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Assessing the Levels of Melanoma Inhibitory Activity Protein 3, E-Selectin, and Cholesteryl Ester Transfer Protein in Patients with Coronary Artery Diseases

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfillment of the Requirements for the Master Degree in **Clinical Chemistry**

By

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بسم الله الرحمن الرحيم

﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا﴾

صَدَقَ اللهُ العَلِيّ العَظِيم

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Supervisor's Certification

We certify that this thesis entitled

Assessing the Levels of Melanoma Inhibitory Activity Protein 3, E-Selectin, and Cholesteryl Ester Transfer Protein in Patients with Coronary Artery Diseases

was prepared by (**Saif Saihood Hateb**) under our supervision at the College of Medicine, University of Kerbala, as a partial fulfillment of the requirement for the Degree of Master in **(Clinical Chemistry).**

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"Assessing the Levels of Melanoma Inhibitory Activity Protein 3, E-Selectin, and Cholesteryl Ester Transfer Protein in Patients with Coronary Artery Diseases"

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Dedication

To the soul of the martyr **(my father)**, who is thanks to his sacrifices I stand before you today

To that soul of the deceased **Falih Saihood (my brother)**

To the great **(my mother)**, my source of force and inspiration

To **my wife** and **children**

To my support in this world, **Haider (my brother)**

To my big family

I extend my thanks to all of them

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Saif

Summary

Coronary artery disease (CAD) is a prevalent cardiovascular condition in the elderly, primarily caused by coronary atherosclerosis. It can lead to complications like artery obstruction, stenosis, and ischemia. Symptoms include angina, which is characterized by chest discomfort. Acute coronary syndromes (ACS) are categorized into ST-elevation myocardial infarction, non-ST elevation myocardial infarction, and unstable angina. Inflammation is a key factor in CAD complications.

This study evaluated the correlation of biochemical markers, such as melanoma inhibitory activity 3 (MIA3), E-selectin, and cholesteryl ester transfer protein (CETP), as novel independent biomarkers with CAD. A case-control study was conducted on 180 subjects aged 41 to 70 years, divided into two groups: stable angina (60) and ACS (60), and the healthy control group (60). Serum biomarker levels were measured using the ELISA technique, and lipid profile levels in human serum were measured using a SMART-120 chemistry analyzer.

Results showed that MIA3, E-selectin, and CETP levels significantly increased in ACS and stable angina cases compared to healthy control cases. A positive and significant correlation was found between MIA3 and BMI, TC, and LDL-C, E-selectin had a negative and significant correlation with waist circumference, CETP had a positive and significant correlation with age and VLDL-C, and MIA3, CETP, and E-selectin had a significant negative correlation with HDL-C.

In conclusion, elevated levels of serum MIA3 are considered an independent diagnostic marker for stable angina and ACS due to their role in promoting monocyte migration across the endothelial layer and smooth muscle cell proliferation. E-selectin is a diagnostic marker for activated

endothelium, crucial in the initiation and progression of atherosclerosis. High levels of serum CETP can be a predictive indicator of the risk of developing CAD due to its impact on reducing HDL-C concentrations and increasing LDL-C and VLDL-C levels, predicting the risk of developing CAD.

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List of Abbreviations

Abbreviations	Complete Names
ACS	Acute coronary syndrome
AC	Atherogenic Coefficient
ACC	American college of cardiology
ADP	Adenosine diphosphate
AIP	Atherogenic Index of Plasma
ATP	Adenosine triphosphate
AUC	Area under curve
BMI	Body mass index
BPI	Bactericidal/permeability-increasing protein
CAD	Coronary artery disease
CC1	coiled-coil domain 1
CCS	Canadian Cardiovascular Society
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CHE	Cholesterol esterase
C-index	Cholesterol Index
COPII	Coat protein II
CPK	Creatine phosphokinase
CR domain	Cysteine-rich domain
CRI-I, CRI-II	Castelli's risk indexes (I & II)
cTAGE5	Cutaneous T-cell lymphoma-associated antigen 5
CVD	Cardiovascular disease
ECM	Extracellular matrix
ER	Endoplasmic reticulum
ERGIC	ER-Golgi intermediate compartment
ES	Embryonic stem cells
ESC	European society of cardiology
ESL-1	E-selectin ligand-1
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase
HDL-C	High-density lipoprotein
HRP-conjugated	Horseradish peroxidase
IL-1b	Interleukin-1b
LCAT	Lecithin: cholesterol acyl transferase
LDL-C	Low-density lipoprotein-cholesterol
LPS	Lipopolysaccharide
MIA3	Melanoma inhibitory activity 3
MMPs	Matrix metalloproteinases

NSTEMI	Non-ST-elevation myocardial infarction
OD	Optical density
PAI-1	Plasminogen activator inhibitor-1
PCr	Phosphocreatine
PRD	Proline-rich domain
PSGL-1	P-selectin glycoprotein ligand-1
ROC	Receiver operating curve
SD	Standard deviation
SMC	Smooth muscles cells
STEMI	ST-elevation myocardial infarction
TALI	TANGO1-Like
TANGO1	Transport and Golgi organization protein 1
TC	Total cholesterol
TF	Tissue factor
TG	Triglyceride
TMB	Tetramethylbenzidine
TNF-α	Tumor necrosis factor-alpha
TRL	Triglyceride-rich lipoprotein
VLDL-C	Very low-density lipoprotein
VSMC	Vascular Smooth muscle cells
v-SNARE	Vesicle-soluble N-ethylmaleimide-sensitive factor activating protein receptor
WHO	World Health Organization

1. Introduction

1.1. Definition of coronary artery disease

Coronary artery disease is a common multiple cardiovascular disease in the elderly and is mainly based on coronary atherosclerosis [1]. As the development of cardiovascular lesions, coronary artery cavity obstruction, and even coronary artery stenosis will occur, further leading to myocardial blood supply insufficiency, ischemia, hypoxia, and even necrosis [2]. A person might have CAD for many years and not have any symptoms until experiencing a heart attack [3]. That is why CAD is a "silent killer."? Particularly, in elderly patients complicated with multiple internal diseases, the morbidity rate of coronary heart disease increases year by year, seriously endangering the health of humans[4]. Therefore, it is very necessary to promptly and effectively diagnose coronary heart disease at an early stage [5]. As shown in Figure (1.1)

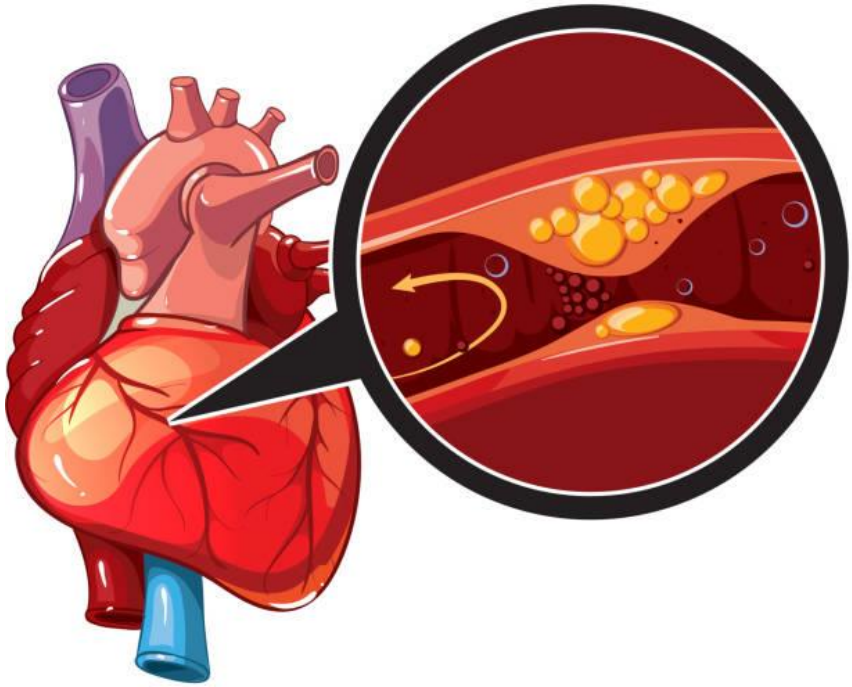


Figure 1-1: Vectorcardiography in acute myocardial infarction [6]

1.1.1. Classification of coronary artery disease

1.1.1.1. Chronic coronary artery disease (stable angina)

Angina is traditionally defined as substernal chest discomfort (pain or tightness) of less than 10 minutes duration [7]. This discomfort is provoked by exertion or emotional stress and is relieved by rest or by administration of nitroglycerin. In this typical form, angina is suggestive of obstructive coronary artery disease [8]. Angina may also be atypical, manifesting with less characteristic symptoms such as dyspnea or jaw pain; atypical presentations are more common among women and elderly persons than among men and younger persons [9]. The severity of angina can be classified with the use of the Canadian Cardiovascular Society (CCS) scale [10]:

Class I Ordinary physical activity does not cause angina: No angina occurs when walking or climbing stairs; angina does occur with strenuous or rapid or prolonged exertion at work or recreation.

Class II Slight limitation of ordinary activity: Angina occurs when walking or climbing stairs rapidly; walking uphill; walking or stair-climbing after meals, in the cold, in the wind, under emotional stress, or only during the first few hours after awakening; walking more than two blocks on the level and climbing more than one flight of ordinary stairs at a normal pace and in normal conditions.

Class III Marked limitation of ordinary physical activity: Angina occurs when walking one or two blocks on the level and climbing one flight of stairs in normal conditions and at a normal pace**.**

Class IV Inability to carry on any physical activity without discomfort: Anginal syndrome may be present at rest.

1.1.1.2. Acute coronary syndrome (ACS)

Acute coronary syndrome is a syndrome (a set of signs and symptoms) due to decreased blood flow in the coronary arteries such that part of the heart muscle is unable to function properly or dies [11]. The most common symptom is centrally located pressure-like chest pain, often radiating to the left shoulder or the angle of the jaw, and associated with nausea and sweating. Many people with acute coronary syndromes present with symptoms other than chest pain, particularly women, older people, and people with diabetes mellitus [12].

ACS is subdivided into three scenarios depending primarily on the presence of electrocardiogram (ECG) changes and blood test results (a change in cardiac biomarkers such as troponin levels: ST-elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), or unstable angina. STEMI is characterized by a complete blockage of a coronary artery resulting in necrosis of part of the heart muscle indicated by ST elevation on ECG, NSTEMI is characterized by a partially blocked coronary artery resulting in necrosis of part of the heart muscle that may be indicated by ECG changes, and unstable angina is characterized by ischemia of the heart muscle that does not result in cell injury or necrosis [13].

ACS should be distinguished from stable angina, which develops during physical activity or stress and resolves at rest [14]. In contrast with stable angina, unstable angina occurs suddenly, often at rest or with minimal exertion, or at lesser degrees of exertion than the individual's previous angina

("crescendo angina"). New-onset angina is also considered unstable angina since it suggests a new problem in a coronary artery [15].

1.2. Causes of coronary artery disease

Atherosclerosis causes coronary artery disease. Atherosclerosis is the gradual buildup of plaque in arteries throughout the body. Plaque consists of cholesterol, waste products, calcium, and fibrin (a substance that helps blood clot). As plaque continues to collect along the artery walls, arteries become narrow and stiff. Plaque can clog or damage the arteries, which limits or stops blood flow to a certain part of the body. When plaque builds up in the coronary arteries, the heart muscle can not receive enough blood. So, the heart can't get the oxygen and nutrients it needs to work properly. This condition is called myocardial ischemia. It leads to chest discomfort (angina) and puts a person at risk for a heart attack [16].

1.3. Symptoms of coronary artery disease

The symptoms of coronary artery disease for a long time. CAD is a chronic condition [16]. Plaque buildup takes many years, even decades. However, as arteries narrow, may notice mild symptoms. These symptoms indicate the heart is pumping harder to deliver oxygen-rich blood to the body [17].

Symptoms of chronic coronary artery disease include:

Stable angina: This is the most common symptom. Stable angina is temporary chest pain or discomfort that comes and goes in a predictable pattern. It is usually noticed during physical activity or emotional distress. It goes away when resting or taking nitroglycerin (medicine that treats angina) [18].

Shortness of breath (dyspnea): Some people feel short of breath during light physical activity due to blockage of vessels leading to a lack of O_2 in the heart muscle [19].

Sometimes, the first symptom of CAD is a heart attack. Symptoms of a heart attack include:

- chest pain: a feeling of pressure, heaviness, tightness, or squeezing across the chest
- pain in other parts of the body: it can feel as if the pain is spreading from the chest to arms (usually the left arm, but it can affect both arms), jaw, neck, back, and tummy
- feeling lightheaded or dizzy
- sweating
- shortness of breath
- feeling sick (nausea) or being sick (vomiting)
- an overwhelming feeling of anxiety (similar to a panic attack)
- coughing or wheezing

1.4. Risk factors of coronary artery disease

1.4.1. Non-modifiable risk factors

The risk factors that cannot be controlled are:

1.4.1.1. Age: Getting older increases the risk of damaged and narrowed arteries [20].

1.4.1.2. Sex: Men are generally at greater risk of coronary artery disease. However, the risk for women increases after menopause [21].

1.4.1.3. Family history: A family history of heart disease is more likely to get coronary artery disease. This is especially true if a close relative

(parent, sibling) develops heart disease at an early age. The risk is highest if the father or a brother had heart disease before age 55 years or if the mother or a sister developed it before age 65 years [22].

1.4.2. Modifiable Risk Factors

Those that can be controlled are:

1.4.2.1. Hypertension: Hypertension is one of the risks associated with the development of CHD. CAD is the most common sequelae for hypertensive patients of all ages [23]. Hypertension predisposes to all clinical manifestations of CHD, including myocardial infarction, angina pectoris, and sudden death. Even high normal BP values are associated with an increased risk of CVD [1].

The presence of other risk factors for CVD, such as high cholesterol, obesity, and diabetes, is seen more in people with prehypertension than in those with normal blood pressure [24]. The CVD risk in prehypertensive increases with the number of associated risk factors present. Therefore, prehypertension confers a greater risk of CVD [25].

1.4.2.2. Hypercholesterolemia: The other major risk for CVD is cholesterol. In 1953, an association between cholesterol levels and CHD mortality was reported in various populations. It was found that its component, low-density lipoprotein cholesterol (LDL-C), which is the principal lipoprotein transporting cholesterol in the blood, was also directly associated with CVD [26].

1.4.2.3. Smoking: The Framingham study showed that smokers were at increased risk of myocardial infarction (MI) or sudden death and that risk

was associated with the number of cigarettes smoked each day [27]. The deleterious effect of smoking on health has been proven in prior studies, in particular on atherosclerosis [28].

1.4.2.4. Diabetes: The role of diabetes in the pathogenesis of CVD was unclear until 1979 when Kannel, *et al.* [29], used data from the Framingham heart study to identify diabetes as a major cardiovascular risk factor. Based on 20 years of surveillance of the Framingham cohort, a two-fold to threefold increased risk of clinical atherosclerotic disease was reported [30]. It was also one of the first studies to demonstrate the higher risk of CVD in women with diabetes compared to men with diabetes [31]. These results have been duplicated by multiple studies. The Kannel article changed the way the medical community thought about diabetes. It is now accepted as a major cardiovascular risk factor. There is a clear-cut relationship between diabetes and CVD [26].

1.4.2.5. Physical inactivity: Myers, J., *et al.*, in their landmark 1953 article in The Lancet, discuss the association between physical activity and coronary artery disease. Since then, many epidemiological studies have confirmed the relationship [32]. The relative risk of death from CHD for sedentary individuals compared with active individuals is 1.9 (95% confidence interval) [33]. The recommendation of physical exercise has become an important element of preventative policies for the general population (including adults, the elderly, and children) [34].

1.4.2.6. Obesity: Obesity is also an independent risk factor for all-cause mortality. It is a metabolic disorder associated with comorbidities such as CHD, type 2 diabetes, hypertension, and sleep apnea [35]. Alterations in metabolic profile and various adaptations in cardiac structure and function occur as excess adipose tissue accumulates [36]. A recent study reported that

a higher body mass index (BMI) during childhood is associated with an increased risk of CHD in adulthood [37]. The prevention and control of overweight and obesity in adults and children has become a key element in the prevention of cardiovascular diseases [38].

1.4.2.7. Unhealthy diet: Eating foods with a lot of saturated fat, trans fat, salt, and sugar can increase the risk of coronary artery disease [39].

1.4.2.8. A lot of stress: Emotional stress may damage the arteries and worsen other risk factors for coronary artery disease [40].

1.5. The Pathophysiology of CAD

1.5.1. The Pathophysiology of stable angina

Atherogenesis, a disease characterized by cholesterol storage, is a complex interaction of risk factors, including artery wall cells and blood, and the molecular messages they exchange. Inflammation plays a major role in all stages of atherogenesis, contributing to local, myocardial, and systemic complications of atherosclerosis [41].

When arterial endothelium encounters bacterial products or risk factors, these cells increase the expression of adhesion molecules, promoting the sticking of blood leukocytes to the arterial wall[42]. The transmigration of adherent leukocytes depends on the expression of chemoattractant cytokines regulated by traditional and emerging risk factors for atherosclerosis[43]. Blood leukocytes, mainly mononuclear phagocytes and T lymphocytes, communicate with endothelial and smooth muscle cells (SMCs) in the arterial intima. Major messages exchanged among cell types involved in atherogenesis depend on mediators of inflammation and immunity, including lipid mediators like prostanoids and leukotrienes[44].

Recent attention has focused on protein mediators of inflammation and immunity, including cytokines and complement components[45]. Virtually unknown by cardiologists a mere decade ago, cytokines have joined the mainstream of specialty [46].

In early atheroma, stem cells migrate from the tunica media to the intima, proliferating and constructing a complex extracellular matrix. They secrete matrix metalloproteinases (MMPs) in response to various signals, which modulate vascular cell functions like activation, proliferation, migration, cell death, and new vessel formation[47] The extracellular matrix's components, particularly proteoglycans, bind lipoproteins, making them more susceptible to oxidative modification and glycation[48]. These products sustain and propagate the inflammatory response. As the lesion progresses, calcification may occur during the initial release of matrix vesicles surrounding the lipid pools of plaques, and cell death, including apoptosis, often occurs in atherosclerotic lesions [49, 50]. The lipid-rich "necrotic" core of the atherosclerotic plaque can form from the extracellular lipid accumulation in the intima[51]. As shown in Figure (1.2).

