Ministry of Higher Education and Scientific Research

University of Babylon

College of Medicine

Chemstiry and Biochemstiry Department

Evaluation of Apelin, Leptin , Homocysteine and Selenium in Sera of Patients with Acute Myocardial Infarction in Babylon Governorate

A Thesis

Submitted to the Council of College of Medicine, University of Babylon in Partial Fulfillment of the Requirements for The Degree of Doctor Philosophy in Siences /Clinical Biochemistry

By

Ahmed Faesel Falih Mahdi

B.Sc in Pathological Analysis Techniques

(Faculty of Health and Medical Technology/Baghadad 2008)

M.Sc in Clinical Biochemstry

(University of Babylon / College of Medicine 2018)

Supervised by

Professor

Dr. Mufeed J. Ewadh

Assistant Professor

Dr.Shokry Faaz Alsaad

2023 A.D. **1445 A.H.**

بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

وَقُل رَّبِّ

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الأية(٨٠)

Dedication

A special dedication to all the martyrs of

Iraq

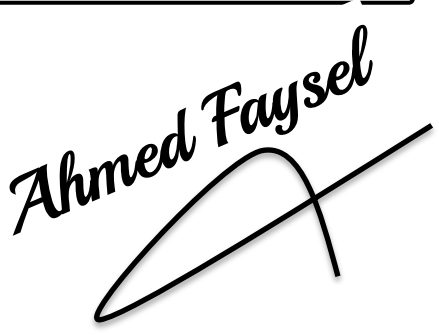
To ..... My idol , My companion in Journey of AL-Hajj.....My Father.

To ..... My two Paradises My two Mothers.

To .....My best Friend ,My Supporter, My Love, My Wife.

To ..... My three Roses My Children , Fatima , Mohamed and Rateel.

To .....My Brothers and Sister



Acknowledgments

Thanks to **Allah** so much for grace and great kindness, who gave me strength and conciliation in order to complete my study and works to the fullest, all of the praise and thanks to **Allah** for all the best blessing bestowed for us.

Firstly, I would like to express my deepest gratitude and extend my sincere thanks with all respect to my supervisors, **Prof. Dr. Mufeed Jalil Ewadh**, for his suggestion of the project, supervision, support, guidance and assistance throughout the work of this study. Also my great thanks extend to **Asst. Prof. Dr. Shokry Faaz Alsaad**, for his restless supervision, assistance and support. Special gratitude is also expressed to **Prof. Dr. Mohend A. AL-Shalah** Dean of College of Medicine, University of Babylon for his kind help. A great thanks goes to the Head of the Chemistry and Biochemistry Department, Professor **Dr. Abdulsamie H. Alta'ee**, for his great assistance and scientific advice

I'd like also to thank all the professors of Biochemistry Department at the College of Medicine, University of Babylon for providing valuable information for me. Thanks and gratitude to the College of Medicine / University of Babylon for providing laboratory instruments and requirements to complete this research.

I'd like also to thank the staff in Shahid al mihrab Center for catheterization and cardiac surgery and the staff at Murjan Teaching Hospital. And special thanks to all people participating in this study as patients and control, without them this study could not be done.

Special gratitude is also expressed to my best friends **Mr. Naser Zeyad** for help, assistance and support.

Supervisors Certification

We certify that this thesis entitled **"Evaluation of Apelin, Leptin , Homocysteine and Selenium in Sera of Patients with Acute Myocardial Infarction in Babylon Governorate "was** carried under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Doctor of Philosophy in Clinical Biochemistry.

Professor **Assistant Professor**

Dr. Mufeed J. Ewadh **Dr.Shokry Faaz Alsaad**

In view of the available recommendation, I forward the present thesis for debate by the examining committee.

Professor

Dr. Abdulsamie H. Alta'ee

Head of Chemistry and Biochemistry Department

College of medicine, Babylon University

Decision of Discussion Committee

We certify that we have read this thesis entitled **" Evaluation of Apelin, Leptin , Homocysteine and Selenium in Sera of Patients with Acute Myocardial Infarction in Babylon Governorate "** and as examining committee examined the student **" Ahmed Faesel Falih Mahdi"** in its content and in our opinion it is adequate with **"Excellent"** rating as a thesis for the degree of Doctor of Philosophy in Science of Clinical Biochemistry.

Professor
Dr. Abdulsamie H. Alta'ee
College of Medicine/University of Babylon
(Chairman)

Prof. Dr.
Hassan Salim Aljumaily
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Rana Abd Al-Aly Khamees
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(Member)

Prof. Dr.
Mufeed Jalil Ewadh
College of Medicine/University of Babylon
(Member and Supervisor)

Assist. Prof. Dr.
Dr.Shokry Faaz Alsaad
College of Medicine/University of Babylon
(Member and Supervisor)

Approved for the College Council

Prof. Dr.
Prof. Dr. Mohend A. AL-Shalah
Dean of College of Medicine/University of Babylon

Summary

Acute myocardial infarction (AMI) is characterized by sudden myocardial tissue death resulting from an imbalance between coronary blood supply and myocardial demand. Typically caused by an occlusive thrombus formation in a ruptured or eroded coronary artery, it triggers an inflammatory response, eventually replaced by granulation tissue and a collagen-based scar.

This case control study was done at the laboratory of Chemistry and Biochemistry Department, College of Medicine, University of Babylon during the period from February 2022 until May 2023. The study aim to determine the rule and diagnostic value of apelin, leptin, homocysteine and selenium among AMI patients. The entire samples collected from patients attending to Marjan Medical City at Al-Hillah city. This study included 120 Participants (48 females, 72 males), who were divided in to three groups, the first group included 27 patients (7 females, 20 males) with mean age (63.3 ±10.5) diagnosed as STEMI, the second group included 33 patients (11 female, 22 males) with mean age (59.5 ±8.0) diagnosed as NSTEMI. And the third group included 60 apparently healthy participant (30 females and 30 males) with mean age (62.4 ±9.4) as control group.

The study concludes that a number of risk factors are associated with AMI, where at the forefront of these factors was family history with an odds ratio (27.8), followed by hypertension with an odds ratio (27.3). Also the results of the current study showed that the combination of multiple risk factors could double the risk of AMI, as well as may contribute to increase the size of the infarction which leads to worse prognosis compared to AMI patients with single risk factor.

The results of the present study were shown a significant increase in the mean levels of apelin, leptin and homocysteine in AMI patients both groups (STEMI and NSTEMI) as compared to the control group, and there was also a clear discrepancy in levels when compare STEMI group with non NSTEMI.

The results of the present study also shown a significant decrease in the mean levels of selenium in AMI patients both groups (STEMI and NSTEMI) in comparison to the control group, with no significant different between the levels of selenium in STEMI group and NSTEMI group.

In this study, there was a significant positive correlation between leptin, homocysteine and apelin with cardiac troponin I in AMI patients in both groups. Also, there was a significant negative correlation between selenium with cardiac troponin I in acute myocardial infraction patients in both groups.

The study concludes that patients with AMI in Babylon Province have high levels of homocysteine, leptin and apelin with higher levels in STEMI group than NSTEMI. This pattern of results could indicate a role for these markers in the formation of thrombosis, atherosclerosis, oxidative stress and the occurrence and prognosis of AMI. Also the study concludes that patients with AMI in Babylon Province have low levels of selenium compared to control group. Which may serve as a biomarker in monitoring the antioxidant status in AMI patient.

In summary, apelin, leptin, homocysteine, and selenium exhibit potential as standard panels for patients suspected of experiencing AMI, serving as confirmatory and monitoring biomarkers. Moreover, the utilization of apelin within medical facilities may serve as a diagnostic biomarker, aiding in categorizing the type of AMI when an electrocardiogram cannot be readily performed.

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List of Abbreviations

Abbreviation	Details
ACS	Acute coronary syndrome
AMI	Acute myocardial infraction
ATP	Adenosine Triphosphate
APLN	Apelin
APJ	Apelin Receptor
AST	Aspartate aminotransferase
BP	Blood Pressure
BBB	Blood–Brain barrier
BMI	Body mass index
Ca2+	Calcium
CNS	Central Nervous System
CAD	Coronary Artery Disease
CRP	C-reactive protein
CK-MB	Creatine Kinase-Myoglobin
cAMP	Cyclic Adenosine Monophosphate
cGMP	cyclic Guanosine Monophosphate
CBS	Cystathionine Synthase
DM	Diabetes Mellitus
DCM	Diabetic cardiomyopathy
DAG	Diacyl Glycerol
ECG	Electrocardiogram
ER	Endoplasmic Reticulum
eNOS	endothelial Nitric Oxide Synthase
ELISA	Enzyme-Linked Immunosorbent Assay

List of Abbreviations

EMT	Epithelial-to-Mesenchymal Transition
EFSA	European Food Safety Authority
FGF-23	Fibroblast Growth Factor 23
GRACE	Global Registry of Acute Coronary Syndrome
GLUT	Glucose Transporter
GPCR	G-protein coupled receptors
GFAAS	Graphite Furnace Atomic absorption spectrophotometer
GTPases	Guanosine Triphosphate Hydrolase
HDL	High-Density Lipoprotein
HDF	High-Fat Diet
Hyc	Homocysteine
HRP	Horseradish Peroxidase
HTN	Hypertension
HHyc	Hyperhomocysteinemia
HIF-1	Hypoxia-Inducible Factor-1
Ins(1,4,5)P3	Inositol 1 4 5-Triphosphate
IFNγ	Interferon Gamma
IL	Interleukin
IBM	International Business Machines
JAK-STAT	Janus kinases signal transducer and activator of transcription proteins
LDH	Lactate dehydrogenase isoenzymes
LV	Left ventricular
LEP	Leptin
LR	Leptin Receptor Gene
mRNA	messenger Ribonucleic acid

List of Abbreviations

MTHFR	Methylenetetrahydrofolate Reductase
CMIA	Monoclonal Chemiluminescent Microparticle Immunoassay
NK	Natural Killer Cells
NO	Nitric Oxide
NSTEMI	non-ST-elevation myocardial infarction
O2	Oxygen
PCI	Percutaneous Coronary Intervention
PPAR	Peroxisome Proliferator-Activated Receptors
PI3K-Akt-mTOR	phosphatidylinositol-3-kinase, mammalian target of rapamycin
PLC	Phospholipase C
PKA	Protein Kinase A
PKC	Protein Kinase C
PLP	Pyridoxal-Phosphate
ROS	Reactive Oxygen Species
RDA	Recommended Dietary Allowance
RLUs	Relative Light Units
RAAS	Renin Angiotensin Aldosterone System
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
Se	Selenium
Sldl	small Low-Density Lipoprotein
SD	Standard Deviation
STEMI	ST-elevation myocardial infarction
SNS	Sympathetic Nervous System

List of Abbreviations

TMB	Tetramethylbenzidine
Th1	T-helper1
TIMI	Thrombolysis in Myocardial Infarction
Tn	Troponin
TNF	Tumor Necrosis Factor
USA	United States of America
β3-AR	β3 Adrenergic Receptors

CHAPTER ONE

Introduction And Literature Review

1. Introduction

1.1 Acute myocardial infarction

1.1.1. Definition

Acute myocardial infarction (AMI) is a clinical problem defined as acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand [1]. It is almost always due to the formation of occlusive thrombus at the site of rupture or erosion of an atheromatous plague in coronary artery causing heart cell to die and without treatment the infarct related artery remains permanently occluded in 30% of patients [2]. Tissue damage suffered during AMI elicits an inflammatory reaction that leads to the necrotic area being replaced with granulation tissue and eventually a collagen-rich scar [3]. As shown in figure 1-1.

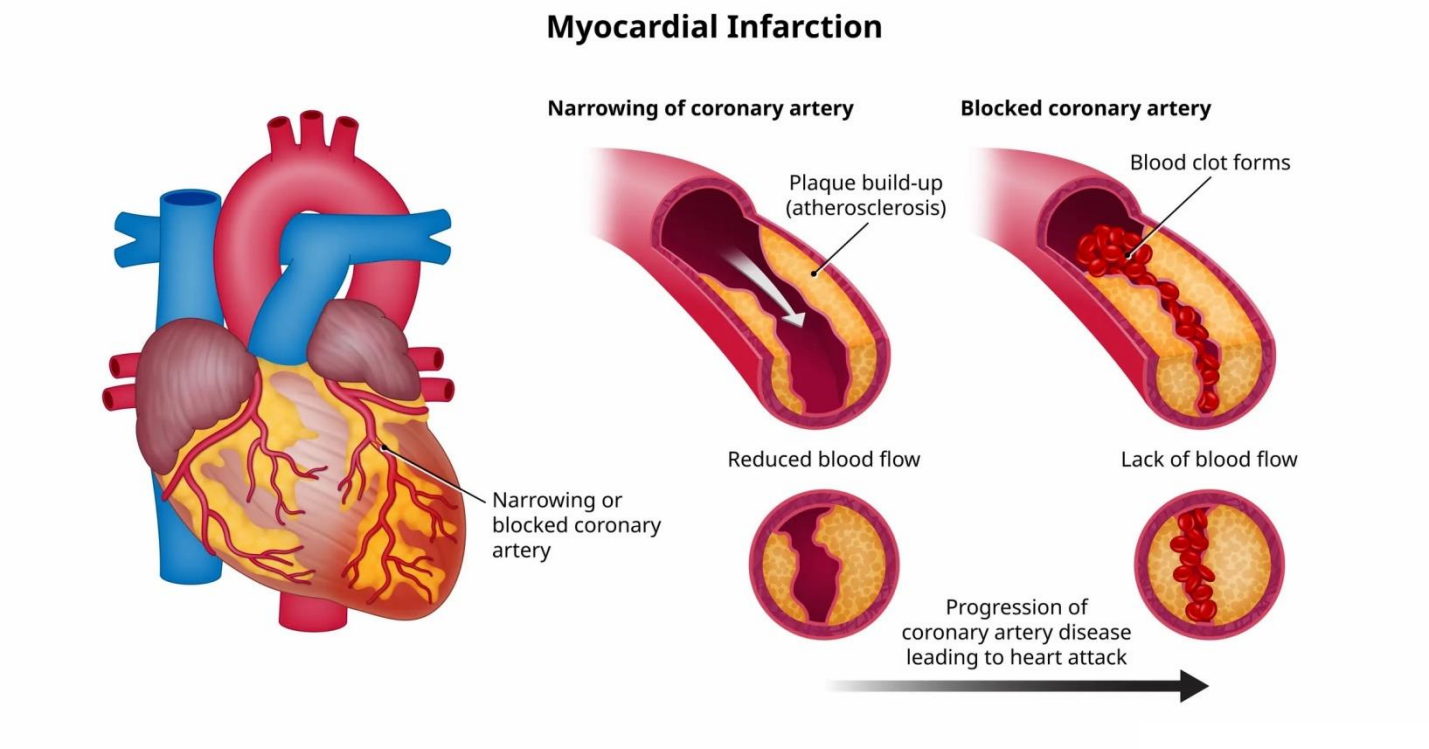

Figure 1-1: Demonstration of Myocardial Infraction [4]

1.1.2 Classification of Acute Myocardial Infarction

According to consensus document of the American faculty of cardiology and the America heart society in 2018 (Fourth Universal Definition of Myocardial Infarction) AMI can be classified into five main types: [5, 6, 7],

Type 1: Spontaneous AMI caused by ischemia owing to a primary coronary event (eg, plaque rupture, erosion, or fissuring; coronary dissection)

Type 2: Ischemia due to increased oxygen demand (eg, hypertension), or decreased supply (eg, coronary artery spasm or embolism, arrhythmia, hypotension)

Type 3: Related to sudden unexpected cardiac death. Patients who suffer cardiac death, with symptoms suggestive of myocardial ischemia accompanied by presumed new ischemic electrocardiogram ECG changes or ventricular fibrillation

Type 4a: Associated with percutaneous coronary intervention

Type 4b: Associated with documented stent thrombosis as detected by coronary angiography or autopsy in the setting of myocardial ischemia in combination with a rise and/or fall of cardiac biomarkers.

Type 5: Associated with coronary artery bypass grafting

1.1.3 Epidemiology

Acute myocardial infarction is a challenging clinical and public health problem among older adults in the world. AMI is one of the most important risk factors for heart failure which contributes substantially to the geographic disparities in life expectancy [8]. AMI occurs once every 40 seconds in the world with an estimated annual incidence of 6,000,000 new cases and 400,000 recurrent cases [9] with a prevalence of 2.8% for Iraq [10].

AMI is more prevalent among older adults with an average age of the first AMI being 65.6 years for males and 72.0 years for females. The incidence of AMI is decreased in the industrialized nations partly because of improved health systems and implementation of effective public health strategies, nevertheless the rates are surging in the developing countries such as South Asia, parts of Latin America, and Eastern Europe [11]

Mortality rates for myocardial infarction are dropping in North America and many North and West European countries, while in Central and East Europe these rates are increasing. It is estimated that around two thirds of the myocardial infarction mortality rates decline in developed countries are due to reduced exposure to risk factors, while the last third is the result of adequate treatment and improved survival [12].

1.1.4 Pathophysiology

Acute myocardial Infarction can be defined as the necrosis of cardiac cells due to reduced oxygen (O_2) supply as a consequence of the occlusion of coronary arteries. Cardiac energy synthesis relies mostly on oxidative metabolism and is therefore highly sensitive to changes in the intracellular O_2 levels. Coronary artery occlusion reduces O_2 concentration and upregulates anaerobic-dependent ATP synthesis, leading to energy starvation and cardiac cell necrosis [13] as shown in figure 1-2.

The general belief is that the stenosing atherosclerotic plaque is the major ischemic cause. These may result in myocardial ischemia or infarction via different pathophysiological mechanisms [14].

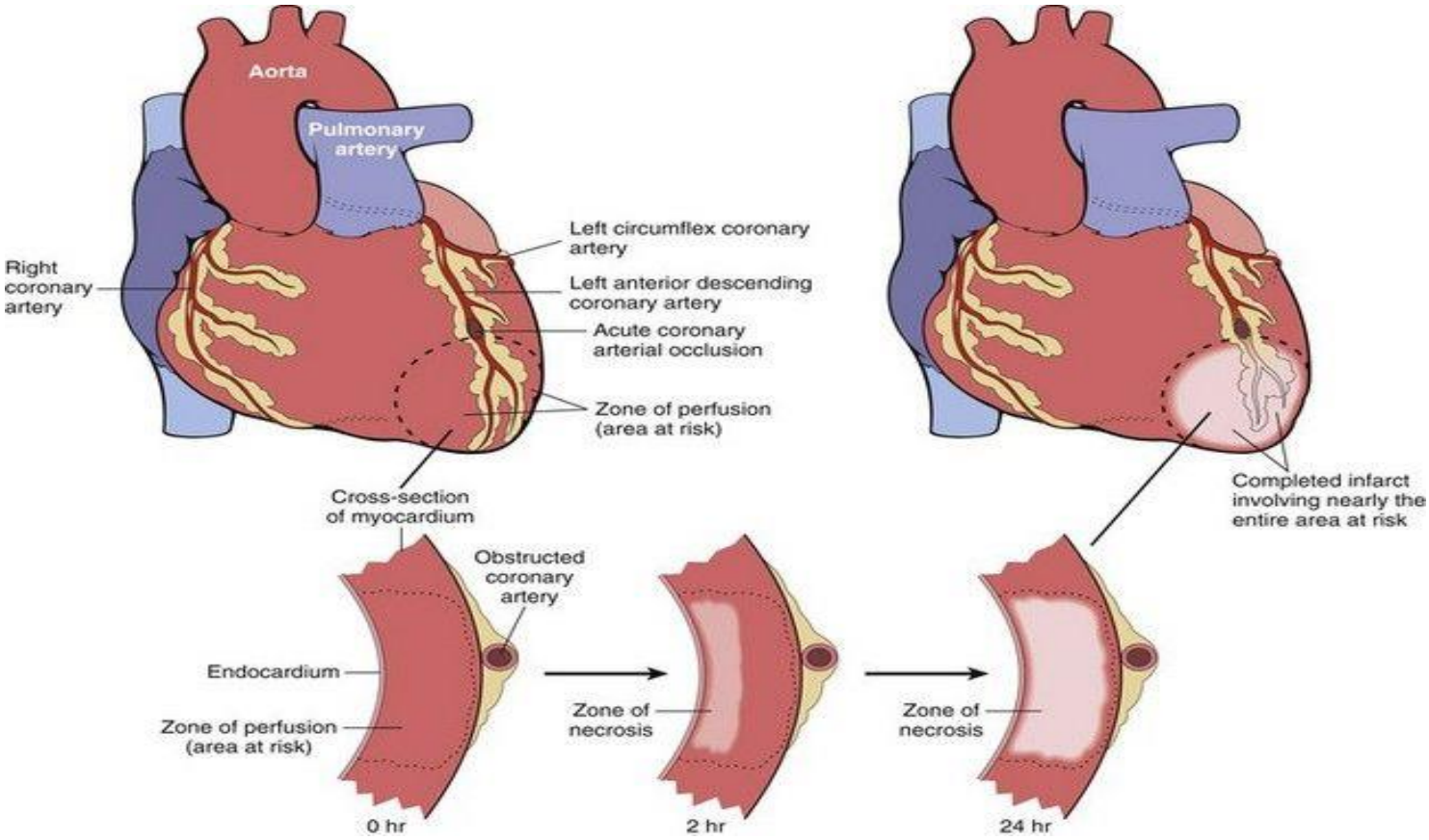

Figure 1-2: Progression of myocardial necrosis after coronary artery occlusion

This combination implies a high wall stress in the fibrous cap, which is conducive to plaque rupture [16], the thrombotic environment, plaque volume and composition, the degree of luminal narrowing, quality of the fibrous cap, and the extent of fibrous cap rupture will collectively determine AMI presentation [17]. In addition to plugging the microvessels, neutrophils could also cause cellular injury and endothelial dysfunction by generating oxygen-derived free radicals. Due to the negligible regeneration capacity of the myocardium, the infarcted area heals by scar formation, and often, the heart is remodeled characterized by dilation, hypertrophy of remaining viable tissue, and cardiac dysfunction [18].

1.1.5 Risk Factors for Acute Myocardial Infarction

There are various risk factors of AMI. Among them, some are modifiable (treatable) and others are non-modifiable (cannot be changed). The major risk factors of AMI are:

a- Physical activity :

Inactive people with multiple cardiac risk factors are more likely to develop AMI. Physical activity may contribute up to 20%-30% reduced risk of AMI [19]. Different types of physical activities may have different effects on the risk of AMI and may interact together. For example, some leisure time activities such as walking, stair climbing, and cycling provide protection against AMI. Whereas others, such as intensive domestic physical activity, may not offer protection against AMI [20].

b- Smoking

Smoking is considered to be strong risk factor of AMI, premature atherosclerosis and sudden cardiac death. Tobacco smoke increases the risk of AMI through many mechanisms. It damages blood vessels, increases the risk of plaques, increases the risk of clots at the site of plaques and reduces the blood's oxygen levels [21]. Smoking, mainly through its nicotine content, activates the sympathetic nervous system (SNS), increasing both heart rate and systolic blood pressure [22]. This increase in the rate-pressure product results in increased myocardial oxygen demands. Increased in activity of SNS also leads to coronary arterial vasoconstriction. In addition to increasing myocardial oxygen demand and reducing coronary blood flow, cigarette smoking also causes increase in the levels of carboxyhemoglobin in the blood, with the potential to further reduce myocardial oxygen delivery from oxyhemoglobin [23].

c- Alcohol Consumption

Alcohol consumption is associated with an acutely higher risk of myocardial infarction in the subsequent hour among people who do not typically drink alcohol daily [24]. Several mechanisms may underlie alcohol's effects on increased risk for AMI. These include impairments in cells that lead to buildup of plaque in arteries (i.e., through alterations in endothelial cell function and nitric oxide availability), and disruptions in arterial-vascular function (i.e., through myogenic mechanisms and changes in baroreceptor function), [25] and hormonal imbalances that control the body's fluid and blood pressure (BP) regulation through the renin–angiotensin–aldosterone system (RAAS) resulting in damaging cardiac muscle cells and cardiovascular system [26].

d- Diabetes Mellitus

It is a chronic condition that occurs when the body cannot produce enough or effectively use of insulin, and are induced by a genetic predisposition coupled with environmental factors, the vascular manifestations associated with diabetes mellitus result from the dysfunction of several vascular physiological components, mainly involving the endothelium, vascular smooth muscle and platelets [27].

Diabetic cardiomyopathy (DCM) is defined as myocardial dysfunction independent of coronary artery disease and hypertension that can lead to heart failure. In its early stages, diabetic cardiomyopathy includes a hidden subclinical period characterized by structural and functional abnormalities, including left ventricular (LV) hypertrophy, fibrosis, and cell signaling abnormalities [28].These pathophysiological changes of cardiac fibrosis and stiffness and associated subclinical diastolic dysfunction often evolve to heart failure with normal ejection fraction and eventual systolic dysfunction accompanied by heart failure with reduced ejection fraction [29].

e- Dyslipidemia

Dyslipidemia, a major risk factor of cardiovascular disease, increased triglyceride levels and dense, small low-density lipoprotein (sLDL) particles act as predisposing risk factors for myocardial infarction. [30]. Also that decreased high-density lipoprotein HDL-C levels and increased triglyceride levels cause metabolic perturbations and thus causing adverse consequences concluded that there is a high frequency of dyslipidemia in young patients presenting with acute myocardial infarction, with descending order hypertriglyceridemia followed by hypercholesterolemia, raised LDLc and low HDLc [31].

f- Hypertension

Both systolic and diastolic hypertension increase the risk of a myocardial infarction and the higher the pressure, the greater the risk. It is major risk factor of causing atherosclerosis in coronary blood vessels, result in heart attack or myocardial infarction [32]. Hypertension and myocardial infarction are closely linked. Hypertension induces endothelial dysfunction by reducing nitric oxide NO. Hypertension was associated with an increased risk for AMI in both men and women [33].

g- Sex

Men tend to have heart attacks earlier in life than women. Women's rate of heart attack increases after menopause but does not equal men's rate [34]. This difference is due to lack or a very low amount of estrogen and particularly 17β-estradiol (E2) in males in comparison to females in premenopausal period. This hormone in addition to its role in sexual development and reproduction is implicated in a large number of physiological processes, particularly in the cardiovascular system [35]. Female patients presenting with AMI are often older, have higher rates of diabetes mellitus, hypertension, and autoimmune disorders, Men with AMI often have higher rates of smoking, peripheral vascular disease ,However, women are less likely to survive a heart attack and more likely to have a second heart attack [36].

h- Family History

Family history of myocardial infarction is an independent risk factor for AMI. Several genetic variants are associated with increased risk of AMI and family history of AMI in a first-degree relative. A past history of cardiovascular disease is an extremely important part of the patient's evaluation and should not be dismissed as "noncontributory [37]. Several genes encoding risk factors for AMI are shown to be related to heart disease and may potentially explain the increased risk of AMI in subjects with family history [38].

