Ministry of Higher Education and
Scientific Research
University of Babylon
College of Medicine
Chemstriy and Biochemistry Department

The Association Of Arginase 1 Gene Polymorphisms And Fibronectin Levels With Lipid Peroxidation In Acute Myocardial Infraction Patients In Babylon Governorate

A Thesis

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

By

Naser Zeyad Naser Hussain

B.S.c in Pathological Analysis Techniques
(Faculty of Health and Medical Technology/Baghadad) (2010)
M.S.C in Clinical Biochemstry
(University of Babylon / College of Medicine 2018)

Supervised by

Professor
Dr. Moaed Emran Al-Gazally

Professor
Dr. Hassan Salim aljumaily

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

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنفُسِهِمْ حَتَّىٰ يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ أَوَلَمْ

يَكْفِ بِرَبِّكَ أَنَّهُ عَلَىٰ كُلِّ شَيْءٍ شَهِيدٌ

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

Acknowledgments

Thanks to **Allah** so much for grace and great kindness, who gave me strength and conciliation in order to complete my study and work 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. Moaed Emran Al-Gazally,** for his suggestion of the project, supervision, support, guidance, and assistance throughout the work of this study. Also, my great thanks extend to **Prof.** ***Dr.Hasan Salim Aljumaily*** also expressed to **Prof. Dr. Mohend A. AL-Shalah,** Dean of College of Medicine, University of Babylon for his kind help و A great thanks goes to the Head of the Chemistry and Biochemistry Branch, 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 are 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 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. Ahmed Faisal** for help, assistance, and support.

DEDICATION

To my Father

My mother, my wife

, My beautiful daughters

Lina & Noor &

my Brothers &

my friends...

Supervisors Certification

We certify that this thesis entitled **"*The association of arginase 1 gene polymorphisms and fibronectin levels with lipid peroxidation in acute myocardial infraction patients 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

Dr. Moaed Emran Al-Gazally

Professor

Dr. Hassan Salim aljumaily

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 **" The Association Of Arginase 1 Gene Polymorphisms And Fibronectin Levels With Lipid Peroxidation In Acute Myocardial Infraction Patients In Babylon Governorate "** and as examining committee examined the student **" Naser Zeyad Naser Hussain"** 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. Khaled Farouk Abdul Ghafoor
College of science/University of Anbar
(Chairman)

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

Assist. Prof. Dr.
Qasim Jawad Fadel
College of Pharmacy /University of Babylon
(Member)

Assist. Prof. Dr.
Antesar Rheem Obead
College of Basic Education /University of Babylon
(Member)

Assist. Prof. Dr.
Zinah Abbass Ali
College of Medicine/University of Babylon
(Member)

Prof. Dr.
Moaed Emran AL-Gazally
College of Medicine/University of Al-Ameed
(Member and Supervisor)

Prof. Dr.
Hassan Salim Aljumaily
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, commonly referred to as a heart attack, is among the most perilous cardiovascular diseases. It is a life-threatening condition that occurs when there is an obstruction in the blood flow to the heart muscle, leading to damage to the heart tissue. Pathologically, Acute Myocardial Infarction is characterized by the death of myocardial cells due to ischemia, which can result from an imbalance in the oxygen supply-demand ratio, either due to a decrease in oxygen supply or an excessive demand. In some cases, myocardial damage may occur even in the absence of blood clot formation, causing permanent damage.

This study was conducted in Hilla city, from 1st of February 2022 until April 2023. The samples were collected from merjan medical city in Hilla city, Babylon Province and all patients of these groups were diagnosed by cardiologist doctors.

In this case control study 90 person were taken as control group and 90 patients were taken as case group and classified as two groups 45 patients with ST elevation myocardial infarction and 45 presented as non-ST elevation myocardial infarction

The sera obtained from the blood of patients and control subjects were used to measure the arginase activity by colorimetric method while concentrations of malondialdehyde and fibronectin by using enzyme-linked immunosorbent assay (ELISA) technique, while whole blood samples from study subjects were used to extract DNA for the genetic study of arginase-1 polymorphisms.

Arginase activity was significantly increase in ST elevation myocardial infarction and non-ST elevation myocardial infarction groups from control group with p-value of less than (0.001) also there were a statically significant relation between arginase activity in ST elevation myocardial infarction groups versus non-ST elevation myocardial infarction group with p-value of (0.001).