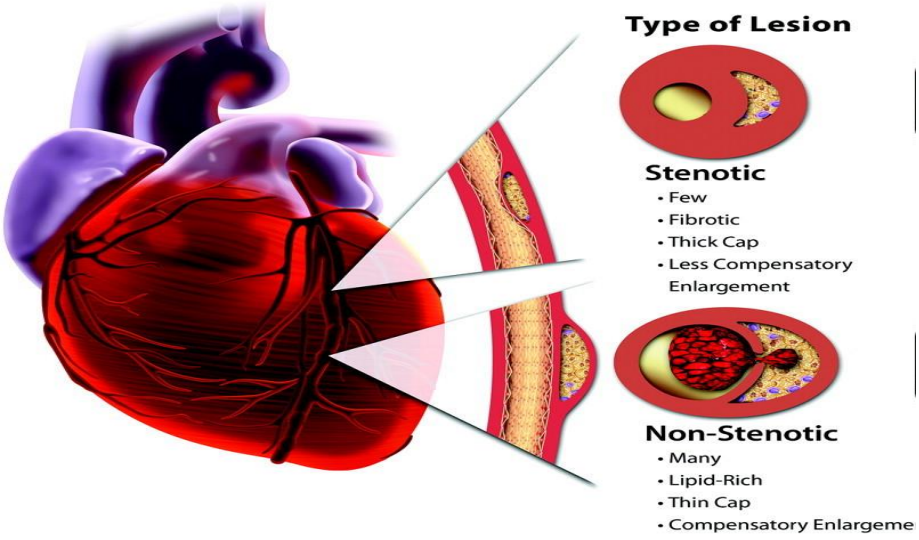


Figure 1-2: Simplified schema of the diversity of lesions in human coronary atherosclerosis[45].

1.5.2. The Pathophysiology of the ACS

As recently as the 1980s, some uncertainty prevailed concerning the causative role of thrombosis in ACS [52]. The role of thrombosis in acute coronary syndrome (ACS) has been established through in *vivo* imaging techniques and the success of antithrombotic and fibrinolytic therapy[53]. The most common cause of lethal coronary thrombosis is through-and-through rupture of the plaque's protective fibrous cap due to blood flow force, with other mechanisms including superficial erosion, intraplaque hemorrhage, and erosion of a calcified nodule [46, 54]. Rupture of the fibrous cap (A) causes some two-thirds to three-quarters of fatal coronary thromboses. Superficial erosion (B) occurs in one-fifth to one-quarter of all cases of fatal coronary thromboses. Certain populations such as diabetic individuals and women appear more susceptible to superficial erosion as the mechanism of plaque disruption and thrombosis. Erosion of a calcium nodule may also cause plaque disruption and thrombosis (C). In addition, friable microvessels in the base of atherosclerotic plaque may rupture and cause intraplaque hemorrhage. Consequent local generation of thrombin may stimulate SMC proliferation, migration, and collagen synthesis, promoting fibrosis and plaque expansion on a subacute basis. Severe intraplaque hemorrhage can cause sudden lesion expansion by mass effect acutely as well [45]. As shown in Figure (1.3).

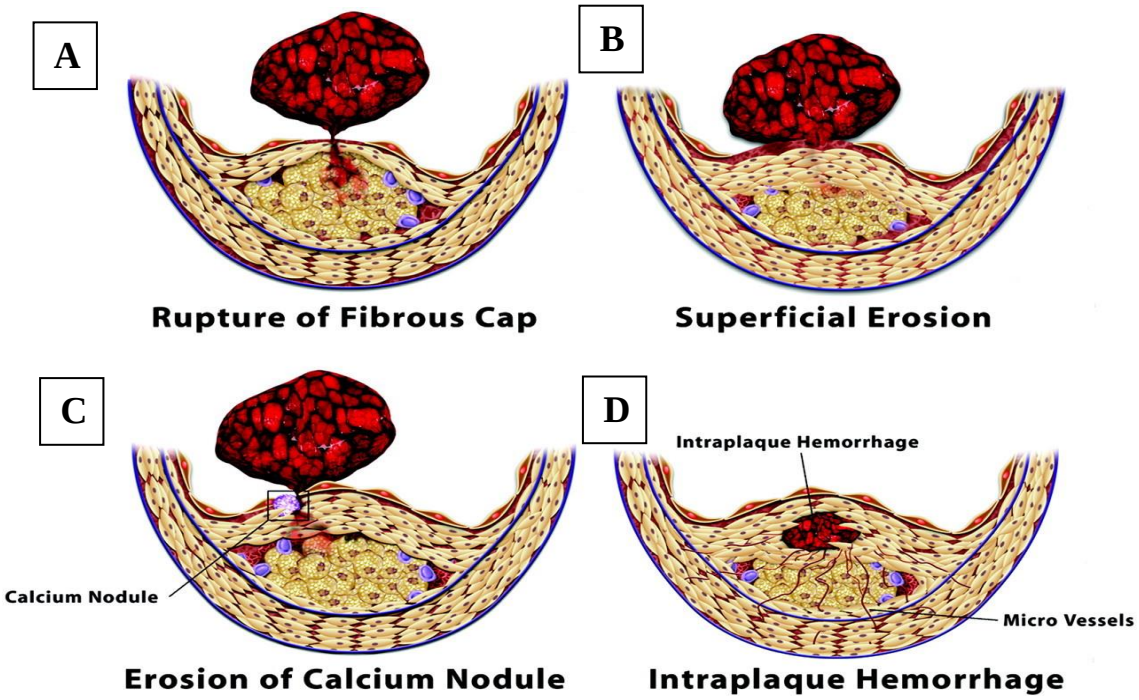


Figure 1.3: Microanatomy of coronary arterial thrombosis and acute occlusion [45].

Disrupted plaques provoke thrombosis through contact with collagen in the plaque's extracellular matrix, Tissue factor (TF) produced by macrophages and SMCs, and the coagulation cascade [55]. The "fluid phase" of blood can also predispose toward coronary thrombosis, with plasminogen activator inhibitor-1 (PAI-1) extinguishing the body's natural fibrinolytic mechanism [45, 52, 56]. Formation, extent, and duration of coronary thrombi produced by mechanisms such as those outlined in Figure 1.3 depend on both solid-state factors in plaque itself and fluid-phase determinants in blood. [45]. As shown in Figure (1.4).

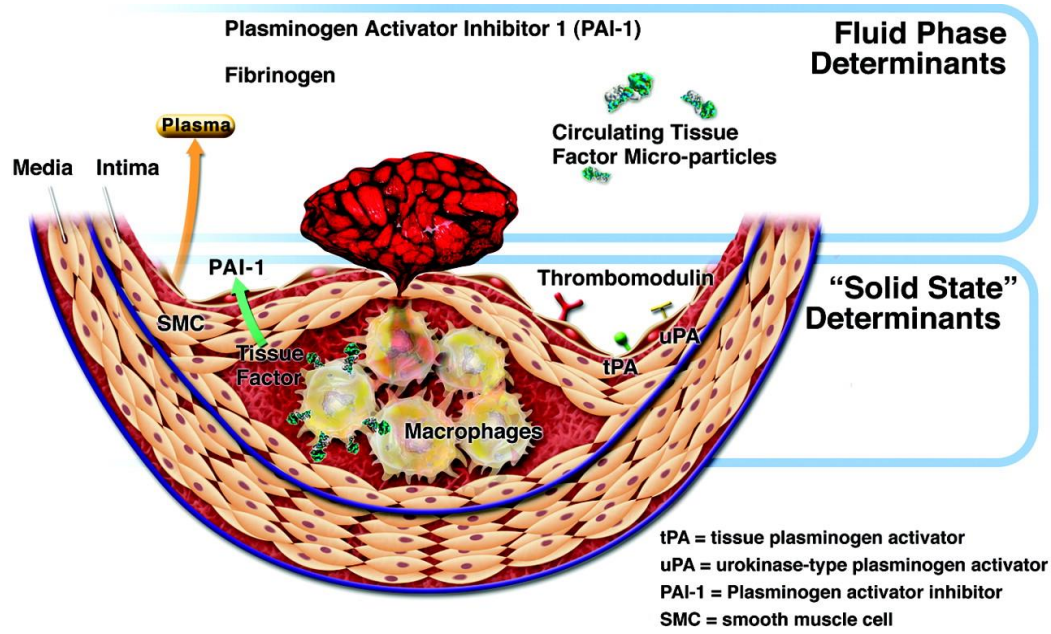


Figure 1.4: Determinants of thrombosis in coronary atherosclerotic plaques [45].

In addition to the solid state of the disrupted plaque, the "fluid phase" of blood can predispose toward coronary thrombosis (Figure 1.4). Plasminogen activator inhibitor-1 (PAI-1) extinguishes the body's natural fibrinolytic mechanism that combats the persistence and accumulation of thrombi by inhibiting urokinase-like and tissue-type plasminogen activators[45]. Circulating levels of PAI-1 increase in diabetes and obesity, and mediators of hypertension such as angiotensin II can augment PAI-1 expression by various cell types. Furthermore, disrupted plaques can elaborate particulate TF, which can heighten the thrombogenicity of blood [57].

Disrupted plaques can elaborate particulate TF, heightening the thrombogenicity of blood. These fluid-phase changes led to the concept of the "vulnerable patient" and the concept of "vulnerable plaque."[57] [58]. In the context of ACS, distal embolization of TF-rich debris may promote distal thrombosis in the microcirculation, causing the "no-reflow" phenomenon [59] [60].

1.6. Epidemiology of coronary artery disease

According to epidemiological reports by the WHO in 2016, the highest prevalence rate of ischemic heart disease was observed in Saudi Arabia (46%). In Iraq, the epidemiological data on the incidence and prevalence of CAD as evidence of awareness are limited due to the unavailability of evidence-based national guidelines for the management of cardiovascular disease and surveillance studies as compared to other Eastern Mediterranean countries [61].

The latest WHO data published in 2020 Coronary Heart Disease Deaths in Iraq reached 36,594 or 24.98% of total deaths. The age-adjusted Death Rate is 227.26 per 100,000 population ranks Iraq 23 in the world [62]. A better understanding of the burden of cardiovascular disease and associated risk factors in this region and increasing the public knowledge and awareness of CAD symptoms and its risk factors are highly imperative to control and prevent this disease.

1.7. Diagnosis of coronary artery disease

1.7.1. Non-biomarker diagnosis

1.7.1.1. Electrocardiogram

This quick and painless test measures the electrical activity of the heart. It can show how fast or slow the heart is beating. Which can look at signal patterns to determine if the patient has had a heart attack [63].

1.7.1.2. Echocardiogram

This test uses sound waves to create pictures of the beating heart. An echocardiogram can show how blood moves through the heart and heart valves[64].

1.7.1.3. Exercise stress test

If signs and symptoms occur most often during exercise, it will be provided to walk on a treadmill or ride a stationary bike during an ECG. If an echocardiogram is done while the person does these exercises, the test is called a stress echo. If a person exercises, he might be given medications that stimulate the heart as exercise does[65].

1.7.1.4. Nuclear stress test

This test is similar to an exercise stress test but adds images to the ECG recordings. A nuclear stress test shows how blood moves to the heart muscle at rest and during stress. A radioactive tracer is given intravenous (IV). The tracer helps the heart arteries show up more clearly in images[66].

1.7.1.5. Heart (cardiac) CT scan

A CT scan of the heart can show calcium deposits and blockages in the heart arteries. Calcium deposits can narrow the arteries.

Sometimes dye is given by IV during this test. The dye helps create detailed pictures of the heart arteries. If dye is used, the test is called a CT coronary angiogram[67].

1.7.1.6. Cardiac catheterization and angiogram

During cardiac catheterization, a heart doctor (cardiologist) gently inserts a flexible tube (catheter) into a blood vessel, usually in the wrist or groin. The catheter is gently guided to the heart. X-rays help guide it. The dye flows through the catheter. The dye helps blood vessels show up better on the images and outlines any blockages[68].

1.7.2. Biomarker diagnosis of CAD

1.7.2.1. Creatine kinase-MB (CK-MB)

It also known as creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme expressed by various tissues and cell types[69]. CK catalyzes the conversion of creatine and uses adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible and thus ATP can be generated from PCr and ADP [70].

In tissues and cells that consume ATP rapidly, especially skeletal muscle, but also the brain, photoreceptor cells of the retina, hair cells of the inner ear, spermatozoa, and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ, as well as for intracellular energy transport by the PCr shuttle or circuit. Thus creatine kinase is an important enzyme in such tissues [71].

Clinically, creatine kinase is assayed in blood tests as a marker of damage of CK-rich tissue such as in myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, autoimmune myositides, acute kidney injury [72].

1.7.2.1.1. Types of CK

In the cells, the cytosolic CK enzymes consist of two subunits, which can be either B (brain type) or M (muscle type). There are, therefore, three different isoenzymes: CK-MM, CK-BB, and CK-MB. The genes for these subunits are located on different chromosomes: B on 14q32 and M on 19q13[73]. In addition to those three cytosolic CK isoforms, there are two

mitochondrial creatine kinase isoenzymes, the ubiquitous form and the sarcomeric form [74]. The functional entity of the mitochondrial CK isoforms is an octamer consisting of four dimers each.

While mitochondrial creatine kinase is directly involved in the formation of phosphocreatine from mitochondrial ATP, cytosolic CK regenerates ATP from ADP, using PCr. This happens at intracellular sites where ATP is used in the cell, with CK acting as an in situ ATP regenerator[75]. Isoenzyme patterns differ in tissues. Skeletal muscle expresses CK-MM (98%) and low levels of CK-MB (1%). The myocardium (heart muscle), in contrast, expresses CK-MM at 70% and CK-MB at 25–30%. CK-BB is predominantly expressed in the brain and smooth muscle, including vascular and uterine tissue [76].

The CPK-MB test (creatine phosphokinase-MB), also known as the CK-MB test, is a cardiac marker used to assist in the diagnosis of acute myocardial infarction, myocardial ischemia, or myocarditis. It measures the blood level of creatine kinase myocardial band (CK-MB), the bound combination of two variants (isoenzymes CKM and CKB) of the enzyme phosphocreatine kinase [77].

In some locations, the test has been superseded by the troponin test. However, recently, there have been improvements to the test that involves measuring the ratio of the CK-MB1 and CK-MB2 isoforms [78].

The newer test detects different isoforms of the B subunit specific to the myocardium whereas the older test detected the presence of cardiac-related isoenzyme dimers [79].

Many cases of CK-MB levels exceeding the blood level of total CK have been reported, especially in newborns with cardiac malformations, especially ventricular septal defects. This reversal of ratios is in favor of pulmonary emboli or vasculitis. An autoimmune reaction creating a complex molecule of CK and IgG should be taken into consideration [80].

1.7.2.2. High-Sensitive Troponin

Troponin, or the troponin complex, is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that are integral to muscle contraction in skeletal muscle and cardiac muscle, but not smooth muscle[81]. Measurements of cardiac-specific troponins I and T are extensively used as diagnostic and prognostic indicators in the management of myocardial infarction and acute coronary syndrome[82]. Blood troponin levels may be used as a diagnostic marker for stroke or other myocardial injuries that are ongoing, although the sensitivity of this measurement is low [83].

Individual subunits serve different functions:

1. **Troponin C** is a protein that is part of the troponin complex. It contains four calcium-binding EF hands, although different isoforms may have fewer than four functional calcium-binding subdomains[84]. It is a component of thin filaments, along with actin and tropomyosin. It contains an N lobe and a C lobe. The C lobe serves a structural purpose and binds to the N domain of troponin I (TnI)[85]. The C lobe can bind either Ca^{2+} or Mg^{2+}. The N lobe, which binds only Ca^{2+}, is the regulatory lobe and binds to the C domain of troponin I after calcium binding. Troponin C binds to calcium ions to produce a conformational change in TnI[86].

2. **Troponin T** (shortened TnT or TropT) is a part of the troponin complex, which are proteins integral to the contraction of skeletal and heart muscles. They are expressed in skeletal and cardiac myocytes [87]. Troponin T binds to tropomyosin and helps position it on actin, and together with the rest of the troponin complex, modulates the contraction of striated muscle [88]. The cardiac subtype of troponin T is especially useful in the laboratory diagnosis of a heart attack because it is released into the bloodstream when damage to the heart muscle occurs [89]. Troponin T binds to tropomyosin, interlocking them to form a troponin-tropomyosin complex[90].
3. **Troponin I** is a cardiac and skeletal muscle protein family. It is a part of the troponin protein complex, where it binds to actin in thin myofilaments to hold the actin-tropomyosin complex in place[91]. Troponin I prevents myosin from binding to actin in relaxed muscles. When calcium binds to troponin C, it causes conformational changes which lead to the dislocation of troponin I[92]. Afterwards, tropomyosin leaves the binding site for myosin on actin leading to the contraction of the muscle. The letter is given due to its inhibitory character[93]. It is a useful marker in the laboratory diagnosis of a heart attack. It occurs in different plasma concentrations but under the same circumstances as troponin T - either test can be performed for confirmation of cardiac muscle damage and laboratories usually offer one test or the other[94].

Troponin I binds to actin in thin myofilaments to hold the actin-tropomyosin complex in place[95].

Certain subtypes of troponin (cardiac I and T) are 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 people with chest pain or acute coronary syndrome [96]. A person who recently had a myocardial infarction would have an area of the damaged heart muscle and elevated cardiac troponin levels in the blood [97]. This can also occur in people with coronary vasospasm, a type of myocardial infarction involving severe constriction of the cardiac blood vessels. After a myocardial infarction troponins release within 6 hours and may remain high for up to 2 weeks [98, 99].

Troponins are also increased in patients with heart failure, where they also predict mortality and ventricular rhythm abnormalities. They can rise in inflammatory conditions such as myocarditis and pericarditis with heart muscle involvement (which is then termed myopericarditis) [100]. Troponins can also indicate several forms of cardiomyopathy, such as dilated cardiomyopathy, hypertrophic cardiomyopathy or (left) ventricular hypertrophy, peripartum cardiomyopathy, Takotsubo cardiomyopathy, or infiltrative disorders such as cardiac amyloidosis [101].

Heart injury with increased troponins also occurs in cardiac contusion, defibrillation, and internal or external cardioversion [102]. Troponins are commonly increased in several procedures such as cardiac surgery and heart transplantation, closure of atrial septal defects, percutaneous coronary intervention, or radiofrequency ablation[103].

1.7.2.3. Melanoma inhibitory activity protein 3

Melanoma inhibitory activity protein 3 (MIA3) also known as transport and Golgi organization protein 1 (TANGO1), MIA3 is a unique 1,907 amino acid protein. is a protein that in humans is encoded by the MIA3 gene on chromosome 1, It is ubiquitously expressed in many tissues and cell types [104]. MIA3 localizes to the endoplasmic reticulum (ER) exit site,

where it binds bulky cargo molecules such as collagens and creates mega transport carriers for the export of cargoes from the ER[105]. TANGO1 is composed of the SH3 domain (N-terminus) that binds collagen molecules, 2 coiled-coil domains (CC1 and CC2), and the proline-rich domain (PRD), which all assist in the formation of collagen-like COPII vesicles (C-terminus) and play a significant role in interactions between the endoplasmic reticulum and the COPII components (Sec23/Sec24) For the secretion of large molecules [106]. This function suggests that it plays a role in the assembly of extracellular matrix (ECM) and bone formation. MIA3 has been demonstrated to contribute to both tumor suppression and progression[107]. As shown in Figure (1.5).

