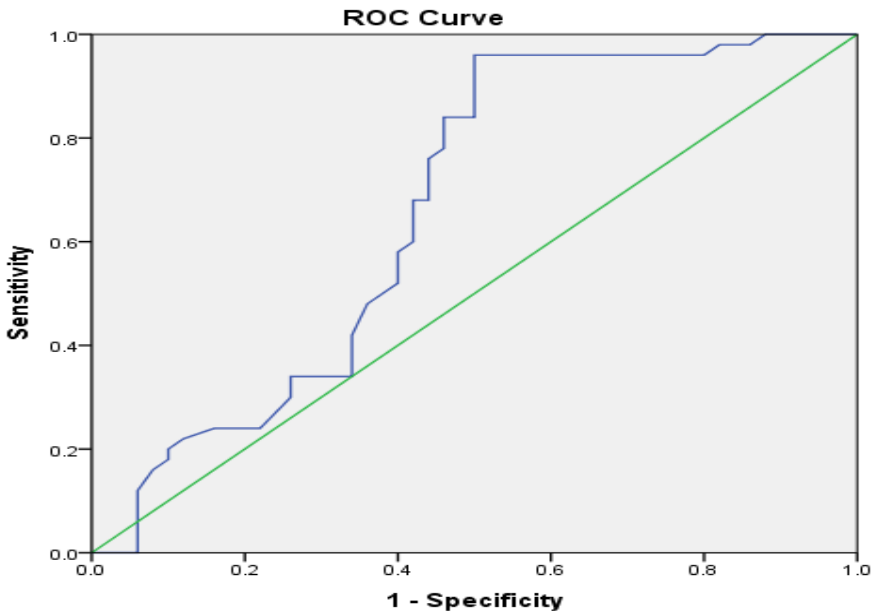


**Figure 3.11 ROC curve for the sensitivity and specificity of Cathepsin K for diagnosis of CCS.**

## 3.6.2 ROC curve for Interleukin-2 (IL-2)

**Table 3.7 ROC curve of Interleukin (IL-2)**

| **Parameter** | **IL2** |
|---|---|
| **Cut-off point** | **0.99** |
| **AUC** | **0.79** |
| **sensitivity** | **78.0 %** |
| **specificity** | **70.0 %** |
| **P value** | **0.00** |

ROC curve for the sensitivity and specificity of Interleukin-2(ng/L**)** for diagnosis of chronic coronary syndrome (CCS), (Cut-off point was 0.99 (ng/L)) , AUC=0.79, P 0.00, the sensitivity and the specificity was 78.0 %, 70.0 % respectively, as shown in figure (3-12).

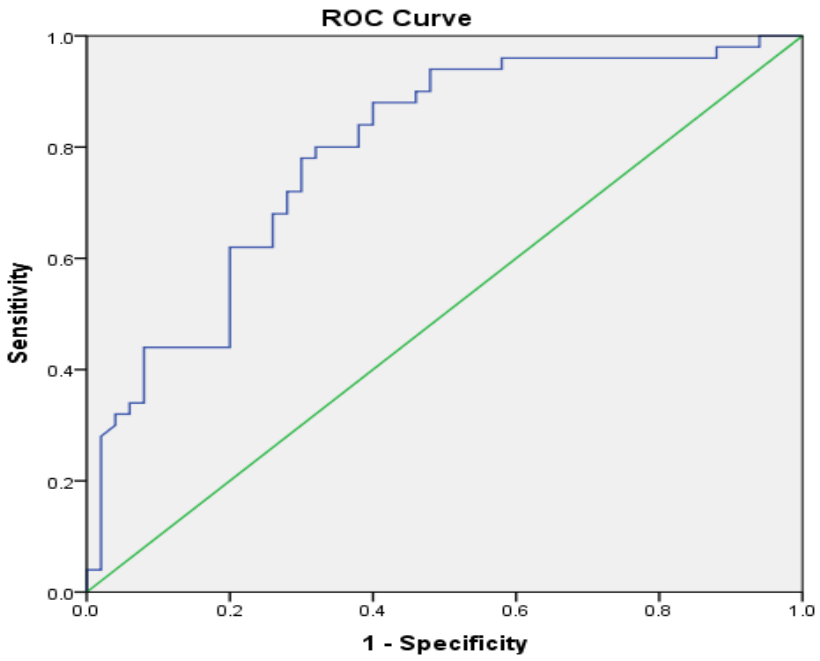


**Figure 3.12 ROC curve for the sensitivity and specificity of IL2 for diagnosis of CCS.**

# Conclusion

1- Elevated cathepsin k (CatK) and Interleukin-2 (IL-2) are significantly associated with chronic coronary syndrome (CCS).
2- Increase concentration of cholesterol and LDL with chronic coronary syndrome, while concentration of triglyceride and VLDL is normal
3- Significant positive correlation between level of cathepsin k and Interleukin 2 with cholesterol.
4- Significant negative correlation between level of Interleukin-2 and HDL.