Fibronectin concentration was statically increase in ST elevation myocardial infarction and non-ST elevation myocardial infarction groups from control group with p-value of less than (0.001) also there was a significantly increase in fibronectin

concentration in ST elevation myocardial infarction group from non-ST elevation myocardial infarction group with p-value (< 0.001).

Malondialdehyde was statically significant increase in ST elevation myocardial infarction and non-ST elevation myocardial infarction groups versus control group with p-value of less than 0.001 while there was no statically significant relation of MDA between ST elevation myocardial infarction and non-ST elevation myocardial infarction patients.

In this study there were a statically significant association of the rs2781666 SNPs in the arginase1 gene with ST elevation myocardial infarction by a p-value of less than (0.001) and with non-ST elevation myocardial infarction with a p-value of (0.001), moreover the ST elevation myocardial infarction and non-ST elevation myocardial infarction groups showed higher frequency of T-allele compared to the healthy group.

In ST elevation myocardial infarction patients there were a strong positive correlation between malondialdehyde and arginase activity with (R=0.774), and weak positive correlation between malondialdehyde and fibronectin with (R=0.225), while in non-ST elevation myocardial infarction patients there were a strong positive correlation between malondialdehyde and arginase activity with (R=0.741), and a weak positive correlation between malondialdehyde and fibronectin with (R=0.258).

This study concludes some risk factors for acute myocardial infarction that the male sex is more affected, the person has high BMI or smoking is more vulnerable for myocardial infarction incidence.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

Abbreviation	Details
$1O_2$	singlet oxygen
4-HNE	4-hydroxy-2-nonenal
ACS	Acute coronary syndrome
ADMA	asymmetric dimethylarginine
AHA	America heart society
AMA	Acute myocardial infarction
ARG	Arginase
AST	aspartate transaminase
BMI	Body Mass Index
bp	base pair
CABG	coronary artery bypass grafting
CK MB	Creatine kinase-MB
CPS1	carbamoyl-phosphate synthase 1
cTnC	cardiac troponin C
cTnI	cardiac troponin I
cGMP	cyclic guanosine monophosphate
DCM	Diabetic cardiomyopathy
DM	Diabetes mellitus
E_2	17β-estradiol
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide
ER	emergency room
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide

FN	**Fibronectin**
H_2O_2	**hydrogen-peroxide**
H-FABP	**Heart-type fatty acid binding protein**
HRP	**horseradish Peroxidase**
iNOS	**Inducible nitric oxide**
LDH	**lactate dehydrogenase**
LO	**Lipid oxidation**
LPO	**Lipid peroxidation**
MDA	**Malondialdehyde**
MI	**myocardial infarction**
NADP	**nicotinamide adenine dinucleotide phosphate**
nNOS	**neuronal nitric oxide synthase**
NO	**nitric oxide**
NOS	**nitric oxide synthase**
$O_2^{\bullet-}$	**superoxide**
OAT	**ornithine amino transferase**
OD	**optical density**
ODC	**ornithine decarboxylase**
$ONOO^-$	**peroxynitrite**
OS	**Oxidative stress**
NSTEMI	**non-ST elevation myocardial infarction**
PCI	**percutaneous coronary intervention**
PCR	**Polymerase Chain Reaction**
PUFA	**polyunsaturated fatty acids**
RAAS	**renin–angiotensin–aldosterone system**
RFLP	**restriction fragment length polymorphism**
ROS	**reactive oxygen species**

RT	**Room temperature**
SNPs	**single nucleotide polymorphisms**
sGC	**guanylate cyclase**
SD	**standard-deviation**
SNS	**sympathetic nervous system**
SOD	**superoxide dismutase**
STEMI	**ST elevation myocardial infarction**
TBE	**Trisbase pH 6.8, Borate and Disodium EDTA**
TnT	**troponin T**
TE	**Tris EDTA**

CHAPTER ONE

Introduction & Literature Review

1. Introduction

1.1 Acute myocardial infarction

1.1.1 Definition

The Acute Myocardial Infarction (AMI), commonly known as a heart attack, stands out as one of the most severe cardiovascular diseases. It poses a life-threatening risk, occurring when there is an obstruction in the blood flow to the heart muscle, leading to tissue damage. From a pathological perspective, AMI results in the death of myocardial cells due to severe ischemia. [1].