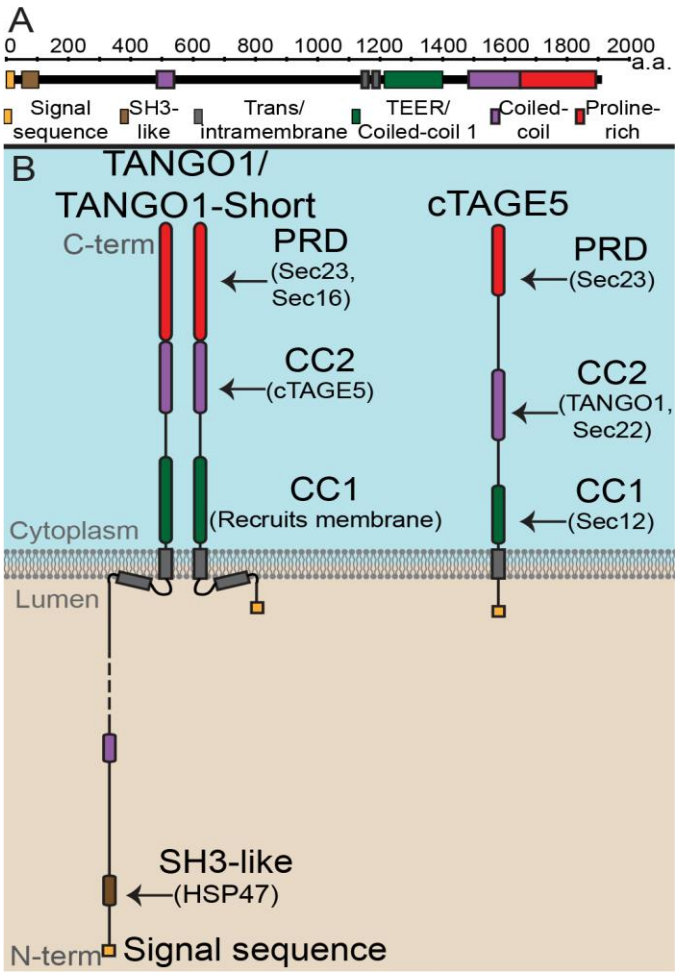


Figure 1.5. The domain architecture and topology of TANGO1 and cTAGE5 [108].

In humans, MIA3 was first discovered as an important constituent in the growth and adhesion of malignant melanoma cells [108]. As it is secreted from both chondrocytes and melanoma cells, it also plays a role in the metastasis of melanomas as well as cartilage development [109]. proved that be MIA3 protein has a major contribution to the aggressive characteristics of malignant melanoma in particular to the formation of metastasis, Treatment of melanoma cells with MIA3 inhibitory peptides almost completely blocked the MIA3 protein uptake into cells [110]. The MIA3 gene also contains one of 27 loci associated with an increased risk of coronary artery disease [111].

The critical function of the inflammatory modes of action in forming atheroma is supported by copious data. This process involves leukocyte mobilization and the pivotal participation of proinflammatory cytokines in the primary phase of atherogenesis [112]. Endothelial cells are activated for inflammation, followed by the entry of monocytes and other leukocytes into the atheroma. The direct binding of TANGO protein occurs to the leukocyte-specific b2-integrin CD11c/CD18, being responsible for leukocyte adherent interplays with vascular endothelium [113]. Experimentally, it is evidenced that the TANGO protein modulates CD11c/CD18 activity, thereby reducing adhesion and promoting monocyte migration through the endothelium [114]. After trans-migration, monocytes are differentiated into macrophages, forming the fatty streak that primarily indicates atherosclerosis in the arterial intima [113, 115].

Vascular injury caused by angioplasty, stenting, or bypass surgery triggers phenotypic switch of vascular smooth muscle cells (VSMCs) and subsequent abnormal proliferation and migration of VSMCs, leading to excessive formation of neointima, which contributes to occlusive vascular diseases such as atherosclerosis, intimal hyperplasia associated with

restenosis [116, 117]. Therefore, unraveling the molecular mechanisms involved in regulating VSMC phenotypic switch, proliferation, and migration is a vital step toward understanding the pathology of restenosis [118]. evidenced that the Knockdown of MIA3 ameliorates femoral artery wire injury-induced neointimal hyperplasia and increases brown-like perivascular adipocytes [119]. As a result, impaired proliferation and migration of VSMCs, whereas MIA3 overexpression promoted VSMC migration and proliferation [120]. The MIA3/TANGO1 protein was found to be involved in the export of collagen VII and ApoB from the endoplasmic reticulum (ER) to the Golgi apparatus and secretion of collagens I, II, III, IV, VII, and IX [121]. recent studies showed that deletion of TANGO1 affected the secretion of ApoB by 44% compared with parental cells indicating that TANGO1 participates in the binding and concentration of ApoB-containing lipid particles for their subsequent export from the ER [122]. TANGO1 and TALI interact with ApoB directly or through an adaptor protein in the lumen of the ER to bring pre-chylomicrons/VLDL-Cs to ER exit sites. On the cytoplasmic side, the TEER domain of TANGO1 recruits ERGIC membranes that fuse to the ER exit site that is now enriched in pre-chylomicrons/VLDL-Cs. The binding of the proline-rich domain (PRD) of TANGO1 and TALI on the cytoplasmic side to Sec23/24 prevents Sec13/31 recruitment, allowing continuous fusion of ERGIC membranes that results in the growth of a mega-bud that is packed with The pre-chylomicrons/VLDL-Cs. Fission is triggered, and a mega carrier, big enough to accommodate bulky pre-chylomicrons/VLDL-Cs, is released from the ER [123]. As shown in Figure (1.6).

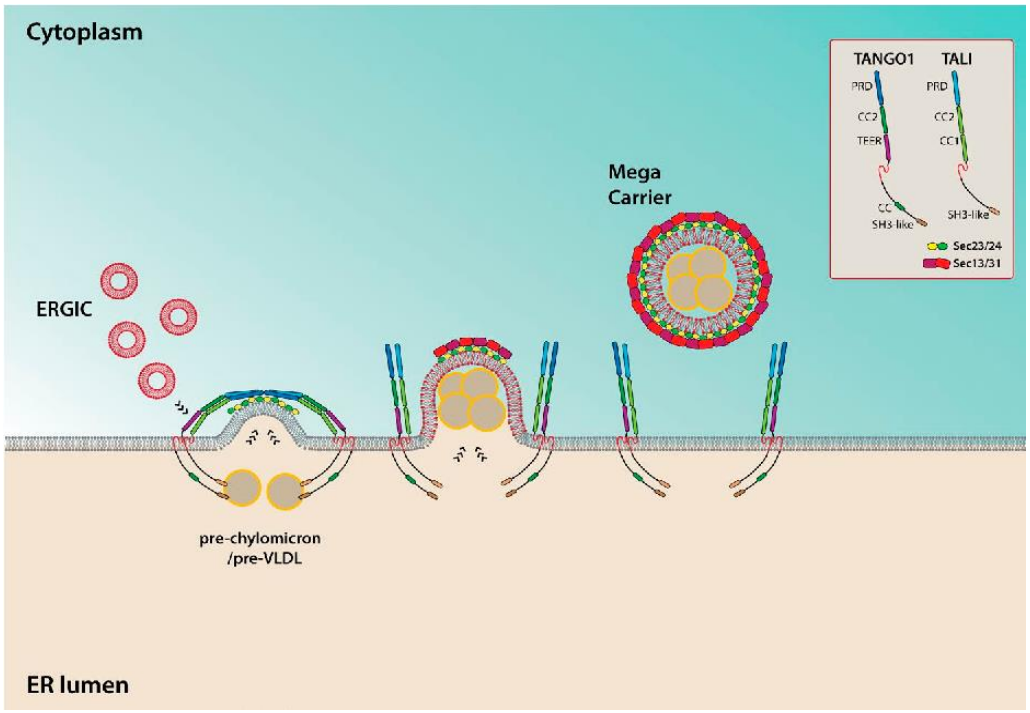


Figure 1.6. Model for the export of pre-chylomicrons/VLDL-Cs from the ER [123].

1.7.2.4. E-Selectin

Selectins are glycoproteins and are important adhesion molecules in the mammalian immune system, especially in the inflammatory response and the healing process of tissues [124]. Selectins are a family of Ca^{2+}-dependent C-type lectins present on the surface of numerous cell types in the cardiovascular system including endothelial cells (E- and P-selectin), platelets (P-selectin), and leukocytes (L-selectin) [125]. They interact with cell surface glycans to promote the adhesion of hematopoietic cells to vascular surfaces and promote the rolling of circulating leukocytes and their delivery to sites of inflammation [124]. Selectins play an important role in a variety of biological processes, including the rolling of leukocytes in endothelial cells, a process known as the adhesion cascade [126]. E-selectin is a 115 kDa adhesion molecule that is expressed exclusively by vascular endothelial cells [127]. The amino acid sequence of the E-selectin has about

six cysteine-rich consensus repeats followed by an N-terminal lectin domain of 119 residues and with a 60–70% identity that effectively binds with carbohydrates, which are responsible for the binding of the oligosaccharide [128]. It is a single-chain transmembrane glycoprotein consisting of an N-terminal calcium-dependent (C-type) lectin domain, an epidermal growth factor domain, a chain of six consensus repeats, a transmembrane domain, and an intracellular cytoplasmic tail [127, 129]. Although E-selectin is constitutively expressed on the surfaces of endothelial cells of bone marrow and skin microvessels in most tissue [130], E-selectin expression is induced in response to inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1b (IL-1b), or lipopolysaccharide (LPS) [131]. E-selectin expression has also been associated with tumor angiogenesis and metastasis in a variety of cancers [132]. E, L, and P selectins contain the N-terminal lectin-like domain with 120 amino acids and with a 60–70% identity that effectively binds with carbohydrates. The adjacent EGF-like domain also shares ~60% sequence similarity. This is followed by the CR domain with a variable number (2–9) of consensus repeats of ~60 amino acids in length, and then a short transmembrane domain and a cytoplasmic [128]. As shown in Figure (1.7).

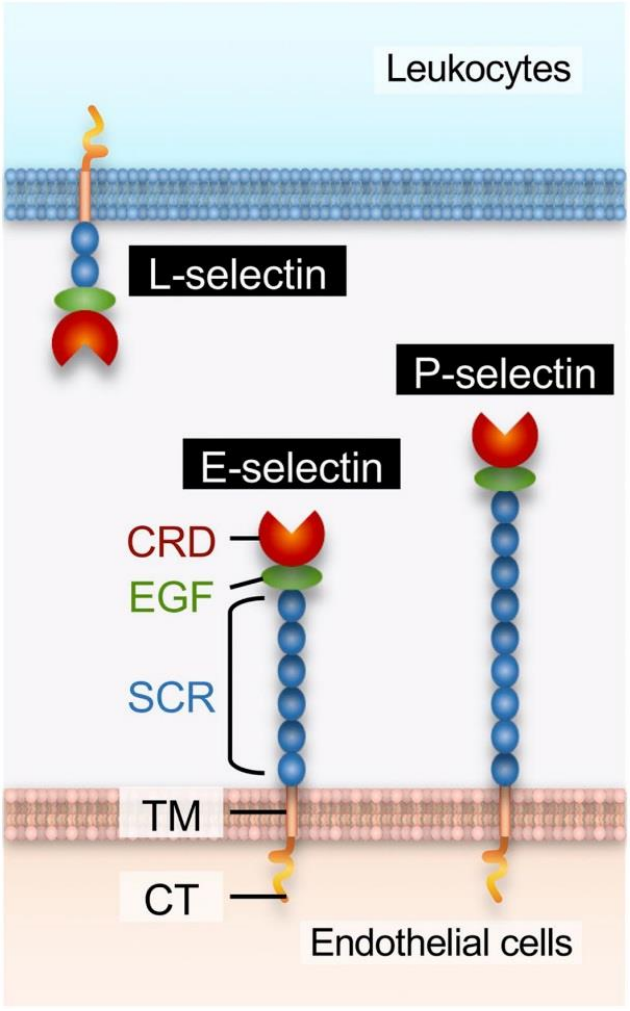


Figure 1.7. Schematic representation of the structure of E-selectin, L-selectin, and P-selectin [128].

Cytokine-activated endothelial cells express E-selectin, which interacts with two major glycoprotein ligands, E-selectin ligand-1 (ESL-1) and P-selectin glycoprotein ligand-1 (PSGL-1), which are both glycoprotein ligands. Among the ligands, ESL-1 is a specific receptor for E-selectin[133]. E-selectin plays an important part in recruiting leukocytes to the site of injury. The local release of cytokines IL-1 and TNF-α by macrophages in the inflamed tissue induces the over-expression of E-selectin on endothelial cells of nearby blood vessels[134]. Leukocytes in the blood expressing the correct ligand will

bind with low affinity to E-selectin, also under the shear stress of blood flow, causing the leukocytes to "roll" along the internal surface of the blood vessel as temporary interactions are made and broken[135].

As the inflammatory response progresses, chemokines released by injured tissue enter the blood vessels and activate the rolling leukocytes, which are now able to tightly bind to the endothelial surface and begin making their way into the tissue[136]. As shown Figure (1.8)

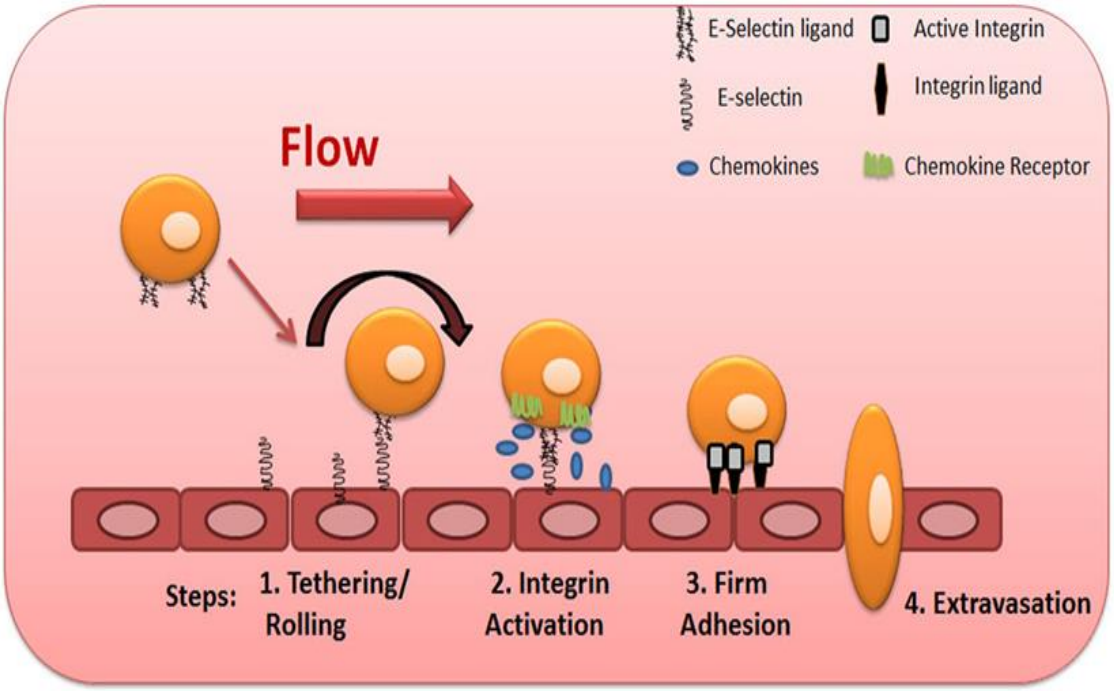


Figure 1.8: Multistep model of circulating blood cell adhesion and migration along the vascular endothelium. Cells make adhesive contacts onto the inflamed endothelial surface through the engagement of their sialofucosylated glycan determinants to vascular E-selectin (Step 1—tethering and rolling). Subsequent engagement of chemokine receptors leads to integrin activation (Step 2) and firm adhesion of leukocytes to the endothelium (Step 3), allowing their transmigration (Step 4) [137].

1.7.2.5. Cholesteryl ester transfer protein

Cholesteryl ester transfer protein is a hydrophobic glycoprotein that is secreted mainly from the liver and that circulates in plasma, bound mainly to high-density lipoprotein (HDL-C) [138]. CETP, containing 476 amino acids, facilitates the transfer of completely water-insoluble lipids between the lipoproteins that transport them through aqueous plasma [139]. The lipoprotein structural organization is designed to shield these lipids (cholesteryl esters and triglycerides) from water by encapsulating them within a coating of polar lipids (mainly phospholipids) and proteins, yet the core lipids can move between lipoprotein particles [140, 141]. First described as a 'high-molecular-weight globulin' that stimulated the transfer of cholesteryl ester between lipoproteins in the plasma of hypercholesterolemic rabbits, CETP was subsequently shown to also transfer triglycerides and phospholipids [139]. As shown in Figure (1.9).

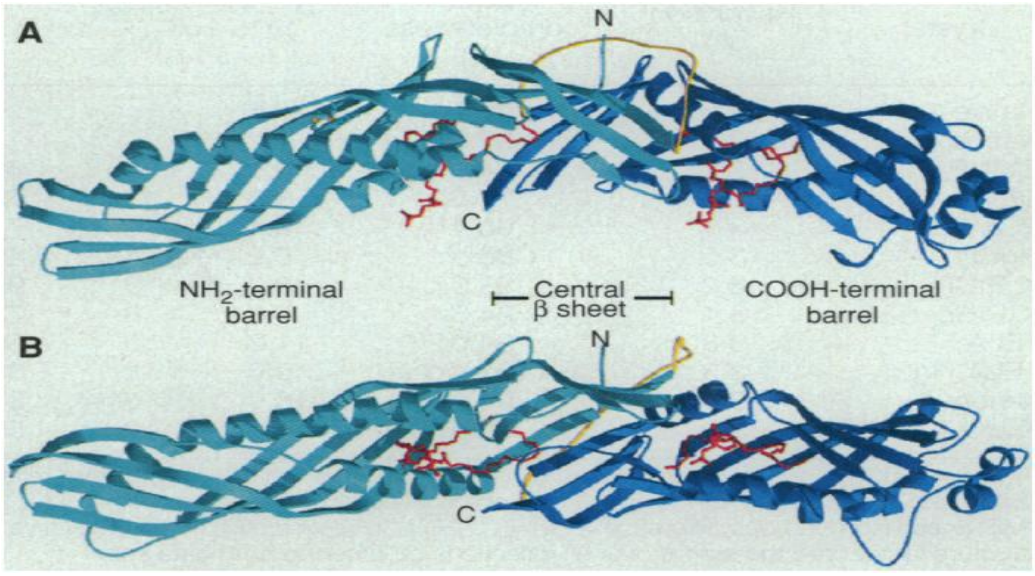


Figure 1.9: (A) ribbon diagram of residues 1 to 456 of BPI, illustrating its boomerang shape. meNH2-terminal domain is shown in green, the COOH-terminal domain in blue, and the two phosphatidylcholine molecules in red. the

linker is yellow, and the disulfide bond is shown as a ball-and-stick model. (B) View after rotating (A) 70" about the long axis of the molecule [142].