1.1.6. Signs and Symptoms

The symptoms of AMI are chest pain, which travels to the left arm or left side of the neck, shortness of breath, sweating, nausea, vomiting, abnormal heart beating, anxiety, fatigue, and other factors. Not a small percentage of people who have AMI do not experience chest pain which is called ‘‘silent’’ AMI [39]. The warning signs of heart attack are in addition to chest pain as fallow: high blood pressure, tightness of chest, squeezing, burning sensations, aching, and heaviness in the chest for more than 10 min, pain in left shoulder or left arm, up into the neck or along the jaw line, shortness of breath, profuse sweating and dizziness, muscles weakness, nausea or vomiting, anxiety or stress, feeling of impending doom and depression. But there are no symptoms for a silent heart attack [40]. Women with an AMI present with chest pain in fact more likely to present with atypical symptoms such as fatigue, sleep disturbance, shortness of breath, back pain, upper abdominal or epigastric pain, and nausea with or without vomiting rather than simply present with chest pain. Some women who have had a heart attack report that their symptoms felt like the symptoms of the flu [41].

1.1.7. Diagnosis of Acute Myocardial Infarction

1.1.7.1 Electrocardiogram (ECG)

Electrocardiogram is a crucial tool in the identification and management of acute myocardial infarction. The electrocardiogram is also crucial for identifying new conduction abnormalities and arrhythmias that influence both short- and long-term outcome, the overall goal of performing an ECG is to obtain information about the electrical functioning of the heart. [42].

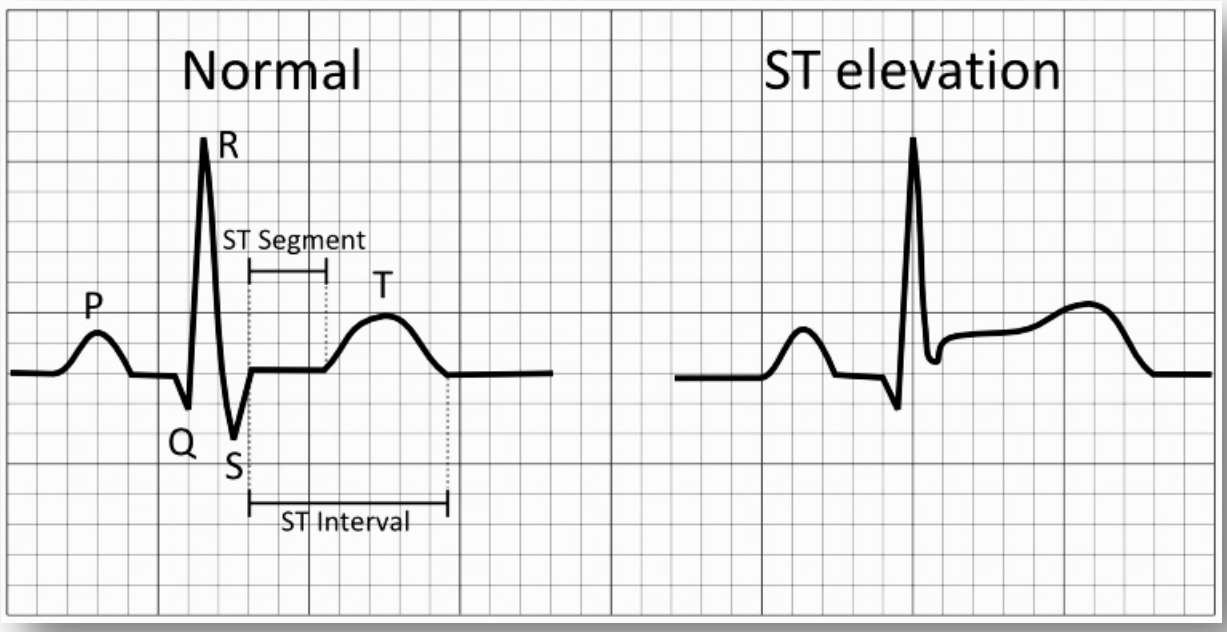

Figure 1-3: Demonstrating of normal vs STEMI heart ECG [42].

AMI can be further divided by ECG into two classes: ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) [43] as shown in figure 1-3.

STEMI is defined as an acute coronary thrombosis or persistent ST-segment elevation of ≥1 mm in ≥2 contiguous electrocardiographic leads. NSTEMI is defined as ischemic symptoms at rest from an acute coronary plaque rupture or erosion, lasting ≥10 minutes, occurring within 24 hours before hospital admission, and displaying either elevated cardiac biomarkers within 24 hours after initial presentation [44].

The most frequently used electrocardiographic criterion for identifying acute myocardial infarction is ST segment elevation in two or more anatomically contiguous leads. The ST segment elevation associated with an evolving myocardial infarction is often readily identifiable, but knowledge of the common "pseudo" infarct patterns is essential to avoid the unnecessary use of thrombolytic treatment [45].

In the early stages of acute myocardial infarction the electrocardiogram may be normal or near normal; less than half of patients with acute myocardial infarction have clear diagnostic changes on their first trace. About 10% of patients with a proved acute myocardial infarction (on the basis of clinical history and enzymatic markers) fail to develop ST segment elevation or depression[46]. In most cases, however, serial electrocardiograms show evolving changes that tend to follow well recognized patterns. The earliest signs of acute myocardial infarction are subtle and include increased T wave amplitude over the affected area. T waves become more prominent, symmetrical, and pointed (hyperacute) as shown in figure 1-4. These changes in T waves are usually present for only five to 30 minutes after the onset of the infarction and are followed by ST segment changes. [47].

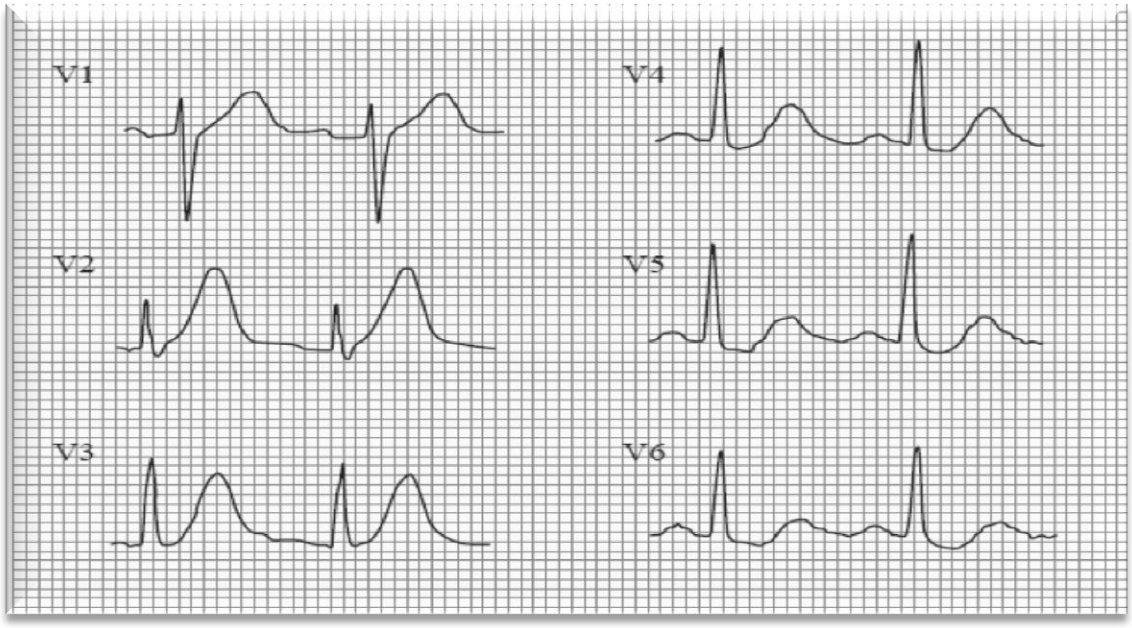

Figure 1-4: Hypercut T wave [48].

As the acute myocardial infarction evolves, changes to the QRS complex include loss of R wave height and the development of pathological Q waves as shown in figure 1-5 [48]. The Q waves are the only firm electrocardiographic evidence of myocardial necrosis. Q waves may develop within one to two hours of the onset of symptoms of acute myocardial infarction, though often they take 12 hours and occasionally up to 24 hours to appear [49,50].

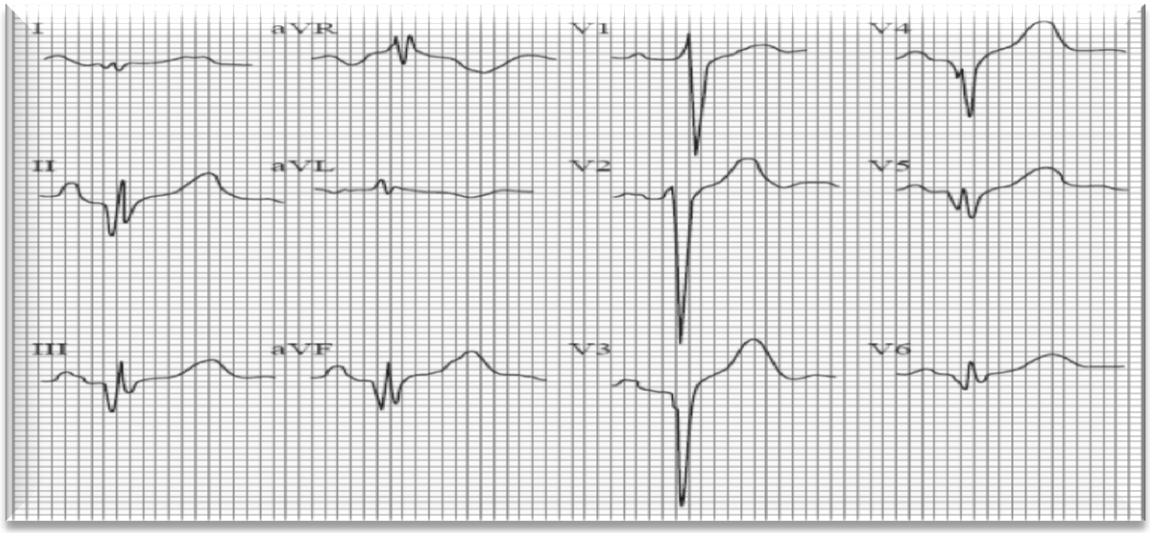

Figure 1-5: Pathological Q wave [48].

1.1.7.2 Cardiac Markers

Blood testing for biomarkers of myocardial injury plays an increasingly important role for the evaluation, diagnosis, and triage of patients with chest pain [51]. The guidelines for the diagnosis of AMI have recently changed and prominently incorporate the results of cardiac marker testing in the clinical definition of AMI. A biomarker is "a characteristic that is objectively measured and quantified as an indicator of normal biological processes, pathogenic processes or pharmacological response to a therapeutic intervention [52].

Several cardiac markers (myocardial enzymes, several myocardial proteins, peptides, and many other molecules) have been used in the diagnosis and management of AMI as shown in figure 1-6. However, a lack of sensitivity and specificity to cardiac muscle necrosis continues to be the need to look for newer specific molecules. Cardiac biomarkers are of great importance in the timely, accurate diagnosis and management of AMI as well as the prognosis. [53].

Lactate dehydrogenase isoenzymes (LDH) (EC 1.1.1.27) were used widely in the past for diagnosis of myocardial infarction, Usually LDH isoenzyme levels (LDH1) increase 24–72 hours following myocardial infarction and reach a peak concentration in 3–4 days. The levels remain elevated for 7 to 12 days, making it a late marker for myocardial infarction [54]. Moreover, LDH is a non-specific marker for myocardial infarction, and its concentration can be elevated in hemolytic anemia, stroke, pancreatitis, ischemic cardiomyopathy, and a variety of other diseases [55].

Aspartate aminotransferase (AST) (EC 2.6. 1.1) is one of the enzymes used in the diagnosis and follow-up of acute myocardial infarction. In acute myocardial infarction, AST start raising 6 to 8 hours after the symptom onset, reaches the peak level at 24 to 36 hours and returns to normal in 3 to 7 days, Reperfusion by thrombolysis or balloon angioplasty shortens the time to AST peak value. The widespread distribution of AST across human tissues (particularly in liver and skeletal muscle) and relatively late increase in serum activity following coronary occlusion are disadvantages of this test with respect to the diagnosis of AMI [56].

Creatine Kinase (CK-MB) (EC 2.7.3.2) It is considered one of the most important biomarkers used in the diagnosis of myocardial infarction, which step down other biomarkers from the competition in the field of laboratory diagnosis The content of CK-MB relative to total CK in myocardial cells is variable; it is low in normal myocardium and increased several-fold in hypoxic myocardium. Elevated serum levels of CK–MB are therefore specific for myocardial cellular injury, but not for acute myocardial infarction. Following onset of symptoms of myocardial infarction CK–MB increase in serum within 3 to 6 hours; the peak levels occur between 16 and 30 hours [57]. CK–MB returns to normal by 24 to 36 hours. This "window" dictates that CK–MB must be determined as soon as possible after the onset of symptoms, and repeated several times in the first 48 hours. Determination of CK–MB isoenzyme has a 98% predictive value for myocardial necrosis with a positive enzyme profile and a 100% negative predictive value for the absence of necrosis with a normal profile. Limitation of CK-MB is that it cannot detect minor myocardial damage, due to its high molecular weight. [58].

Myoglobin is a low molecular- weight haem-containing protein found in both skeletal and cardiac muscle. Because of its low molecular weight, it is rapidly released from the myocardium upon damage, and a typical rise occurs within 2–4 h after the onset of acute myocardial infarction [59]. This is useful for the early diagnosis of acute myocardial infarction, as this rise is generally earlier than that of the other currently used cardiac markers. Unfortunately, myoglobin is not cardiac specific, being also found in skeletal muscle, and thus is less useful in the diagnosis of acute myocardial infarction unless used in conjunction with other markers [60].

Troponins are regulatory proteins that assist in cardiac and skeletal muscle contraction [61]. Skeletal and cardiac troponin isoforms differ in structure, with the cardiac troponin complex comprised of troponin C (TnC), troponin I (TnI), and troponin T (TnT). Troponin C binds to calcium ions, TnI binds to actin and inhibits the interaction of actin and myosin, and TnT binds to tropomyosin and assists with contraction. Cardiac troponin I is an extremely sensitive biomarker for AMI and is the current biomarker of choice for the diagnosis, risk-stratification, and clinical management of AMI [62].

Troponin I and TnT appear in the plasma 4–8 h or earlier with high-sensitivity troponin assays after symptoms of acute myocardial infarction, and are best measured 12 h after the start of chest pain [63]. They are therefore not early markers of acute myocardial infarction, but they do stay elevated for about 7–10 days in plasma, which makes them useful in the late presentation of chest pain. Troponin T may be elevated in patients with chronic kidney disease, subarachnoid haemorrhage (due to vasoactive peptide release affecting the myocardium), hypertension, tachyarrhythmias, cardiac surgery, sepsis, congestive cardiac failure, pulmonary embolism and hypothyroidism [64].

Cardiac troponin measurements are particularly useful in excluding the diagnosis of myocardial damage, particularly after 12 h following chest pain or other symptoms, and in patients who are likely to have concurrent cardiac and skeletal muscle damage [65].

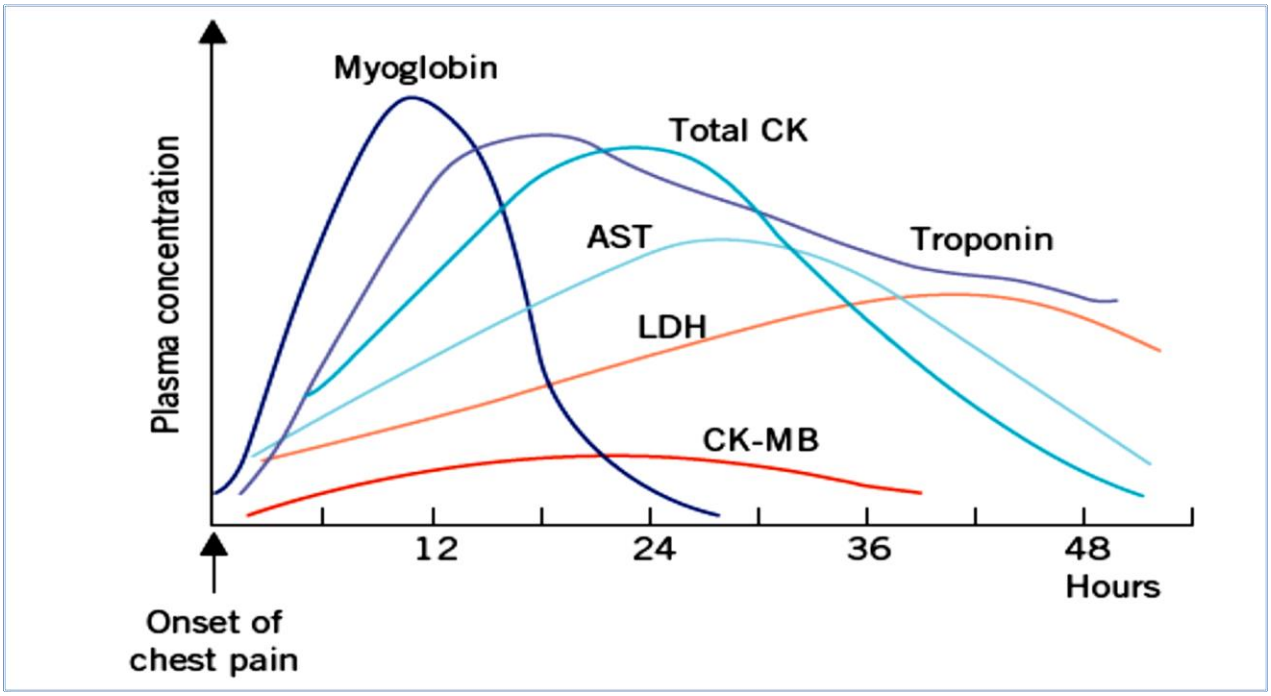

Figure 1-6: The Time Sequence of Changes in Plasma Cardiac Markers after Acute Myocardial Infarction [66].

1.1.8. Management of Acute Myocardial Infarction

Early risk stratification of patients with myocardial infarction allows for prognostication and triage via initiation of one of several vital treatment pathways. The Thrombolysis in Myocardial Infarction (TIMI) risk score is easiest to use, whereas the Global Registry of Acute Coronary Syndrome (GRACE) is more accurate, comprehensive, and applicable to both NSTEMI and STEMI [67].

In NSTEMI, antithrombotic therapy is thought to stabilize the vulnerable plaque and allow endogenous fibrinolysis to restore patency. The artery is usually patent but severely stenosed with a ruptured plaque [68].

The goal is to prevent progression of the thrombus to complete occlusion. Percutaneous coronary intervention (PCI) is usually pursued to improve blood flow and prevent recurrent ischemia. PCI should be done within 24 h of NSTEMI if possible. PCI could be done in low-risk patients up to 48−72 h without clinical consequence. However, doing PCI after 24 h has been associated with longer hospitalization [69].In STEMI, priority should be given to immediate reperfusion to limit infarct size, and antithrombotic therapy is used adjunctively. Patients usually have complete arterial occlusion, and as such reperfusion is needed to restore patency as quickly as possible (eg, within 60−90 min) [70]. Patients who undergo fibrinolysis often have residual stenosis, and a reduction in this stenosis with subsequent angioplasty or stenting, or both, improves perfusion and prevents acute re-occlusion [71].

1.2. Biochemical Parameters

1.2.1. Apelin

1.2.1.1 Definition

Apelin (APLN) is an endogenous peptide hormone composed of 35 amino acid and identified as a ligand of the G protein-coupled apelin receptor (APJ). Apelin belongs to the family of adipokines, which are bioactive mediators released by adipose tissue [72]. Apelin was identified in 1998 by Masahiko Fujino and his colleagues at Gunma University and Takeda Pharmaceutical Company [73].

Apelin and APJ are expressed in the central nervous system, particularly in the hypothalamus and in many peripheral tissues. Apelin has been shown to be involved in the regulation of cardiovascular and fluid homeostasis, food intake, cell proliferation, and angiogenesis [74].

1.2.1.2 Physiology of Apelin

The apelin gene produces a pre-proprotein (preproapelin) with 77 amino acids. After being translocated to the endoplasmic reticulum and the signal peptide being broken down to 36 amino acid and different smaller fragments. Several cell types in the body can synthesize apelin protein, for example central nervous system, pituitary gland, lungs, cardiac muscle, gastrointestinal tract and mammary glands, with lower values in kidneys and skeletal muscles. The highest apelin concentrations have been found in adipose tissue, gastric exocrine and endocrine cells [75]. APJ (apelin receptor) is a typical 380-amino-acid G protein-coupled receptor with 7 transmembrane domains showing close sequence homology to the angiotensin II receptor type 1 [76].

APJ receptor expression has been found in the pancreas (alpha and beta cell), stomach, intestine, liver, APJ is strongly expressed in the hepatocytes. Also, APJ is found in, cardiomyocytes and vascular endothelial and smooth muscle cells. In addition, apelin receptor mRNA is present in lung endothelial cells, kidneys and mammary gland, during pregnancy and lactation [77]. Apelin has a different functions that affect more than one axis within the body. To explain this concept, it must be taken into account that apelin binds to different subfamilies of G-protein coupled receptors which differ according to the mediator acting, and thus the effect of the hormone on the cellular level varies. For example of these subfamilies: Gαs, Gαi/o, Gαq/11, and Gα12/13 [78].

As shown in figure 1-7 each family, in turn, is associated with a hallmark cellular effect: adenylyl cyclase activation for Gαs; Adenylyl cyclase inhibition for Gαi/o; phospholipase C- activation and increased intracellular Calcium (Ca2+) for Gαq/11; and regulation of Rho guanosine triphosphate hydrolase (GTPases), which are known for their role in regulating the actin cytoskeleton, for Gα12/13 [79].

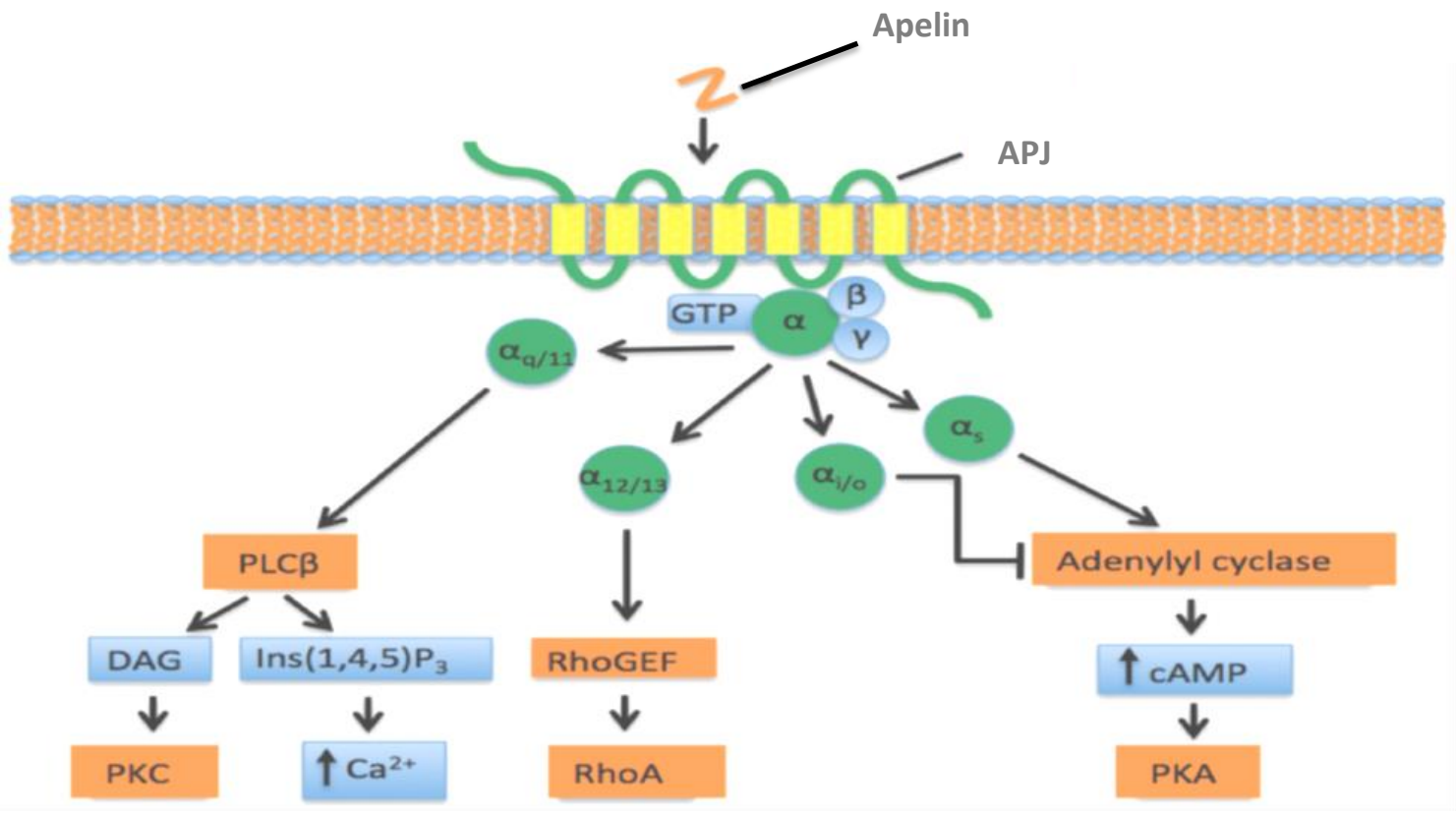

Figure 1-7: Mechanism of action of apelin and different subfamilies of G-protein coupled receptors (GPCR), protein kinase A (PKA), phospholipase C (PLC), diacyl glycerol (DAG), 1,4,5-triphosphate (Ins1,4,5P3), calcium (Ca2+), , protein kinase C (PKC), cyclic adenosine monophosphate (cAMP).[80,81].

1.2.1.3 Clinical Significance of Apelin

Apelin is one of the most potent known inotropic agents , have been shown to increase myocardial contraction and reduce cardiac load without inducing pathological hypertrophy, Apelin signalling is also reported to have cardioprotective effects; such as reduce cardiomyocyte apoptosis. Low apelin levels are associated with greater mortality rates and less effective cardiac remodelling post-injury [82].

As long as apelin secreted by adipose tissue, leading to its description as an adipokine. Correspondingly, elevated levels of apelin in the blood are correlated to obesity; insulin and apelin have been shown to have coregulatory effects. Thus, it has been proposed that the APJ that present at the membrane of islet cells, with its activation by apelin leading to reduced insulin secretion and to the resulting impairment of glucose elimination, apelin increase glucose transporter (GLUT2) so increase intestinal glucose absorption, increase glucose uptake by skeletal muscle and increase myocardial glucose uptake. Insulin, on the other hand, binds its receptor on adipocytes, inducing apelin expression and providing a negative feedback mechanism for insulin production [83].

Both apelin and its receptor are present in high levels in the central nervous system. In particular, highly present in the paraventricular nucleus and supraoptic nucleus of the hypothalamus bodies of neurons that project into the pituitary gland, producing secretory hormones to be released into systemic circulation. Production of the antidiuretic hormone vasopressin is a hallmark action of these nuclei, and the co-localization of apelin with vasopressin here suggests a potential shared regulatory role of these peptides in fluid balance [84].