This condition prompts extensive structural changes in the heart, characterized by the accumulation of fibrous tissue. This accumulation distorts the tissue structure, increases its rigidity, and ultimately results in a disruption of ventricular function. The blockage of coronary arteries can be attributed to the formation of a thrombus [2] as seen in figure (1.1).

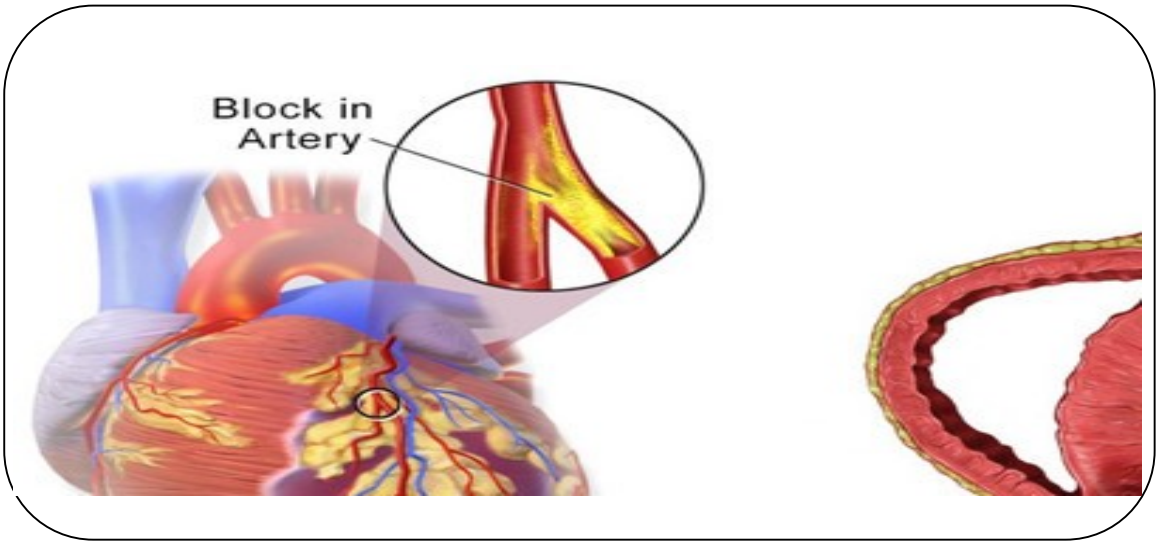

Figure (1-1): Myocardial Infarction[1]

Sometimes, an imbalance in the oxygen ratio (either due to a decreased supply or excessive demand by myocardial cells) can cause myocardial damage. This can occur in conditions such as Tachyarrhythmia (excessive demand) or with a decrease in blood pressure (limited supply) without the formation of a blood clot, resulting in permanent damage (myocardial cell death). Therefore, early detection through laboratory tests and medical methods is crucial to prevent complications [3][4].

The patient in the emergency room presenting with myocardial infarction typically manifests as acute coronary syndrome (ACS). ACS is classified into several distinct categories, including ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina. Nevertheless, there are similarities in clinical signs and symptoms, such as the presence of chest pain [5].

1.1.2 Classification of Acute myocardial infarction:

The AMI is classified into 5 categories which updated by the fourth universal definition of (MI) in 2018 by American faculty of cardiology and the America heart society (AHA) [6].

Type 1: - infarction due to blockage of the coronary arteries caused by acute rupture of cholesterol rich atherosclerotic plaque (coronary atherothrombosis), type 1 is classified according to electrocardiogram (ECG) to STEMI or STEMI in order to use suitable therapeutic protocol [7].

Type 2: - In this type the pathophysiological mechanism that responsible for ischemic myocardial injury is due to decrease in supply or excessive demand

of oxygen by myocardiocytes that happen secondary to renal or heart disease [8].

Type 3: - the myocardial infarction in this type causes immediate death before the opportunity of biomarker study for blood sample drawn or electrocardiogram study for confirmation of MI [9].

Type 4a: - this type is describing the myocardial infarction that happens after percutaneous coronary intervention (PCI) [10].

Type 4b: - describe the myocardial infarction that happens after thrombosis of a coronary stent [11].

Type 5: - this type happens after the coronary artery bypass grafting (CABG) which is consider the most operation done in heart surgery part in the world [12].