# Recommendations

- Recognize that serum cathepsin k should be evaluated in conjunction with established cardiovascular biomarkers.
- The study of cathepsin k in terms of genetic variance to understand the causes of CCS patients in a study similar to ours.
- All investigations should be available at the research center and supported by scientific bodies.
- A future study is to be done on larger sample size to give results that are more accurate.
- Diet regulation significantly reduces the risk of heart disease

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**الخلاصة:**

تشير متلازمة الشريان التاجي المزمنة (CCS) إلى مجموعة من أمراض القلب والأوعية الدموية الناتجة عن تضيق أو انسداد الشرايين التاجية المسؤولة عن إمداد عضلة القلب بالدم. تتطور الحالة عادةً تدريجيًا بمرور الوقت ، وتتميز بألم أو انزعاج مستمر أو متكرر في الصدر (الذبحة الصدرية) يحدث أثناء النشاط البدني أو الإجهاد العاطفي. قد تشمل الأعراض الشائعة الأخرى لـ CCS ضيق التنفس ، والتعب ، والشعور بالضيق أو الضغط في الصدر.

الهدف من دراسة الحالة الحالية - التحكم:

تظهر هذه الدراسة ارتباطًا بين أي حدث مناعي يبدأ بتدمير الأنسجة وتحلل البروتين الذي يؤدي إلى استجابة مناعية التهابية يمكن أن تنطوي على احتمال حدوث متلازمة الشريان التاجي المزمنة

تضمنت هذه الدراسة (١٠٠) عينة ضمن أعمار تزيد عن ٤٠ عامًا ، (٥٠) مريضًا تشمل الذكور (٢٨) والإناث (٢٢) بمتوسط ± الانحراف المعياري (SD) للعمر (٥٥,٤٣ ± ٥,١٧) ، تم قبول هؤلاء المرضى في أمراض القلب وحدة الرعاية بمستشفى الإمام الصادق التعليمي للمصابين بمتلازمة الشريان التاجي المزمنة. تم أخذ جميع العينات في غضون اثنتي عشرة ساعة من القبول. المجموعة الثانية تضم (٥٠) عينة من المجموعة الضابطة ، ويبدو أن الضبط الصحي يشمل الذكور (٢٨) والإناث (٢٢) بمتوسط SD ± للعمر (٥٣,١٣ ± ٥,٠٩). تم مطابقة المجموعة الضابطة مع مجموعات المرضى في الجنس والعمر لزيادة دقة النتائج وتم جمع عينات الدم من الأقارب والطاقم الطبي. تم جمع عينات المشاركين من الأول من (سبتمبر ٢٠٢٢ حتى يناير ٢٠٢٣). تم انجاز الجزء الذي يتناول الجانب العملي من الدراسة في معمل قسم الكيمياء الحيوية ، كلية الطب ، جامعة بابل. تم تحديد مصل cathepsin k ، Interleukin-2بتقنية .(ELISA) تم تحديد ملف تعريف الدهون بواسطة مقياس الطيف الضوئي كما تم تحديد مؤشر كتلة الجسم.

أظهرت النتيجة أن مستويات الكاتيبسين k تزداد بشكل ملحوظ في مجموعة المرضى عند مقارنتها مع المجموعة الضابطة (p 0.01) ، كما أظهرت النتيجة أن مستويات Interleukin-2 تزداد بشكل ملحوظ في مجموعة المرضى عند مقارنتها مع مجموعة التحكم ( ص ٠,٠٠١). أظهر تقدير الكوليسترول الكلي (TC) أن نتائج الدراسة الحالية كان متوسط قيمة TC بشكل ملحوظ في مجموعات المرضى الذكور والإناث (القيمة ($p \leq 0.001$ و SD لمجموعة الضابطة (٣,٥ ± ٠,٥٥ (، SD للمجموعة المرضى (٤,٤ ± ٠,٨٠) ، أظهر تقدير الدهون الثلاثية (TG) أن نتائج الدراسة الحالية لم يكن متوسط قيمة TG معنوياً في مجموعات المرضى الذكور والإناث و (القيمة (p 0.45 ، تقدير الكوليسترول الدهني عالي الكثافة (C- (HDL) أظهرت نتائج الدراسة الحالية أن متوسط قيمةHDL-C كان معنويا في المرضى الذكور ومجموعات المرضى الإناث (القيمة (p (0.001 و SD لمجموعة