The actions of apelin through the APJ (apelin receptors) have been shown to be angiogenic. Both apelin and the APJ were found expressed in biopsy samples taken from different types of cancer such as colon and breast cancer. The role of this system in tumor angiogenesis has led to identification of apelin and the APJ as anticancer therapeutic targets or, at the very least, as potential diagnostic biomarkers [85].

Apelin considered to be a potent vasodilator by stimulate endothelial nitric oxide synthase (eNO), eNO stimulate soluble guanylate cyclase result in increased cyclic guanosine monophosphate [cGMP] that lead to vasodilation effect and regulation of blood pressure. Moreover, apelin receptor is also present on muscle stem cells and promotes proliferation and differentiation of these cells into mature muscle cells participating to muscle regeneration [86].

1.2.2. Leptin

1.2.2.1. Definition

Leptin is a 146-residue polypeptide hormone that is normally produced by adipocytes. It was discovered by studying genetically obese mice in 1960s [87]. Adipocyte secreted protein cytokine was named leptin. It was derived from greek word “leptos” for thin that coded by the single leptin gene (LEP) which identified in 1995 [88].

Leptin acts on cell receptors in the arcuate and ventromedial nuclei, as well as other parts of the hypothalamus and dopaminergic neurons of the ventral tegmental area. Leptin is thought to serve as an indicator of energy stores (lipostat), as well as a modulator of energy balance [89]. Circulating leptin serves to communicate the state of body energy repletion to the central nervous system (CNS) in order to suppress food intake and permit energy expenditure [90].

Adequate leptin levels permit energy expenditure on the processes of reproduction, tissue remodeling, and growth and similarly regulate the autonomic nervous system, other elements of the endocrine system, and the immune system Conversely, [91], lack of leptin signaling due to mutation of leptin gene or the leptin receptor gene (LR) in humans results in increased food intake in combination with reduced energy expenditure and a phenotype reminiscent of the neuroendocrine starvation response (including hypothyroidism, decreased growth, infertility, and decreased immune function) in spite of obesity [92].

1.2.2.2 Physiology of Leptin

Leptin is secreted by adipose tissue and regulates energy homeostasis, neuroendocrine function, metabolism, immune function and other systems through its effects on the central nervous system and peripheral tissues. Circulating leptin levels are directly in proportion to the amount of body fat, thereby reflecting the status of long-term energy stores. In addition, leptin levels fluctuate according to changes in calorie intake with a marked decrease during starvation [93]. Leptin levels exhibit sexual dimorphism. Although leptin levels decline significantly after the menopause, women tend to have higher levels than men even after controlling for body fat mass, suggesting a role of sex steroids. Subcutaneous fat produces more leptin than visceral fat, and this may, in part, contribute to higher leptin levels in women compared to men [94].

To fulfill its physiological role, leptin must bind to type R leptin receptors (LepRb) which is highly enriched in the hypothalamic region of the brain and to a lesser extent in peripheral tissues and macrophages, once leptin binds to the LepRb, a series of intracellular reactions will be activated, thereby inducing a reduction in food intake and an increase in energy expenditure [95].

The major two classic signaling pathways related to leptin functions have been discovered [96] which include:

1-JAK-STAT (Janus kinases signal transducer and activator of transcription proteins)

2-PI3K-Akt-mTOR (phosphatidylinositol-3-kinase, mammalian target of rapamycin).

1.2.2.3. Clinical Significance of Leptin

Many Studies demonstrated that the concentration of circulating leptin decreases during fasting or energy restriction. But increases during refeeding, overfeeding, as well as during surgical stress. These effects provide an overview of how various pathways regulate the leptin signaling system to maintain body mass. Leptin action in the brain potently suppresses hepatic glucose production while increasing tissue glucose uptake [97].

When the fat cells increase, leptin levels increase proportionally, then bind to leptin receptors (LEP-R) in the brain that send signals to inhibit food intake and increase energy expenditure. However, when a positive energy balance (i.e., caloric intake exceeds energy expenditure) is sustained for critical periods, weight is gained; Thus, leptin is considered a satiety signal that any defect in its pathways can cause obesity [98].

This multiplicity of intracellular mechanisms gives leptin more than one function. Leptin has an effect on diabetes mellitus (DM) and its mechanism, Leptin has beneficial effects on the glucose-insulin metabolism, by decreasing glycemia, insulinemia and insulin resistance [99]. Leptin deficiency associated with insulin resistance and DM. Because insulin is required for the synthesis and storage of triacylglycerol into adipose tissue, in the absence of insulin therapy in DM there is a depletion of body fat stores that result in leptin deficiency [100].

Serum leptin levels had positive associations with serum follicle-stimulating hormone, luteinizing hormone, and prolactin levels and abnormal sperm morphology and had negative associations with serum testosterone levels and sperm parameters including sperm concentration and motility [101]. An abnormal level of leptin had a significant correlation with sperm disorders in infertile men. The leptin hormones played an important role in transferring energy to the reproductive system and providing the sperms incapacitated with the energy it consumes in a process capacitation [102].

Leptin has a dual role as a hormone and a cytokine. As a cytokine Leptin regulates both innate and adaptive responses through modulation of immune cells survival and proliferation as well as its activity. In innate immunity, leptin increases the cytotoxicity of natural killer (NK) cells and promotes the activation of granulocytes, and macrophages [103]. In adaptive immunity, leptin increases the proliferation of immature T cells and B cells while it reduces that of regulatory T cells. Leptin promotes the switch towards a pro-inflammatory T-helper1 (Th1) which secretes interferon gamma (IFNγ) rather than anti-inflammatory Th2 which secretes IL-4 phenotype, finally, leptin activates B cells to secrete cytokines and modulates B cell development [104].

Elevated levels of circulating leptin in obese patients contribute significantly to the low-grade inflammatory state that makes those individuals more susceptible to develop autoimmune disease. Conversely; reduced levels of leptin such as those found in malnourished individuals have been linked to increased risk of infection and reduced cell-mediated immunity [105].

Pleiotropic nature of leptin, since its discovery in 1994 in human, several physiological functions have been attributed to leptin, such as modulation of bone metabolism, inflammation, via activation of leptin receptor [106].

The leptin receptor can be found in adult primary osteoblasts and chondrocytes, suggesting that the effects of leptin on bone growth and metabolism may be direct [107]. Leptin may impact bone growth through the activation of fibroblast growth factor 23 (FGF-23) [108]. Leptin also impacts and regulates osteocalcin, which in turn regulates not only bone metabolism [109].

Leptin exerts physiologic effects that may be detrimental in states of cardiac dysfunction or heart failure. Leptin's hemodynamic effects generally increase myocardial workload via activation of the sympathetic nervous system. These effects include increasing resting heart rate and blood pressure [110]. Elevated leptin levels have been reported in patients with dilated cardiomyopathy Leptin has peripheral actions to stimulate vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy that may contribute to pathogenesis of hypertension, atherosclerosis, and coronary heart disease [111].

1.2.2.4. Leptin Resistance

Mechanisms of leptin resistance include genetic mutation, leptin self-regulation, limited tissue access and cellular or circulating molecular regulation. Food intake and metabolism are regulated by different hormones, such as leptin, whose circulating levels must be regulated very precisely and are often altered in obesity [112]. Produce important changes in the level of leptin in the blood–brain barrier BBB as well as in different regions of the brain, especially in the regions of neuronal populations with high metabolic demands, such as the hippocampus. Feeding with a HFD produces neuronal loss in the arcuate nucleus and hypothalamus [113,114].In addition to causing a decrease in the integrity of the BBB because of the loss of tanycytes (specialized ependymal cells in the median eminence) and transporters at the level of the BBB [115].To exert leptin action, it must pass through the BBB through a specific and saturable transporter. As adiposity increases, serum leptin levels also increase, which can lead to the development of resistance at the level of the BBB transporter. This implies that a lesser amount of leptin will reach the brain, thereby leading to reduced activation of the signaling pathway for body weight regulation [116].

A second mechanism has been proposed to explain leptin resistance; which include, alterations in cellular leptin receptors signaling. The concept of leptin resistance is analogous to the syndrome of insulin resistance, in which elevated levels of insulin are required to mediate adequate glucose disposal and metabolic control. In the case of insulin resistance, a number of intracellular pathways contribute to the attenuation of insulin signaling in insulin-responsive tissues such as muscle and liver ,Indeed, diet-induced obese (in which consumption of a palatable, calorically dense diet promotes obesity) are may cause attenuation in leptin receptors signaling [117].

Several examples of these proposed mechanisms lead to a decrease in the response of leptin receptors, such as decreased STAT3 phosphorylation and neuropeptide release , binding of JAK2 with inhibitor complex protein and over expression of Suppressor Of Cytokine Signaling 3 gene which responsible of production ubiquitination and subsequent proteasome degradation of the leptin receptors [118]. The leptin axis has functional interactions with elements of metabolism, such as insulin, and inflammation, including mediators of innate immunity such as interleukin-6. and C-reactive protein (CRP), resulting in leptin resistance, atherothrombosis and myocardial injury [119].

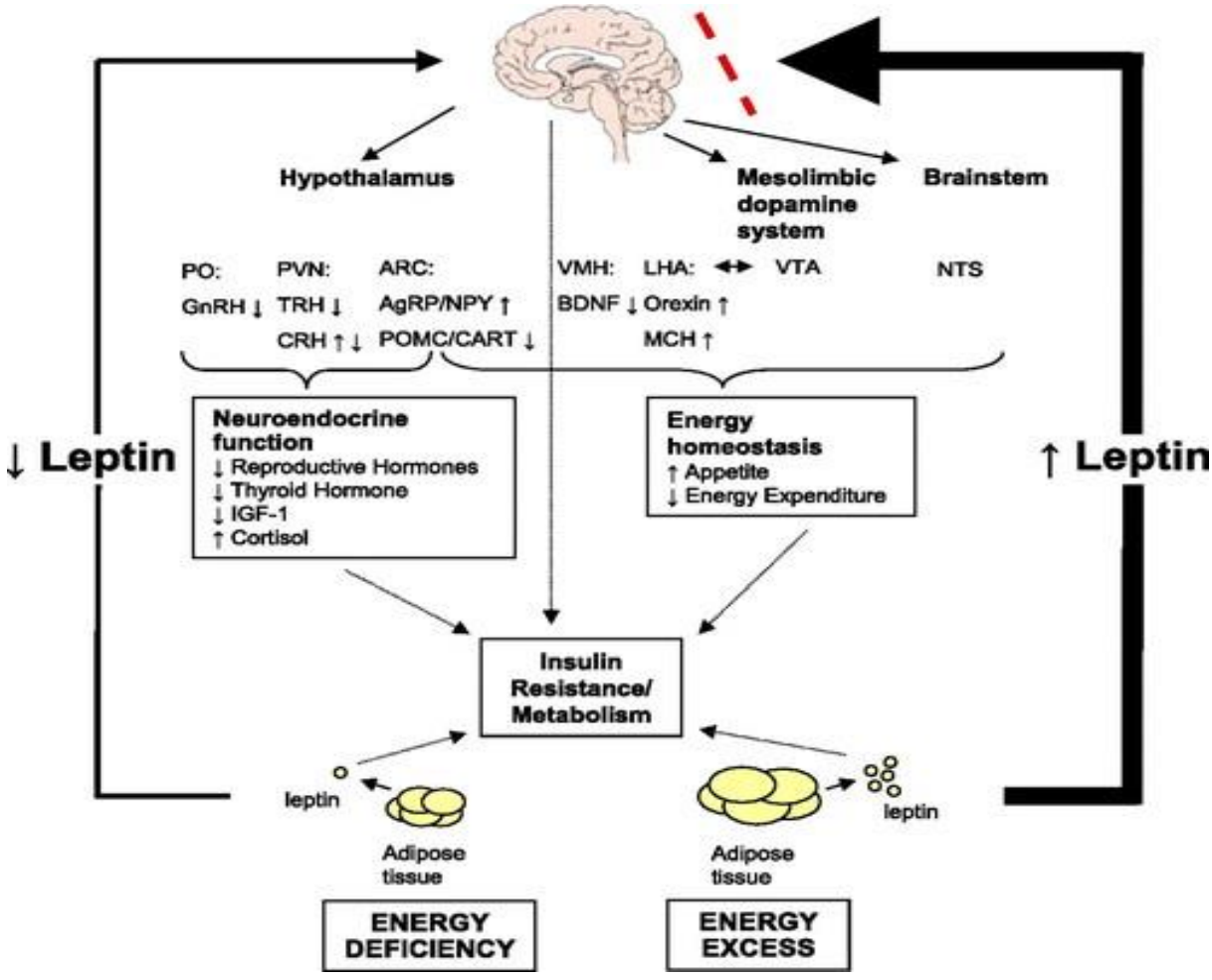

Figure 1-8: Effects of Leptin [116].

1.2.3. Homocysteine

1.2.3.1 Definition

Homocysteine (Hcy) is a sulfur nonproteinogenic amino acid whose metabolism stands at the intersection of two pathways: remethylation to methionine, which requires folate and vitamin B12; and transsulfuration to cystathionine, which requires pyridoxal phosphate [120]. Its chemical properties showed a similarity to cysteine, hence the name homocysteine differing by an additional methylene bridge [121].

Homocysteine is produced via demethylation of dietary methionine, Homocysteine present in plasma in four different forms: around 1% circulates as free, 70–80% remains bound to plasma proteins, mainly albumin and 20–30% combines with it to form the dimer homocysteine or with other thiols. Homocysteine was first described by Butz and du Vigneaud in 1932 [122].

1.2.3.2 Metabolism of Homocysteine

Homocysteine represents a point of intersection of two pathways: the methionine cycle and the transsulfuration sequence. The methionine cycle is formed by the synthesis of S-adenosylmethionine (SAM); the numerous specific transmethylation reactions that yield S-adenosylhomocysteine (SAH) as a product. The remethylation of homocysteine which requires 5-methyltetrahydrofolate as the methyl donor and methylcobalamin as the coenzyme. This basic methionine cycle occurs in all normal mammalian cells [123].

A second homocysteine methylase, which employs betaine as the methyl Donor, has been found in liver of all mammalian species and in primate kidney. The two pathways are coordinated by S-adenosylmethionine, which acts as an allosteric inhibitor of the methylenetetrahydrofolate reductase reaction and as an activator of cystathionine-synthase [124]. The product of these methylation reactions SAH, is subsequently hydrolyzed, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer. It is important to note that this hydrolysis is a reversible reaction that favors the synthesis of SAH, and that elevated cellular concentrations of this metabolite are likely to precede and accompany all forms of hyperhomocysteinemia [125].In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal-phosphate (PLP)–containing enzyme, cystathionine –synthase. Cystathionine is hydrolyzed by a second PLP-containing enzyme, γ-cystathionase, to form cysteine and α-ketobutyrate [126]. Excess cysteine is oxidized to taurine or inorganic sulfates or is excreted in the urine. Thus, in addition to the synthesis of cysteine, this transsulfuration pathway effectively catabolizes excess homocysteine, which is not required for methyl transfer [127].

Homocysteine metabolism by the transsulfuration and remethylation pathways as shown in figure 1-9 is nutritionally regulated. This capacity of the body to discriminate between the remethylation and transsulfuration pathways as a way to adapt to varying amounts of methionine in the diet strongly implies the existence of a coordinate regulation between these two pathways [128].

This regulation is achieved by at least two mechanisms. The first mechanism is a function of SAM's propensity to act as an allosteric inhibitor of methylenetetrahydrofolate reductase (MTHFR) and as an activator of cystathionine –synthase [129].

As such an effector, SAM suppresses the synthesis of an important substrate (N-5-methyltetrahydrofolate) required for remethylation and promotes the initial reaction of transsulfuration (cystathionine synthesis). Thus, intracellular SAM concentration is an important determinant of the fate of homocysteine molecules [130].

The second mechanism by which remethylation and transsulfuration are coordinated consists of the regulation of intracellular SAM concentration, itself. In the liver, SAM synthesis is catalyzed by two enzymes peculiar to this organ that are immunologically similar but different in other respects [131].

One enzyme, a tetramer of high molecular weight, exhibits a high affinity for methionine and is thought to function at normal physiological conditions. The second enzyme is a dimer of a lower molecular weight, has a low affinity for methionine, and is thought to function under conditions of high methionine intake. Thus, changes in intracellular methionine, particularly due to dietary intake, will affect the rate of SAM synthesis based on the activity of the SAM synthetase enzymes. [132]. When the two mechanisms of regulation are considered together, the following scenarios can be predicted:

1- When dietary methionine is high, the low-molecular-weight SAM synthetase will rapidly convert the incoming methionine to SAM. The resulting rise in intracellular SAM concentration will be associated with a) inhibition of methylenetetrahydrofolate reductase (MTHFR), b) activation of the

cystathionine -synthase enzyme, thus increasing the rate of homocysteine catabolism. In this way, homocysteine transsulfuration is promoted over remethylation (133).

2- Conversely, when the dietary methionine supply is low, SAM concentration is insufficient for the inhibition of MTHFR, resulting in an elevated rate of N-5-methyltetrahydrofolate production. The resulting rise in intracellular N-5-methyltetrahydrofolate concentration will be associated with a) increase production of SAM, and b) an increase in the availability of substrate for homocysteine remethylation [134].Thus, remethylation will be favored over transsulfuration because the concentration of SAM is too low to activate the cystathionine -synthase enzyme [135].

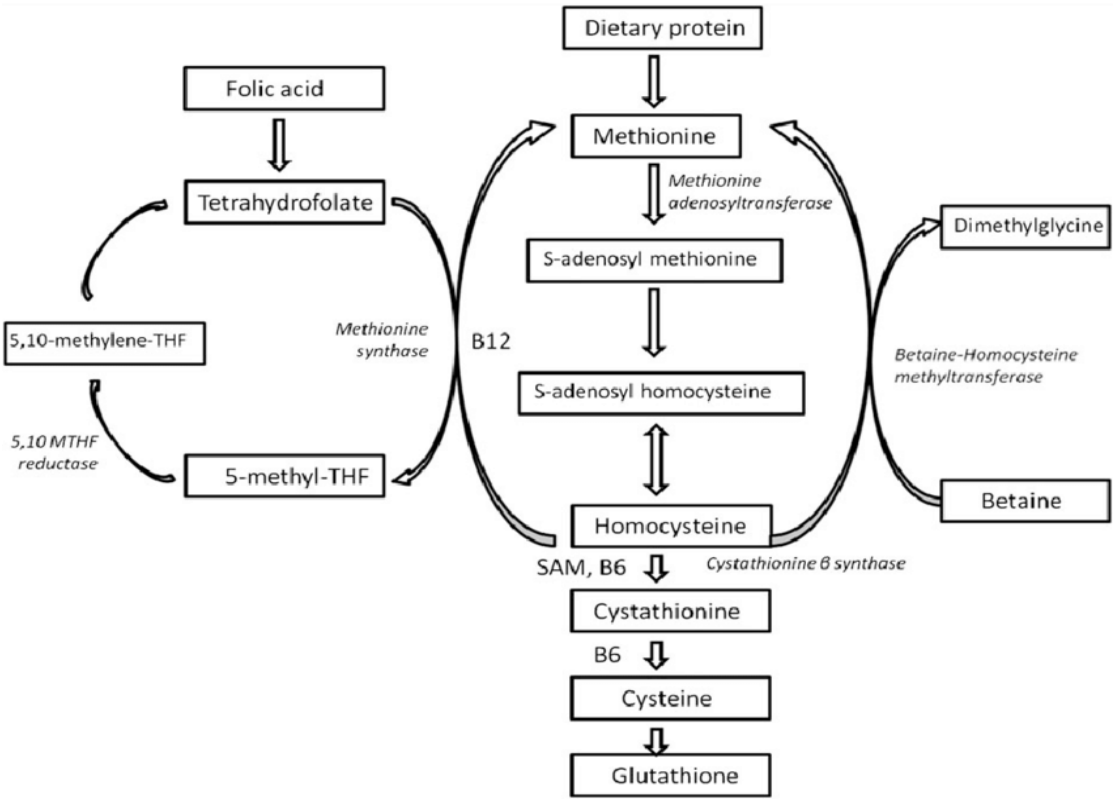

Figure 1-9: Homocysteine cycle [132].

1.2.3.2 Clinical Significance of Homocysteine

The physiologic levels of Hcy in a healthy population are determined primarily by the dietary intakes of methionine, folate, and vitamin B12. Lifestyle conditions, s such as smoking, alcohol consumption, and physical inactivity, may increase the elevation of Hcy [136]. Normal levels of Hcy range between 5-and 15 micromol/L and in physiological conditions, plasma Hcy. Increased homocysteine levels is an independent risk factor for vascular diseases including stroke and dementia [137].

Hyperhomocysteinemia [HHcy] induced oxidative stress, endothelium dysfunction, inflammation, smooth muscle cell proliferation, and endoplasmic reticulum [ER] stress have been considered to play an important role in the pathogenesis of several diseases including atherosclerosis [138]. One of the most important proposed mechanisms of how homocysteine causes diseases is homocysteinylation. homocysteinylation alters the properties of the protein and has been associated with several disease outcomes. This process is irreversible and cumulative [139].

Elevated Hcy may cause coronary artery disease (CAD) through various mechanisms such as the increased proliferation of muscle cells that cause narrowing of vessels, alter blood coagulant properties, cause oxidant injury to the vascular endothelium, and damage arterial walls [140] High level of Hcy in diabetes is a biomarker of microvascular complications like diabetic neuropathy, retinopathy, and nephropathy. [141].Hcy toxicity can cause kidney damage. Imbalanced homeostasis and increased oxidative stress and reactive oxygen species (ROS) which caused glomerular endothelial dysfunction also resulted in a change in glomerular filtration rate, which induced renal dysfunction [142].

Hcy associated with overall risk of cancer. Cancer cells derived had a deficit in the ability to remethylate Hcy. This changed metabolic condition results in elevated levels of serum Hcy [143]. Elevated level of Hcy found in vitiligo patients as Hcy increased oxidative stress and disrupted melanocytes. Hcy inhibit melanin synthesis enzyme (tyrosinase) [144].

1.2.4. Selenium

1.2.4.1. Definition

Selenium (Se) is a naturally occurring metalloid with many chemical and physical properties similar to those of sulfur. Selenium is an essential trace element and a major constituent of 40 minerals and a minor constituent of 37 others [145]. Most processed selenium is used in the electronics industry; however, other uses include nutritional supplements, pigments, pesticides, rubber production, anti-dandruff shampoos, and fungicides [146].

Selenium was discovered by the Swedish chemist Jöns Jakob Berzelius in 1817 and was considered a toxic element for humans for nearly 150 years. However, in 1957, the benefits of selenium for humans and other mammals were revealed in landmark studies by Klaus Schwartz and Calvin Foltz [147].

1.2.4.2. Chemistry of Selenium

Selenium is a chemical element with the atomic number 34 and atomic weight 78.96. it is found in group 16 of the periodic table. Se is a nonmetal [more rarely considered a metalloid] with properties that are intermediate between the elements above and below in the periodic table, sulfur and tellurium. It seldom occurs in its elemental state or as pure ore compounds in the Earth's crust [148].

Selenium is usually an amorphous, brick-red powder. When rapidly melted, it forms the black, vitreous form, usually sold commercially as beads. In living systems, Se is found in the amino acids selenomethionine, selenocysteine, and methylselenocysteine [149].

1.2.4.3. Nutritional Sources of Selenium

Selenium is accumulated in the human organism to the largest extent mainly through ingestion. Dietary selenium comes from meat, nuts, cereals, mushrooms, and seafood. Brazil nuts are the richest dietary source [150]. The Recommended Dietary Allowance (RDA) of selenium for adults is 55 µg/day. Selenium as a dietary supplement is available in many forms, including multi-vitamins/mineral supplements, which typically contain 55 or 70 µg/serving. Selenium-specific supplements typically contain either 100 or 200 µg/serving [151].

1.2.4.4. Absorption and Excretion of Selenium

Selenium is well absorbed from the gastrointestinal tract (approximately 50%). Selenium is absorbed mainly from the duodenum and is transported actively across the intestinal brush border particularly in the form of methionine analogue. Selenium after absorption is transported bound to proteins particularly Selenoprotein P in plasma and Selenoprotein W in skeletal muscle. selenomethionine can be deposited directly in tissues and taken up also by myoglobin, cytochrome C, myosin, and aldolase [152]. Selenium exposure occurs primarily from diet but can be found in drinking water, usually in the form of inorganic sodium selenate or sodium selenite. Selenium balance is largely achieved by excretion through urine and stool. Other routes of elimination include sweat and, at very high intakes, exhalation of volatile forms of selenium [153].

1.2.4.5 Functions of Selenium in Biology System

Selenium has gone through different stages to explain its effect on the human body. In the 1930s, selenium was considered a toxic element; in the 1940s, a carcinogen; in the 1950s, it was declared as an essential element; and since the 1960s and especially the 1970s, it has been viewed as an anticarcinogen [154].

As shown in figure 1-10 Selenium enters as an essential part and cofactor in number of important enzymes, such as Glutathione peroxidase (in the form of selenocysteine) is part of the cellular antioxidant defense system against free radicals. Selenium is also involved in the metabolism of thyroid hormones such as deiodinase enzymes and thioredoxin reductase according to what mention above Se play important rule as antioxidant and prober function of thyroid gland [155].

Selenium act as immune stimulator that modulates different immune response mechanism such as T cell proliferation, NK cell activity, and innate immune cell functions. Vaccine responses against pathogens such as poliovirus have been shown to improve with selenium supplementation [156].

Selenium was shown to modulate the inflammatory response in respiratory distress syndrome patients by restoring the antioxidant capacity of the lungs, which moderated the inflammatory responses through interleukin such as IL-1 and IL-6 levels [157]. Selenium has an important role as an anti-cancer that can be explained by more than one mechanism. One of the direct anti-cancer effects of selenium is related to the ability of seleno-compounds to induce oxidative stress and DNA damage to cancer cells and, consequently, apoptosis. Also an inhibitory effect of selenium on the epithelial-to-mesenchymal transition (EMT) that drives metastasis of cancer lesion [158].

Selenium has an important role in protecting heart cells from oxidation by reducing lipid peroxidation and preventing apoptosis. It also has a role in preventing the formation of fibrosis. Se Prevents atherosclerosis includes monocyte adhesion and migration, foam cell formation, vascular apoptosis, endothelial dysfunction, and vascular calcification [159].

It is noteworthy that Se and vitamin E have a potential synergistic effect when the two agents were used in combination. It is possible that the antioxidant actions of both selenium-containing glutathione peroxidase and vitamin E synergize to retard formation of cytosolic lipid peroxides; this may explain why selenium deficiency is usually associated with vitamin E deficiency [160].