The universal define of AMI classified the type 1 and type 2 into subgroups although its differ in the presence of obstruction of the coronary artery they were divided to subgroups according to the pathophysiology of (MI) the type1 is divided to four types (plaque rupture/erosion with thrombus, spontaneous coronary artery dissection, coronary embolisim and vasospasm or microvascular dysfunction) while type two was divided into two subgroups (with fixed obstructive coronary artery disease and without fixed obstructive coronary artery disease) as shown in figure (1-2) [13,14].

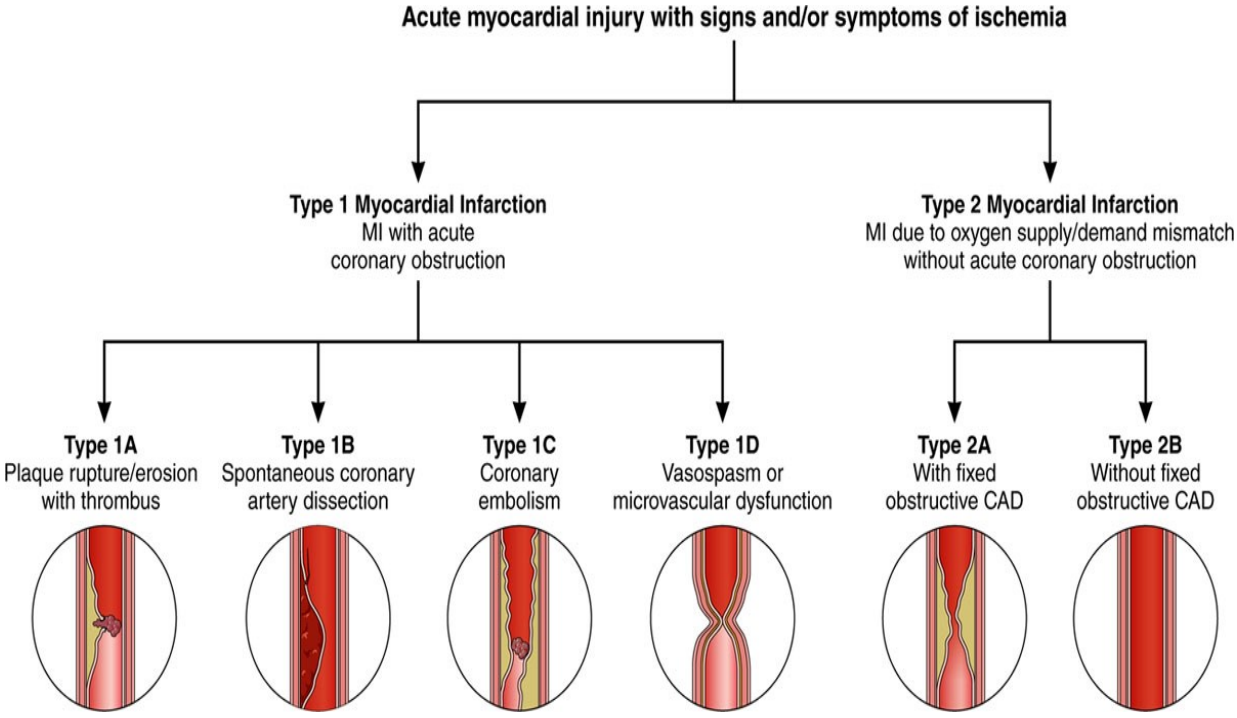

Figure (1-2): Classification of universal defines of acute MI [14]

1.1.3 Epidemiology

Most patient in the world enter emergency room (ER) are presented with chest pain which consider the more symptoms appear in cardiovascular disease, myocardial infarction consider one of the most reason of death around countries, ten percent of patient that enter the emergency room consultant have symptoms that diagnosed with acute myocardial infarction [15].

Several Middle East and North Africa nations have demonstrated progress in age-adjusted CVD mortality rates despite the overall increase in the total number of CVD deaths from 1990 to 2015, ranging from 29% (Somalia) to 42% (United Arab Emirates). This increase can be attributed to

population growth and the aging population. It has been reported that the Middle East boasts the lowest median age for first MI (51 years), which is approximately a decade younger compared to North America (59 years) and Western Europe (63 years). In addition, exhibit the highest percentage (11%) of individuals aged 40 or younger with MI, compared to 4% in North America and 3% in Western Europe [16].