The exchanges occur on a much faster timescale than does the catabolism of the lipoproteins, leading to changes in composition while the lipoproteins are still in circulation [143]. Most of the cholesteryl esters in plasma originate in HDL-C in the reaction catalyzed by lecithin: cholesterol acyl transferase (LCAT), and the majority of the triglycerides enter the plasma as a component of chylomicrons and very low-density lipoproteins (VLDL-Cs) that are called triglyceride-rich lipoproteins (TRLs) [144]. The overall effect of CETP is a net mass transfer of cholesteryl esters from HDL-Cs to TRLs and low-density lipoproteins (LDL-Cs) and of triglycerides from TRLs to LDL-Cs and HDL-Cs [145]. Thus, CETP-mediated transfers from HDL-C to VLDL-C and LDL-C provide a potential indirect pathway by which HDL-C cholesteryl esters can be delivered to the liver [146].

The rate of cholesteryl ester transfer to TRLs and LDL-Cs and the mass of CETP is increased in patients with a range of atherogenic dyslipidemias [147]. Effects of CETP in normotriglyceridemia (Normo TG) and hypertriglyceridemia (Hyper TG). The magnitude of the net flux of cholesteryl esters (CE) and TGs between lipoproteins is dependent, in large part, on the relative sizes of the TRL, LDL-C, and HDL-C pools. In Normo TG, net CE flux to LDL-Cs from HDL-Cs predominates, with minor transfer to TRLs. In contrast, in Hyper TG, increased particle numbers of large VLDL-Cs exhibit elevated acceptor activity for CETP. Under these conditions, there are high net transfer rates of CEs from HDL-Cs to TRLs and of TGs from TRLs to both HDL-Cs and LDL-Cs. TG-enriched LDL-Cs and HDL-Cs are substrates for hepatic lipase (HL) that hydrolyzes

phospholipid (PL) and TGs to form small, dense LDL-C and small, dense HDL-C, respectively[148]. As shown in Figure (1.10).

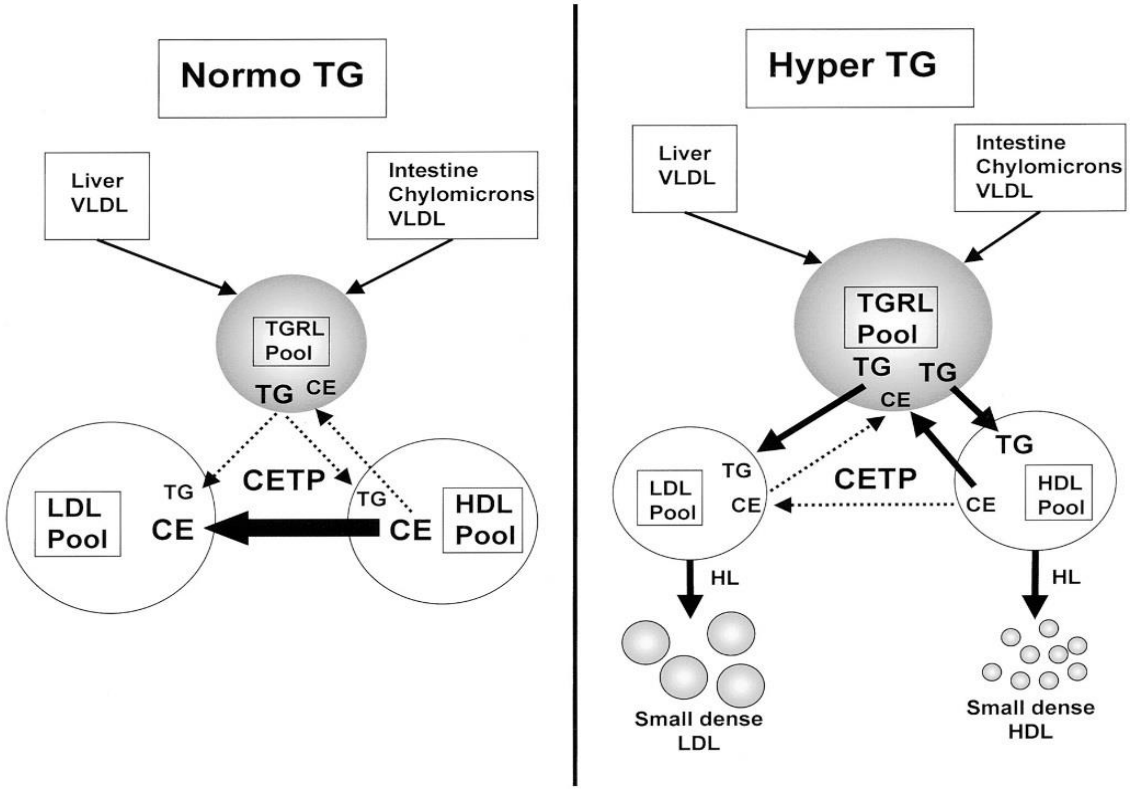


Figure 1.10: Effects of CETP in normotriglyceridemia (Normo TG) and hypertriglyceridemia (Hyper TG)[148].

1.8. Aim of study:

The presented study aimed to assess the serum levels of MIA3, CETP, and E-selectin in CAD Iraqi patients with the following objectives:

1. To investigation the serum levels of (MIA3), E-selectin, and CETP in Iraqi coronary artery patients and to compare it with the healthy group.
2. To determination Lipid profiles and atherogenic index in patients and control group.
3. To show the correlation analysis between serum levels of MIA3, E-selectin, and CETP with other biochemical parameters study.
4. Shed light on the receiver operating characteristic analysis (ROC) to get the best diagnostic ability for MIA3, E-selectin, and CETP for Iraqi patients with CAD

Chapter Two

Materials and Methods

2. Materials and method

2.1. Study Design and Ethical Approval

A case-control research approach was used to collect samples from 180 subjects between December 2022 and April 2023. and they are classified as shown in Fig (2-1). The study's ethical approval was confirmed by the Kerbala College of Medicine, Kerbala University (3544 on 29/12/2022), and Kerbala Health Directorate. After describing the nature of the study and objectives to each patient, the management of the Kerbala Center for Cardiac Diseases and Surgery and the male patients and control themselves gave their consent. The questionnaire included name, age, sex, under treatment, history of other diseases (mentioned in Appendix).

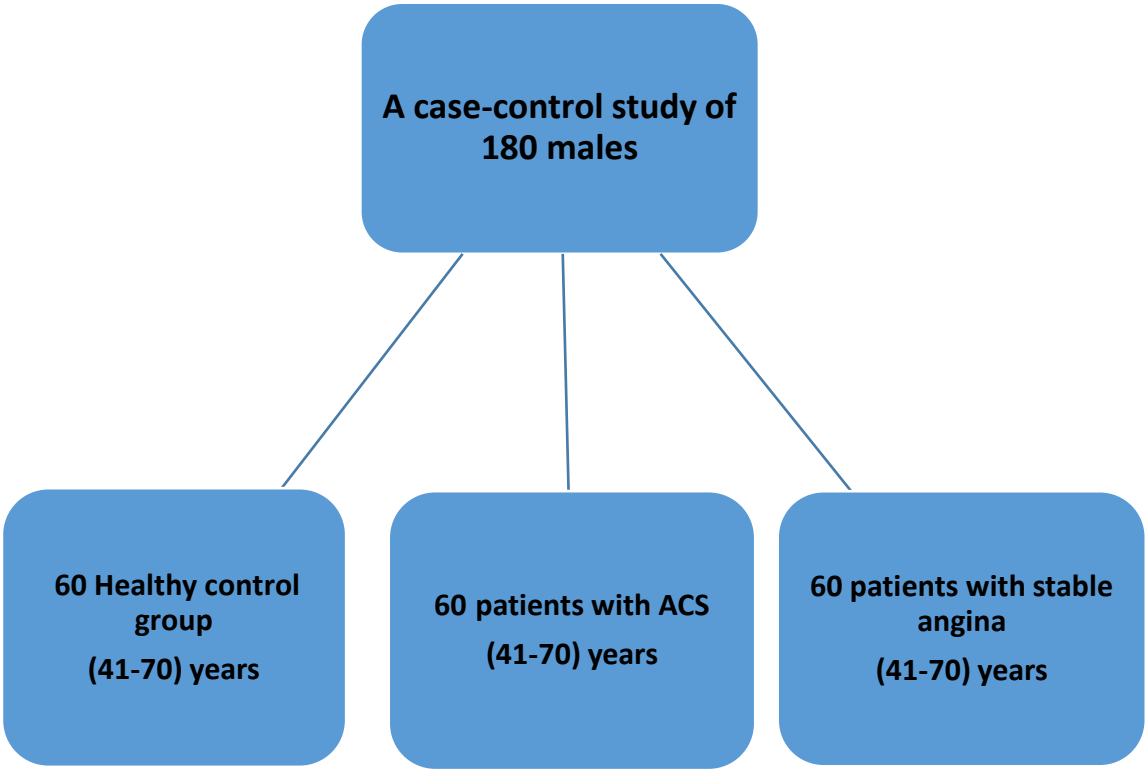


Figure (2.1): Flow chart of study design.

2.2. Subjects

They included:

2.2.1. Patients

The study was conducted on 120 patients aged between 41-70 years old as shown in Fig (2-1). The patients were divided into two subgroups: 60 stable angina, and 60 ACS. They have been observed and diagnosed by specialist physicians. Patients were collected from the Kerbala Center for Cardiac Diseases and Surgery. A particular questionnaire form including descriptive information was designed and filled with each patient.

2.2.1.1. Patient Inclusion Criteria

All male patients underwent a thorough clinical history, physical examination, and pertinent laboratory tests. According to the most recent clinical practice guidelines published by the ACC and ESC, the diagnosis of the clinical states associated with coronary artery disorders (stable angina, unstable angina, and myocardial infarction) was established. Based on the signs and symptoms, evaluation of the ECG, and laboratory measures for the clinical assessment, the etiology of the cases was determined.

2.2.1.2. Patient Exclusion Criteria

Patients with the Presence of cirrhosis, Diabetes, end-stage renal disease, Acute Heart Failure, stroke, skeletal muscle injury, malignancy, Ongoing infectious diseases, hormonal, and other metabolic dysfunctions that may affect the results were excluded from this study.

2.2.2. Healthy Control

Sixty males who were apparently healthy were selected from the list of volunteers accompanying the patients and from outside the hospital. Volunteers' blood was obtained and none of them had a history of heart disease. In the entire study group, the ages of the participants were also close together. Through a self-report method, demographic data of the participants were also collected.

2.2.3. Blood sample collection and Storage

The patient's blood samples were taken from the aforementioned facility. Venipuncture was used to obtain five-milliliter blood samples, which were then put in gel tubes at room temperature for ten minutes. Approximately 3,000 x g centrifugation was used to separate the serum for 10 minutes. Serum samples were divided into two samples in Eppendorf, and kept at -20 °C, to be used later for additional testing.

2.3. Chemicals and Kits

The kits used in this study are summarized in Table (2-1)

Table 2-1: kits which are used in this study

No.	Kits	Suppliers
1	Total Cholesterol Kit	AFLO/ Germany
2	Triglycerides Kit	AFLO/ Germany
3	HDL-Cholesterol Kit	AFLO/ Germany
4	MIA3 ELISA Kit	SunLong / China
5	E-Selectin (ES) ELISA Kit	SunLong / China
6	CETP ELISA Kit	SunLong / China

2.4. Instruments and Lab Equipment

The Instrument used in this study are summarized in Table (2-2)

Table 2-2: The instruments used in the study

NO.	Instrument	Suppliers
1	Centrifuge	HETTICH / Germany
2	Deep Freeze	COOLTECH / China
3	Microplate reader	UNO/HUMAN / Germany
4	Roller Mixer	China
5	SMART-120 chemistry analyzer	AFLO / Germany

2.5. Methods

2.5.1. Calculation of Body Mass Index

Weight in kilogram (kg) and height in meters (m) were recorded. Body mass index (BMI) was calculated by the following equation[149]:

BMI (kg/m^2) = weight / $(height)^2$

Patients were classified into normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese ($\geq$ 30.0 kg/m^2) depending on reference ranges [150].

2.5.2. Measurement of Serum Lipid Profile

2.5.2.1. Measurement of Serum Total Cholesterol Concentration

Principle: Esterified cholesterol is hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CHE). 4-Cholesten-3-one and hydrogen peroxide (H_2O_2) are then formed from the released free cholesterol by the action of cholesterol oxidase. In the presence of peroxidase (POD), hydrogen peroxide reacts with a derivative of phenol and 4-amino antipyrine (4-AAP) to produce a colored complex whose color intensity is directly proportional to the total cholesterol concentration in the sample [151, 152].

The schematic representation of the reaction is shown in the following equations:

$$\text{Cholesterol ester} \xrightarrow{\text{CHE}} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol} \xrightarrow{\text{CHOD}} \text{4-Cholesten-3-one + } H_2O_2$$

$$H_2O_2 + \text{Phenol + 4-AAP} \xrightarrow{\text{POD}} \text{colored complex + } H_2O$$

Reagents

Table 2-3: Reagents used for total cholesterol assay.

	Reagents	Concentration
Reagent A (100 ml)	4-AAP	1mmol/l
	CHE	300 U/l
	CHOD	300 U/l
	POD	1500 U/l
	Derivative of phenol	1mmol/l
Standard (5 ml)	Cholesterol	200 mg/dl

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase; POD, peroxidase.

Procedure

Concentrations of total cholesterol were measured by using a SMART-120 chemistry analyzer and the procedure for blank, standard, and sample measurement is demonstrated. The mixture was prepared and incubated at 37°C for 5 minutes. The sample-to-reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

Tale 2-4: The procedure of total cholesterol assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	1000	1000	1000
Water	10	-	-
Sample	-	10	-
Standard	-	-	10

Cholesterol (mg/dl) = Ax/As × 200 (standard Conc.)

2.5.2.2. Measurement of Serum Triglyceride Concentration

Methods for triglyceride determination generally involve enzymatic hydrolysis of triglycerides to glycerol and free fatty acids followed by enzymatic measurement of the glycerol released [153]. The glycerol participates in a series of coupled enzymatic reactions, in which glycerol kinase (GK) and glycerol phosphate oxidase (GPO) are involved, and hydrogen peroxide (H_2O_2) is generated. Produced H_2O_2 reacts with TOOS and 4-AAP to form a colored complex, whose absorbance is directly proportional to the concentration of triglycerides in the sample [154].

The schematic representation of the reaction is shown in the following equations:

$$\text{Triglyceride} \xrightarrow{\textbf{LPL}} \text{Glycerol + Fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\textbf{GK}} \text{Glycerol-1-phosphate + ADP}$$

$$\text{Glycerol-1-phosphate + } O_2 \xrightarrow{\textbf{GPO}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$H_2O_2 + \text{4-AAP + TOOS} \xrightarrow{\textbf{POD}} \text{colored complex + } H_2O$$

Reagents

Table 2-5: Reagents used for triglycerides assay.

	Buffer	Concentration
Reagent A (100 ml)	Magnesium chloride	15 mmol/l
	ATP	4 mmol/l
	4-AAP	1 mmol/l
	TOOS	0.1 mmol/l
	LPL	2500 U/I
	POD	1800 U/I
	G**K**	1000 U/I
	GPO	5500 U/I
Standard (10 ml)	Glycerol	200 mg/dl

ATP, adenosine triphosphate; 4-AAP, 4-amino antipyrine; LPL, lipoprotein lipase; POD, peroxidase; GK, glycerol kinase; GPO, glycerol phosphate oxidase.

Table 2-6: The procedure of triglycerides assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	1000	1000	1000
Water	10	-	-
Sample	-	10	-
Standard	-	-	10

Triglycerides (mg/dl) = Ax/As × 200 (standard Conc.)

Procedure

Concentrations of triglycerides were measured by using a SMART-120 chemistry analyzer and the procedure for blank, standard, and sample measurement. The mixture was prepared and incubated at 37°C for 5 minutes. The sample-to-reagent ratio was 1:100, and the absorbance of the

sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

2.5.2.3. Measurement of Serum High-Density Lipoprotein Cholesterol

Very Low-Density Lipoprotein-cholesterol (VLDL-C) and Low-Density Lipoprotein- cholesterol (LDL-C) are quantitatively precipitated and after centrifugation, the cholesterol bound to High-Density Lipoprotein-cholesterol (HDL-C) is measured in the supernatant. The intensity of the color is directly proportional to the HDL-C in the sample [155].

A schematic representation of the reaction is shown in the following equations:

HDL-C + LDL-C + VLDL-C + Chylomicron (CM) → HDL-C + (LDL-C +VLDL-C +CM)

$$\text{HDL-C} \xrightarrow{\text{CHE, CHOD}} \text{Fatty acids} + H_2O_2$$

H_2O_2 + 4-AAP + HDAOS → colored complex +H_2O

Reagents

Table 2-7: Reagents used for high-density lipoprotein cholesterol assay.

	Reagents	Concentration
Reagent A (90 ml)	Polyanions	1 mmol/l
	4-AAP	4 mmol/l
Reagent B (30 ml)	CHE	800 U/l
	CHOD	500 U/l
	Peroxidase	1500 U/l
	HDAOS	1 mmol/l
	Detergent	4 mmol/l

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase.

Procedure

The mixture was prepared and incubated at 37 °C for 5 minutes, and the absorbance of the blank sample (Abx) was read against a blank reagent at 600 nm. Shown Table (2.8)

Table 2-8: The procedure of high-density lipoprotein cholesterol assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	300	300	300
Water	4	-	-
Sample	-	4	-
Standard	-	-	4
Reagent (B)	100	100	100

HDL-C (mg/dl) = Ax / As × 40(Standard Conc.)

2.5.2.4. Calculation of Low-density lipoprotein cholesterol (LDL-C)

LDL-C (mg/dl) = Total Cholesterol – HDL-C – (Triglycerides/5)

2.5.2.5. Calculation of Very low-density lipoprotein cholesterol (VLDL-C)

VLDL-C (mg/dl) = Triglycerides/5 [156]

2.5.3. Calculation of Atherogenic Index

2.5.3.1. Atherogenic Coefficient

The atherogenic coefficient (AC) is the ratio of non-high-density lipoproteins cholesterol (non-HDL-C) to high-density lipoproteins cholesterol (HDL-C) [157]. It is a diagnostic alternative, which has been used in predicting the risk of developing cardiovascular events [158].

AC = non-HDL-C / HDL-C

Non- HDL-C = TC – HDL-C

2.5.3.2. Atherogenic index of Plasma (AIP)

The atherogenic index of plasma (AIP) is an unconventional lipid ratio representing the logarithm of the molar ratio of TG to HDL-C [159]. AIP has been shown to exhibit a strong association and inverse correlation with the diameter of LDL-C particles, serving as an indirect indicator of small, low-density lipoprotein (sdLDL) levels [160]. Thus, AIP may be a predictor of CAD.