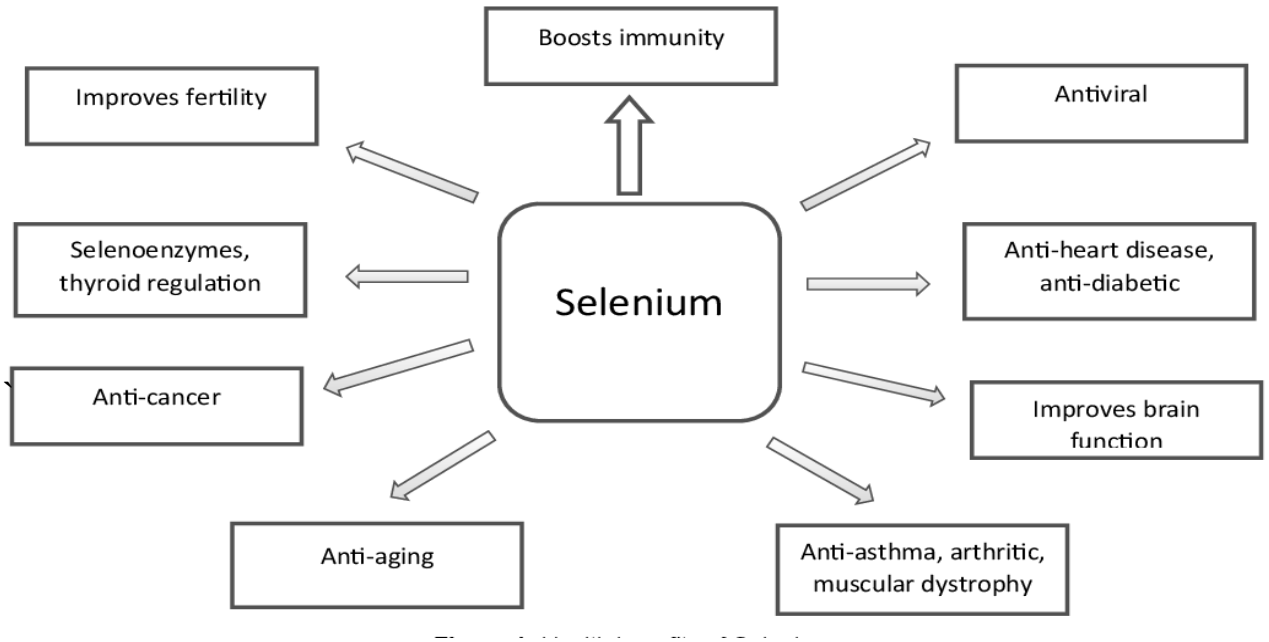

Figure 1-10: Selenium Functions [153].

1.2.4.6 Selenium Deficiency and Toxicity

Selenium deficiency has been associated with cardiomyopathy, skeletal muscle weakness, and osteoarthritis. A significant negative correlation was observed between selenium intakes and the rate of cancer of the large intestine, rectum, prostate, breast, ovary, and lungs and leukemia [161]

Keshan disease, an endemic cardiomyopathy that affects mostly children and women in childbearing age in certain areas in China, has been associated with selenium deficiency. Symptoms include dizziness, malaise, loss of appetite, nausea, chills, abnormal electrocardiograms, cardiogenic shock, cardiac enlargements, and congestive heart failure [162]. Kashin-Beck disease, an endemic osteoarthritis that occurs during adolescent and preadolescent years, is another disease linked to low selenium status in northern China, North Korea, and eastern Siberia [163].

Acute oral exposure to extremely high levels of selenium may produce gastrointestinal symptoms (nausea, vomiting, and diarrhea) and cardiovascular symptoms such as tachycardia. Chronic exposure to very high levels can cause dermal effects, including nails discoloration and hair loss, as well as neurologic problems such as Imbalance in walking or paralysis. The loss of hair and nails, skin lesions, tooth decay, and anomalies of the nervous system were the most typical symptoms of selenium overdose [164].

The European Food Safety Authority (EFSA) determined Dietary Supplements require for selenium to be manufactured and labelled so that the recommended daily dose is no more than 150µg. A maximum safe daily dietary intake has been estimated at 400µg. At an intake of 750-850µg functional signs of toxicity can be expected [165].

1.3. Literature Review

Acute myocardial infarction had been a very interesting topic to many researchers for a long time. The cornerstone of most of the researchers that involve AMI is how to choose a specific and sensitive marker that may predict the occurrence, ensure the diagnosis and Monitor the progression. Recently OttoMayerJr , *et al* 2022 conclude that high leptin concentration entails an increased risk of mortality in patients with acute coronary syndrome (ACS) as leptin connect directly to obesity and metabolic syndrome [166].also S Attia, *et al* 2022 study the prognostic value of leptin in the different types of CAD [167].

Homocysteine one of the most important parameters that studied its association with a large number of diseases such as ACS As a risk factor and causative agent of disease development. Soderstrom E , *et al* 2022 has been shown that high levels of homocysteine increase the risk of coronary heart disease in certain groups, such as smokers [168].Begum R, *et al* 2022 link between high levels of homocysteine and coronary heart disease even in young people due to the ability of homocysteine induce endothelial dysfunction and stimulate vascular smooth muscle cell proliferation, both important events in the pathogenesis of atherothrombotic disease [169].

Selenium and its effect on coronary heart disease have long been studied due to its antioxidant effect and its reduction in oxidative stress. L Yang, *et al* 2022 in meta-analysis study found lower selenium levels in patients with different types of heart disease such as ACS [170]. Filonenko M, *et al* 2022 found significance correlation between low selenium levels and the high levels of different types of cardiac markers [171].

Apelin is one of the most important and promising new parameters currently being studied in patients with myocardial infarction and coronary heart disease. Oliveira AA, *et al* 2022 found that there is an important intersection between the apelin pathways and the events leading to myocardial infarction [172]. Yumun G, *et al* 2022 concluded that apelin could be considered as an independent risk factor for coronary heart disease [173].

A lot of researches over the years that has studied myocardial infarction has tried, as much as possible, to determine the parameters directly related to the occurrence of myocardial infarction, diagnosis, or follow-up of the progression of the disease. One of the most important goals of these researches is to identify high-sensitivity and specific tests that can be used in emergency and critical care unit, or specify one of the parameters that can match or come close to some extent to the electrocardiogram.

Aims of Study

1- Determine the extent to which some risk factors relate to acute myocardial infarction in patients in Babylon Province.
2- Study the role of leptin, homocysteine, selenium and apelin in the occurrence of acute myocardial infarction among patients in Babylon Province.
3- Evolution of leptin, homocysteine, selenium and apelin levels for the diagnosis and progression of acute myocardial infarction among patients in Babylon Province.
4- Study the correlation between leptin, homocysteine, selenium, and apelin with cardiac troponin I in patients with acute myocardial infarction in Babylon Province.
5- Study the possibility of using leptin, homocysteine, selenium, and apelin as a biomarker by which to determine the type of acute myocardial infarction (STEMI or NSTEMI).

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and kits

Chemicals and kits that were used in this study are listed in the Table 2-1

Table 2 -1: The chemicals and kits used in the study

Chemicals	Company and Country
Apelin (ELISA)	Human (Germany)
Cardiac Troponin I titer (Chemiluminescence)	Roche (Switzerland)
Homocysteine (Chemiluminescence)	Abbott (United States)
Leptin (ELISA)	Human (Germany)
Nitric acid (10%)	Merck (Germany)
Selenium Standards Solutions	Merck (Germany)

2.1.2 Instruments

All instruments and tools which were used in this study are in the Table 2-2

Table 2-2: Instruments and Tools

No.	Instruments and Equipments	Company and Country
.1	Architect i1000 sr	Abbott (United States)
.2	Atomic Absorption Spectroscopy	PG 990 Instruments Ltd (UK)
.3	Centrifuge EBA 20	Hettich (Germany)
.4	Cobas e411	Roche (Switzerland)
.5	Deep Freezer	Samsung/Korea
.6	Distillator	Bibby science (England)
.7	ELISA system	Bio Tech (USA)
.8	Incubator	Fisher scientific (USA)
.9	Multiple micropipettes (10-100 µL)	Watson Nexty (Japan)
.10	Plate shaker	Biosan (Latvia)
.11	Vortex (Electronic)	Bionex (Korea)
.12	Water bath	Grant (England)

2.1.3 Patients Groups

This case-control study was done at the laboratory of Biochemistry Department, College of Medicine, and University of Babylon. The study was conducted during the period from February 2022 until May 2023.The entire samples collected from patients attending to Marjan Medical City. The patient's groups who subjected to this study were 60 patients divided into two groups depend on ECG stratification:-

The First Group: patients with STEMI, this group including 27 patients in the age ranging from 48-81 years, the mean of their ages were (63.3± 10.5 years).**The Second Group:** patients with non-STEMI, this group include 33 patients their age ranging from 50-78 years, the mean of their ages was (59.5 ± 8 years).

2.1.3.1 Inclusion Criteria

All patients with acute myocardial infraction were inclusive in this study diagnosed by ECG and cardiac troponin I (qualitative); all of theme underwent an overall questionnaire about the following: smoking and alcohol history, family history of myocardial infraction, chronic diseases, age, body mass index, and address.

2.1.3.2 Exclusion Criteria

Patients with congestive heart failure, all types of cancer, liver diseases. diabetes mellitus type 1 or 2, and renal diseases.

2.1.3.3 Ethical Issues

The study was conducted in compliance with ethical principles based on the Declaration of Helsinki. Patients provided both verbal and written consent before the sample was taken. The study protocol, as well as the subject information and consent form, were reviewed and approved by a local Ethics Committee, as evidenced by document number fifth session, which includes the approval date of 23/11/2021.

2.1.3.4 Control group

This includes 60 apparently healthy subjects with the mean age 62.4± 9.4 years. None of these subjects had a history of AMI, liver disease, renal disease and diabetes mellitus.

Table 2-3: Age and sex for patients and control

	Subjects	Gender	No.	Age (years) Mean ± SD	P value
Study Groups	STEMI (27)	Female	7	64.1±9.7	> 0.05
		Male	20	62.5±11.3	
	non-STEMI (33)	Female	11	60.8 ± 8.0	> 0.05
		Male	22	58.3±8.1	
	Control (60)	Female	30	65.5±10.0	> 0.05
		Male	30	59.3±8.8	

2.2 Methods

2.2.1 Collection of Blood and Sample Preparation

Five to eight milliliters of blood was drawn from all patients and healthy subjects participating in the study collected in gel tubes and were stand at room temperature for 15 minutes to clot. After that, the blood specimens were centrifuged at 2200 RCF for approximately 10-15 minutes. The separated serum was divided into 3 parts and transferred to Eppendorf tube and stored at -20 Celsius until time of use.

2.2.2. Determination of Apelin

2.2.2.1 Principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Human Apelin. During the reaction, Human Apelin in the sample or standard competes with a fixed amount of Human Apelin on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Human Apelin. Excess conjugate and unbound sample or standard are washed from the plate, and Streptavidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well [174].

The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450. The concentration of Human Apelin in the samples is then determined by comparing the OD of the samples to the standard curve. Components of the apelin ELISA kit mentioned in Table 2-4.

Table 2-4: Components of the Apelin ELISA kit

No.	Description	Quantity
1.	Human Apelin coated strip plate.	96 wells
2.	Human Apelin Std. A (0 pg/ml).	0.5 ml 1 vial
3.	Human Apelin Std. B (62.5 pg/ml).	0.5 ml 1 vial
4.	Human Apelin Std. C (125 pg/ml).	0.5 ml 1 vial
5.	Human Apelin Std. D (250 pg/ml).	0.5 ml 1 vial
6.	Human Apelin Std. E (500 pg/ml).	0.5 ml 1 vial

7.	Human Apelin Std. F (1000 pg/ml).	0.5 ml 1 vial
8.	Human Apelin Std. G (2000 pg/ml).	0.5 ml 1 vial
9.	Human Apelin Std. H (4000 pg/ml).	0.5 ml 1 vial
10.	Anti-Apelin-Biotin ready to use.	10 ml 1 vial
11.	Streptavidin-HRP Conjugate concentrated (50X).	0.5 ml 1 vial
12.	Assay buffer ready to use.	20 ml 1 vial
13.	Wash buffer concentrated. (10X solution).	50 ml 1 vial
14.	TMB substrate	10 ml 1 vial
15.	Stop solution	6 ml 1 vial

2.2.2.2 Reagents preparation for the assay

1-Streptavidin-HRP conjugate was diluted by 1:50 with assay buffer.

2-Wash buffer was diluted by 1:10 with distilled water.

2.2.2.3 Procedure

1- Fifty µl of standards and samples had been added into appropriate wells. This had been followed by adding 50 µl of Biotin-Anti-Apelin conjugate into each well. They had been mixed gently for 5-10 seconds.

2-The wells plate had been covered and incubated for 45 minutes at 37°C

3-The wells had then been aspirated and washed 5 times by 350 µl of diluted wash buffer using an automated washer.

4-One hundred µl of diluted Streptavidin-HRP conjugate had been added into each well. It had been mixed gently and incubated for 30 minutes at 37°C.

5- The wells had then been aspirated and washed 5 times by 350 µl of diluted wash buffer using an automated washer

6- Ninety µl of (TMB) substrate had been added into each well. It had been mixed gently, and then the plate had been covered and incubated for 15 minutes at 37°C.

7- The reaction had been stopped by adding 50 µl of stop solution into each well.

8- The well absorbance had been measured by an ELISA reader at 450 nanometers (nm).

2.2.2.4 Calculation

The standard curve can be plotted as the relative absorbance at 450 nm of each standard solution (Y) vs. the respective concentration of the standard solution (X). The apelin concentration of the samples can be interpolated from the standard curve.

2.2.3. Determination of Leptin

2.2.3.1. Principle

Human leptin ELISA kit is based on binding of human leptin from standards or samples to the anti-human leptin coated on the microwell plate and biotinylated antibody and subsequent detection of biotin-antibody by Streptavidin-HRP Conjugate (sandwich method). After a washing step, chromogenic substrate tetramethylbenzidine (TMB) is added and colors (blue) developed. Higher concentrations of leptin in the sample result in higher binding of antibody-enzyme horseradish peroxidase (HRP) to the antibody coated plate [175].The enzymatic reaction (color) is directly proportional to the amount of leptin present in the sample. Adding stopping solution terminates the reaction (blue color turns yellow). Absorbance is then measured using an ELISA reader at 450 nm. And the concentration of leptin in samples and control is read off the standard curve. Co1mponents of the Leptin ELISA kit mentioned in Table 2-5.

Table 2-5: Components of the Leptin ELISA kit

No.	Description	Quantity
1.	Anti-Human Leptin coated strip plate	96 wells
2.	Human Leptin Std. A (0 ng/ml)	0.5 ml 1 vial
3.	Human Leptin Std. B (1 ng/ml)	0.5 ml 1 vial
4.	Human Leptin Std. C (10 ng/ml)	0.5 ml 1 vial
5.	Human Leptin Std. D (20 ng/ml)	0.5 ml 1 vial
6.	Human Leptin Std. E (50 ng/ml)	0.5 ml 1 vial
7.	Human Leptin Std. F (100 ng/ml)	0.5 ml 1 vial
8.	Anti-Leptin-Biotin Conjugate	10 ml 1 vial
9.	Streptavidin-HRP Conjugate concentrated	1 ml 1 vial

10.	Assay buffer	20 ml 1 vial
11.	Wash buffer concentrated.	50 ml 1 vial
12.	TMB substrate	16 ml 1 vial
13.	Stop solution	6 ml 1 vial

2.2.3.2 Reagents preparation for the assay

1- Streptavidin-HRP conjugate was diluted by 1:50 with assay buffer.

2- Wash buffer was diluted by 1:10 with distilled water.

2.2.3.3 Assay Procedure

1- Twenty µl of standards, and samples were added into appropriate wells. Follow by addition of 80 µl of Biotin-Anti-leptin conjugate into each well. Mixed gently for 5-10 seconds.

2- The well plate had been covered and incubated for 1 hour at room temperature (25-28°C) and had been placed on a plate shaker.

3- The wells had been aspirated and washed 3 times with 300 µl of diluted wash buffer using an automated washer.

4- One hundred µl of diluted Streptavidin-HRP conjugate had been added to each well. They were gently mixed and incubated for 30 minutes at room temperature (25-28°C) on a plate shaker.

5- The wells had then been aspirated and washed 3 times with 300 µl of diluted wash buffer using an automated washer.

6- One hundred µl of (TMB) substrate had been added to each well. They were gently mixed, and the plate had been covered and incubated for 15 minutes at room temperature (25-28°C) on a plate shaker.

7- The reaction had been stopped by adding 50 µl of stop solution to each well. The well plate had then been gently mixed by a plate shaker for 2 minutes.

8-The well absorbance had been measured by an ELISA reader at 450 nanometers (nm).

2.2.2.4 Calculation

The standard curve can be plotted as the relative absorbance at 450 nm of each standard solution (Y) vs. the respective concentration of the standard solution (X). The leptin concentration of the samples can be interpolated from the standard curve Figure 2-2.

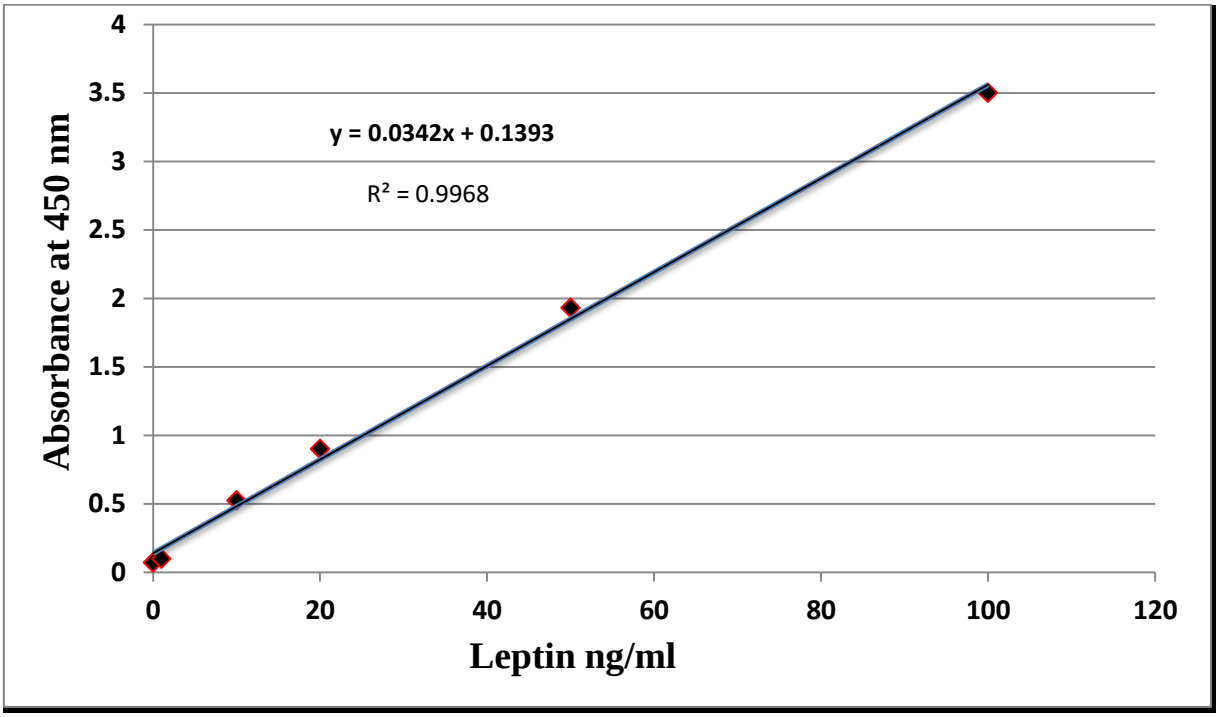

Figure 2-1: Standard Curve for Leptin.

2.2.4. Determination of Homocysteine

2.2.4.1. Principle

The Abbott Architect Hcy test is a two-stage immunoassay for quantitative identification of Hcy in human serum using double-monoclonal chemiluminescent microparticle immunoassay (CMIA) technology for the detection of Hcy. The principle based on competition of analyte in sample with acridinium-labeled analogue [176]. Components of the Homocysteine kit mentioned in Table 2-6.

Table 2-6: Components of the Homocysteine Abbott kit

No.	Description	Quantity
1.	Reagent 1 Reaction Buffer ready to use	1 vial 35 ml
2.	Reagent 2 Dilution Buffer ready to use	1 vial 35 ml
3.	Reagent 3 Immunoparticle Suspension (ready-to use suspension of micro particles coated with monoclonal antibodies to Hcy)	1 vial 7 ml
4.	Pre-trigger solution ready to use	1 Package 975 ml
5.	Trigger solution ready to use	1 Package 975 ml
6.	Standard A 0 µmol/L	1 vial 1 ml
7.	Standard B 5 µmol/L	1 vial 1 ml
8.	Standard C 10 µmol/L	1 vial 1 ml
9.	Standard D 40 µmol/L	1 vial 1 ml
10.	Standard E 80 µmol/L	1 vial 1 ml
11.	Standard F 100 µmol/L	1 vial 1 ml
12.	Control level 1 ready to use	1 vial 0.5 ml
13.	Control level 2 ready to use	1 vial 0.5 ml
14.	Control level 3 ready to use	1 vial 0.5 ml
15.	Control level 4 ready to use	1 vial 0.5 ml

2.2.4.2. Procedures

The procedure was automated in Abbott ARCHITECT i1000SR. Including two steps:-

* In the first step sample and dilution buffer had been mixed to produce a diluted sample of 1:10. An aliquot of the pre-diluted sample, reaction buffer and anti Hcy- for the detection of Hcy coated with micro-particles is combined. The sample binds to anti-Hcy for the detection of Hcy by coated micro-particles.

* In the second step, the conjugate marked with anti-Hcy-acridinium had been added. Pre-trigger and trigger solutions are added to the reaction mixture after another wash cycle. As relative light units (RLUs), the resulting chemiluminescent reaction is evaluated. There is a connection between the quantity of Hcy in the sample and the RLUs detected by the optics of the Abbott architect System.

The device has gone through all stages of daily, weekly and monthly routine maintenance before conducting the test. The Hcy kit was calibrated using Abbott's multiple calibration materials, and the validity of the kit was tested using internal quality control from Bio-Rad Lyphochek Controls.

Table 2-7: Controls results for Homocysteine

	Control kit result	Target result
Control 1	5.68 µmol/L	4.98- 5.70 µmol/L
Control 2	22.44 µmol/L	21.33-22.50 µmol/L
Control 3	50.65 µmol/L	49.81-50.91 µmol/L
Control 4	91.36 µmol/L	90.21-92.11 µmol/L

2.2.4.3 Calculation

The standard curve can be plotted as the relative absorbance at 450 nm of each standard solution (Y) vs. the respective concentration of the standard solution (X). The homocysteine concentration of the samples can be interpolated from the standard curve Figure 2-3

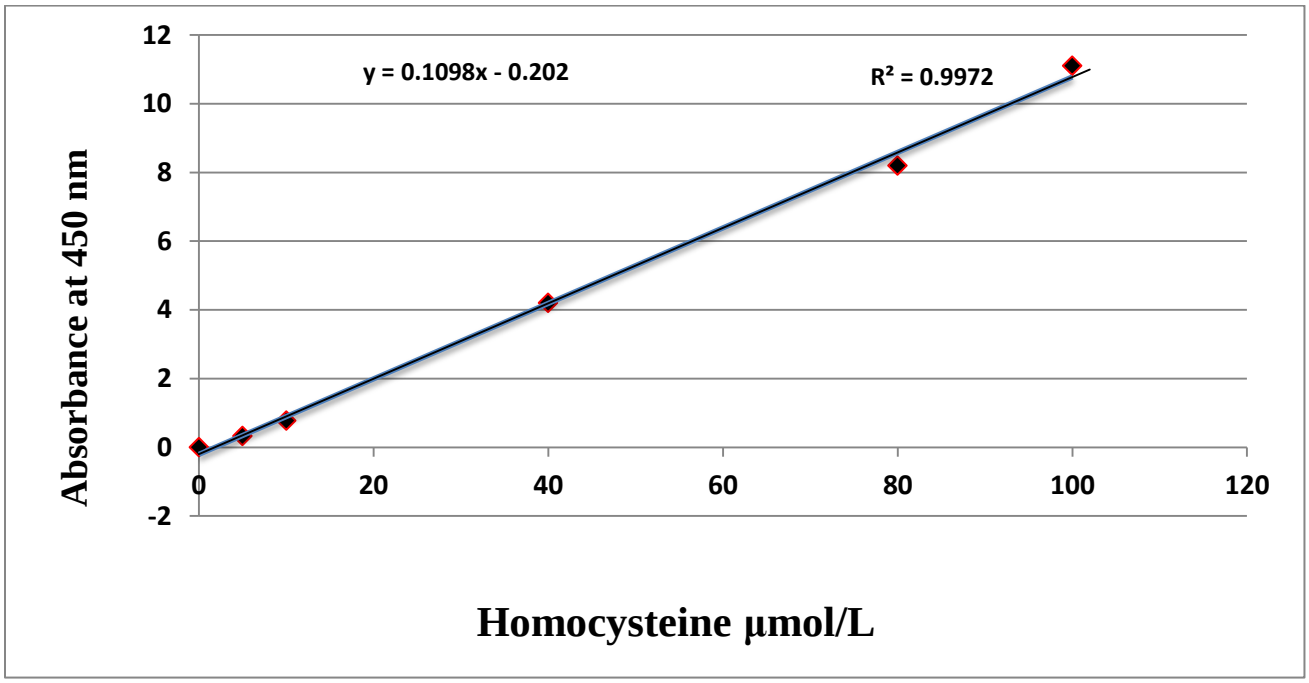

Figure 2-2: Standard Curve for Homocysteine.

2.2.5. Determination of Selenium

Serum Selenium was determined by graphite furnace atomic absorption spectrophotometer.

2.2.5.1. Principle

The instrument PG 990 Atomic Absorption Spectrophotometer was used for the determination of selenium by the technique of graphite furnace (GFAAS).

GFAAS is one of the most important techniques of the atomic absorption spectrometry in which it has the higher sensitivity that can be reached to the lower detection limits. This technique is a type of spectrometry that uses a graphite furnace tube to vaporize the sample in three stages, drying, ashing, and atomizing [177].

The principle of this technique is based on that free atoms of element absorb light produced from the specific cathode lamp at specific wavelengths characteristic of the interest element. Within certain limits, the amount of light absorbed reflect the concentration of analyte present and can be linearly correlated to this concentration [178].

In GFAAS, very small size of samples (10μL-20μL) is injected in paralytic carbon coated graphite tube, which can then be heated by a wide range of temperature to vaporize and atomize the analyst. The atoms absorb the electromagnetic radiation in the ultraviolet or visible region resulting in transitions of electrons to higher electronic energy levels to the excited state and then back to the ground state by emitting its specific characteristic light which can be measured to determine the samples concentrations. The temperature of the graphite tube increases over a matter of seconds and can reach up to 3000ºC depending on the element being analyzed [179].

2.2.5.2. Standard Solutions

Five selenium standards had been provided from the manufacturer company Merck (Germany) labeled from A to E with desirable concentration (ready to use) with concentration (5,10,40,80,100) μg/l to facilitate accurate selenium concentration measurements in various analytical. These standards had been already diluted with nitric acid to preserve the integrity of selenium.