The greatest numbers of patient with acute coronary syndrome is diagnosed as non ST-segment elevation myocardial infarction and it's keep related to death until the present day, despite the development work by angiography [17].

1.1.4 Pathophysiology

Myocardial infarction is happening by the death of the myocardiocytes which is called necrosis, that result from the decrease of oxygen supply to the cell which may result from thrombus that blockage the coronary artery (type 1) or from the demand abnormality for example in severe hypertension or supply abnormality for example in severe anemia (type 2) from both reasons the cell necrosis resulted by decreased oxygen supplement [18].

During ischemia, when there is a lack of oxygen and reduced ATP production, the sodium-potassium pump (Na+/K+ pump) becomes less active. As a result, sodium ions (Na+) start to accumulate inside the cell because they cannot be efficiently pumped out, activation of Na/H antiport that cause decrement in intracellular pH , also will make cells shift from the aerobic manner to anaerobic respiration that will result from the energy imbalance, this scenario will cause harmful effect which include the increase

of intracellular calcium influx and acidosis which with one another cause defect in membrane integrity that result in damage of cellular membrane and release of intracellular content out of cell, all of these will led the myocardiocytes to be necrotic and death [19].

The major effect of myocardiocytes necrosis are the sudden lowering of cardiac output so progress of severe cardiac arrhythmias results from the lack of functional contractile muscle mass [20].

1.1.5 Risk factors for Acute Myocardial Infarction

Myocardial infarction has different risk factor there is modified which can be treat and non-modified which cannot be reflect these risk factors include: -

a) Physical activity:

The people with low activity consider more likely to have acute myocardial infarction in their life, the physical activity has advantage to decrease the rate of acute myocardial infarction incidence up to 20%-30% [21, 22].

The decrement effect of physical activity on acute myocardial infarction can be result from different types of physical activities for example; walking, clamping and play with bicycle on leisure time provide protection against acute myocardial infarction, while others activity, like doing intensive physical activity, have no protection effect against AMI [23,24].

However, there are relation between sedentary life and exercise there are also relationship between lack of exercise and sitting at work on the association with increased risk of acute myocardial infarction [25].

b) Smoking

Smoking represents the strong risk factor of acute myocardial infarction, premature atherosclerosis and sudden cardiac death [26].

Acute myocardial infarction risk is increase as smoking tobacco increase through many mechanisms. It causes deterioration of blood vessels, increase the incidence of plaque formation, have role in decrease the bloods oxygen and increases the risk of clots formation at the site of plaques [27].

Some tobacco ingredient, mainly nicotine has role in increase the activates of the sympathetic nervous system (SNS), that result increasing both heart rate and systolic blood pressure [28].

The Increment in SNS activity cause increase in blood pressure result in increased the myocardiocyte oxygen demands also leads to coronary arterial vasoconstriction [29].

However, cigarette smoking in addition to increasing myocardiocyte oxygen demand and decrease coronary blood flow and causes increase in the levels of carboxyhemoglobin in the blood [30].

c) Alcohol Consumption

The risk of acute myocardial infarction is highly related to people after couple's hours of alcohol consumption in people who do not typically drink alcohol daily [31].

chronic alcohol abuse can lead to endothelial dysfunction through multiple mechanisms, including oxidative stress, inflammation, decreased NO bioavailability, ER stress, hypertension, vasoconstriction, and altered lipid metabolism [32,33].

These changes collectively contribute to impaired endothelial cell function, disrupt the delicate balance of vascular regulation, and increase the risk of cardiovascular diseases, including atherosclerosis, hypertension, and heart disease [34].

d) Diabetes Mellitus

Diabetes mellitus (DM) is a chronic condition happens when the body produces insufficient insulin or don't respond normally to it, DM induced from a genetic predisposition in relation with environmental factors [35].

Diabetes cause deterioration effect on the vascular physiological components, that is mainly include the endothelium, vascular smooth muscle and platelets [36].

Diabetic cardiomyopathy (DCM) is defined as myocardial dysfunction leads to heart failure that happen despite of absence of other cardiac risk factors like coronary artery disease and hypertension, In the early stages, DCM involves a hidden subclinical period characterized with structural and functional defect, including left ventricular hypertrophy, fibrosis, and cell signaling dysfunction [37,38].