المرضى (٠,٩٠ ±٠,١٦ )SD (للمجموعة الضابطة (١,٢٠ ± ٠,٢٣) ) ، انخفض في متوسط مستوى HDL-Cفي مجموعات المرضى المتوافقين مع المجموعة الضابطة. بالإضافة إلى ذلك ، أظهر تقدير كوليسترول البروتين الدهني منخفض الكثافة (LDL-C) أن نتائج الدراسة الحالية كانت القيمة المتوسطة لـ LDL-C بشكل ملحوظ في المرضى الذكور ومجموعات المرضى الإناث (القيمة (0.001 p ومجموعة المرضى SD (2.66 ± 0.50) SD للمجموعة الضابطة (١,٩٨ ± ٠,٦٧) ، زاد في متوسط مستوى LDL-C في مجموعات المرضى ، أظهر تقدير كوليسترول البروتين الدهني منخفض الكثافة (VLDL)أن نتائج الدراسة الحالية لم يكن متوسط قيمة VLDL معنوياً. في المرضى الذكور ومجموعات المرضى الإناث (القيمة (0.71 p و SD لمجموعة المرضى (٠,٦٣ ± ٠,٤٦ SD ) لمجموعة التحكم (٠,٥٩ ± ٠,٣٦) ، طبيعي في متوسط مستوى VLDL في مجموعات المرضى. كانت هناك علاقة ارتباط موجبة معنوية بين العمر والكولسترول والكاثيبسين ك = (p=0.01, r=0.0328 (p=0.003, r =0.294)( على التوالي . وانترلوكين -٢ ارتباط موجب معنوي بالعمر والكوليسترول (p=0.00, r= o.363)(p=0.034 , r=0.212) ، في حين أن إنترلوكين -٢ علاقة سلبية معنوية مع HDL (p= ٠,٠٠٣، r=٠,٢٩٨ ).

.وأظهر الاستنتاج في هذه الدراسة أن ارتفاع كاثيبسين K وإنترلوكين ٢ مرتبطان بمتلازمة الشريان التاجي المزمنة ، حيث يساهم ارتفاعهما في تدمير الأنسجة وتحلل البروتين ، مما يؤدي إلى استجابة مناعية التهابية قد تنطوي على حدوث متلازمة الشريان التاجي المزمنة.

وزارة التعليم العالي
والبحث العلمي
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# رابطة مصل كاتيبسين ك ، انترلوكين ٢ وملف الدهون بين مرضى متلازمة الشريان التاجي المزمنة في محافظة بابل

رسالة

مقدمة إلى عمادة كلية الطب في جامعة بابل وهي جزء من متطلبات نيل
درجة الماجستير في العلوم / الكيمياء الحياتية السريرية

من قبل

**علياء محمد عبد الحسن هادي**

بكالوريوس علوم كيمياء / كلية العلوم للبنات/ جامعة بابل ( ٢٠١٦)

إشراف

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أ.م. أمير احمد الجباوي

١٤٤٤ ٢٠٢٣

# Appendix

# Chronic coronary syndrome questionnaire

## Patient information:

| | | |
|---|---|---|
| **Name:** | | **Age:** |
| **Height:** | **Weight:** | **BMI:** |
| **Gender:**<br>Male: Female: | | **Family History of disease:** |
| **Smoking:**<br>Yes: No: | | **Duration of disease:**<br>DM:<br>Hypertension: |
| **Treatment:**<br>DM:<br>Hypertension:<br>CS: | | |
| **Electrocardiogram:** | | |
| **Other diseases:** | | |

## Biochemical Tests

| | | | | | |
|---|---|---|---|---|---|
| **Lipid Profile:** | **Cho:** | **TG:** | **HDL:** | **LDL:** | **VLDL:** |

## Other Notes:

| |
|---|
| |