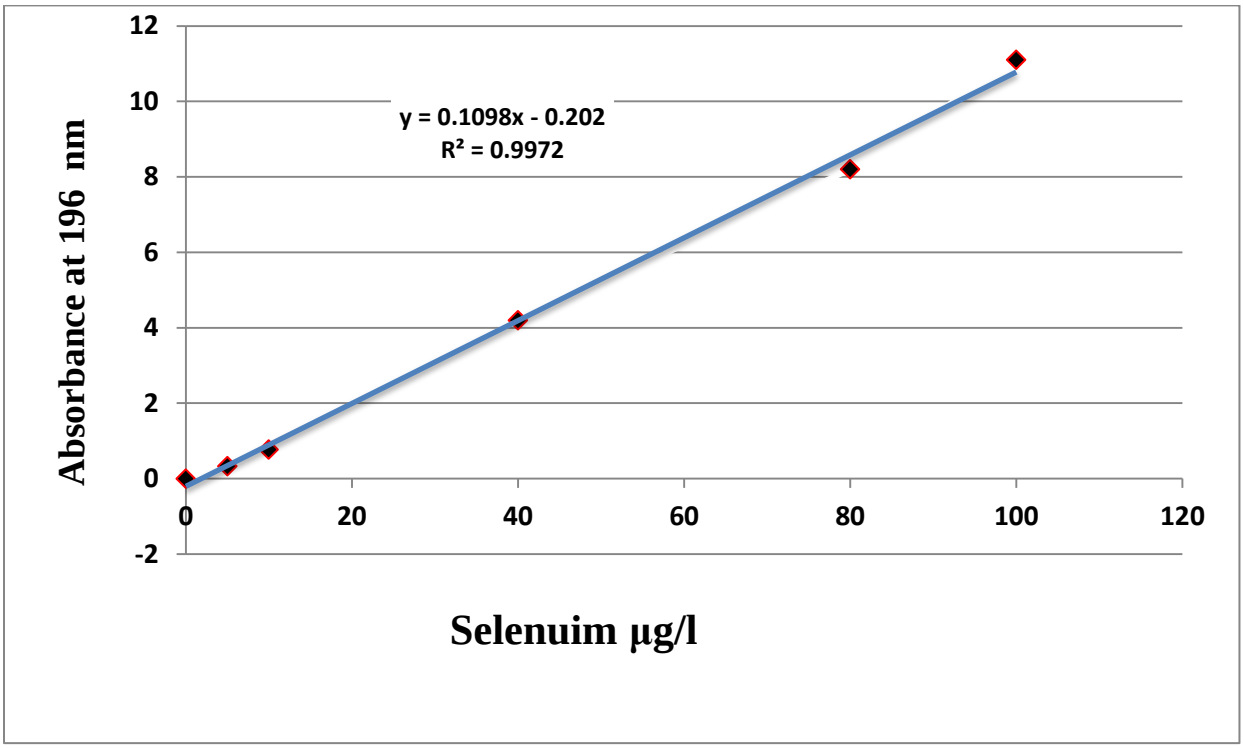

Figure 2-3: Standard Curve for Selenium.

2.2.5.3 Sample Preparation

Samples were digested by transferring (20µL) of serum into Eppendorf tube, then (40µL) of 10% nitric acid was added and mixed well for 10 minutes, finally complete dilution volume to (100µL) with deionized water. Then appropriate solution volume of (20µL) was injected into the graphite tube for reading.

2.2.5.4 Procedures

The concentrations of Selenium in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for selenium determination: the ideal condition of selenium determination was listed in Table 2-6.

Table 2-8: Ideal Conditions for Selenium Determination.

	Variable	Ideal condition
1	Lamp current	23 Ma
2	Wavelength	196.0 nm
3	Slit width	0.5nm
4	Lighting mode	BGC-D2
5	Sample Size	20 µl

2.2.6 Determination of Troponin I titer

2.2.6.1 Principle

This test is done by cobas e411 instrument from Roche diagnostic. Its principle based on the competition of analyte in sample with a ruthenium-labeled analogue (Sandwich principle). A voltage is applied and electrochemiluminescence signal is detected [180]**.** Testing was performed according to the manufacturer's instructions.

The troponin titer by cobas e411 assays were calibrated as per manufacturer's instruction by a 2-point calibration using calibrators traceable to pure standard materials. Components of the troponin I titer kit mentioned in Table 2-9.

Table 2-9: Components of the troponin I titer kit

No.	Description	Quantity
1.	Streptavidin-coated microparticles	1 bottle, 6.5 Ml
2.	Anti-cardiac troponin I-Ab (biotinylated monoclonal anti-cardiac troponin I-antibodies)	1 bottle, 10 mL
3.	Anti-cardiac troponin I-Ab (monoclonal anti-cardiac troponin I-antibodies labeled with ruthenium complex)	1 bottle, 10 Ml
4.	Procell (wash buffer)	1 bottle 380 ml
5.	Cleancell (wash clean)	1 bottle 380 ml
6.	Universal diluent	1 bottle 16 ml
7.	Precicontrol multimarker level 1 (low) ready to use	2 vial 3.0 ml
8.	Precicontrol multimarker level 2 (high) ready to use	2 vial 3.0 ml
9.	Cardiac Troponin I STAT calset 1	1 vial 3.0 ml
10.	Cardiac Troponin I STAT calset 2	1 vial 3.0 ml

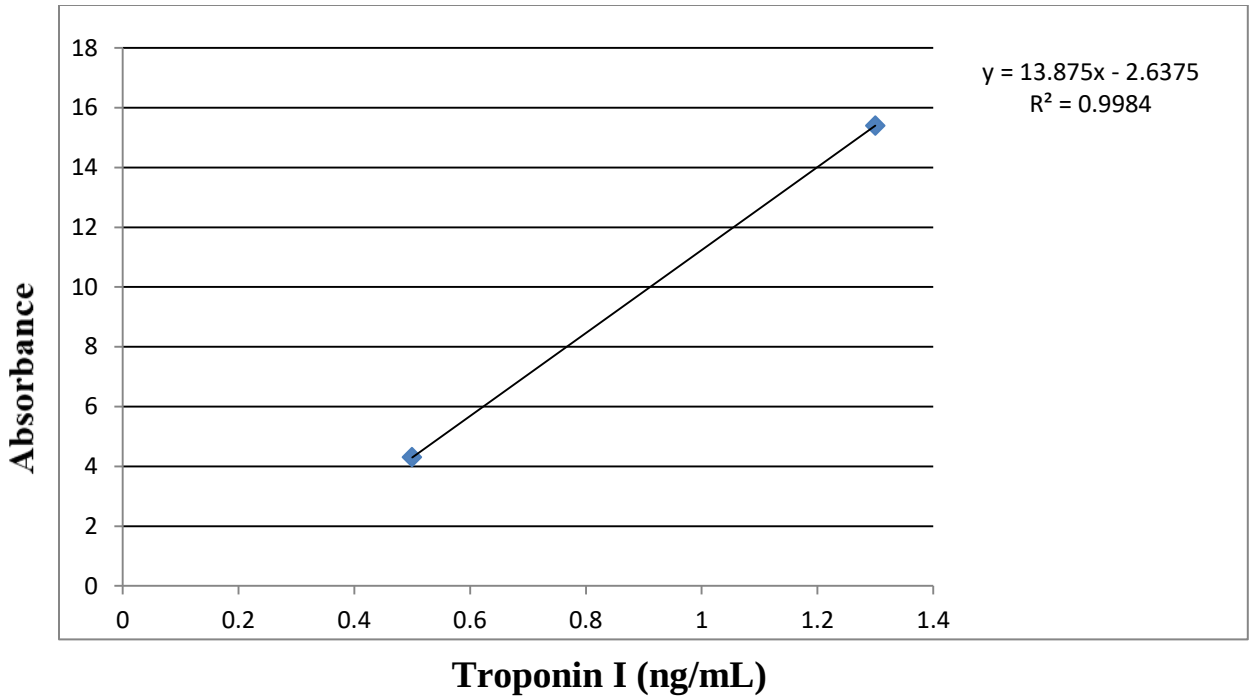

Figure 2-4: Standard Curve for cardiac troponin I

2.2.6.2. Procedure

The procedure was automated in cobas e411 including to steps:-

*First step include automated mixing of 30 µL of sample with 60 µL of biotinylated monoclonal anti-cardiac troponin I antibodies and 60 µL of monoclonal anti-cardiac troponin I antibodies labeled with a ruthenium complex incubated for 5 min to react and form a sandwich complex.

*Second step include automated addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and Streptavidin (4 min incubation).

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Pro-Cell/Clean-Cell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by 2-point. Note that the device has gone through all stages of daily, weekly, monthly routine and maintenance before conducting the test. And the validity of the kit was tested using precicontrol multimarker from roche diagnostic as shown in Table 2-10.

Table 2-10: Precicontrol multimarker results for cardiac Troponin I

	Kit result	Target result
Precicontrol level 1 (low)	1.72 ng/ml	1.69 - 1.78 ng/ml
Precicontrol level 1 (high)	4.51 ng/ml	4.12 - 4.83 ng/ml

2.3 Statistical Aanalysis

All statistical calculations were carried out by the aid of SPSS software (International Business Machines IBM Corp. Released 2022. IBM SPSS Statistics for Windows, version 23. Armonk, NY: IBM Corp. United states of America USA) and Microsoft Excel (2013 Microsoft Corp. USA). The results were expressed as mean ± SD. P value < 0.05 is considered as statistically significant. Student's t-test has been used to determine the significant difference between the study groups. While Pearsone correlation test was employed to assess the correlation between study parameters. Odd ratio for risk factors estimated using logistic regression (cross tabulation).

CHAPTER THREE

Results and Discussion

3.1 General Characteristic of AMI Patients

3.1.1 Age

The mean age ±SD of AMI patients were (61.4±9.2 years). Age distributions of AMI patients were demonstrated in figure 3-1

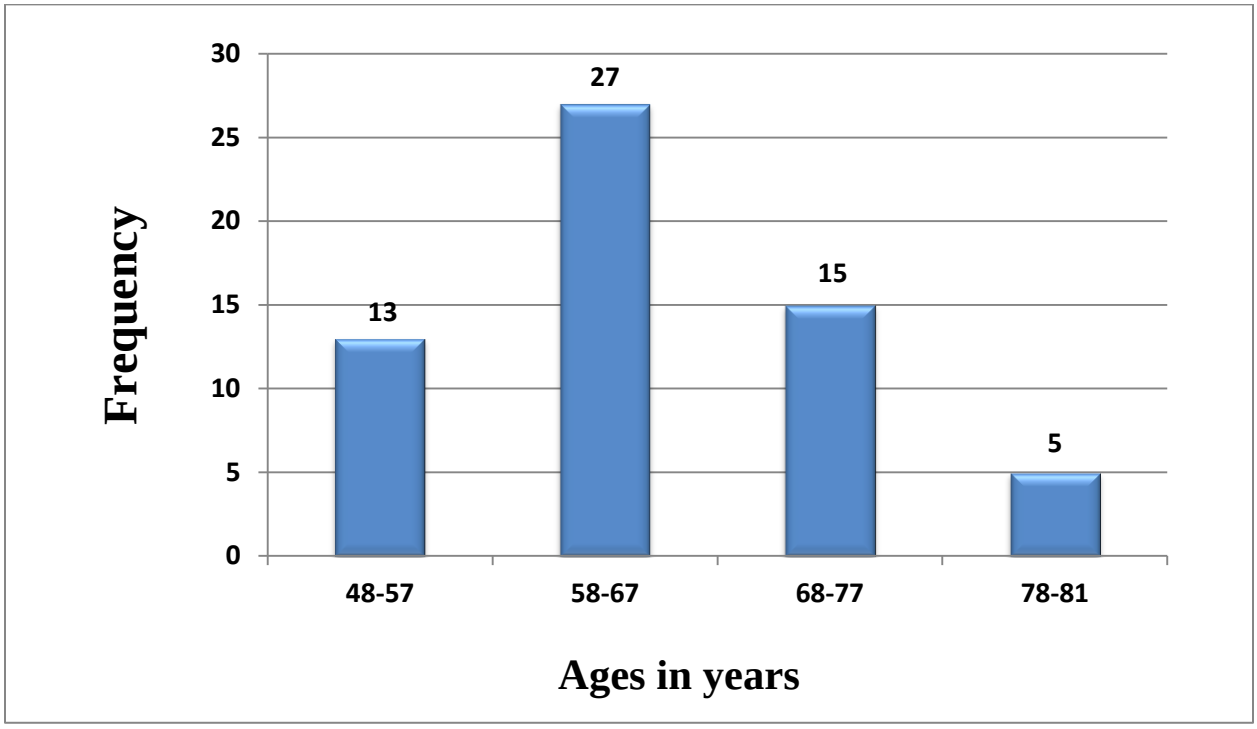

Figure 3- 1:- Age Distributions among Patients with AMI

As illustrated in Figure 3-1, the distribution of age among AMI patients in this study reveals a higher imcedence occurring between the ages of 58 to 67 years, while the lowest incidence is observed within the age range of 48 to 57 years. These observations in agreement with the findings of a pervious study conducted in Iraq by Shwan Othman Amen *et al.* in 2020, which also reported the highest occurrence of acute myocardial infarction within the same age category [181].

The high incidence of acute myocardial infarction in interval between 58 to 67 years age group founded in current study is lower than the age groups category concluded in previous studies, such as Mustafa Kemal Erol, *et al.*2020. in Turkey [182] and Elizabeth R C Millett, *et al.*2018. in United Kingdom [183].

This difference in high prevalence of AMI with specific age category in the present study when compare to previous studies in other countries The younger age of AMI patients in Iraq than in other countries may be due to higher prevalence of risk factors (smoking, obesity), unhealthy diets, genetic predispositions, limited healthcare access, and environmental stressors. These factors collectively contribute to earlier onset of heart conditions in the Iraqi population.

There was a no significant difference in this study in the mean age between AMI patients and control group (P value > 0.05) as shown in Table 3-1.

Table 3-1: Age Means of AMI Patients Compared to Control.

	Group	No.	Mean ± SD
Age (years)	STEMI	27	61.4 ± 9.2
	non-STEMI	33	60.8± 8.0
	Control	60	62.4 ±9.4
P-value	**STEMI versus Control group (P > 0.05)** **non-STEMI versus Control group (P > 0.05)** **STEMI versus non-STEMI group (P > 0.05)**		

3.1.2 Body Mass Index

The mean of body mass index (BMI) ±SD of AMI patients were (29.7±3.2) Kg/m2 while for controls group was (28.7±2.1) Kg/m2. As shown in Table 3-2.

Table 3-2: BMI Means of AMI Patients Compared to Control.

	Group	No.	Mean ± SD
BMI Kg/m2	STEMI	27	30.2 ± 2.7
	non-STEMI	33	29.2± 3.3
	Control	52	28.7+2.1
P-value	**STEMI versus Control group (P > 0.05)** **non-STEMI versus Control group (P > 0.05)** **STEMI versus non-STEMI group (P > 0.05)**		

These finding in agreement with pervious study done in Sulaimani City-Iraq by Bayan Omar Sharif, *et al.*2021. That also found significant difference in BMI between AMI patients and control group [184]. Another study done by Hind S Ahmed, *et al.* 2020. also concluded a higher mean of BMI in AMI patients when compared to healthy control category [185]. BMI is often used to quantify overweight and obesity owing to a high fat percentage. Excessive visceral fat is associated with an increased risk of developing metabolic syndrome, atherosclerosis and consider primary risk factor for AMI. Overweight and obesity are contributed to different chronic disease such as heart disease, nonalcoholic fatty liver disease and inflammatory bowel disease [186].

3.2 Risk Factors

3.2.1 Modifiable Risk Factors

3.2.1.1 Hypertension

According to the American Heart Association, hypertension (HTN) is characterized by a chronic elevation of systemic arterial pressure above a certain threshold value (≥130 mm Hg systolic or ≥80 mm Hg diastolic) [187]. In the current study, 47 out of 60 cases were suffering from hypertension as shown in figure 3-2 and in Table 3-3.

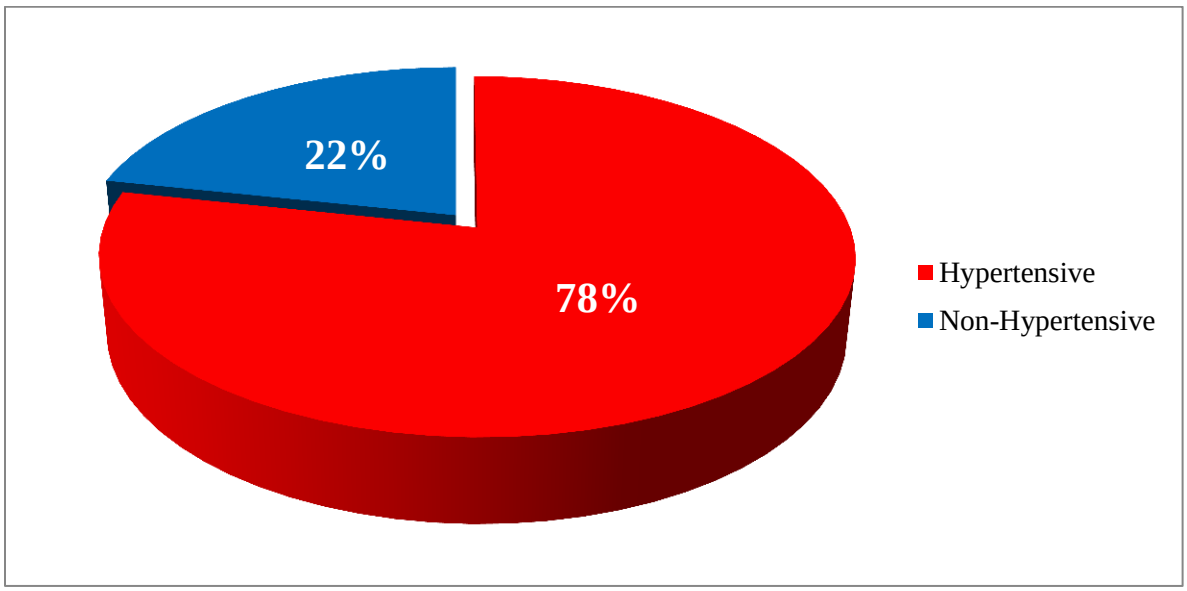

Figure 3- 2: Hypertensive Distribution among Patients with AMI

The percentage of cases with AMI accompanied with hypertension accounted for 78% of the total cases included in the current study. These results were in agreement with Amen SO, *et al.* 2021.which found approach result when study association of hypertension with myocardial infraction in Iraqi patients [188]. Another previous study done by SH Yousif, *et al.* 2020. That also found approach result while studying different risk factors of AMI among Iraqi patients [189].

Hypertension is regarded as one of the main factors leading to atherogenesis and the development of atherogenic plaques which in turn results in thrombosis or vascular rupture and leads to develop AMI [190]. Hypertension accelerates the effects on atheroma, increases stress on plaques, exerts adverse functional effects on the coronary circulation, and impairs endothelial function and control of sympathetic tone [191].

A number of previous studies in different regions of the world found lower percentages of the association of high blood pressure with myocardial infarction such as Afreen, *et al.*2019 in Pakistan (37.7%) [192], Aydın G, *et al.*2020 in Tturkey (49%)[193] and SITEPU R, *et al.*2019 in Indonesia (42.6%) [194]. the findings of current study confirm an alarming high prevalence of Hypertension and its strong association with increasing risk of AMI among Iraqi patients.

Table 3-3: Means of BP of AMI Patients Compared to Control.

	Group	No.	Systolic mean ± SD	Diastolic mean ± SD
BP mmHg	STEMI (27)	25	169.5 ± 3.4	106.1 ±2.5
	non-STEMI (33)	22	164.9± 2.7	103.3±3.1
P-value	**STEMI versus non-STEMI group (P > 0.05)**			

3.2.1.2 Smoking

Heavy smokers define as those who smoke greater than or equal to 25 or more cigarettes a day for more than ten years [195]. According to the history of AMI patients participated in this study 38 patients out of 60 were current heavy smoker for more than ten years. The percentage of smokers to non-smoker is represented in the following Figure 3-3.

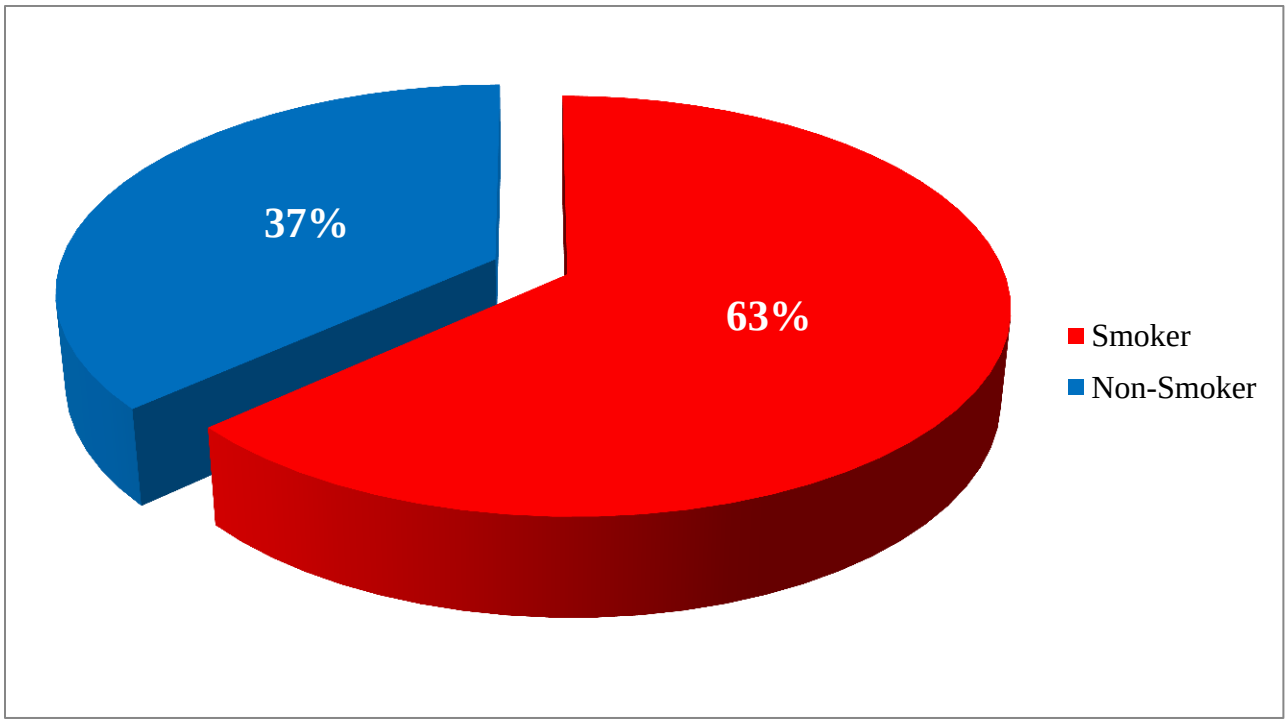

Figure 3- 3:- Smoking Distribution among Patients with AMI

This finding of current study as shown in Table 3-4. is in agreement with Gisela Feltes, *et al.*2020[196] and K. Sushritha, *et al.*2020[197] were they found higher percentage of AMI infraction was classify as a heavy smokers.

Smoking, a significant modifiable risk factor for AMI, exerts its influence on the cardiovascular system through diverse mechanisms. It plays a pivotal role in AMI development by disrupting the usual equilibrium between oxidants and antioxidants, instigating systemic oxidative stress. Smoking directly damages cells and tissues deactivates defensive mechanisms, initiates inflammation, thereby setting off a sequence of events culminating in plaque formation and hastening atherogenesis. This process, coupled with heightened platelet aggregation and a hypercoagulable state, contributes to the pathogenesis of AMI [198]. Moreover, smoking triggers an increase in heart rate and blood pressure by activating the sympathetic nervous system. Simultaneously, it elevates oxygen demand while inducing vasoconstriction, subsequently reducing oxygen supply [199].

Table 3-4: Number of Smoker of AMI Patients Compared to Control.

	Group	No.
Smoking	STEMI (27)	18
	non-STEMI (33)	20
	Control (60)	9
P-value	**STEMI versus Control group (P < 0.001)** **non-STEMI versus Control group (P < 0.001)** **STEMI versus non-STEMI group (P > 0.05)**	

Also, smoking increases oxidation of LDL cholesterol and interferes with endothelial function. Increase in inflammatory factors and acceleration of atherogenesis in combination with increased platelet aggregation and hypercoagulable state contribute to pathogenesis of coronary disease in smokers [200].

3.2.1.3 Physical Inactivity

According to the data collecting in current study 50 patients with AMI participating in the study were not regularly engaged in any physical activity in the form of sports activity, leisure time physical activity, walking, occupational activity or house holding activity as shown in Table 3-5 and figure 3-4.

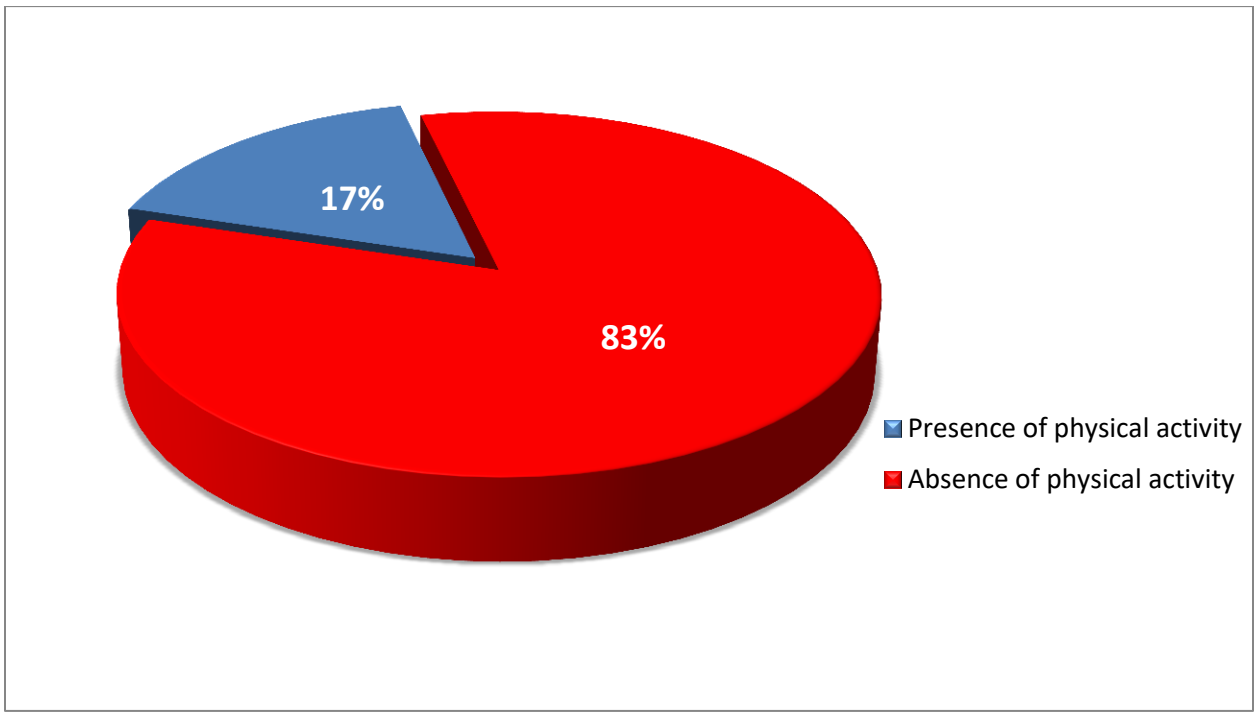

Figure 3- 4:- Physical Inactivity Distribution among Patients with AMI

A large number of previous studies have found the importance of physical activity for preventing different type of CAD such as AMI. EB Winzer, *et al.*2018. found that leisure-time physical activity is effective in the primary prevention of AMI[201]. M Hamer, *et al.*2019 concludes that Physical activity was associated with reduced risk of AMI and there is negative relationship between AMI and physical activity [202].