AIP = log (TG/ HDL-C)

2.5.3.3. Castelli's Risk Indexes (I & II)

Castelli's risk indexes (I & II) also called cardiac risk indexes) are two lipid ratios, the CRI-I is the ratio of TC to HDL-C, while the CRI-II is the ratio of LDL-C to HDL-C. They were reported by William Castelli, at the end of the past century [161].

CRI-I= TC/ HDL-C ratio

CRI-II = LDL-C / HDL-C ratio

2.5.3.4. Cholesterol Index

The cholesterol index (C-index) is a simple index that predicts the probability of developing CAD with greater accuracy than the other indices [162].

C- index= (LDL-C) – (HDL-C)

2.5.4. Measurement of MIA3 using Sandwich-ELISA Technique:

Principle

This ELISA kit uses Sandwich-ELISA as a method. The Micro ELISA ribbon plate provided in this kit is pre-coated with an antibody specific to MIA3. Standards or samples are added to the appropriate wells of a Microelisa strip plate and combined with the specific antibody. A horseradish peroxidase (HRP)-conjugated antibody specific for MIA3 was then added to each well of a Microelisa strip plate and incubated. Complimentary components are washed. TMB substrate solution is added to each well. Only those wells containing HRP-conjugated MIA3 and MIA3 antibodies will appear blue and then turn yellow after the addition of the stop solution. Optical density (OD) is measured in the optical spectrum at a wavelength of 450 nm. The OD value is proportional to the MIA3 concentration. The concentration of MIA3 in the samples can be calculated by comparing the OD of the samples to the standard curve.

Table 2-9: Materials provided with the ELISA kit

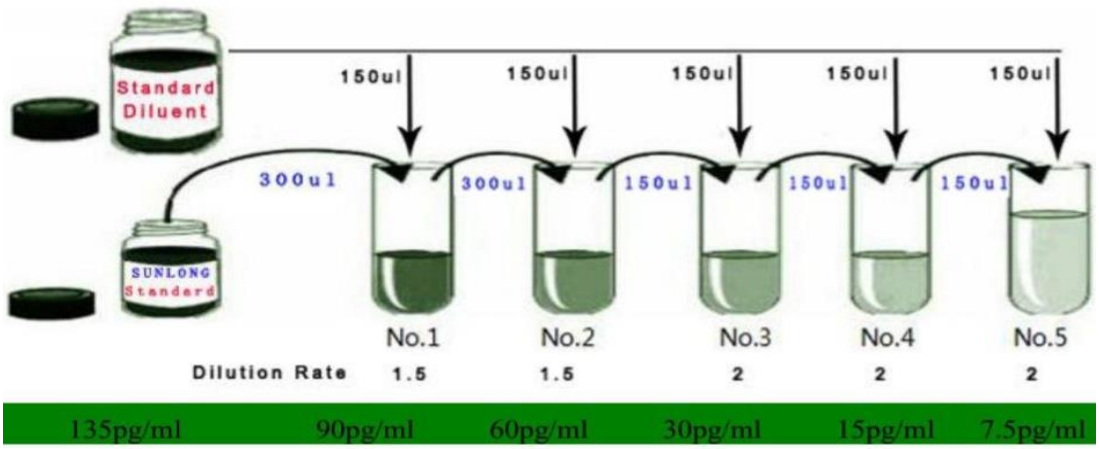
NO.	Materials provided with the kit	Volume & NO.
1	Closure plate membrane	2
2	Sealed bags	1
3	Micro ELISA strip plate	1
4	Standard：135 pg/ml	0.5ml×1 bottle
5	Standard diluent	1.5ml×1 bottle
6	HRP-Conjugate reagent	6ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	Chromogen Solution A	6ml×1 bottle
9	Chromogen Solution B	6ml×1 bottle
10	Stop Solution (sulphuric acid)	6ml×1 bottle
11	wash solution	20ml (30X) ×1bottle

Procedure

1. Dilution of Standards

The standard was diluted in small tubes first, then pipette 50 µl from each tube onto a microplate, each tube using two wells, for a total of ten wells.

120 pg/ml	**Standard No.1**	**300µl Original Standard + 150µl Standard diluents**
60 pg/ml	Standard No.2	300µl Standard No.1 + 150µlStandard diluents
30 pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
15 pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
7.5 pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



2. In the Microelisa strip plate, the well was left empty as an empty control. In the sample wells, 40 sample buffers and 10 µl samples were added (dilution factor is 5). Samples should be loaded at the bottom without touching the well wall. Mix well with light shaking.
3. Incubation: Incubated for 30 minutes at 37 °C after sealing with a lock plate.
4. Dilution: Concentrated laundry detergent was diluted with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: The membrane of the sealing plate was carefully peeled off, aspirated, and then refilled with the washing solution. The wash solution was discarded after resting for 30 seconds. Then I repeated the washing process 5 times.
6. 50 µl of HRP-conjugate reagent was added to each well except for the empty control well.
7. Incubation is described in Step 3.
8. Washing was done as described in step 5.
9. Coloring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, mixed with gentle shaking, and incubated at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: 50 µl of stop solution was added to each well to terminate the reaction. The color of the well should change from blue to yellow.

11. The O.D. absorbance was read. at 450 nm using a microtiter plate reader. The OD value of the blank control well was set as zero. The assay should be performed within 15 minutes after the addition of the stop solution.

Calculation

In Figure (2-2), concentrations of standards, the corresponding OD values, and the linear regression equation of the standard curve were calculated. Additionally, according to the OD value of the samples, the concentration of the corresponding sample was calculated, and the best-fit line can be determined by regression analysis.

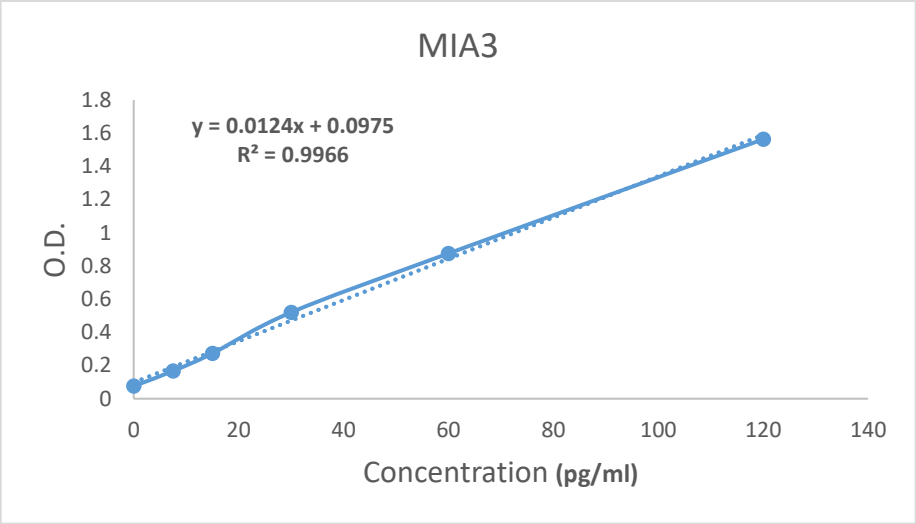


Figure (2-2): Standard curve of Melanoma inhibitory activity 3 concentration (pg/ml).

2.5.5. Measurement of E-Selectin by using Sandwich- ELISA Technique:

* Principle and procedure are mentioned in melanoma inhibitory activity 3, section 2.5.4.

Calculation of Results

As mentioned in 2.5.4.

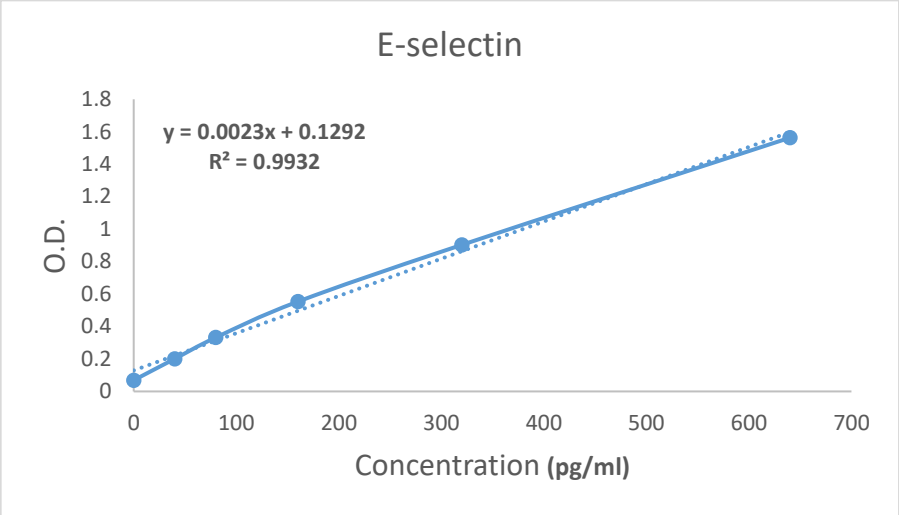


Figure (2-3): Standard curve of E-selectin concentration (pg/ml).

2.5.6. Measurement of CETP by using Sandwich- ELISA Technique:

* Principle and procedure are mentioned in melanoma inhibitory activity 3, section 2.5.4.

Calculation of Results

As mentioned in 2.5.4.

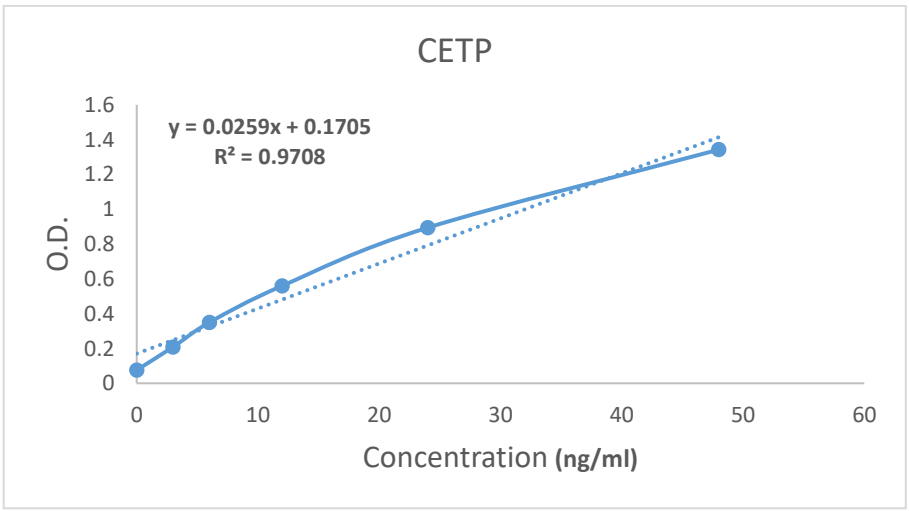


Figure (2-4): Standard curve of Cholesteryl ester transfer protein concentration (ng/ml).

2.6. Statistical Analysis

Information from the questionnaire and all test results from study group samples were entered into a data sheet. The data analysis for this work was generated using the Statistical Package for the Social Sciences software, version 22.0 (IBM, SPSS, Chicago, Illinois, USA). Values were illustrated by n (%) for categorical, Scale variables were presented by mean ± standard deviation for normal data. The distribution of the data was checked using the Shapiro-Wilk test as a numerical means of assessing normality. Biomarkers were compared using the one-way analysis of variance (ANOVA) was done to compare the means of different groups. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values ≤ 0.05 (two-tail) were considered to be statistically significant. The optimal threshold with high specificity and sensitivity for critical cases was detected using receiver operating characteristic (ROC) analysis. It was found that all the values of P were two-sided, and a $P \leq 0.05$ was considered to be statistically significant.

Chapter Three

Results

3. Results

3.1. Demographic Characteristics

The demographic characteristics of patients and healthy control groups are summarized in Table 3-1. The age range of participants was between 41 and 70 years old, with 33.3% of patients in the 41-50 age group, 35.0% in the 51-60 age group, and 31.7% in the 61-70 age group. Overall, the results indicate that most of the patient samples were overweight or obese. The medical history of the patients was collected through a self-report questionnaire, revealing that approximately 36.7% have hypertension, 28.3% have a family history of the disease, and 90% have hypercholesterolemia.

Table (3-1): Data sets according to different factors in patients and healthy control groups

Factors		Patients No.=120		Healthy control No.=60	
		No.	(%)	No.	(%)
Age group	(41-50)	40	33.3%	15	25.0%
	(51-60)	42	35.0%	22	36.7%
	(61-70)	38	31.7%	23	38.3%
BMI	Normal	34	28.3%	60	00.0%
	Overweight	57	47.5%	0	0.0%
	Obese	29	24.2%	0	0.0%
Family History	No	86	71.7%	60	00.0%
	Yes	34	28.3%	0	0.0%
HTN	No	76	63.3%	60	00.0%
	Yes	44	36.7%	0	0.0%
Hyper Cholesterolemia	Yes	108	90.0%	0	0.0%
	No	12	10.0%	60	00.0%

BMI: body mass index, HTN: hypertension

3.2. Examination of the data in the studied groups.

3.2.1. Anthropometric Characteristics

Table (3-2) and Figure (3-1) show the age of the stable angina group shows no significant differences compared to the other study groups (ACS and control) but ACS was a significant difference compared to the healthy control group. BMI and waist of patients show highly significant differences compared to the healthy control group.

Table (3-2): Comparison between patient and control groups in Age, Waist, and BMI

Groups	Mean ± SD		
	Age (year)	Waist (cm)	BMI (kg/m^2)
ACS	57.82 ±8.00 **a**	106.23 ±6.66 **a**	27.40 ±3.75 **a**
stable angina	56.31 ±7.91 **ab**	105.70 ±6.26 **a**	28.90 ±4.11 **a**
Healthy Control	54.08 ±8.71 **b**	96.85 ±11.82 **b**	23.70 ±1.80 **b**
P-value	0.106	0.001	0.001
Using the Post Hoc test, the mean's vertically different in letters differ significantly, while the mean having (ab) did not significantly differ with other groups vertically.			

One-way ANOVA was significant at 0.05; SD: Standard deviation, S: Significant; BMI: Body mass index; ACS: Acute Coronary Syndrome

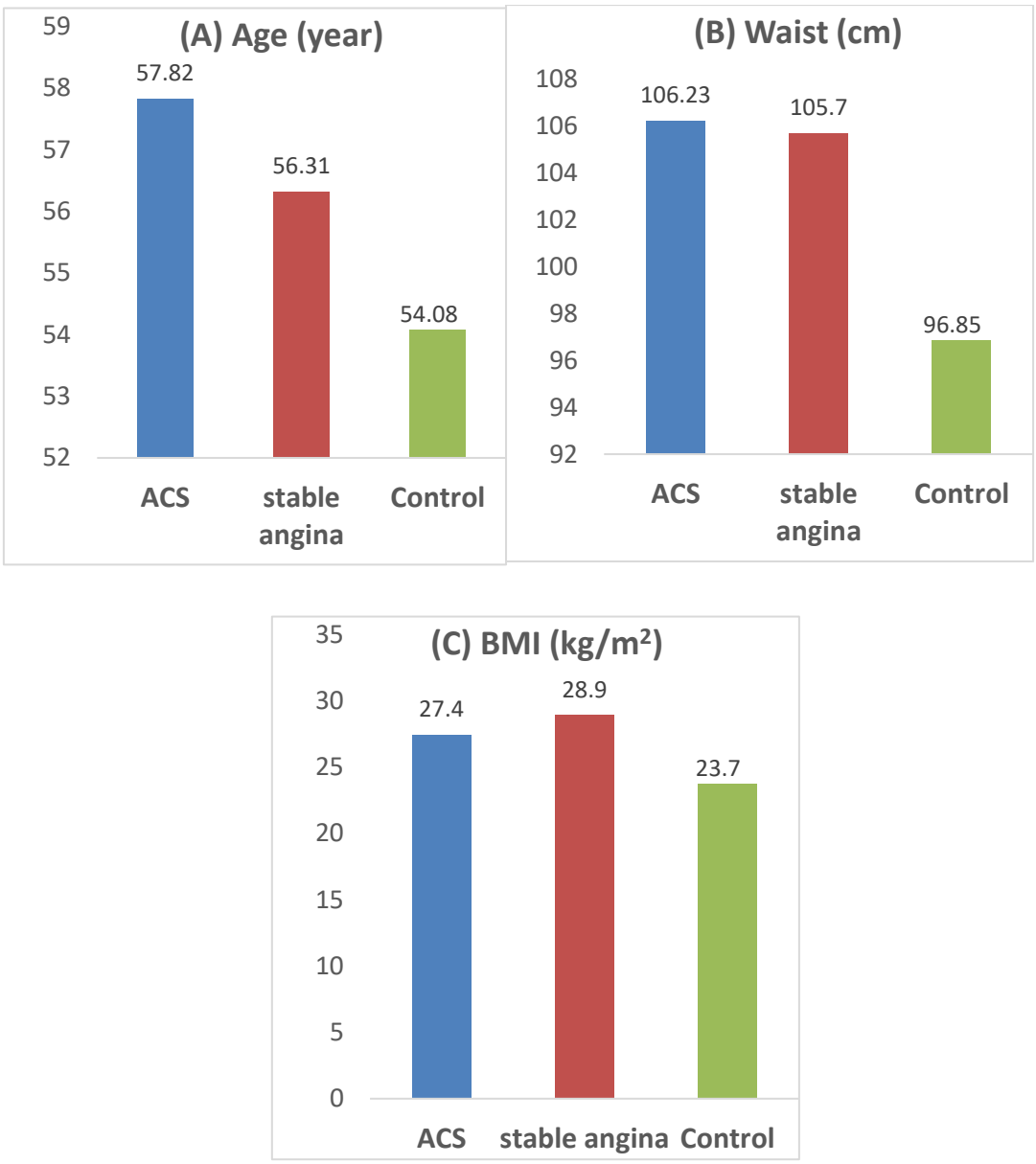


Figure (3-1): A, B, and C Comparison between patient and control groups in Age, Waist, and BMI.

3.2.2. Comparison of Lipid Profile between patients and healthy groups.

Table (3-3) and Figure (3-2) demonstrated the distribution of serum levels of the lipid profile in the patient's groups and the healthy control group. Patients were divided into two subgroups (ACS and stable angina).