Diabetic cardiomyopathy is the main causes of both atherosclerotic plaque formation and thrombosis that consider the main causes of myocardial infarction; that is, myocardial infarction is more often fatal in people with diabetes [39].

e) Dyslipidemia

Dyslipidemia, is consider one of the important risk factor of cardiovascular disease, people with increment concentration of triglyceride

and small dense LDL (more atherogenic particle) are more prone to have myocardial infarction [40].

Post-meal measuring of triglyceride level appears to be more benefit to predict a future risk of acute myocardial infarction, mainly when total cholesterol concentration is also increased [41].

That happen because the decrement of HDL-C levels and increased triglyceride levels result in metabolic disturbance result in bad consequences, therefore most of the young patients presenting with acute myocardial infarction have dyslipidemia with hypertriglyceridemia, hypercholesterolemia, raised LDL and low HDL [42].

f) Hypertension

The risk of a myocardial infarction is increase by both systolic and diastolic hypertension and as the blood pressure increase, so hypertension is a major risk factor of atherosclerosis in coronary blood vessels and heart attack or myocardial infarction [43].

Hypertension and myocardial infarction have positive relationship and closely linked, hypertension prompt endothelial dysfunction through reducing nitric oxide (NO), this may be related to increase calcium ions by reducing NO synthetase or excess production of free radicals which inhibit NO production, hypertension was associated with an increased risk for AMI in both male and female [44,45].

g) Sex

Men more prone to have heart attacks earlier in their lifetime than women, women's average of heart attack raise after menopause however does not equal men's average [46].

This distinction is consequent from the lack or a very low amount of estrogen and especially 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 also play role in many physiological functions, especially in the cardiovascular system [47].

Female patients with acute myocardial infarction are often older, higher rates with diabetes mellitus, hypertension, and autoimmune disorders, while men with acute myocardial infarction often times have higher rates of smoking, peripheral vascular disease, yet women are less probable to survive a heart attack also more likely to have a second heart attack [48].

h) Family History

Family history of myocardial infarction is a distinct risk factor for acute myocardial infarction; some genetic variants are related with increased risk of AMI specially family history of AMI in the first-degree relative, in the evaluation of patient's case the past-history of cardiovascular disease is very important and ought not to be dismissed [49].

Expression of Some genes promoting the risk factors for acute myocardial infarction and shown to be associated with heart disease and several studies elucidated the increased risk of AMI in subjects with family history of disease [50].

1.1.6 Signs and Symptoms

The symptoms of AMI are chest pain that transport to the left arm or left side of the neck, shortness of breath, sweating, nausea, vomiting, abnormal heart beating, anxiety, fatigue, and other factors, around sixty-four

percent of people who have MI do not experience chest pain which is called silent AMI [51].

The danger signs of heart attack are also the following: high blood pressure, tightness of chest, squeezing, burning sensations, aching and heaviness in the chest for more than ten minute, pain in left shoulder or left arm, and up till the neck or along the jaw line, shortness of breath, plenteous sweating and dizziness, muscles weakness, nausea or vomiting, anxiety or stress, feeling of impending doom and depression. But sometime there are no symptoms for a silent heart attack like in elderly and diabetic patients [52, 53].

Approximately only one-half of women with an AMI present with chest pain. In fact, women are more likely to present with irregular symptoms such as fatigue, sleep trouble, shortness of breath, back pain, upper abdominal or epigastric pain, and nausea together with or without vomiting rather than present with chest pain, several women who have had a heart attack describe that their symptoms felt like the symptoms of the flu [54].

1.1.7 Diagnosis

1.1.7.1 Electrocardiogram (ECG)

The standard Diagnosis for acute myocardial infarction is done by ECG. However, the development technology in ECG devices had role in the early diagnosis, twelve lead ECG data used for diagnosis of myocardial infarction so other heart diseases [55].

That ECG have role to determine the presence and the local site for myocardial infarction which have characteristic pattern that start by the

elevate of ST-wave then decrement of (R) wave then increment of the (Q) wave thereafter the (T) wave reversed, however this change may still even after the ST increment wave return to normal as shown in figure (1-3) [56].

The detection usually better to observe in the lead which site against the infracted part when there is an anteroseptal infarction change in electrocardiogram are seen in leads from V(1) to V(4), while anterolateral infarction make variation in electrocardiogram read from V(4) to V(6), VL also lead (I), and inferior infarction anomaly is observed in leads (II), (III) also VF, while when there is infarction happen on the posterior wall of the left ventricle it could me detect from the reversible appearance S-T diminuend and increment R wave that appear in the leads between V1 to 4 [57].