There are many desired benefits of physical activity and its effect on the heart muscle by protecting it from myocardial infarction such as: - increases cardiac contractility to supply the raised demand of oxygenated blood, enhanced proliferation and division of differentiated cardiomyocytes, increases in cardiac mitochondrial energy capacity [203].

Physical activity has demonstrated the ability to elevate circulating catecholamines, thereby enhancing the expression of β3 adrenergic receptors (β3-AR). Activation of β3-AR subsequently triggers endothelial nitric oxide synthase phosphorylation, elevating cardiac NO metabolite levels (such as nitrite and nitrosothiols), contributing significantly to the cardioprotective effects during ischemic heart conditions [204]. Additionally, physical activity induces the peroxisome proliferator-activated receptors (PPAR) pathway, essential for regulating cardiac hypertrophy and modulating fatty acid metabolism. This exercise-induced increase in PPAR-alpha levels diminishes the inflammatory response, reducing tumor necrosis factor (TNF-alpha) and nuclear factor kappa (NF-kB) levels. Crucially, PPAR-alpha activation downregulates inflammatory molecules, diminishes infarct size, and boosts fatty acid oxidation. The collective outcome encompasses reduced serum triglycerides (TG), elevated high-density lipoprotein (HDL), and enhanced cholesterol efflux [205].

Table 3-5: Physical Inactivity of AMI Patients Compared to Control**.**

	Group	Physical Inactivity
Study Groups	STEMI (27)	23
	non-STEMI (33)	27
	Control (60)	16
P-value	**STEMI versus Control group (P < 0.05)** **non-STEMI versus Control group (P < 0.01)** **STEMI versus non-STEMI group (P < 0.05)**	

3.2.2 Non-Modifiable Risk Factors

3.2.2.1 Sex

The number of males patients in this study was 42 while the females number was 18 the percentage of males to females in AMI patients in this study represented in the following figure (3-5) and in Table 3-6.

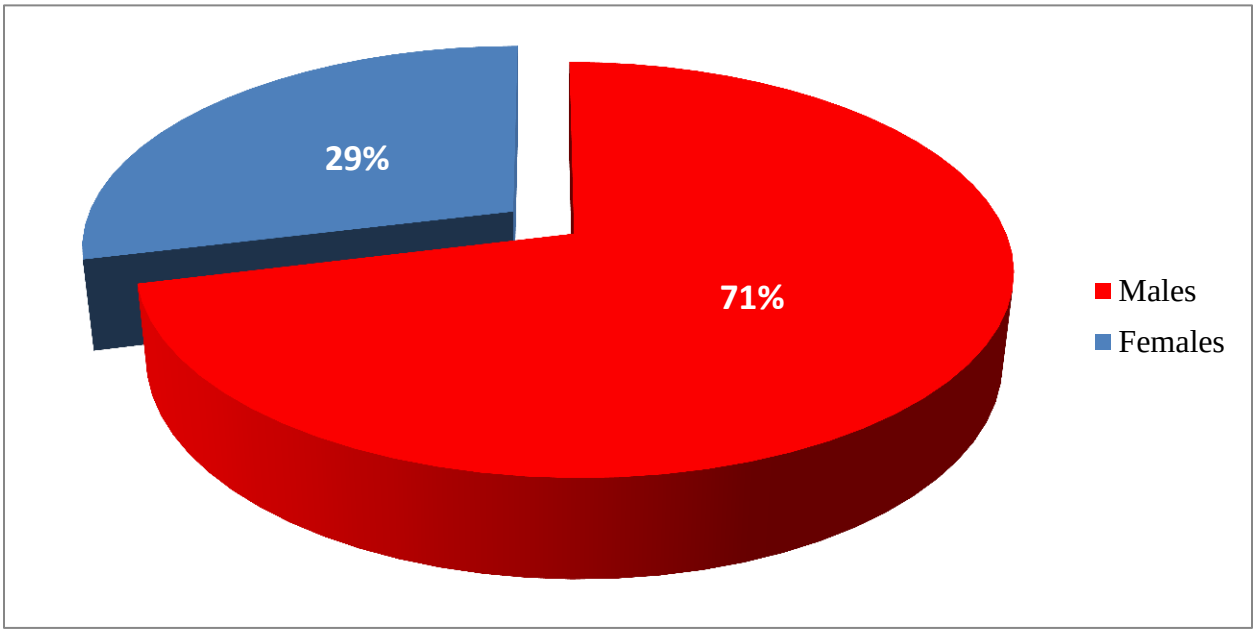

Figure 3- 5:- Sex Distribution among Patients with AMI

In this study, males exhibit a higher percentage of patients compared to females, consistent with findings from other studies conducted in Iraq such as Ali BM, *et al.* 2021 [206] in Al-Najaf Governorate and Albustany O, *et al.* 2021 [207] in Kirkuk Governorate, which similarly reported a greater prevalence of males among AMI patients. This trend of male predominance in myocardial infarction cases is observed consistently across various countries. A meta-analysis conducted by Tayyab Shah, *et al.* 2021 [208], incorporating data from 56 previous studies, consistently reveals a majority of studies demonstrating analogous outcomes.

Many factors may influence the initiation and prognosis of AMI and that factors differ from males to females. Saraschandra Vallabhajosyula, *et al*.2020 suggest that gender is a factor that independently influences the incidence, and progression AMI [209]. Sex disparity in the incidence of AMI in this study could be back to hormonal differences between male and female which suggest that estrogen have a protective role in females as cardiomyocyte cell express different types of estrogen receptors [210] while testosterone increase risk for AMI as testosterone was positively associated with thromboembolism [211].

Table 3-6: Sex Distribution among patients Group

	Group	Males	Females	P value
Sex	STEMI (27)	20	7	P < 0.001
	non-STEMI (33)	22	11	P < 0.001

3.2.2.2 Family History

According to the data collecting in current study 42 patients with AMI participating in current study had a positive family history from first-degree relative or positive family history from second-degree relative with 18 patients had negative family history the percentage of positive to negative family history shown in figure (3-6) and in Table 3-7.

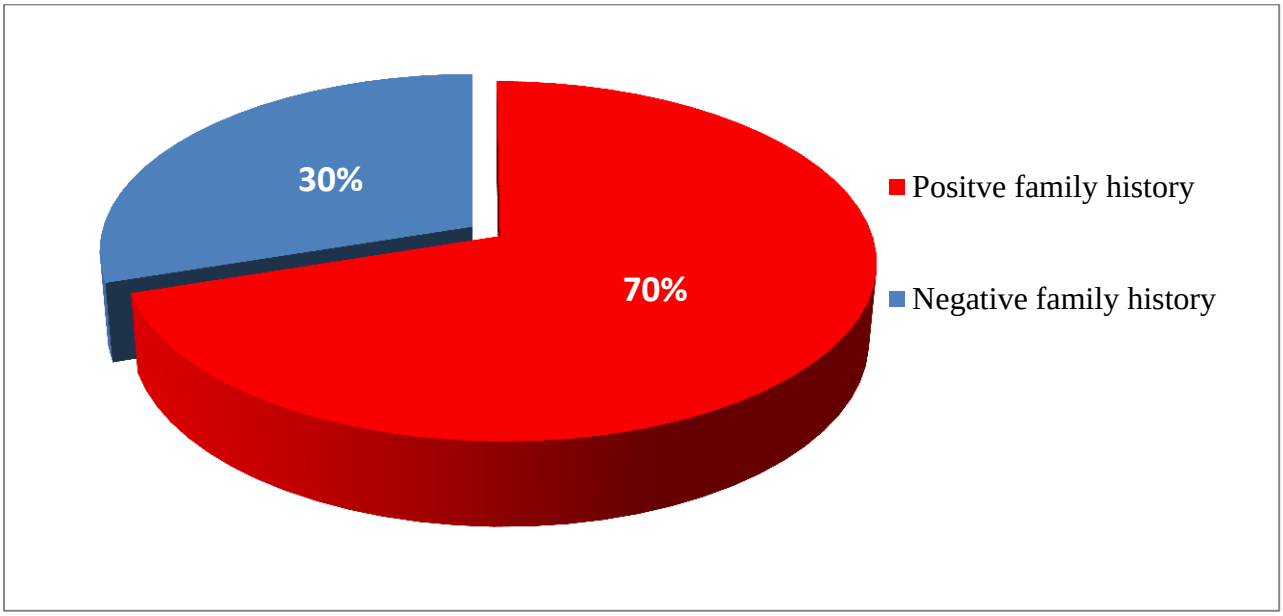

Figure 3- 6:- Family History Distribution among Patients with AMI

Family history is an independent risk factor for AMI. Several genetic variants are associated with increased risk of AMI and family history of AMI in a first-degree relative doubles AMI risk [212]. Family history of AMI is an independent of established risk factors such as high blood pressure and elevated plasma lipid levels. DNT Idris, *et al.* 2020 Also conclude that family history is independent risk factor for AMI when they study the characteristics of AMI Patients [213].

Genome studies have identified more than 40 common genetic variants associated with AMI risk. Each individual variant is only modestly associated with disease risk, with each variant estimated to increase risk 6–92%, but it is estimated that most individuals carry 20–40 risk alleles [214,215].Said M, *et al*. 2021 conclude that AMI patients with positive family history have double probability of having more than two affected coronary vessels through the infraction [216].

According to what was reached in the previous studies mentioned above, the proposed interpretation suggesting that AMI have inherited causes in addition to environmental causes. And that family history not only increases the risk of AMI, but may increase the severity of infarction already.

Table 3-7: Family History of AMI Compared to Control

	Group	Positive Family History
Study Groups	STEMI (27)	19
	non-STEMI (33)	23
	Control (60)	5
P-value	**STEMI versus Control group (P < 0.001)** **non-STEMI versus Control group (P < 0.001)** **STEMI versus non-STEMI group (P > 0.05)**	

Table 3-8: Risk factors among study groups

	Parameter	Numbers			P value
		STEMI	NSTEMI	Control	
.1	**Hypertension**	25	22	0	STEMI vs non-STEMI **(P > 0.05)**
.2	**Smoking**	18	20	9	STEMI vs Control **(P < 0.001)** non-STEMI vs Control **(P < 0.001)** STEMI vs non-STEMI **(P > 0.05)**
.3	**Physical Inactivity**	23	27	16	STEMI vs Control **(P < 0.05)** non-STEMI vs Control **(P < 0.01)** STEMI vs non-STEMI **(P < 0.05)**
.4	**Sex (male)**	20	22	30	STEMI **(P < 0.001)** non-STEMI **(P < 0.001)**
.5	**Family History**	19	23	5	STEMI vs Control **(P < 0.001)** non-STEMI vs Control **(P < 0.001)** STEMI vs non-STEMI **(P > 0.05)**

3.2.3 Multiple Risk Factors

Different previous studies suggested that AMI is a multifactorial condition that more than on risk factor combined and contributed to the occurrence and outcome of the disease [217,218].in the current study 40 out of 60 patients had more than one risk factor. The percentage of single to multiple risk factors demonstrating in figure 3-7 and in Table 3-9.

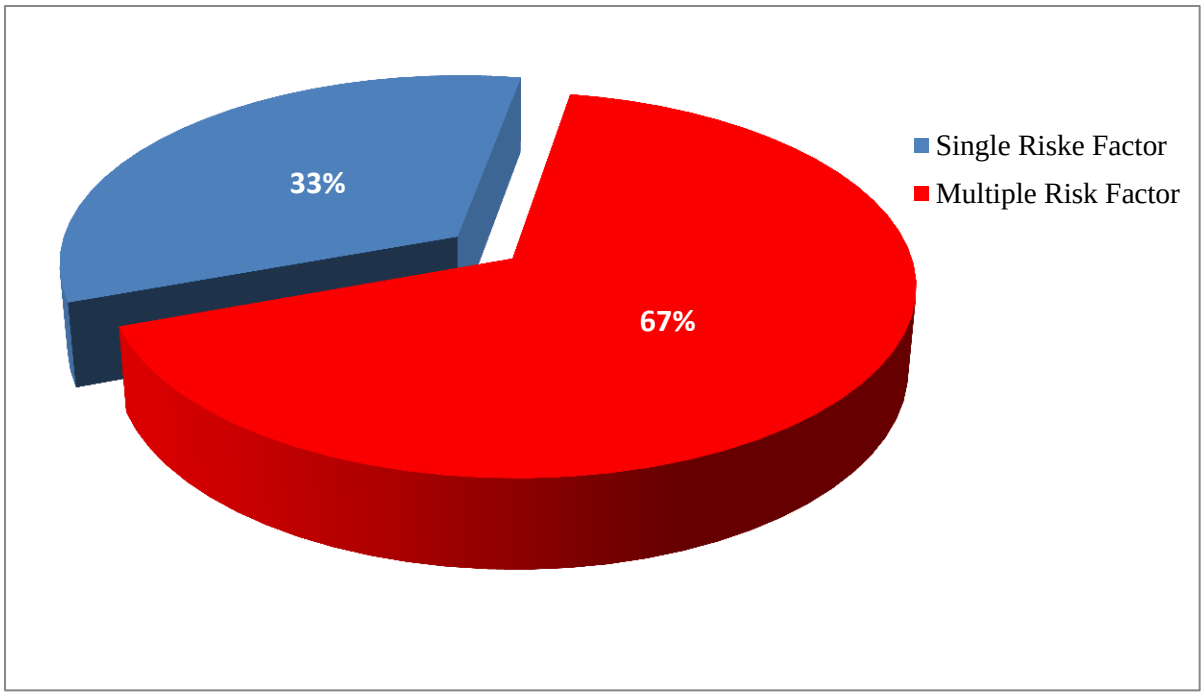

Figure 3- 7:- Single and Multiple Risk Factor Distribution among Patients with AMI

S Kaptoge, *et al.*2019 conclude in there pervious study that the combination of multiple risk factors can double the effect of the risk factor and even increase the risk of having AMI in younger age [219]. Y Zhao, *et al.*2019. also concluded that a combination of more than one risk factor could be directly proportional to the size of the myocardial infarction and thus could lead to worse prognosis than a single risk factor case [220].

In current study major cases with multiple risk factor had STEMI while major cases with single risk factor had NSTEMI , suggesting that multiple risk factor may contribute to complete and prolonged occlusion of coronary blood vessels and/or accelerate ruptured plaque completely blocks a major coronary artery, resulting in extensive heart damage. Among all risk factors studied in this study, hypertension and family history had the highest odd ratio while male gender had the lowest odd ratio as shown in Table 3-9.

Table 3-9: Multiple Risk Factors among AMI Patients.

	Group	Single Risk Factor	Multiple Risk Factor	P value
Multiple Risk Factor	STEMI (27)	2	25	P < 0.001
	non-STEMI (33)	18	15	P > 0.05

Table 3-10: Odd Ratio of Risk Factors for AMI among Study Groups

	Risk factors		AMI	Odd ratio	95% Cl	P value
1.	Hypertension	Yes	47	27.3	10.0-74.3	< 0.001
		No	13			
2.	Smoking	Yes	38	9.7	4.0-23.67	< 0.05
		No	22			
3.	Physical inactivity	Yes	50	13.7	5.6-33.4	< 0.001
		No	10			
4.	Male gender	Yes	42	2.0	0.96- 4.3	> 0.05
		No	18			
5.	Family history	Yes	42	27.8	9.5-81.4	< 0.001
		No	18			

3.3 Biochemical Parameters

3.3.1. Apelin

In this study, serum apelin was significantly higher in AMI patients groups compared with normal subjects (Control group) P-value < 0.001. Serum apelin was significantly higher in STEMI group when compared to non-STEMI group (P-value < 0.001) , as shown in Table 3-11.

Table 3-11: The Mean of Apelin in AMI Compared to Control Group**.**

Parameter	Subjects	No.	Mean	Standard Deviation
Apelin (pg/ml)	STEMI	27	389.3	19.5
	Non-STEMI	33	220.6	16.3
	Control	60	70.7	8.2
P-value	**STEMI versus Control group (P < 0.001)** **non-STEMI versus Control group (P < 0.001)** **STEMI versus non-STEMI group (P < 0.001)**			

Strohbach A,*et al*. 2021 approach a similar result (higher apelin levels in AMI patients) and suggested that apelin had a positive effect on AMI patients by reducing the vascular tone and enhancing cardiac contractile function. Therefore, apelin was found to be increased as a response to severe necrosis and ischemia [221].

Previous study conclude that myocardial cells increase expression of hypoxia-inducible factor-1 (HIF-1) as response to APJ activation by apelin [222],also another previous study demonstrated that apelin and APJ levels are rise during AMI [223]. This suggests that apelin increases in ischemic myocardium and decreases back to its normal level after reperfusion.

High levels of apelin found in STEMI group higher than NSTEMI group; this is consistent with what was previously mentioned about the fact that an increase in apelin production is a normal response during events that lead to AMI, as apelin mediated cell surviving through different mechanisms. apelin also production of endothelium-derived hyperpolarizing factor mechanism such as increase expression of certain type of potassium channel and production of C-type natriuretic peptide. These factors causing hyperpolarization of endothelium cells causing relaxation and prevent them from contraction. These factors represent an alternative mechanism for vasodilation when NO vasodilation is inhibited [224]. The larger the infarction, the greater response of apelin releasing, and consequently, the higher levels of apelin.

Guzelburc O,*et al.*2021. Also found higher apelin levels in patients with AMI in a study include different types of ACS in University of Health Sciences Haydarpasa Numune, Turkey [225]. Current study suggested that higher apelin levels represent a reflection for complete occlusion of one or more than one of the coronary arteries, faster cell apoptosis and a higher degree of necrosis of cardiac muscle cells, this gives the apelin great diagnostic and categorical value.

3.3.2 Leptin

In this study, Serum leptin was significantly higher in AMI patients groups compared with normal subjects (Control group) P-value < 0.001. Serum leptin was significantly higher in STEMI group when compared to non-STEMI group (P-value < 0.05), as shown in Table 3-12.

Table 3-12: The Mean of Serum Leptin in AMI Compared to Control Group

Parameter	Subjects	No.	Mean	Standard Deviation
Leptin **(ng/ml)**	STEMI	27	23.5	±2.4
	Non-STEMI	33	15.3	±3.1
	Control	60	6.8	±1.8
P-value	**STEMI versus Control group (P < 0.001)** **non-STEMI versus Control group (P < 0.001)** **STEMI versus non-STEMI group (P < 0.05)**			

Leptin has a motivational effect on the expression of Tumor necrosis factor-alpha (TNF-α).TNF s an important pro-inflammatory mediator and is linked with the risk of recurrent myocardial infarction [226,227].

Evidence suggests that hyperleptinemia can trigger an inflammatory response by activating TNF-α via MAPK (mitogen-activated protein kinases) pathway in adipose tissues especially in multiple risk factors patients. the inflammatory response by TNF-α contribute to cardiomyocyte apoptosis and cardiotoxicity [228].

Another possible mechanism to explain the high leptin levels in AMI patients is the role of leptin in atherogenesis. Leptin transmits a signal by binding to its receptor LepR, which is mainly found in the hypothalamus but is also expressed in macrophage, endothelial cell, and smooth muscle cells, leptin increased accumulation of cholesteryl ester via upregulation of acyl CoA:cholesterol acyltransferase-1 (ACAT-1), an enzyme catalyzing cholesteryl ester synthesis. Leptin was also found to suppress HDL-mediated cholesterol efflux [229].The leptin results in this study is in agreement with AH Syed, *et al*. 2020 that also found high leptin result in AMI patients [230]. Leptin in addition to its angiogenic activity, leptin increases oxidative stress in endothelial cells by induce reactive oxygen species (ROS) [231].

Leptin also stimulates platelet aggregation and atherothrombosis platelets recently were shown to express the long form of the leptin receptor , thus, it hypothesized that leptin altered the thrombotic response to injury, effecting on the levels of plasminogen activator inhibitor-1 suggest that both impaired fibrinolysis and enhanced coagulation may contribute to platelet aggregation [232].

Leptin decreases arterial distensibility, and contributes to obesity-associated hypertension. These factors correlate negatively with vascular health and are strongly involved in the pathophysiology of atherosclerosis and the development of AMI [233].another previous study done by Faulkner JL,*et al.*2019 conclude that leptin increase secretion of aldosterone hormone causing hypertension in patients with hyperleptinemia as adrenal gland specially zona glomerulosa express plenty numbers of leptin receptors. what makes leptin directly contribute to the emergence of one of the most important risk factors of AMI which is hypertension [234].

In current study there was significance difference (P value < 0.05) in the level of leptin between STEMI and non-STEMI, suggesting that higher leptin level is associated with complete and prolonged occlusion of a coronary blood vessel that cause STEMI. While moderate elevation level of leptin associated with NSTEMI usually results from severe coronary artery narrowing, transient occlusion, or microembolization of thrombus and/or atheromatous material. These results are in concordant with E Belik, *et al*.2021 which found similar association while studying degree of lesion of AMI [235].

Overall, the inflammatory response arising from the effect of leptin to cardiomyocytes, endothelial cells, fibroblasts, and smooth muscle cells and by infiltrating leukocytes, platelets, increased sympathetic activity and macrophages can impact the structure and function of the myocardium, and systemic inflammation also impacts vascular function, Which is the starting spark for the sequential events of AMI, The higher the leptin concentration, the stronger the inflammatory response, the larger the infarct size [234,235].

3.3.3 Homocysteine

In this study, homocysteine was significantly higher in AMI patients groups compared with normal subjects (control group) P-value < 0.001. Homocysteine was significantly higher in STEMI group when compared to non-STEMI group (P-value < 0.001), as shown in Table 3-13.

Table 3-13: The Mean of Serum Homocysteine in AMI Compared to Control Group**.**

Parameter	Subjects	No.	Mean	Standard Deviation
Homocysteine (µmol/L)	STEMI	27	45.7	±7.4
	Non-STEMI	33	27.5	±4.3
	Control	60	9.6	±3.1
P-value	**STEMI versus Control group (P < 0.001)** **non-STEMI versus Control group (P < 0.001)** **STEMI versus non-STEMI group (P < 0.001)**			

Previous studies connect between elevated homocysteine level and ACS. Karger AB, *et al*.2020 shown that homocysteine promote osteogenic differentiation and calcium deposition in coronary artery, and homocysteine was found at higher levels in calcified human atheroma, suggesting that elevated homocysteine is a contributor to this pathophysiologic process of vascular inflammation and damage that leads to vascular calcification which accelerate the occurrence of AMI [236].

The findings in this present study in agreement with those of a pervious study by Boras MM, et al. in 2018, which similarly identified elevated levels of Hcy in patients compared to controls while examining the association between Hcy and AMI [237].

One of the most deleterious effects of elevated Hcy is homocysteinylation of Proteins, which are mainly involved in the formation of coronary artery walls. This cause altering the structure of these arteries and this knowing as the athreognic effect of Hcy [238]. Previous study attributed the role of homocysteine in stimulating the production of inflammatory protein such as neoptrin, induce endothelial dysfunction, causing oxidative stress and contributed to plaque raptured, lesions in the coronary arteries, peripheral vasculature and initiating of AMI. Hcy also can impair NO synthesis by complex mechanism which involves inhibition of dimethylarginine dimethylaminohydrolase the enzyme responsible of degradation of dimethylarginine an analogue of L-arginine [239].

In current study there was significance difference (P value < 0.001) in the level of Hcy between STEMI and non-STEMI group. This result is in agreement with A Calim, *et al.*2020 which found higher Hcy in STEMI group than non-STEMI group in study done in Istanbul, turkey [240].

This result shows that an extreme elevation in Hcy levels can contribute to a complete closure of the coronary arteries, and its effect may extend to more than one artery, which leads to an increase in the size of the infarction in the myocardium. According to this concept, the severity of the myocardial infarction can be expected by observing the high concern levels (moderate or severe).

3.3.4 Selenium

In this study, selenium was significantly lower in AMI patients groups compared with normal subjects (Control group) (P-value < 0.001). There was no significantly different in Se level between STEMI and NSTEMI groups, (P-value > 0.05), as shown in Table 3-14.

Table 3-14: The Mean of Selenium in AMI Compared to Control Group**.**

Parameter	Subjects	No.	Mean	Standard Deviation
Selenium (µg/l)	STEMI	27	19.3	± 3.5
	Non-STEMI	33	17.5	±2.9
	Control	60	55.4	±8.6
P-value	**STEMI versus Control group (P < 0.001)** **non-STEMI versus Control group (P < 0.001)** **STEMI versus non-STEMI group (P > 0.05)**			

Selenium has always been an interesting topic for its important role as an antioxidant, and as a cofactor for a large number of enzymes like thioredoxin reductase (TrxRs), which are a family of enzymes involved in cellular redox processes. Se is found in the form of the amino acid selenocysteine within the active site of these enzymes. The presence of selenium is crucial for the enzymatic activity of thioredoxin reductases. Decrease activity of these enzymes as a result of low Se levels causing low anti-oxidant ability of the body in addition to inactivation of virous transcription factor such as Nuclear factor erythroid 2-related factor1 and 2 that involve in production of anti-oxidant protein and mediate cardiac hypertrophy [241].

Another previous study done by Shimada BK,*et al*. 2021 also found lower Se levels in different types of ACS including AMI group when compared to control group [242]. As a cofactor Se have very important rule that protects cells from oxidative damage through its collaborative role via Se-dependent glutathione peroxidases. Se depletion is observed with the decreased activities of glutathione peroxidase inclusive of platelets and arterial partitions. This enzyme has critical capabilities in removing free radicals protecting the coronary epithelium from oxidative harm [243]. Another suggested mechanism explain how low Se levels contribute to the initiation of cascade events which leading to AMI had been described by Sun H,*et al.*2021. Which states that low selenium in cardiomyocytes results in decreased expression of potassium channels, reduced mitochondrial activity and decreased mitochondrial function, which in turn promotes cardiomyocyte apoptosis [244].

Therefore, selenium deficiency can lead to a number of scenarios that contribute significantly to the occurrence of infarction or poor resistance to necrosis. a suggested scenarios is inhibition of normal immune response, increase production of ROS and lipid peroxidation leading to decrease resistance of cardiomyocytes cells to oxidative stress and more prone to artery thrombosis , cell necrosis and accelerated apoptosis [243,244].

3.3.5 Cardiac Troponin I Titer

As a certified biochemical marker, in this study measuring troponin I titer (quantitative) to determine mean ±SD among AMI groups for statistical purposes and to clarify the correlation between cardiac troponin I and the rest of the study parameters.

3.4. Correlation of Cardiac Troponin I Titer with Study Parameters

3.4.1. Correlation between Cardiac Troponin I Titer and Apelin in AMI Patients.

The correlation of cardiac troponin I and apelin was determined by Pearson correlation coefficient (r), the results of the current study were found toward strong positive correlations in STEMI and NSTEMI groups between cardiac troponin I and apelin among AMI patients, figure (3-8) and figure (3-9) showed the correlations between cardiac troponin I and apelin for patients groups (STEMI and NSTEMI), respectively.