Table (3-3): Comparison between patient and control groups in Lipid profile

Groups	Mean ± SD				
	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
ACS	247.38 ±44.36 **a**	221.29 ±82.93 **a**	34.44 ±2.48 **a**	170.58 ±39.97 **a**	42.07 ±17.75 **a**
stable angina	241.03 ±39.29 **a**	191.07 ±82.65 **a**	35.03 ±3.14 **a**	158.88 ±33.09 **a**	39.65 ±16.77 **a**
Healthy control	176.50 ±14.50 **b**	128.53 ±29.67 **b**	53.85 ±7.52 **b**	98.03 ±42.80 **b**	21.70 ±5.76 **b**
P-value	0.001	0.001	0.001	0.001	0.001
Using the Post Hoc test, the mean's vertically different in letters differ significantly					

One-way ANOVA was significant at 0.05; SD: standard deviation, S: significant; ACS: Acute Coronary Syndrome, TC: Total Cholesterol, TG: Triglycerides, HDL-C: High-Density Lipoprotein, LDL-C: Low-Density Lipoprotein, VLDL-C: Very Low-Density Lipoprotein

Total cholesterol levels were increased markedly in patient groups (ACS and stable angina). The mean levels of cholesterol in the patient's group were (247.38, and 241.03 mg/dl) respectively, while in the control group, the mean level of cholesterol was (176.50 mg/dl). on the other hand, TG in the patient's groups was (221.29, and 191.07 mg/dl) compared to (128.53 mg/dl) in the healthy control group. The HDL-C was significantly decreased in patient groups compared to the control group. Furthermore, the mean differences in LDL-C were also examined, and results indicated that there was an increase in the LDL-C levels in patients compared to the healthy control group. The mean of LDL-C in the control group (98.03) mg/dl was significantly lower than in patient subgroups, (170.58, and 158.88 mg/dl) respectively. The VLDL-C was also measured and that showed increases markedly in patient groups compared to the control groups

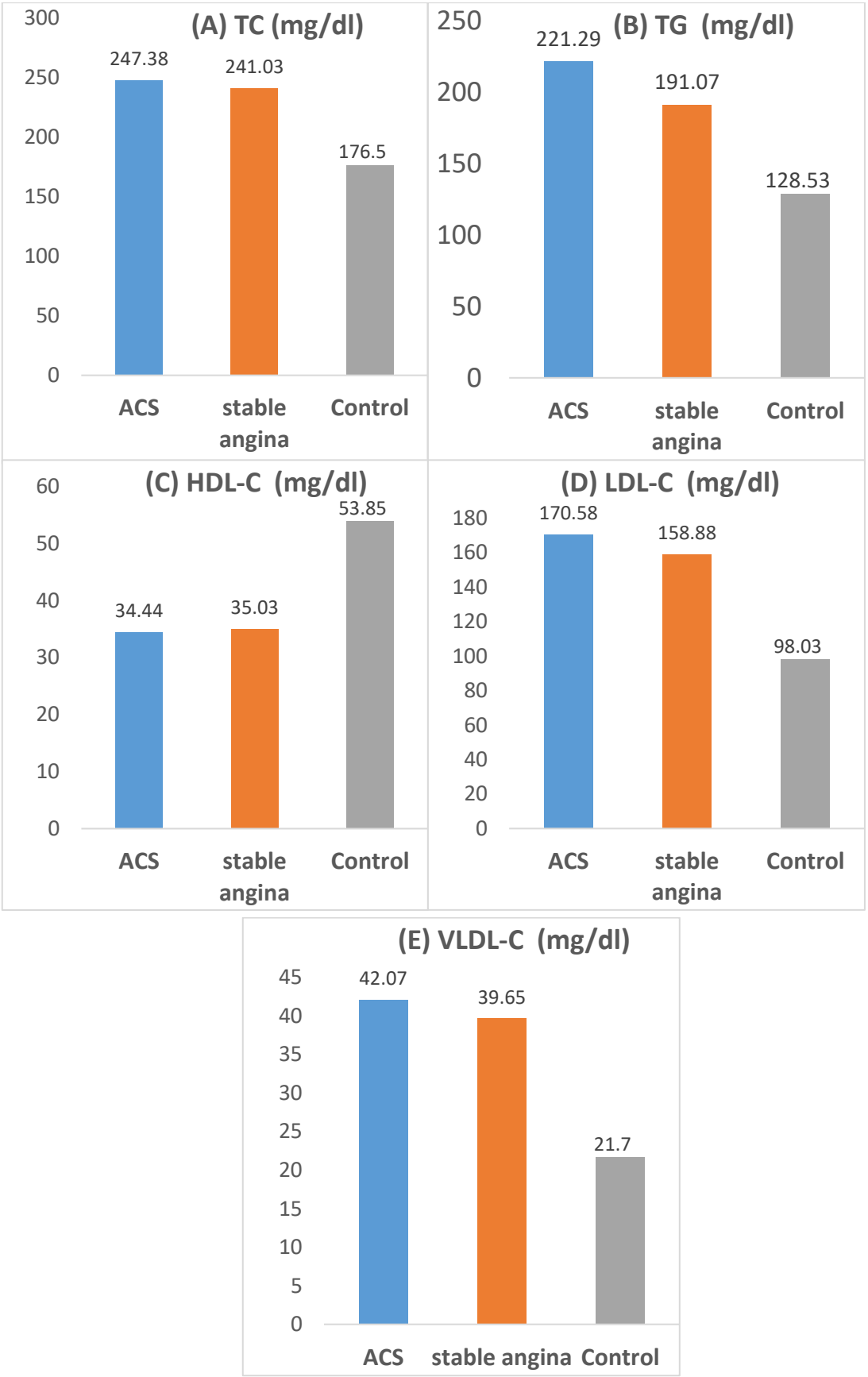


Figure (3-2): A, B, C, D, and E comparison between patient and control groups in lipid profile

3.2.3. Distribution of Atherogenic Index

Table (3-4) and Figure (3-3): The levels of AC in patient's groups (ACS and stable angina) were (6.24, and 5.99) respectively, and (2.33) in the healthy control group. Levels of AIP were (0.78, 0.69) respectively and (0.37) in the healthy control group. CR-I levels were (7.23, 6.99) while (3.33) in a healthy control group, and the levels for CR-II were (4.93, 4.87) and (1.84) in the control group. Finally, the levels of the C-index were significantly higher in-patient groups (134.24, 132.74) compared to the control group (43.09).

Table (3-4): Comparison between patient and control groups in Atherogenic index

Groups	Mean ± SD				
	AC	**AIP**	**CR-I**	**CR-II**	**C-index**
ACS	6.24 ±1.46 **a**	0.78 ±0.17 **a**	7.23 ±1.46 **a**	4.93 ±1.24 **a**	134.24 ±4.44 **a**
stable angina	5.99 ±1.56 **a**	0.69 ±0.23 **b**	6.99 ±1.56 **a**	4.87 ±1.17 **a**	132.74 ±32.37 **a**
Healthy control	2.33 ±0.49 **b**	0.37 ±0.12 **c**	3.33 ±0.49 **b**	1.84 ±0.45 **b**	43.09 ±19.40 **b**
P-value	0.001	0.001	0.001	0.001	0.001
Using the Post Hoc test, the mean's vertically different in letters differ significantly					

One-way ANOVA was significant at 0.05; SD: standard deviation, S: significant; ACS: Acute Coronary Syndrome, AC: Atherogenic Coefficient; AIP: Atherogenic Index of Plasma; CR-I, CR-II: Castelli's Risk Indexes; C-index: Cholesterol Index

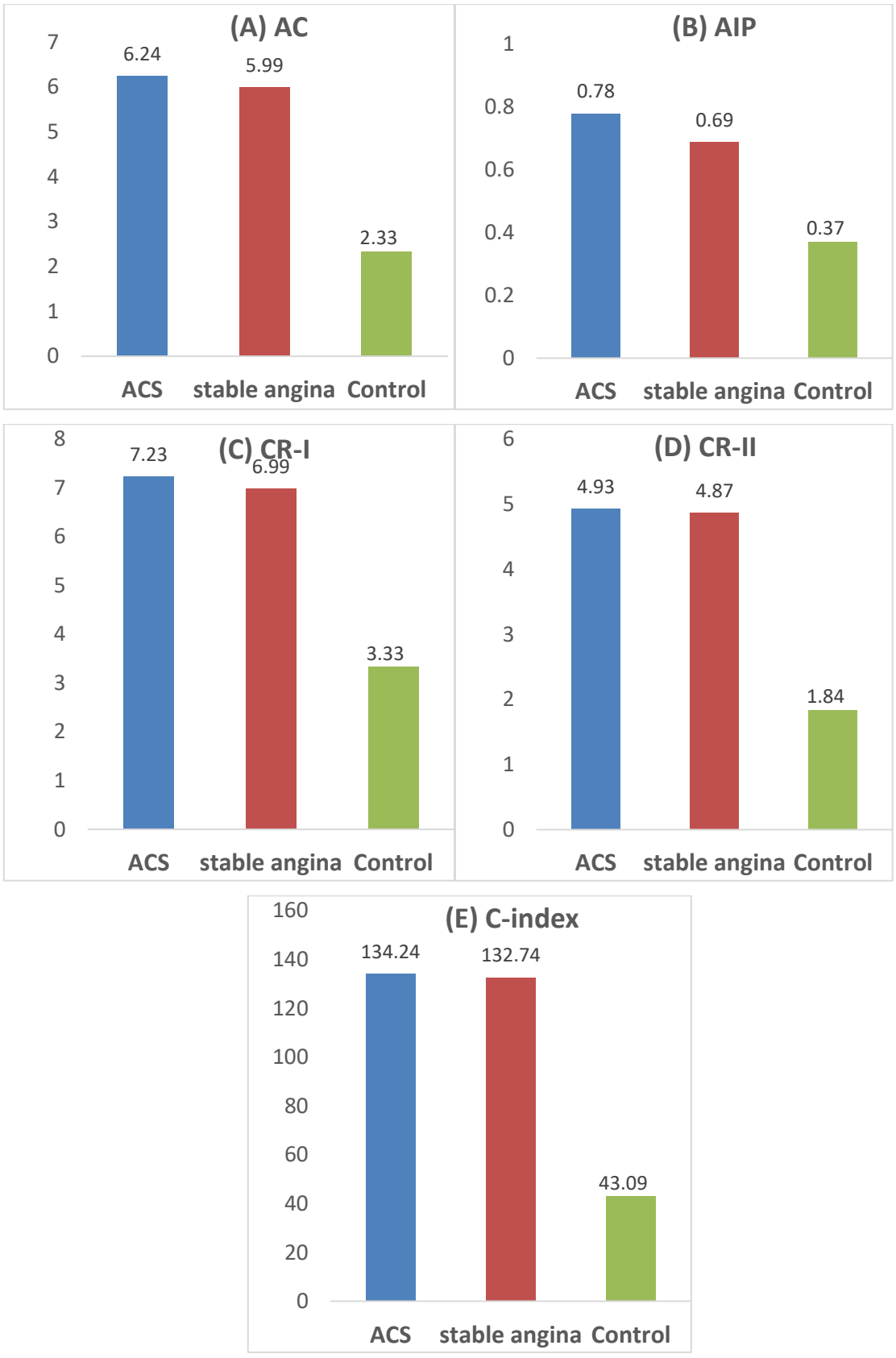


Figure (3-3): A, B, C, D, and E Comparison between patient and control groups in Atherogenic index

3.2.4. Distribution of MIA3, E-Selectin and CETP.

These are shown in Table (3-5) and Figure (3-4) which consists of the means of MIA3, E-Selectin, and CETP respectively for the study groups (ACS, stable angina, and healthy control).

Table (3-5): Comparison between patient and control groups in MIA3, E-Selectin, and CETP

Groups	Mean ± SD		
	MIA3 (pg/ml)	E-Selectin (pg/ml)	CETP (ng/ml)
ACS	65.89 ±12.55 **a**	216.07 ±20.26 **a**	17.53 ±8.26 **a**
stable angina	55.87 ±10.77 **b**	188.42 ±37.10 **b**	16.87 ±7.65 **a**
Healthy control	38.86 ±2.42 **c**	179.74 ±53.14 **b**	10.00 ±3.59 **b**
P-value	0.001	0.0002	0.001
Using the Post Hoc test, the mean's vertically different in letters differ significantly			

One-way ANOVA was significant at 0.05; SD: standard deviation, S: significant; ACS: Acute Coronary Syndrome, MIA3: Melanoma Inhibitory Activity protein 3, CETP: Cholesteryl Ester Transfer Protein

A significantly higher level of MIA3 in patient groups (65.89 pg/ml), (55.87 pg/ml) respectively compared to the control group (38.86 pg/ml), the levels of MIA3 means a significant difference between patient groups (ACS and stable angina) with (65.89 pg/ml) and (55.87 pg/ml) respectively.

A significant difference means levels of E-selectin between patient's groups (216.07 pg/ml), (188.42 pg/ml) respectively. A significantly higher level of E-selectin is noticed in ACS compared to the control group (216.07 pg/ml, 179.74 pg/ml) respectively, the levels of E-Selectin had no significant

difference between stable angina in the control groups with (188.42 pg/ml) and (179.74 pg/ml) respectively.

The CETP means for the study groups given in the same table show a significantly higher level of CETP in patient groups (17.53 ng/ml), (16.87 ng/ml) respectively compared to the control group (10.00 ng/ml), but no significant difference is noticed within patient's groups.

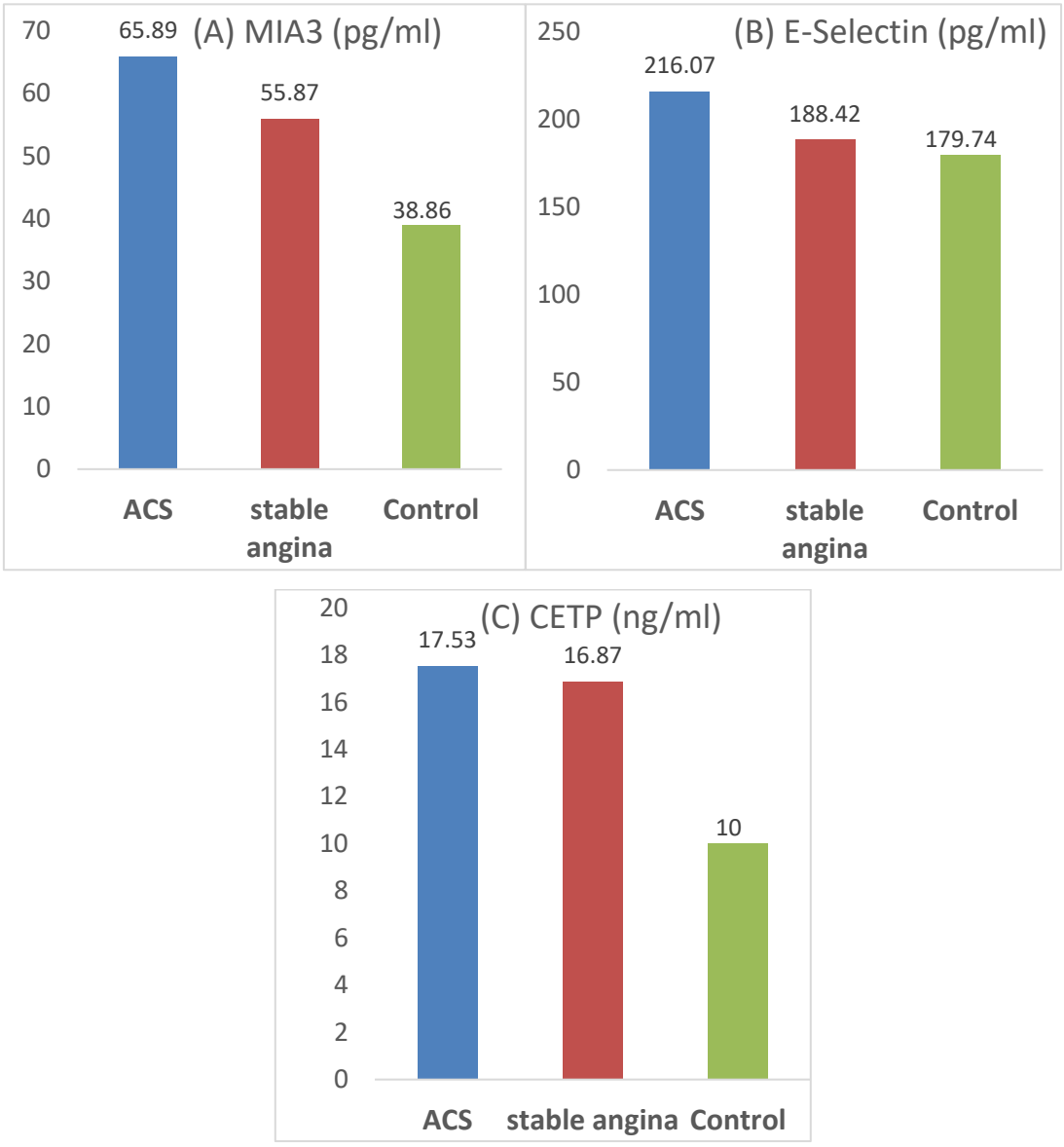


Figure (3-4): A, B, and C Comparison between patient and control groups in MIA3, E-Selectin, and CETP

3.3. Correlation between Biomarkers and studied parameters in patient groups.

3.3.1. Correlation between MIA3 and parameters

The correlation between serum levels of MIA3 and biochemical parameters and anthropometric characteristics in patient groups is shown in Table 3-6.

The results indicate a significant positive correlation between MIA3 levels and BMI (P=0.001), TC (P=0.009), LDL-C (P=0.017), AC (P=0.023), CR-I (P=0.023), CR-II (P=0.041), C-index (P=0.029), and CETP (P=0.001) in the ACS group. Age, TG, HDL-C, VLDL-C, AIP, and E-Selectin show a non-significant positive correlation, while waist has a non-significant negative correlation.

In the stable angina group, there is a significant positive correlation between serum MIA3 levels and age, waist, BMI, TC, TG, VLDL-C, AC, AIP, CR-I, CR-II, and C-index ($P \leq 0.0001$) respectively. There is also a non-significant positive correlation with CETP. MIA3 shows a significant negative correlation with HDL-C ($P \leq 0.010$).

Table (3-6): Correlation coefficient of MIA3 with other studied Parameters in patient groups

Parameters	MIA3 (pg/ml)			
	ACS		stable angina	
	r	P-value	r	P-value
Age, (year)	0.21	0.101	0.76**	0.0001
Waist, (cm)	-0.17	0.192	0.57**	0.0001
BMI, (kg/m^2)	0.41**	0.001	0.44*	0.0001
TC, (mg/dl)	0.34**	0.009	0.69**	0.0001
TG, (mg/dl)	0.20	0.121	0.64**	0.0001
HDL-C, (mg/dl)	0.04	0.756	-0.50**	0.0001
LDL-C, (mg/dl)	0.31*	0.017	0.33**	0.010
VLDL-C, (mg/dl)	0.25	0.056	0.69**	0.0001
AC	0.29*	0.023	0.68**	0.0001
AIP	0.12	0.373	0.62**	0.0001
CR-I	0.29*	0.023	0.68**	0.0001
CR-II	0.26*	0.041	0.62**	0.0001
C-index	0.28*	0.029	0.61**	0.0001
E-Selectin, (pg/ml)	0.17	0.180	0.44*	0.0001
CETP, (ng/ml)	0.43**	0.001	0.23	0.078

r: Pearson correlation coefficient; **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative; S: significant; BMI: Body mass index; MIA3: Melanoma Inhibitory Activity protein 3, CETP: Cholesteryl Ester Transfer Protein, TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein; AC: Atherogenic coefficient; AIP: Atherogenic index of plasma; CR-I, CR-II: Castelli's risk indexes; C-index: Cholesterol index

3.3.2 Correlation between E-Selectin and studied parameters.

In Table (3-7), correlations of serum E-Selectin with biochemical parameters and anthropometric characteristics in patient groups are shown.