Type 1 myocardial infarction could be classified by ECG into two classes: STEMI and NSTEMI, STEMI is known as an acute coronary thrombosis or persistent ST-segment elevation of ≥1 mm in ≥2 contiguous electrocardiographic leads, NSTEMI is known as ischemic symptoms like chest pain at rest resulted from an acute coronary plaque rupture or erosion and partial blockage of coronary artery, lasting ≥10 minutes, occurring within 24 hours before hospital admission, and displaying elevated cardiac biomarkers (either creatine kinase or cTn) [58-61].

Electrocardiogram sensitivity and specificity is not high for the detection of acute myocardial infarction so European society of cardiology state that a patient with acute myocardial infarction should have at least 2 of the next criteria: ideal myocardial infarction symptoms (like chest pain that the pain may radiate to the arms commonly the left arm, shoulders, neck and/or jaw. or discomfort with or without dyspnea, nausea), an increment in

cardiac markers like Creatine kinase-MB (CK MB), serum cardiac troponins (cTnI or cTnT), or an ideal ECG with Q waves as seen in figure (1-3) [62]

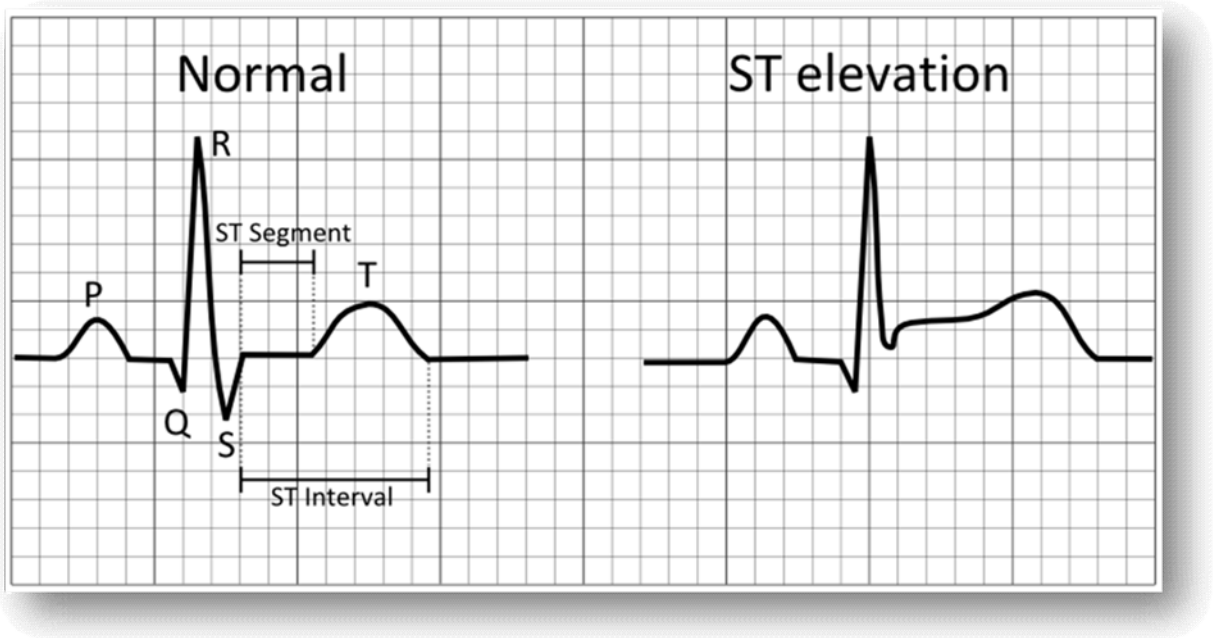

Figure (1-3) demonstrating of heart ECG with MI [63]

1.1.7.2 Cardiac markers

Use of cardiac biomarkers is very important in MI diagnosis that when the cardiac cell death and loss of cell integrity result in release of intracellular component from the myocardium into the circulation and the gradient increment in the concentration of some component reflect the damage and timing of MI [64].