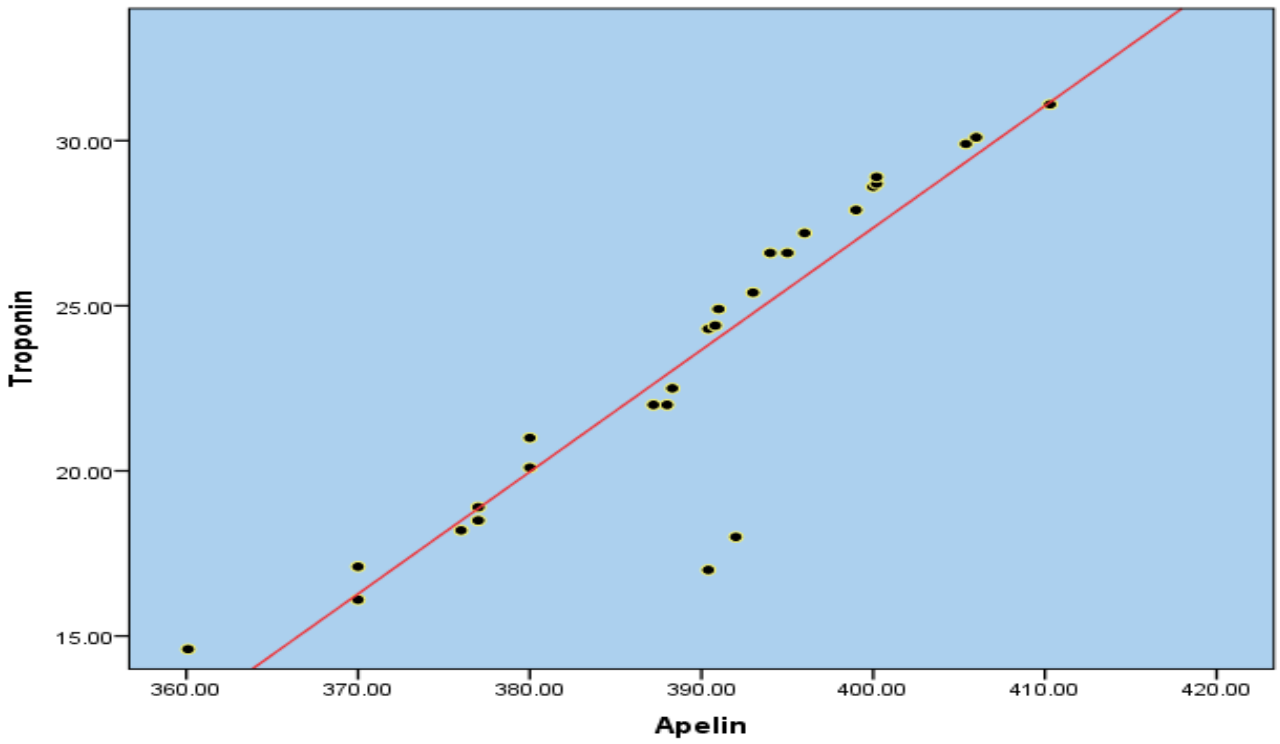

Figure 3-8: Correlation between cardiac Troponin I and Apelin in STEMI Patients. (R=0.910, P value < 0.001)

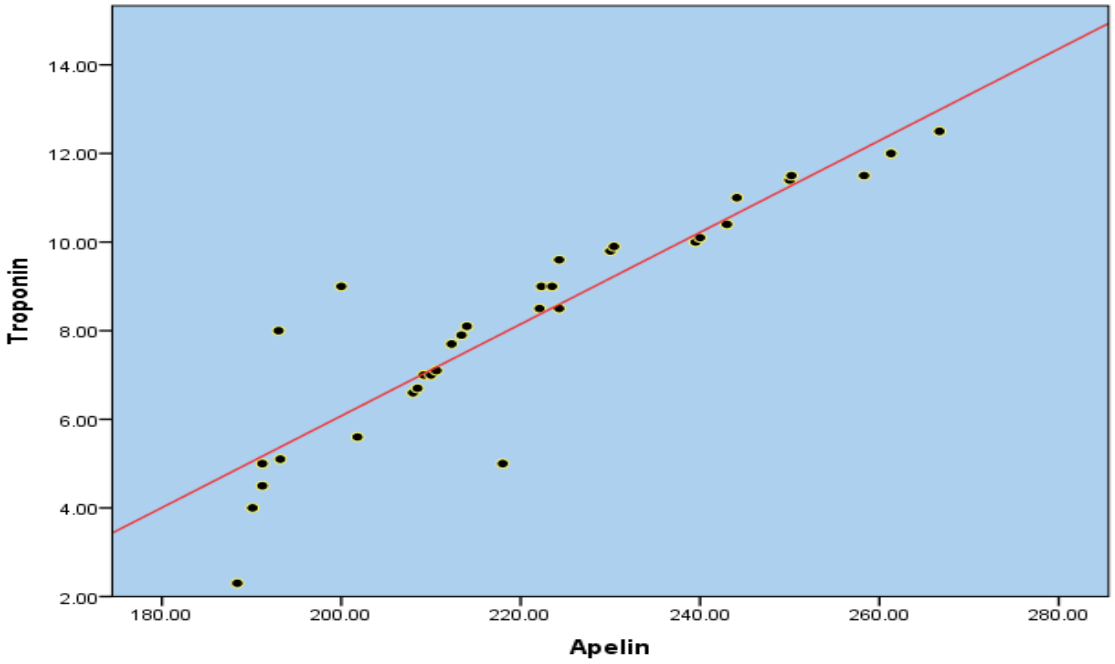

Figure 3-9: Correlation between cardiac Troponin I and Apelin in NSTEMI Patients. (R= -0.902, P value < 0.001)

This strong positive correlation can explain as follow: High levels of cardiac troponin I express greater necrosis in the myocardium, which leads to release higher levels of apelin. Apelin had protective rule for the coronary artery system, such as vasodilation, and Anti-fibrotic. Endothelial damage caused by ischemia leads to the increased vascular permeability, which increases not only water permeability but also protein leakage, and enhances inflammation. Apelin found to alleviate myocardial injury by inhibiting vascular permeability [245]. And for all these benefits, the release of apelin increases from endothelial cell after myocardial damage as a natural response for the cardiomyocyte against necrosis and infraction.

3.4.2. Correlation between Cardiac Troponin I Titer and Leptin in AMI Patients.

The correlation of cardiac troponin I and Leptin was determined by Pearson correlation coefficient (r), the results of the current study were found toward moderate positive correlations between cardiac troponin I and Leptin among AMI patients, P-value < 0.05. figure (3-10) and figure (3-11) showed the correlations between cardiac troponin I and Leptin for patient groups (STEMI and NSTEMI), respectively.

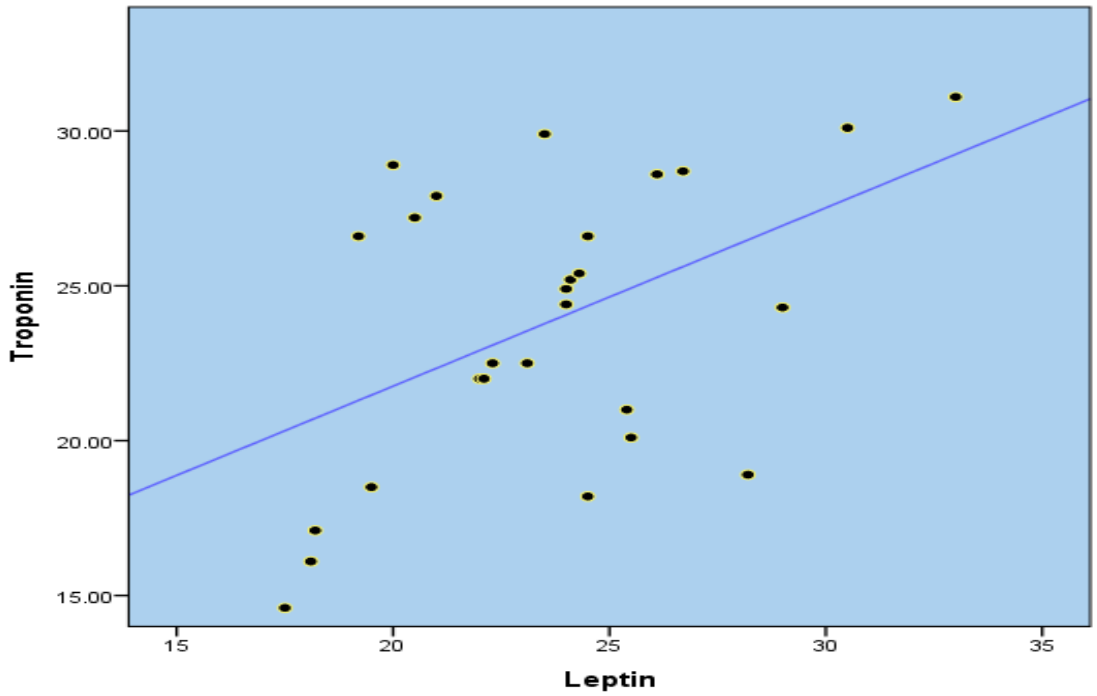

Figure 3-10: Correlation between Troponin I and Leptin in STEMI Patients. (R= 0.476, P value < 0.05).

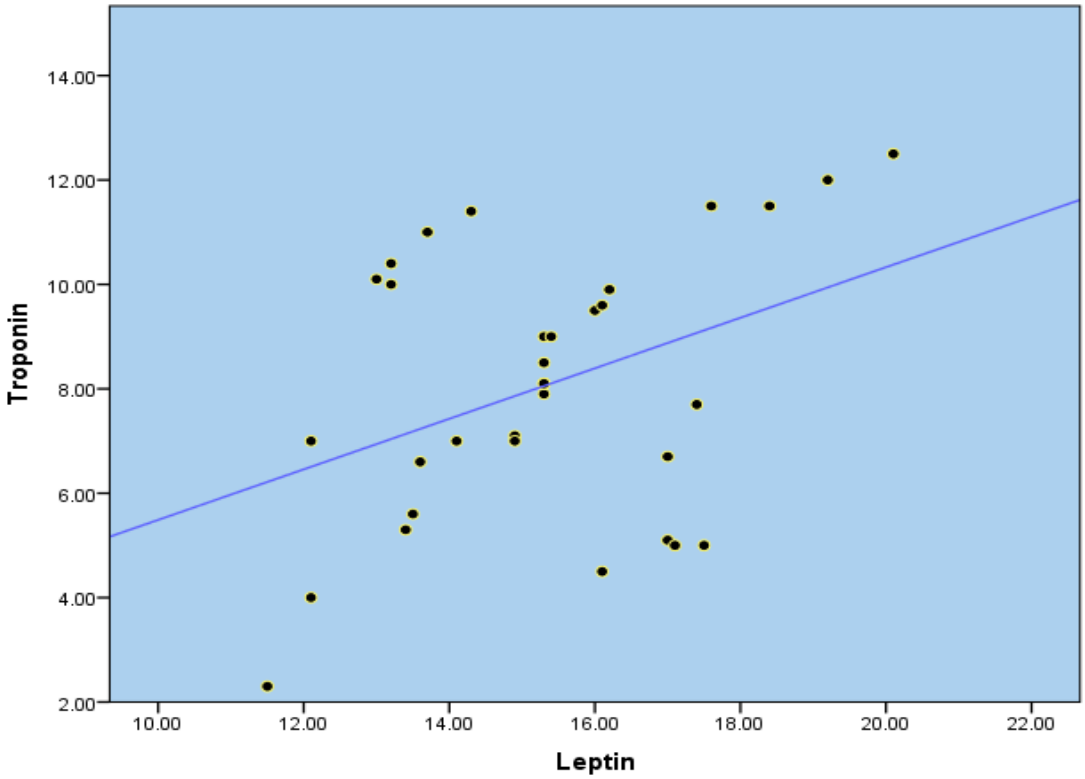

Figure 3-11: Correlation between Troponin I and Leptin in NSTEMI Patients. (R= 0.389, P value < 0.05)

This moderate positive correlation can explain as follow: higher leptin levels are associated with higher cardiac troponin I levels as leptin increase production of pro-inflammatory cytokine and different types of acute phase protein triggering an inflammatory process and contributing to atherosclerosis and plaque formation, this elevation in leptin levels may contribute to an exacerbation of necrosis volume and elevated cardiac troponin I levels (246). STEMI group had higher leptin and cardiac troponin I levels as observed in this study.

3.4.3. Correlation between Cardiac Troponin I Titer and Homocysteine in AMI Patients.

The correlation of cardiac troponin I and homocysteine was determined by Pearson correlation coefficient (r).

The results of the current study were found toward moderate positive correlations between cardiac troponin I and homocysteine among AMI patients, P-value < 0.05. Figure 3-12 and Figure 3-13 showed the correlations between cardiac troponin I and homocysteine for patients groups (STEMI and NSTEMI), respectively.

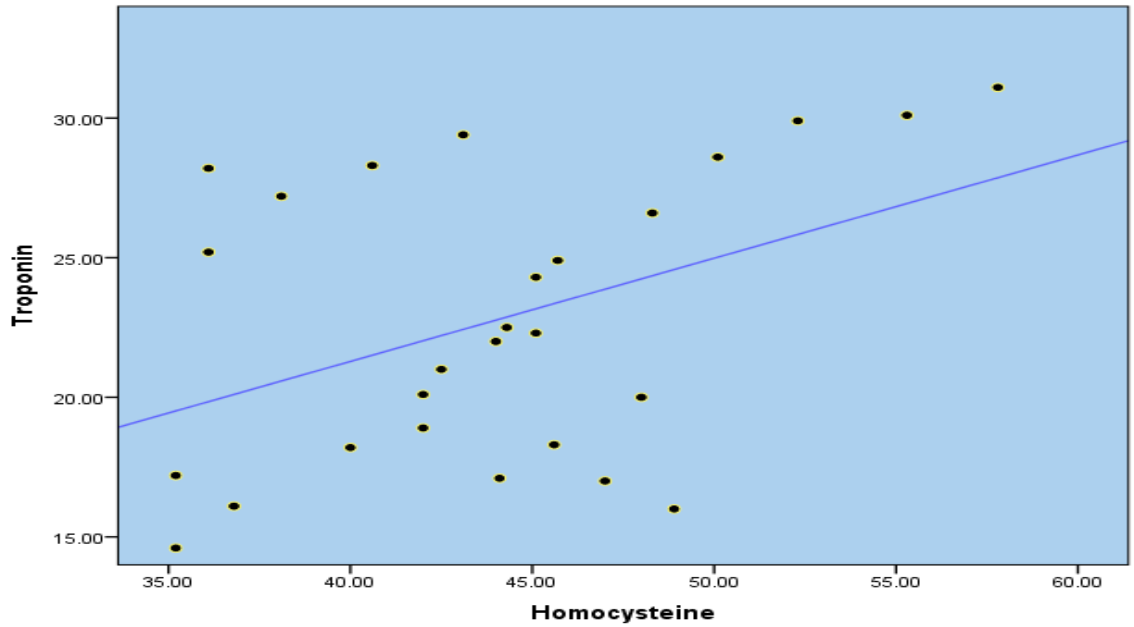

Figure 3-12: Correlation between cardiac Troponin I and Homocysteine in STEMI Patients. (R= 0.424, P value < 0.05)

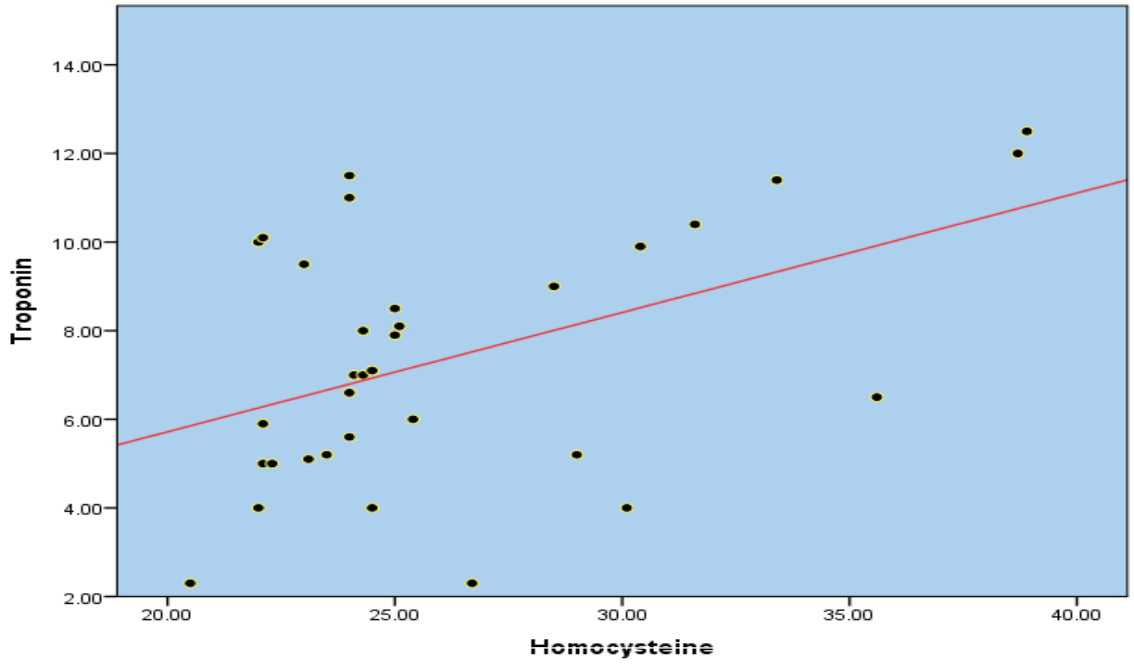

Figure 3-13: Correlation between cardiac Troponin I and Homocysteine in NSTEMI Patients. (R= 0.458, P value < 0.05)

This moderate positive correlation can explain as follow: higher homocysteine levels are associated with higher troponin levels. increased HCY level associated with more rapid development of thrombotic states ,which explains the levels in patients with AMI, Hyperhomocysteinemia and because of its prothrombotic effect is associated with platelet reactivity, and increases arterial stiffness , reduces the possibility of methylation, leading to endothelial dysfunction and proliferation of smooth muscle cells in blood vessels, oxidative stress occurrence, inflammation, and inhibition of nitric oxide synthesis in the endothelium [247,248].

3.4.4. Correlation between Cardiac Troponin I Titer and Selenium in AMI Patients.

The correlation of cardiac troponin I and selenium was determined by Pearson correlation coefficient (r), the results of the current study were found toward moderate negative correlations in STEMI group and weak negative correlation in NSTEMI group between cardiac troponin I and selenium among AMI patients, figure (3-14) and figure (3-15) showed the correlations between cardiac troponin I and selenium for patients groups (STEMI and NSTEMI), respectively.

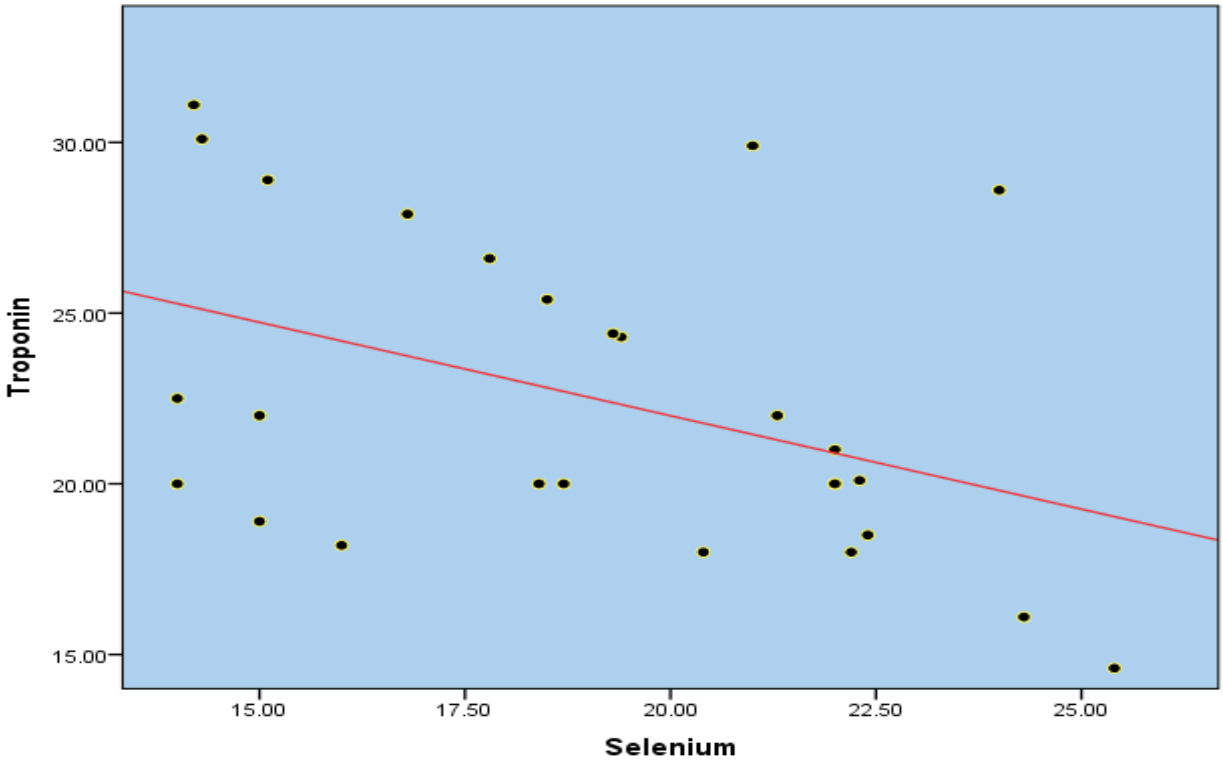

Figure 3-14: Correlation between cardiac Troponin I and Selenium in STEMI Patients. (R= -0.417, P value < 0.05)

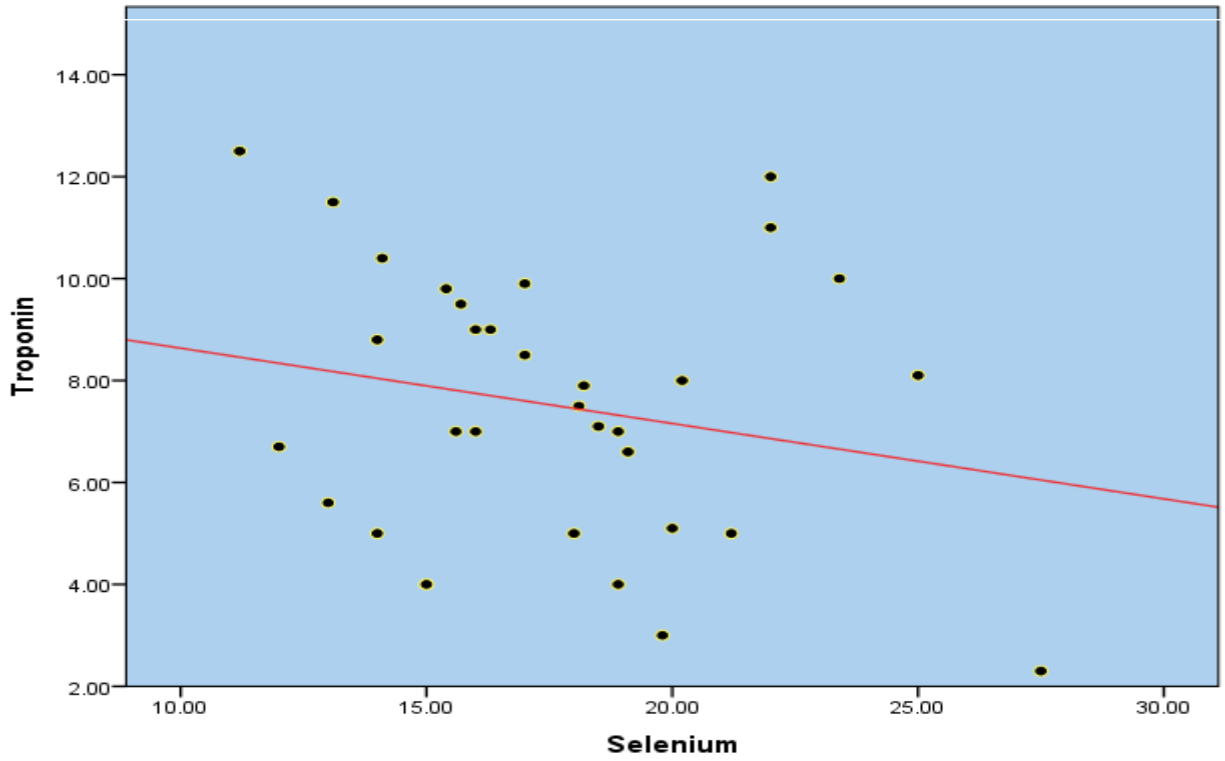

Figure (3-15): Correlation between Troponin I and Selenium in NSTEMI Patients. (R= -0.208, P value > 0.05)

This negative correlation can explain as follow: High levels of cardiac troponin I express greater necrosis in the myocardium, which leads to a higher consumption of selenium, given its important role as an antioxidant such its role as cofactor glutathione peroxidases and as immune stimulator in a number of seleanoprotien that can be highly active as a natural immune response against necrosis. Lower selenium levels in this study had been found in STEMI group as this group had higher levels of cardiac troponin I titer [249].

Table 3-15: Correlation of study parameters with cardiac troponin I among AMI patients.

Parameter	STEMI	R value	P value	NSTEMI	R value	P value
Apelin	Positive	0.910	< 0.001	Positive	0.902	< 0.001
Leptin	Positive	0.476	< 0.05	Positive	0.389	< 0.05
Homocysteine	Positive	0.424	< 0.05	Positive	0.458	< 0.05
Selenium	Negative	-0.417	< 0.05	Negative	-0.208	> 0.05

3.5 Receiver Operating Characteristic curve of study markers

Receiver Operating Characteristic curve analysis was done compared between AMI patients with healthy control. Depends on these parameters and ROC curve analysis apelin, leptin, and homocysteine were found to have significant diagnostic ability for AMI by ROC curve analysis as shown in figure 3-16. The AUC for apelin was highest and statistically significant when compared to other parameters (0.971) as shown in Table 3-16.