The results of Table (3-7) revealed that in the ACS group, there was a non-significant positive correlation between the level of serum E-Selectin and Age, BMI, TC, TG, LDL-C, VLDL-C, AC, AIP, CR-I, CR-II, C-index, MIA3, and CETP levels, while HDL-C had a non-significant negative correlation with serum E-Selectin in patients of the ACS group. Waist had a significant negative correlation with E-selectin (P=0.049).

In the stable angina group, there was a significant positive correlation between the level of E-Selectin with age (P=0.008), waist (P≤0.0001), BMI (P=0.005), TC (P=0.002), TG (P=0.014), VLDL-C (P=0.007), AC (P= 0.005), AIP (P=0.010), CR-I (P=0.005), CR-II (P=0.009), C-index (P=0.006), and MIA3 (P<0.0001). LDL-C and CETP levels had a non-significant positive correlation, while HDL-C had a non-significant positive correlation with serum E-Selectin in patients of the stable angina group.

Table (3-7): Correlation coefficient of E-selectin with other studied Parameters in patient groups.

Parameters	E-Selectin (pg/ml)			
	ACS		stable angina	
	r	P-value	r	P-value
Age, (year)	0.15	0.253	0.34**	0.008
Waist, (cm)	-0.25*	0.049	0.47**	0.0001
BMI, (kg/m^2)	0.21	0.109	0.36**	0.005
TC, (mg/dl)	0.22	0.09	0.40**	0.002
TG, (mg/dl)	0.13	0.333	0.31*	0.014
HDL-C, (mg/dl)	-0.04	0.777	-0.16	0.233
LDL-C, (mg/dl)	0.16	0.215	0.23	0.074
VLDL-C, (mg/dl)	0.18	0.171	0.34**	0.007
AC	0.20	0.122	0.35**	0.005
AIP	0.10	0.429	0.33**	0.010
CR-I	0.20	0.122	0.35**	0.005
CR-II	0.19	0.148	0.33**	0.009
C-index	0.19	0.137	0.35**	0.006
MIA3, (pg/ml)	0.1	0.180	0.44**	0.0001
CETP, (ng/ml)	0.27*	0.035	0.06	0. 658

r: Pearson correlation coefficient; **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative; S: significant; BMI: Body mass index; MIA3: Melanoma Inhibitory Activity protein 3, CETP: Cholesteryl Ester Transfer Protein, TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein; AC: Atherogenic coefficient; AIP: Atherogenic index of plasma; CR-I, CR-II: Castelli's risk indexes; C-index: Cholesterol index

3.3.3. Correlation between CETP and studied parameters

Correlations between CETP and biochemical parameters and anthropometric characteristics in patient groups are displayed in Tables 3-8.

The results indicated a significant positive correlation between CETP levels and age (P=0.003), AC (P=0.048), AIP (P=0.034), CR-I (P=0.048), MIA3 (P=0.001), and E-Selectin (P=0.035) in the ACS group. BMI, TC, TG LDL-C, VLDL-C, CR-II, C-index, and showed non-significant positive correlations, while Waist and HDL-C showed a non-significant negative correlation.

In the stable angina group, there was a significant positive correlation between CETP levels and age, TG, VLDL-C, and AIP at ($P \leq 0.0001$), and (AC, CR-I) at (P=0.018). Waist, BMI, TC, LDL-C, CR-II, C-index, MIA3, and E-Selectin showed non-significant positive correlations, while HDL-C showed a significant negative correlation (P=0.005).

Table (3-8): Correlation coefficient of CETP with other studied Parameters in patient groups.

Parameters	CETP (ng/ml)			
	ACS		stable angina	
	r	P-value	r	P-value
Age, (year)	0.38**	0.003	0.52**	0.0001
Waist, (cm)	-0.06	0.624	0.17	0.199
BMI, (kg/m^2)	0.08	0.556	0.15	0.253
TC, (mg/dl)	0.21	0.110	0.21	0.109
TG, (mg/dl)	0.20	0.132	0.49**	0.0001
HDL-C, (mg/dl)	-0.22	0.09	-0.35**	0.005
LDL-C, (mg/dl)	0.15	0.267	0.005	0.971
VLDL-C, (mg/dl)	0.33	0.055	0.44**	0.0001
AC	0.26*	0.048	0.30*	0.018
AIP	0.27*	0.034	0.44**	0.0001
CR-I	0.26*	0.048	0.30*	0.018
CR-II	0.24	0.068	0.18	0.174
C-index	0.18	0.177	0.08	0.547
MIA3, (pg/ml)	0.43**	0.001	0.23	0.078
E-Selectin, (pg/ml)	0.27*	0.035	0.06	0. 658

r: Pearson correlation coefficient; **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative; P : P value; S: significant; BMI: Body mass index; MIA3: Melanoma Inhibitory Activity protein 3, CETP: Cholesteryl Ester Transfer Protein, TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein; AC: Atherogenic coefficient; AIP: Atherogenic index of plasma; CR-I, CR-II: Castelli's risk indexes; C-index: Cholesterol index

3.4. Receiver operating characteristic (ROC)

ROC curve and AUC analysis were performed for MIA3, E-Selectin, and CETP in the patient groups. The results of the receiver operating curve (ROC) and AUC analysis as a diagnostic parameter showed that MIA3 has good performance. The data was presented in Table 3-9 and Table 3-10.

Table (3-9): Area under the curve (AUC), Cut-off point, sensitivity, and specificity of MIA3, E-Selectin, and CETP obtained by ROC curve in patients with ACS.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CI	
MIA3 (pg/ml)	≥ 50.80	%97	%88	0.957	0.0001	0.919	0.995
E-Selectin (pg/ml)	≥197.37	%85	%70	0.768	0.0001	0.673	0.863
CETP (ng/ml)	≥11.86	%68	%73	0.767	0.0001	0.666	0.868

In the ACS group, MIA3 had the highest AUC with a value of 0.957 [95% CI (confidence interval) =0.919 – 0.995, Sensitivity = %97, Specificity= %88, Cut-off point = 50.80 pg/ml]. E-Selectin had an AUC of 0.768 [95%CI (confidence interval) = 0.673-0.863, sensitivity= %85, Specificity = %70, Cut-off point = 197.37 pg/ml]. CETP had an AUC of 0.767 [95%CI (confidence interval) = 0.666-0.868, sensitivity = %68, Specificity = %73, Cut-off point = 11.86 ng/ml].

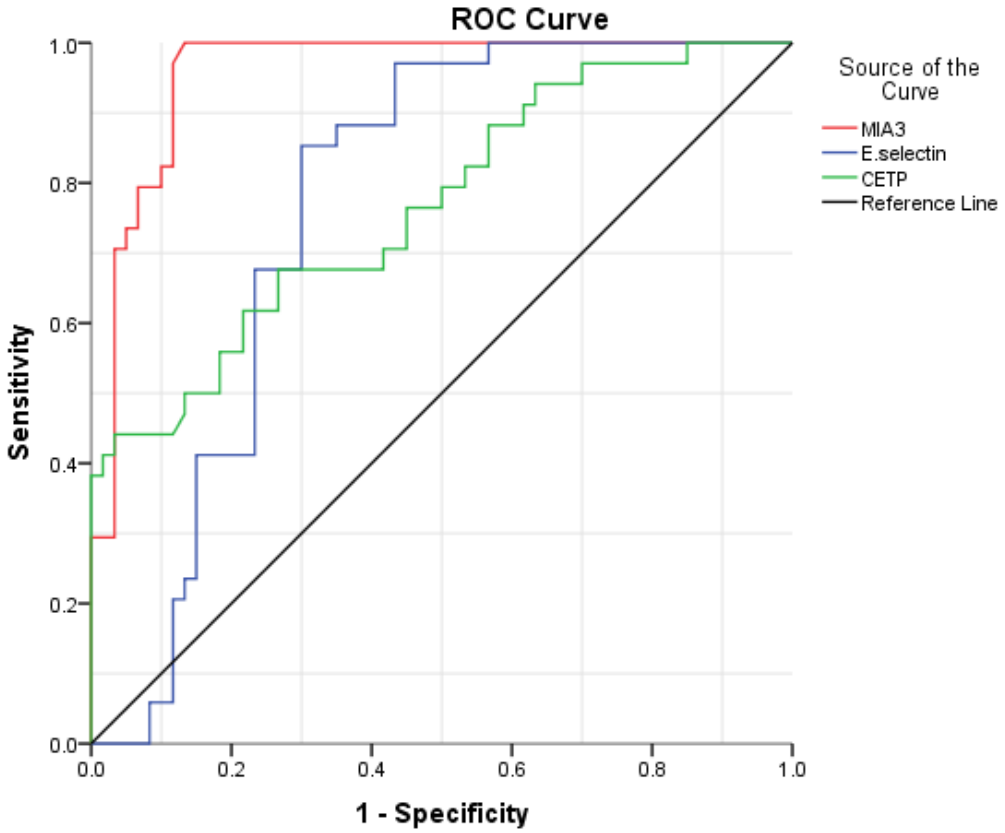


Figure (3-5): Receiver Operating Characteristic (ROC) curve of serum MIA3, E-Selectin, and CETP levels as discriminators of patients with ACS.

Table (3-10): Area under the curve (AUC), Cut-off point, sensitivity, and specificity of MIA3, E-Selectin, and CETP obtained by ROC curve in patients with stable angina.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CI	
MIA3 (pg/ml)	≥44.29	%92	%70	0.859	0.0001	0.780	0.939
E-Selectin (pg/ml)	≥174.94	%73	%52	0.596	0.161	0.475	0.716
CETP (ng/ml)	≥11.86	%73	%73	0.773	0.0001	0.662	0.885

In the stable angina group, the AUC of MIA3 was 0.859 (95% CI = 0.780-0.939, Sensitivity = %92, Specificity = %70, Cut-off point = 44.29 pg/ml). For E-Selectin, it was 0.596 (95% CI = 0.475-0.716, Sensitivity = %73, Specificity = %52, Cut-off point = 174.94 pg/ml), and for CETP, it was 0.773 (95% CI = 0.662-0.885, Sensitivity = %73, Specificity =%73, Cut-off point = 11.86 ng/ml).

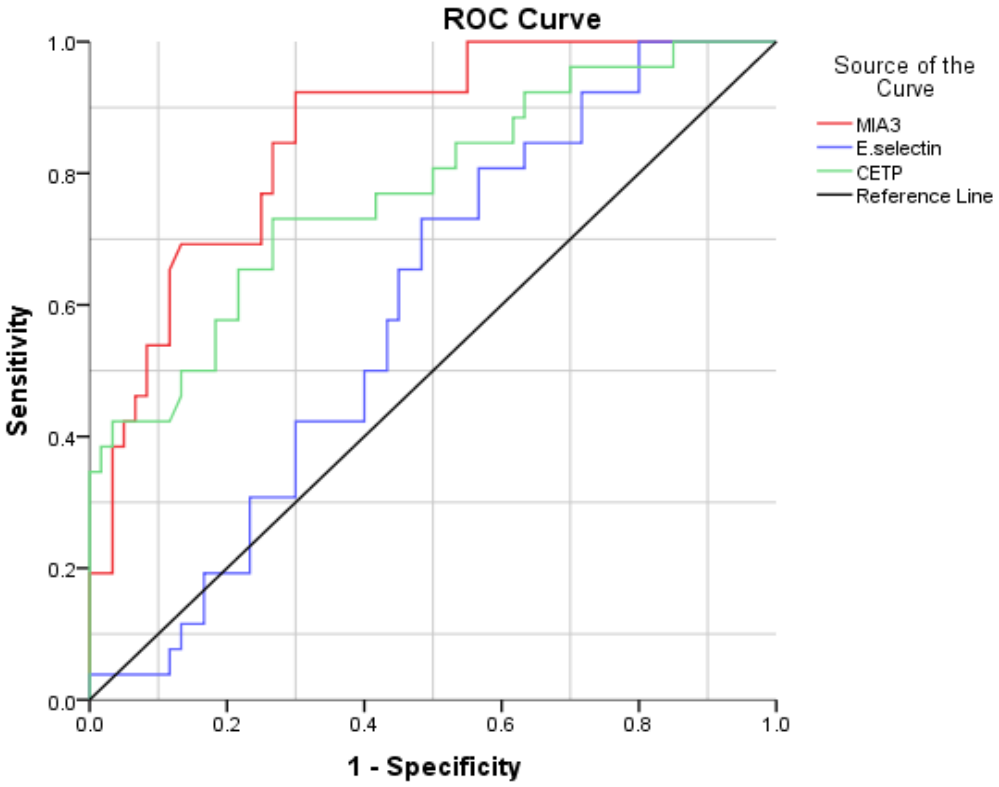


Figure (3-6): Receiver Operating Characteristic (ROC) curve of serum MIA3, E-Selectin, and CETP levels as discriminators of patients with stable angina.

Chapter Four

Discussion

4. Discussion

4.1. Demographic and anthropometric characteristics

In the present study, it was noticed that most patients were in the age groups 51–61 (35.0%) and 61–70 (35.6%). Age is a strong non-modifiable risk factor for (CAD) including stroke, MI, and peripheral arterial disease[163]. The prevalence of CAD increases with age, regardless of gender, in both biological men and women[164]. Age is a significant independent risk factor for CAD due to the increased likelihood of having any additional cardiac risk factors, such as diabetes and obesity [165]. Numerous variables, including elevated oxidative stress, inflammation, apoptosis, and general cardiac degradation and degeneration, have been linked to the high frequency of CAD in this population. Additionally, age is linked to a higher risk of diabetes, obesity, and frailty [166, 167].

In the current study, it was noticed that a large percentage of patients were overweight (45.0%) and obese (30.0%). In previous reports, 26% of adults worldwide are overweight (body mass index [BMI] 25–29.9 kg/m^2) and 13% are obese (BMI ≥30 kg/m^2) [168]. Obesity has been recognized as an independent risk factor for coronary artery disease (CAD) [169]. In a large multicenter registry of patients without known CAD disease undergoing computed tomography (CT) coronary angiography, BMI was independently associated with the presence of any CAD and obstructive CAD [170]. Obesity is also associated with a clustering of other risk factors for CAD, such as diabetes mellitus (DM), hyperlipidemia, and hypertension (HTN) [171]. Although previous studies strongly suggest that obesity promotes CAD, the association between BMI and age of presentation with symptomatic CAD is not well understood [172].

By measuring the waist for patients and healthy control groups, this study showed that most patients suffer from central obesity in the abdominal area compared to healthy controls. Some studies reported that the association between BMI and CAD was similar to measures of central obesity. Greater central obesity is associated with systemic inflammation, which directly contributes to CAD risk [173]. Hence, measures that account for the accumulation of excess abdominal fat would report stronger associations and are desirable for assessing adiposity [174]. The addition of central obesity measures to BMI has also been shown to improve the accuracy of stratifying participants into lower and higher risk categories for mortality Hypertension (HTN) is an established risk factor for cardiovascular disease (CVD) [175].

In the current study, 36.7% of patients have hypertension. Moreover, patients with HTN have an increased risk of cardiovascular mortality compared with patients who do not have the condition [176]. Current evidence suggests that hypertension, via increased oxidant stress, damages the endothelium [177]. This evokes several responses that disrupt the balance between processes such as constriction/dilation, proliferation/antiproliferation, thrombosis/antithrombosis, and fibrinolysis/antifibrinolysis [178]. These consequences of endothelial dysfunction may lead to transient ischemia (through hypoperfusion) or ischemia-related adverse outcomes (coronary thrombosis, sudden death) [178]. Also, the percentage of patients with coronary arteries who have a family history of the disease was 28.3%. The study refers to a family history of CAD as an independent long-term risk factor for CAD, indicating a genetic predisposition for CAD beyond the heritability of traditional risk factors [179].

4.2. Serum lipid Profile and atherogenic index

In this study, the results indicated that the levels of lipid profiles were elevated in patient groups (ACS and stable angina). The obese and CAD groups showed a dyslipidemic profile compared to the healthy control group, as shown in Table (3-3) and Fig (3–2).

A previous study indicated that Jordanian patients with CAD have higher cholesterol, LDL-C, and triglyceride levels and lower HDL-C levels than the control group, which comes from other studies [180]. Hyperlipidemia remains the strongest risk factor for CAD Abnormalities in the lipid profile, specifically hypertriglyceridemia and low levels of HDL-C, are a strong predisposing issue for many diseases, including obesity, diabetes, and cardiovascular diseases [181]. Despite some controversy, elevated levels of triglycerides, fasting as well as nonfasting, also appear to be an independent risk factor for CAD [182]. Evidence from epidemiologic studies suggests that the co-occurrence of low HDL-C and elevated triglyceride levels is a strong risk factor for CAD [181]. while post hoc analyses of several studies have shown that patients with low HDL-C and elevated triglycerides have the highest rate of major coronary events [183]. Whether an increased level of small, dense LDL-C represents an independent risk factor remains somewhat controversial, it is associated with an increase in CAD risk [184]. Serum cholesterol is an established CAD risk factor in Europeans as well as Asians Elevated levels of plasma LDL-C are major CAD risk factors, as therapy with LDL-C drugs has reduced CAD risk, and the reduction is proportional to a decrease in LDL-C levels [185, 186].

As expected, already established, obesity and CAD risk factors had a high prevalence in the patients in the current study as well. It was clear that the lipid profile of patients was more atherogenic, and patients exhibited a

significantly higher prevalence of CAD risk compared to age- and sex-matched controls. The findings are from other studies carried out in other developed countries[184].

Hypercholesterolemia, either genetic or acquired, is an independent CAD risk factor. It is estimated that 56% of total heart diseases may be due to hypercholesterolemia (> 200 mg/dl) alone [187]. Many authors have reported lipid levels to be similar among Asian and Western countries and attributed the increased risk of CAD to insulin resistance and central obesity [184].

The ratio of TC/HDL-C (the atherogenic coefficient) is considered to be a sensitive predictor of cardiovascular disease risk, especially if the values are ≥6 [188]. In our study, the TC/HDL-C ratio is significantly higher than controls, and the values are elevated or equal to this cutoff. In the USA, however, the TC/HDL-C ratio above 5 is considered to be atherogenic.

In the current study, increased TG concentrations were consistently accompanied by low HDL-C concentrations. Further lowering in HDL-C concentration results from its conversion by hepatic lipase into smaller particles, which are rapidly cleared from plasma, resulting in VLDL-C particles forming cholesteryl ester-depleted, small, dense LDL-C particles that are taken up by arterial wall macrophages, causing atherogenesis [184].