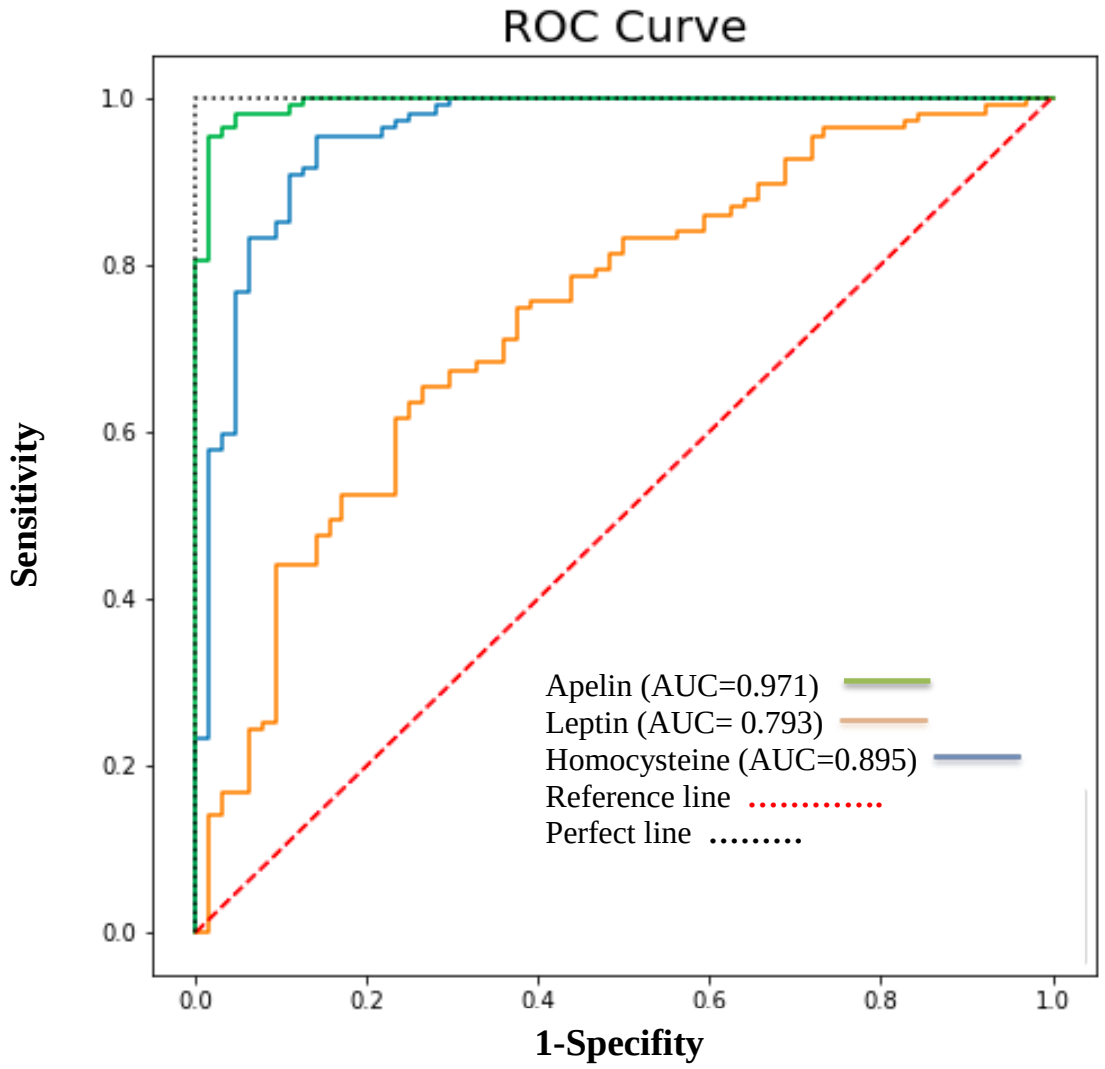

Figure 3-16: ROC curve analysis of apelin, leptin and homocysteine in AMI patients compared to control group.

Table 3-16: Area under the curve for study parameters in AMI patients compared to control group.

Area under the curve AMI with healthy control				
Test Result	**AUC**	**Asymptotic Sig.[b]**	**Asymptotic 95% confidence interval**	
			Lower Bound	**Upper Bound**
Apelin	0.971	0.000	0.913	0.987
Leptin	0.793	0.000	0.610	0.884
Homocysteine	0.895	0.000	0.710	0.923

a-Under the nonparametric assumption

b-Null hypothesis: true area = 0.5

Conclusions

1- Family history and hypertension have strong association with AMI in.

2- The combination of more than one risk factor (multiple risk factors) can contribute to worse prognosis which leads to increase necrosis and thus increase the size of the infarction.

3- The observed rise in biochemical markers in this study suggests their involvement in thrombosis, atherosclerosis, oxidative stress, and their significance in both the onset and prognosis of myocardial infarction. These markers potentially play a crucial role in understanding and managing AMI related conditions.

4- Selenium may serve as a biomarker in monitoring the antioxidant status in AMI patient.

5- The identified correlation between leptin, homocysteine, and cardiac troponin I implies possible diagnostic importance for leptin and homocysteine in evaluating and detecting acute myocardial infarction.

6- The significant correlation between apelin and cardiac troponin I suggests a potentially valuable and definitive role of apelin in diagnosing acute myocardial infarction (AMI) and categorizing patients into either ST-segment elevation myocardial infarction (STEMI) or non-ST-segment elevation myocardial infarction (NSTEMI).

Recommendations

1- Insert new laboratory test panel in the medical center and hospitals including the measuring of leptin, homocysteine and selenium. using this panel as a routine work requested for patients suspected having AMI as confirmatory biomarkers.

2- Using apelin in hospitals and medical centers as a diagnostic and categorical biomarker to determine the type of acute myocardial infarction as an alternative protocol if it is not possible to perform an electrocardiogram.

3- Study the patterns of leptin, homocysteine and apelin as prognosis markers for AMI.

4- Further study including examination of apelin on larger sample size to determine the sensitivity and specificity as marker for AMI.

5- Study different subtypes of apelin and their association with other diseases.

6- Genetic study on leptin and apelin receptors gene polymorphism in AMI infraction among Iraqi population.

7- Study the adipocytokines profile in AMI infraction among Iraqi population.

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Appendix

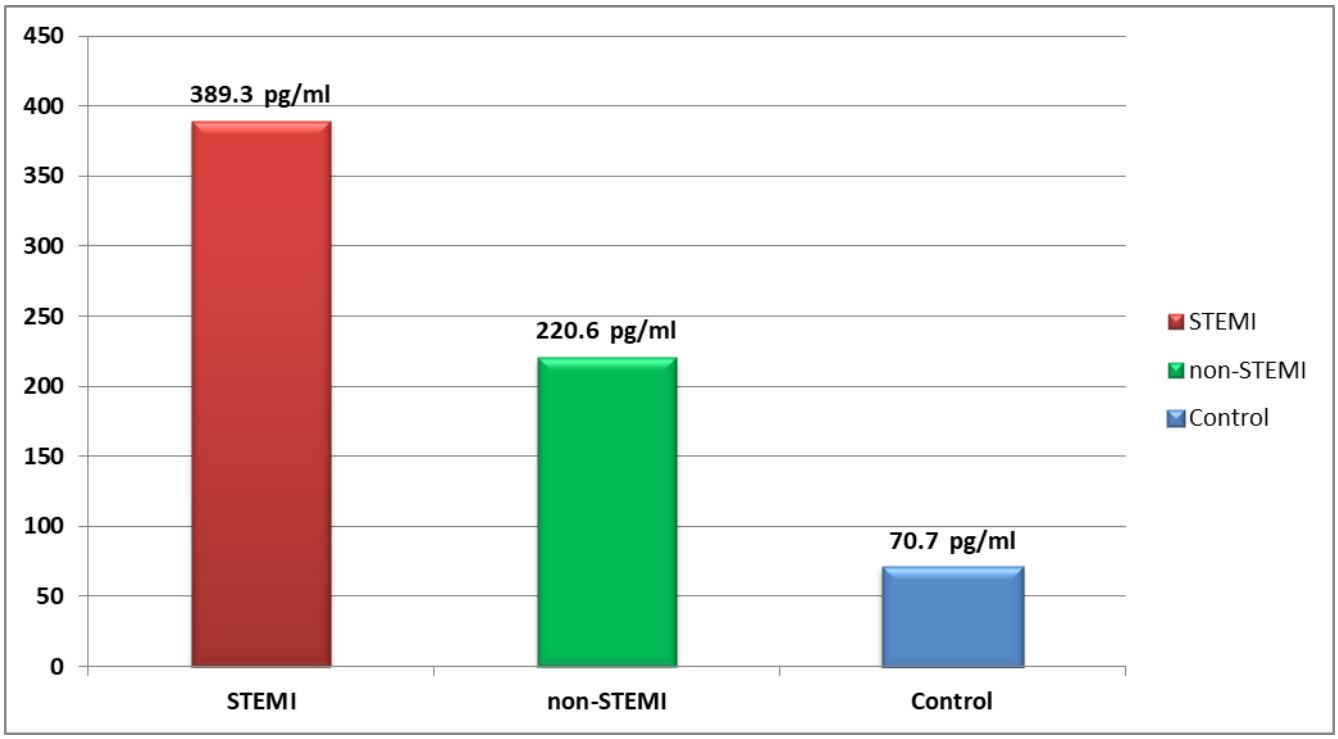

Figure 4-1: The Mean of Apelin in AMI (STEMI and non-STEMI) Compared to Control Group

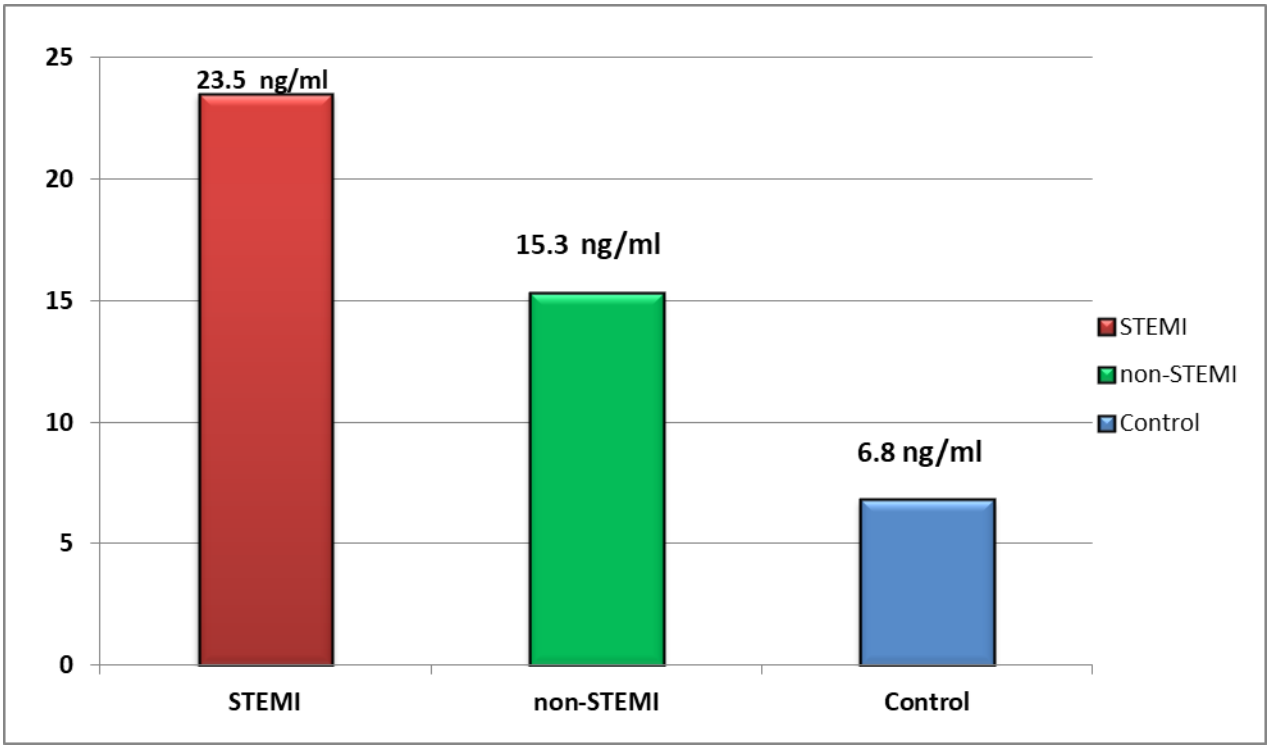

Figure 4-2: The Mean of Leptin in AMI (STEMI and non-STEMI) Compared to Control Group

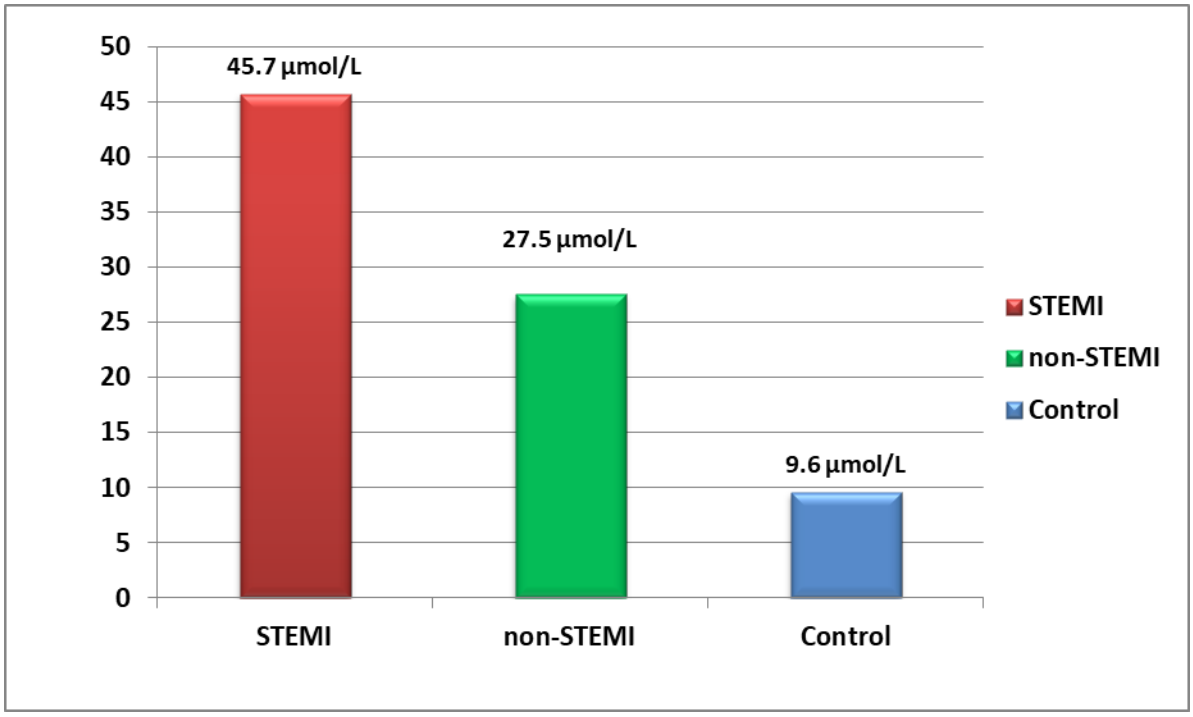

Figure 4-3: The Mean of Homocysteine in AMI (STEMI and non-STEMI) Compared to Control Group

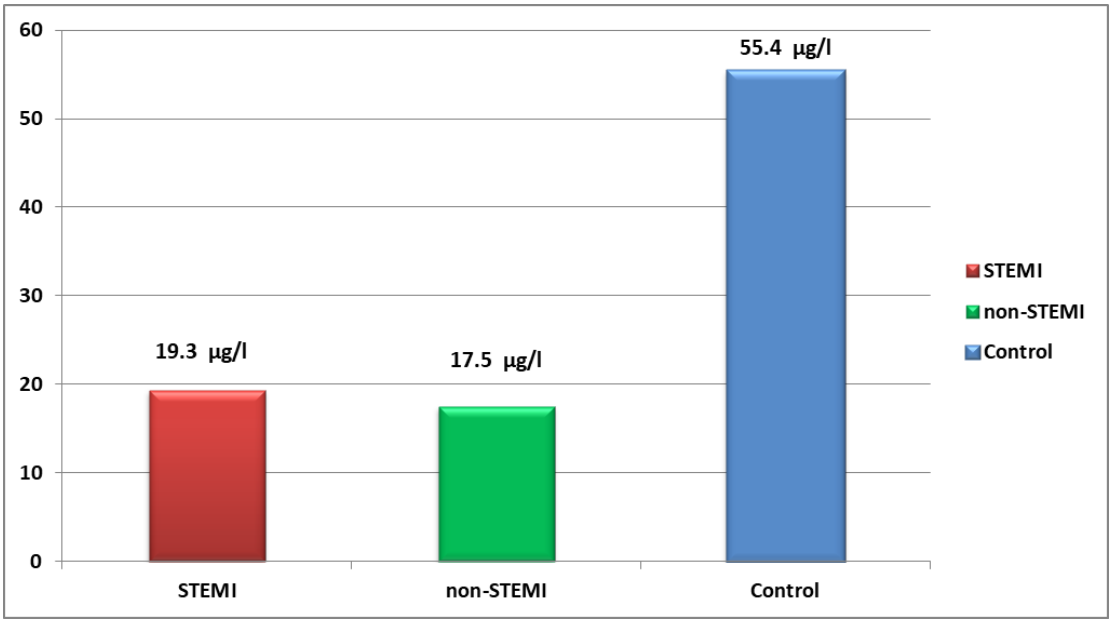

Figure 4-4: The Mean of Selenium in AMI (STEMI and non-STEMI) Compared to Control Group

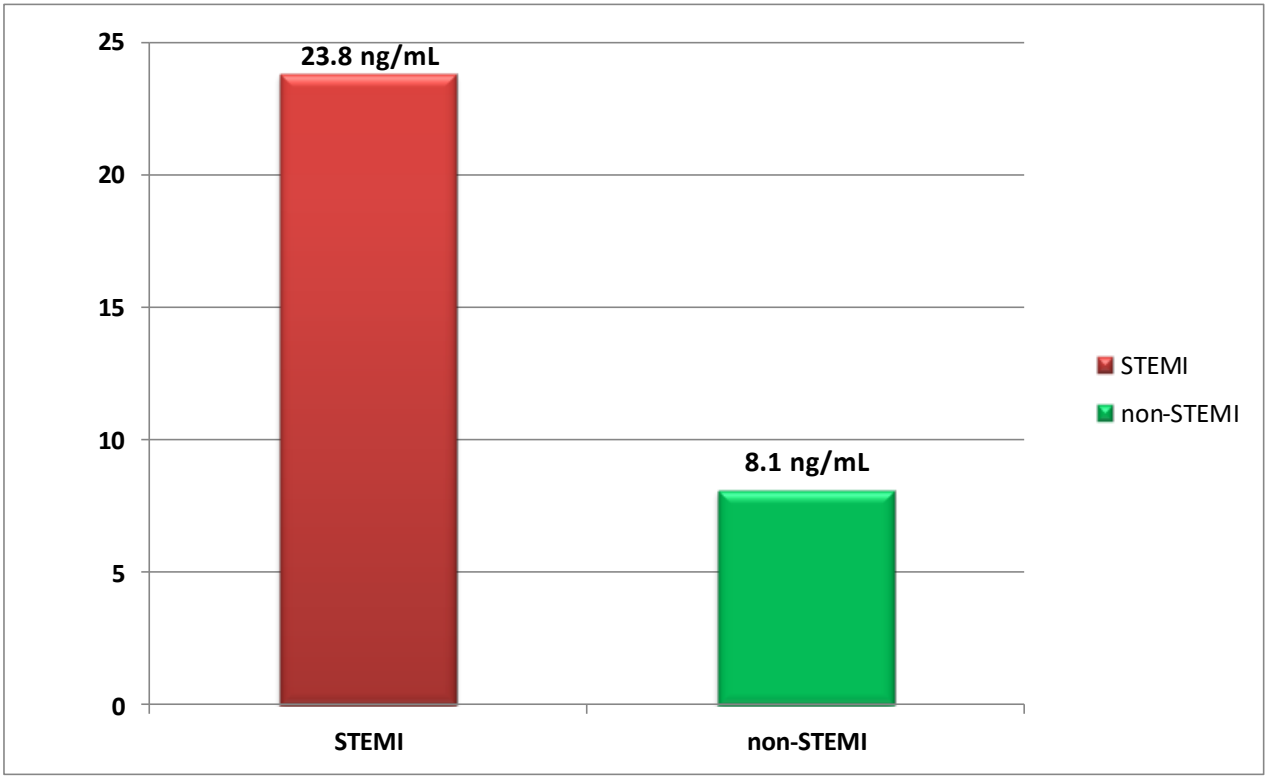

Figure 4-5: The Mean of cardiac Troponin I in AMI (STEMI and non-STEMI).

Questionnaire

Form number…… **Date of diagnoses / /**

Name: …… **Age:** **Sex: male / female**

Address : …… **Mobile Number:**

Hypertension : yes / no **DM: yes / no** **Heart diseases:**

BMI:……. **Smoking : yes/ no**

Family history:…… **History of cancer yes/ no**

Residency : urban /rural

Symptoms:

ECG findings: STEMI / non- STEMI

cTroponin I:

Other laboratory tests:

الـــخلاصـــــة

يوصف احتشاء عضلة القلب الحاد بكونه موت أنسجة عضلة القلب المفاجئ نتيجة عدم التوازن بين إمدادات الدم الشرايين التاجية واحتياج الدم في عضلة القلب. يحدث عادةً بسبب تكوين خثرة انسدادية في الشريان التاجي الممزق أو المتآكل، مما يؤدي إلى استجابة التهابية، يتم استبدالها في النهاية بنسيج حبيبي وندبة تحتوي على الكولاجينز

اجريت هذه الدراسة ذات النوع الحالة-السيطرة في مختبرات قسم الكيمياء والكيمياء الحياتية,كلية الطب,جامعة بابل خلال الفترة من شباط ٢٠٢٢ لغاية مايس ٢٠٢٣.حيث كان الهدف من الرسالة تحديد القيمة التشخصية للابلين ,اللبتين, الهوموسستين والسيلنيوم لدى مرضى احتشاء عضلة القلب الحاد. جمعت العينات من المرضى المراجعيين في مدينة مرجان الطبية في مدينة الحلة .تضمنت هذه الدراسة ١٢٠ مشاركاً (٤٨ اناث و ٧٢ ذكور) والذين تم تقسيمهم الى ثلاث مجموعات,المجموعة الاولى تضمنت ٢٧ مريضاً (٧ اناث و ٢٠ ذكور) بمتوسط عمر (٦٣,٣±١٠,٥) تم تشخيص اصابتهم باحتشاء العضلة القلبية الحاد المصحوب بارتفاع مقطع اس تي,المجموعة الثانية تضمنت ٣٣ مريضا (١١ اناث و٢٢ ذكور) بمتوسط عمر (٥٩,٥±٨,٠) تم تشخيص اصابتهم باحتشاء العضلة القلبية الحاد غير المصحوب بارتفاع مقطع اس تي. والمجموعة الثالثة تضمنت ٦٠ مشاركاً من الاصحاء (٣٠ اناث و ٣٠ ذكور) بمتوسط عمر (٦٢,٤±٩,٤) كمجموعة سيطرة.

توصلت هذه الدراسة الى ان عدداً من عوامل الخطورة مرتبطة بأحتشاء العضلة القلبية الحاد, وكان في مقدمة تلك العوامل التاريخ العائلي بمعامل ارجحية (٢٧,٨) ,تبعه ارتفاع ضغط الدم بمعامل ارجحية (٢٧,٣). اظهرت نتائج الدراسة الحالية ايضا ان مزيج من عوامل خطورة متعددة يمكن ان يضاعف من خطورة الاصابة باحتشاء العضلة القلبية الحاد ,وكذلك قد يساهم بزيادة حجم الاحتشاء وبالتالي يؤدي الى تكهن اسوأ مقارنة بمرضى احتشاء العضلة القلبية بمعامل خطورة مفرد.

اظهرت نتائج الدراسة الحالية زيادة معنوية في متوسط الابلين ,اللبتين و الهوموسستين لدى كلتا مجموعتي مرضى احتشاء العضلة القلبية الحاد مقارنةً بمجموعة السيطرة ,وهذه المستويات تزداد اكثر لدى مجموعة احتشاء العضلة القلبية الحاد المصحوب بارتفاع مقطع اس تي عند المقارنة بمجموعة احتشاء العضلة القلبية الحاد غير المصحوب بارتفاع مقطع اس تي.

اظهرت نتائج الدراسة الحالية ايضا انخفاض معنوي بمتوسط مستويات السيلنيوم لدى كلتا مجموعتي مرضى احتشاء العضلة القلبية الحاد مقارنة بمجموعة السيطرة, مع عدم وجود اختلاف معنوي في مستويات السيلينيوم بين مجموعة احتشاء العضلة القلبية الحاد المصحوب بارتفاع مقطع اس تي و و احتشاء العضلة القلبية الحاد غير المصحوب بارتفاع مقطع اس تي.

في هذه الدراسة كان هناك ارتباط معنوي موجب بين اللبتين, الهوموسستين والابلين مع تروبونين القلب اي لدى مرضى احتشاء العضلة القلبية الحاد في كلتا المجموعتين .ايضا كان هناك ارتباط معنوي سالب بين السيلينيوم مع تروبونين القلب اي لدى مرضى احتشاء العضلة القلبية الحاد في كلتا المجموعتين.

توصلت الدراسة الى ان لدى مرضى احتشاء العضلة القلبية الحاد في محافظة بابل مستويات مرتفعة من اللبيتن ,الهوموسستين والابلين مع مستويات اكثر ارتفاعاً لدى مرضى احتشاء العضلة القلبية الحاد المصحوب بارتفاع مقطع اس تي مقارنةً بمرضى احتشاء العضلة القلبية الحاد غير المصحوب بارتفاع مقطع اس تي. هذا النمط من النتائج يستنتج وجود دور لهذه المعلمات في تشكيل الجلطة,تصلب الشرايين,الجهد التأكسدي وحدوث وتطور احتشاء العضلة القلبية الحاد. توصلت الدراسة ايضا الى ان لدى مرضى احتشاء العضلة القلبية الحاد في محافظة بابل مستويات منخفضة من السيلينيوم مقارنة بمجموعة السيطرة.والذي قد يكون بمثابة دلالة حيوية لمراقبة حالة مضادات الاكسدة لدى مرضى احتشاء العضلة القلبية الحاد.

ختاماً ,يمكن استخدام اللبتين, الهوموسستين والسيلينيوم كقائمة عمل روتيني تطلب للمرضى المشتبه اصابتهم باحتشاء العضلة القلبية الحاد كدلالات حيوية للتأكيد والمراقبة. كذلك يمكن استخدام الابلين في المستشفيات والمراكز الطبية كدلالة تشخيصية وفئوية لتحديد نوع احتشاء العضلة القلبية الحاد كسياق بديل اذا تعذر اجراء تخطيط القلب الكهربائي.

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وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية الطب

فرع الكيمياء و الكيمياء الحياتية

تقييم الابلين, اللبتين , الهوموسيستين والسيلينيوم في امصال مرضى احتشاء عضلة القلب الحاد في محافظة بابل

رسالة

مقدمة الى مجلس كلية الطب في جامعة بابل

كجزء من متطلبات نيل شهادة الدكتوراه فلسفة في العلوم / الكيمياء الحياتية السريرية

من قبل

احمـد فيـصل فـالح مهـدي

بكالوريوس تقنيات التحليلات المرضية/ كلية التقنيات الصحية و الطبية – بغداد (٢٠٠٨)

ماجستير كيمياء حياتية سريرية /جامعة بابل / كلية الطب

(٢٠١٨)

إشـــراف

الأستاذ الدكتور

مفـيد جلـيل عـوض

الأستاذ المساعد الدكتور

شـكري فـائز السـعد

٢٠٢٣م

١٤٤٥هـ