The current study gives basic information about dyslipidemia in the obese and CAD populations in Kerbala and can be helpful in advanced research about lipid profiles and establishing a correlation between lipid profile parameters and dyslipidemia-associated abnormalities in the Iraqi population.

4.3. Serum Melanoma inhibitory activity protein 3 level

The results of this study showed a significant increase in MIA3 levels in both groups of patients (ACS and stable angina) compared to the control group (mean = 65.89, 55.87, and 38.86 pg/ml, respectively) with a P-value of 0.0001, as described in Table (3-5) and Fig (3-4).

The present study is considered the first to investigate MIA3 levels in the circulation and its relationship to coronary artery disease, whether acute or chronic. The current data demonstrated a high serum MIA3 levels may be associated with the risk of developing CAD and may be an independent marker for its diagnosis, but more research is needed to determine whether other confounding factors influence its high levels.

It has also been reported that inhibition of MIA3 in the injured arteries can prevent postangioplasty restenosis, supporting a potential role for MIA3 and its target genes in a variety of proliferative vascular diseases. These findings may have extensive implications for the treatment of occlusive vascular diseases [119].

The reasons for the increase in MIA3 levels may be related to previous studies genome-wide association studies have found a link between elevated coronary artery disease susceptibility and particular SNPs within the genome that play a role in combination with other established CAD risk factors, but the exact mechanism remains unknown [119, 189]. Atherosclerosis is one of the key pathophysiological causes of CAD, according to clinical observation [190]. In a meta-analysis, on the other hand, Xiuchun Li *et al*. presented evidence of a case-control association in the Chinese Han population with 2503 CAD patients and 2920 controls. Their report suggested a significant association of the SNP rs17465637 in MIA3 with CAD (p-value = 0.01,

OR = 1.11), with rigorous confirmation by follow-up meta-analyses in five admixed Asian populations with 7263 CAD cases and 8347 controls for CAD. Highly significant relationships were reported between the SNP rs17465637 and CAD in the populations from Asia (p-value = 4.97 *10¯5, OR = 1.11). These data are strong supporters of the reality that the SNP rs17465637 in MIA3 populations in Asia confers a substantive risk of CAD [191]. Luo, Chunyan, *et al*., Their study they conducted in 2017, Confirmed the expression of the MIA3 gene in coronary artery disease leads to accelerated disease progression, which is consistent with our results [121].

MIA3 protein is an evolutionarily conserved endoplasmic reticulum-resident transmembrane protein and is required for the export of collagen VII from the endoplasmic reticulum is encoded by the TANGO gene referred to later (MIA3)[192]. Moreover, TANGO can supposedly influence an increase in the risk of CAD via inflammatory procedures [113]. The critical function of the inflammatory modes of action in forming atheroma is supported by copious data, This process involves leukocyte mobilization and the pivotal participation of proinflammatory cytokines in the primary phase of atherogenesis [193, 194]. Endothelial cells are activated for inflammation, followed by the entry of monocytes and other leukocytes into the atheroma [195]. After transmigration, monocytes are differentiated into macrophages, forming the fatty streak that primarily indicates atherosclerosis in the arterial intima [196]. Thus, atherosclerosis is driven by inflammation, eventually triggering thrombotic plaque consequences, often causing myocardial infarction (MI) and CAD [197].

In a prior study that demonstrated the relationship between VSMC and CAD, it was shown that MIA3 is a novel regulator that promotes proliferation and migration during neointimal formation [119]. VSMCs are

the major cell types of medial layer arteries and play a pivotal role in regulating the remodeling process of the vessel wall [198]. Subsequent excess proliferation and migration result in an accumulation of synthetic SMCs in the stented artery, which contributes to in-stent restenosis [199]. Thus, inhibiting the proliferation and migration of intravascular SMC is the predominant therapeutic strategy to prevent the excessive formation of neointima [200]. Knockdown of MIA3 reduces proliferation and migration of SMC. In contrast, MIA3 overexpression promoted VSMC migration and proliferation. VSMCs are a significant source of chemokines and cytokines ، and found that knockdown MIA3 in VSMC decreased IL-1β, IL18, CCL7, and CxCL8 expression. The knockdown of MIA3 results indicated that proliferation and migration, which are the critical cellular events in vascular neointimal lesion formation, were regulated, at least in part, by MIA3 [119].

The results of these studies provide clear evidence of the involvement of MIA3 in CAD and support the findings of the present study

4.4. Serum E-selectin level

The results showed an increased level of E-Selectin in patient groups, as described in Table (3-5) and Fig (3-4).

The current study showed that E-selectin was elevated means of levels was higher in both groups of patients compared with controls (ACS, 216.07 ± 20.26 pg/ml; stable angina, 188.42 ± 37.10 pg/ml; controls, 179.74 ± 53.14 pg/ml; one-way ANOVA, P = 0.0002), but there was no significant difference between patients with stable angina and healthy control groups. It may be that soluble E-selectin exacerbates symptoms in inflammatory disease through the activation of β2 integrins, and modulation of leukocyte movement [201].

Expression of E-selectin can be induced by *de novo* synthesis on the endothelium, peaking within 2–6 hours in response to inflammatory stimuli, including IL-1, lipopolysaccharide, or TNF-α, and subsiding to basal levels within 10–24 hours [202], this supports the results shown by this study. Smyła, W., *et al.*, showed in their study that the serine/arginine-128 polymorphism in E-selectin was associated with a greater risk for early severe atherosclerosis and was the reason for incorrect recognition of heparin. A role for E-selectin in atherosclerosis [203].

Prior human studies on E-selectin concentrations in cardiovascular disorders have been conducted. E-selectin is a cell adhesion molecule that mediates leukocyte adhesion to vessel walls in response to inflammation. E-selectin enables rolling in monocytes, neutrophils, effector T cells, B cells, and natural killer cells [124]. Recruiting these cells, especially pentrax monocytes, begins the formation of atherosclerotic plaques [203]. E-selectin is expressed on the vascular endothelium and is responsible for the adhesion and transendothelial migration of circulating leukocytes [204]. E-selectin is important and unique because it is expressed only by endothelial cells of the intima associated with atherosclerotic lesions. The circulating level of the soluble form of E-selectin reflects its endothelial expression and indicates the presence of systemic inflammation and, consequently, endothelial activation [203]. Elevation of E-selectin is considered a specific marker of endothelial activation reactive protein and dysfunction [205].

Prior studies have indicated that many adhesion molecules are increased in the presence of chronic and acute atherosclerotic conditions. Thus, the elevation of serum E-selectin is considered to be a specific marker of endothelial activation. Inflammation appears to play an important role throughout all stages of atherosclerosis and has recently been implicated in

the pathogenesis of ACS-induced plaque rupture [206]. In the present study, in keeping with this background, we have demonstrated that serum E-selectin levels are increased in the presence of clinically relevant atherosclerosis. Histologically, it is very difficult to detect such a small number of cells using conventional histopathology. A biologically relevant increase in the number of E-selectin-positive cells would still be difficult to detect histologically, whereas an elevation in serum E-selectin may be more relevant [207]. Furthermore, serum E-selectin appears to be a very sensitive marker of endothelial activation, given the fact that it produces elevated preclinical conditions such as dyslipidemia, decreasing rapidly following aggressive treatment with cholesterol-lowering drugs [208].

4.5. Serum Cholesteryl ester transfer protein level

The current study showed a significant increase in serum CETP levels in coronary artery disease patients for both groups (ACS and stable angina) compared to its levels in the healthy group, as revealed in Table (3-5) and Fig (3-4).

The increased level of serum CETP protein may be returned to the role of CETP as a key plasma glycoprotein that is primarily produced by the liver and affects circulating HDL-C levels by transporting esterified cholesterol from HDL-C to APO-B-containing particles instead of receiving triglycerides [209]. CETP promotes the exchange of neutral lipids between HDL-C and non-HDL-C lipoproteins, consisting of lipoprotein particles containing apolipoprotein B100 (apoB100) [210]. The non-HDL-C lipoproteins include VLDL-C, their remnants, and LDL-C particles, all of which are pro-atherogenic [211]. The net effect is a mass transport of cholesteryl esters (CE) from HDL-C to VLDL-C and LDL-C, with a reverse movement of triglycerides (TG) to HDL-C [212]. This neutral lipid transfer

is most commonly believed to occur by a conformational change in CETP upon CE binding, leading to the formation of a continuous tunnel across CETP through which CE and TG transfers occur [213]. Indirectly, esterified cholesterol is transported to VLDL-C and LDL-C lipoproteins by the action of cholesteryl ester transfer protein (CETP) and finally to endocytosis by liver cells [209]. As a result of the above CETP-mediated process, the overall effect of CETP inhibition should be to increase HDL-Cholesterol (HDL-C) levels and, conversely, to decrease the cholesterol. In more detail, it mediates the transport of CE from HDL-C to VLDL-C/chylomicrons and TG from VLDL-C/chylomicrons to HDL-C and low-density lipoproteins (LDL-C), thus regulating HDL-C plasma levels [214].

Additionally, in this study, there was an inverse relationship between CETP and HDL-C (ACS, r = -0.25; P= 0.09; stable angina, r = -0.35**; P=0.005). Giammanco, A., *et al.*, in the Honolulu Heart Program, examined 3469 Japanese male subjects, carriers of two different CETP gene mutations, and were evaluated for correlations between CETP deficiency, HDL-C levels, and cardiovascular diseases [215]. It was found that male heterozygous carriers of CETP gene mutations with low or slightly increased HDL-C levels (1.0–1.6 mmol/l) exhibited a higher cardiovascular risk than non-carriers matched for gender and HDL-C levels [216]. On the other hand, males with considerably elevated HDL-C levels (>1.6 mmol/l), regardless of their CETP gene status, had a low frequency of cardiovascular disease (coronary heart disease) [217]. Furthermore, analysis of cardiovascular outcomes in a Japanese cohort of 19,044 males and 29,487 females showed that subjects with both markedly elevated and mild-to-moderate HDL-C levels experienced fewer cardiovascular diseases independently of the status of the CETP gene mutation carrier. that according to Japanese epidemiological data, 27.6% of Japanese subjects with HDL-C > 60 mg/dl

and 31.4%–32.5% of those with HDL-C > 80 mg/dl are carriers of CETP gene mutations [215]. The lack of CETP activity is responsible for the accumulation of CE in HDL-Cs that becomes larger (>11 nm); on the other hand, LDL-C levels tend to be low, and LDL-C particles are rich in triglycerides and polydisperse with a subpopulation of small LDL-Cs [218].

The evidence of an inverse association between CETP and HDL-C activity, along with a direct association between HDL-C deficiency and increased susceptibility to atherosclerosis, In the direct pathway, HDL-C binds to liver receptors (SR-BI), and fat enters the liver cells [219]. Through participation in the RCT route, cholesterol homeostasis in the body is maintained by cholesteryl ester transfer protein (CETP). As a result of this transfer, APO-B-containing particles that are atherogenic increase and HDL-C levels decrease. In some studies, there have been reports of declining levels of high-density lipoprotein cholesterol (HDL-C) and a raised risk of CAD caused by the elevated activity of the CETP [113]. This supports the findings of our study

In addition, there is an additional hypothesis (but not alternative) that high CETP concentrations may promote atherogenesis by impairing other HDL-C beneficial functions (e.g., antioxidant and anti-inflammatory) [220].

Colombo, G.I., *et al*., found the reduction in CETP activity and the increase in HDL-C levels induced by using CETP inhibitors are paralleled by a significant increase in the CETP concentration. Such an increased CETP concentration may have a potential pro-atherogenic effect outweighing the atheroprotective function of high HDL-C levels [221]. Therefore, reducing both the CETP concentration and activity could be a promising anti-atherosclerotic strategy, as has been reported for CETP antisense oligonucleotide inhibitors, Raising HDL-C using cholesteryl ester transfer

protein (CETP) inhibitors failed to show a clinically relevant risk reduction of cardiovascular disease in clinical trials, inviting reconsideration of the role of CETP and HDL-C in human physiology [222].

Even though CETP deficiency is associated with high HDL-C and decreased LDL-C, its role in atherosclerotic cardiovascular diseases has been controversial [223].

Conclusions and Recommendations

Conclusions

1. Elevated MIA3 Levels: Serum MIA3 is identified as an independent diagnostic marker for ACS, due to its role in promoting monocyte migration and smooth muscle cell proliferation.
2. Diagnostic Marker E-selectin: Elevated E-selectin serves as a diagnostic marker for activated endothelial, which is crucial in the initiation and progression of atherosclerosis.
3. CETP as a Predictive Indicator: High levels of serum CETP in the stable angina group can predict the risk of developing CAD by impacting HDL-C concentrations and increasing LDL-C and VLDL-C levels.

Recommendations

The present study has some recommendations:

1. It's necessary to study serum CETP activity, which is a more reliable measure of CE transport than the CETP concentration.
2. It is recommended to study MIA3 and CETP gene variants, which might have compromised the possibility of better evaluating the relationship between them and atherosclerosis.
3. It is not excluded that there is a residual confounding effect due to additional variables (e.g., socio-environmental and behavioral factors) beyond those included in the multivariable analyses cannot be excluded.
4. It's necessary to investigate serum MIA3 and E-selectin with diabetic complications, renal diseases, cancer, and other metabolic diseases.
5. Further studies are needed to answer whether an increase in plasma HDL-C levels following a reduction in CETP mass and activity could prevent atherosclerosis progression and related cardiovascular events.

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Appendixes

University of Kerbala – College of Medicine

Department of Chemistry and Biochemistry

Student Name: **Saif Saihood Hateb**

Supervisors:

Professor Dr. Fadhil Jawad Al-Tu'ma - College of Medicine – University of Kerbala

Professor Dr. Hanaa Adai Al-Sultani – Faculty of Science – University of Kufa

((Experimental Data))

Assessing the Levels of Melanoma Inhibitory Activity Protein 3, E-Selectin, and Cholesteryl Ester Transfer Protein in Patients with Coronary Artery Diseases
Sample NO
Inclusion Criteria: Stable angina, Acute Coronary Syndrome
Exclusion Criteria: Diabetes Mellitus, Advanced liver disease, End-stage renal disease (ESRD), Acute Heart Failure, STEMI received thrombolytic
Risk Factors of Coronary Artery Disease
Age:
sex:
Family History:

Hypertension	**Yes**	**No**
hypercholesterolemia	**Yes**	**No**
Sedentary Life Style	**Yes**	**No**
<u>Investigation</u>		
Coronary Angiogram:		
CCTA:		
ECHO:		
Melanoma Inhibitory Activity Protein 3 (MIA3):		
E-Selectin:		
Cholesteryl Ester Transfer Protein (CETP):		
<u>Lipid Profile test</u>		
Total Cholesterol:		
Triglycerides:		
HDL-Cholesterol:		
LDL-Cholesterol:		
VLDL-Cholesterol:		
Height:	**Weight:**	
BMI:		
Waist:		

الملخص

مرض الشريان التاجي: هو من الامراض القلبية الوعائية الشائعة لدى كبار السن، ويحدث في المقام الأول بسبب تصلب الشرايين التاجية. ويمكن أن يؤدي إلى مضاعفات مثل انسداد الشرايين، وتضيقها، ونقص التروية. وتشمل الأعراض الذبحة الصدرية، التي تتميز بعدم الراحة في الصدر. وتصنف متلازمة الشريان التاجي الحادة إلى احتشاء عضلة القلب مع ارتفاع القطعةST ، واحتشاء عضلة القلب بدون ارتفاع القطعةST ، والذبحة الصدرية غير المستقرة. والالتهاب هو عامل رئيسي في مضاعفات مرض الشريان التاجي.

قامت هذه الدراسة بتقييم ارتباط العلامات الكيميائية الحيوية، مثل نشاط تثبيط الورم الميلانيني 3 (MIA3)، وE-selectin، وبروتين نقل إستر الكوليسترول(CETP) ، كعلامات حيوية مستقلة جديدة مع مرض الشريان التاجي. أجريت دراسة مقارنة بين المرضى والاصحاء على 180 شخصًا بالغا من الرجال تتراوح أعمارهم بين 41 و70 عامًا، مقسمين إلى ثلاث مجاميع: الذبحة الصدرية المستقرة (60) مريضا، متلازمة الشريان التاجي الحادة (60) مريضا، ومجموعة السيطرة الصحية (60) شخصا. تم قياس مستويات المؤشرات الحيوية في المصل باستخدام تقنيةELISA ، وتم قياس مستويات الدهون في مصل الإنسان باستخدام محلل كيميائي.SMART-120

أظهرت النتائج أن مستويات MIA3 و E-selectinو CETP زادت بشكل ملحوظ في حالات متلازمة الشريان التاجي الحادة والذبحة الصدرية المستقرة على حد سواء مقارنة بمجموعة السيطرة الصحية. تم العثور على ارتباط إيجابي وهام بين MIA3 ومؤشر كتلة الجسم و TCوLDL-C، وكان لـ E-selectin ارتباط سلبي وهام بمحيط الخصر، وكان لـ CETP ارتباط إيجابي وهام بالعمر وVLDL-C، وكان لـ MIA3 و CETPو E-selectinارتباط سلبي كبير بـHDL-C.

وفي الختام، تعتبر المستويات المرتفعة من MIA3 في المصل علامة تشخيصية مستقلة للذبحة الصدرية المستقرة ومتلازمة الشريان التاجي الحادة بسبب دورها في تعزيز هجرة الخلايا الوحيدة عبر الطبقة البطانية وانتشار الخلايا العضلية الملساء. يعد E-selectin علامة تشخيصية للبطانة النشطة، وهو أمر بالغ الأهمية في بدء وتطور تصلب الشرايين. يمكن أن تكون المستويات المرتفعة من CETP في المصل مؤشرا تنبؤيا لخطر الإصابة بأمراض الشريان التاجي بسبب تأثيرها على تقليل تركيزات HDL-C وزيادة مستويات LDL-C و VLDL-C، مما يتنبأ بخطر الإصابة بأمراض الشريان التاجي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء - كلية الطب

فرع الكيمياء والكيمياء الحياتية

تقييم مستويات بروتين النشاط المثبط للورم الميلانيني 3، ي-سيليكتين وبروتين نقل الكولستريل استر لدى المرضى المصابين بأمراض الشريان التاجي

رسالة ماجستير

مقدمة إلى مجلس كلية الطب/ فرع الكيمياء والكيمياء الحياتية / جامعة كربلاء كجزء من

متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل:

سيف صيهود حطيب الباوي

بكالوريوس علوم الكيمياء -كلية العلوم– جامعة بغداد / (2017 – 2018)

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كلية الطب – جامعة كربلاء

أ. د. هناء عداي علي السلطاني

كلية العلوم – جامعة الكوفة

حزيران 2024 ميلادية

ذو الحجة 1445 هجرية