The aspartate transaminase is the first cardiac markers discovered in 1954 and start to detect for myocardial infarction diagnosis however the lack of sensitivity that happen from high false negative results make the test to be replaced by lactate dehydrogenase (LDH) [65].

lactate dehydrogenase (LDH) is exist in five isoforms that the LDH-1 isoform is found in the heart tissue, lactate dehydrogenase is preferred compared to aspartate transaminase (AST) because of high sensitivity, but because of that this enzyme have role in most cell of body in glycolysis pathway and its increment in other pathological disease like many types of cancer or in anemia, LDH is replaced later by other cardiac markers like creatine kinase and troponin [66,67].

a- Creatine kinase

Creatine kinase (CK) was the marker that being interest after the LDH, creatine kinase exist in approximately all cells, however not like LDH, creatine kinase stimulate a reaction necessary for energy production (the turning of creatine to creatine phosphate), that highly up-regulated in the muscle cells and brain, so the highest concentration of creatine kinase exist in all muscle cells, especially in striated muscle, when there is damage of muscle or brain there will be increment in creatine kinase levels, at short times after muscle damage [68,69].

There are three isoenzymes (creatine kinase-MM, creatine kinase-BB and creatine kinase-MB), highest level of creatine kinase-MM or creatine kinase-BB happen in case of muscle or brain cell damage, respectively while, fifteen to thirty percent creatine kinase exist in the myocardium is creatine kinase-MB, while only one to three percent in normal striated muscle that made the elevated level of CK-MB to be highly specific for myocardial damage, also increment of serum creatine kinase-MB levels observed at four to six hours after the onset of myocardial infarction symptoms [70,71].

b- Troponin

Troponin is regulatory protein that exists in 3 types, troponin T (TNT), troponin I (TNI) and troponin C (TNC), which play important role in transfer of calcium and trigger the process of muscle contraction [72].

The troponin isoforms encoded from different gene so that reflect the specificity of cardiac troponin detection that the serum normal range is less than 10 ng/ml while when there is acute myocardial infarction the serum concentration may be more than ten times the normal range, however because of that the troponin of skeletal muscle and cardiac have different amino-acid so the development of sensitive lab method made the cardiac troponin test is the preferable marker for myocardial infarction detection [73,74].

Measurement of troponins have another feature that's the time of secretion, creatine kinase-MB concentration increase quickly after myocardial infarction by 4-6 hours and regress to normal concentration after 2-4 days; so creatine kinase-MB can use just as through little period of time after a suspicious myocardial infarction [75].

On the contrary, LDH concentration stay rise for up to one week however its need 24 to 48 hours after myocardial infarction to be increased, while troponin is raise in the plasma at 4-6 hours after MI, peak level will reach at (12 – 24) hours and stay raise for more than 7 days that Troponin-T still for (8 – 21) days while Troponin-I for (7 – 14) days [76].

So as mentioned the troponin consider more important as cardiac marker by wide window-time and more sensitivity and specificity than other cardiac marker, but also in some condition other than AMI cardiac troponin

increase like Strenuous exercise, acute pericarditis, cerebrovascular accidents, chronic obstructive pulmonary disease, chronic renal failure, tachycardia, inflammatory heart disease, acute pulmonary embolism, systemic inflammation (sepsis) [77-85].

C- Heart-type fatty acid binding protein

Heart-type fatty acid binding protein (H-FABP) is a tiny cytosolic protein which role as the principal carrier of long-chain fatty acids at myocardiocytes, it is existing in plenty in the cardiomyocyte also sent out quickly at circulation at myocardial defect [86].

Heart-type fatty acid binding protein is fundamentally existing in the myocardiocyte and has a molecular weight of 15 kDa so in myocardiocyte injury it will quickly be released in the circulation and then be discarded by the renal [87].

The interest of heart-type fatty acid binding protein used for the early detection of acute myocardial infarction, also the sensitivity for the diagnosis of myocardial infarction has been found to be more than cardiac troponin, but the cardiac troponin is more specific than H-FABP [88].

When checked the levels of heart-type fatty acid binding protein, creatine kinase-MB, myoglobin, and cardiac troponin through patients have acute myocardial infarction and seen that heart-type fatty acid binding protein have notable preferable sensitivity to any of the other parameters detected that the sensitivity and specificity of heart-type fatty acid binding protein are preferable than those of creatine kinase-MB and myoglobin at first three hours and (3–6) hours after a claim of chest pain, but, the specificity of heart-type fatty acid binding protein is not as high as that of

the cardiac troponin a group of heart-type fatty acid binding protein and other markers are suggested for appropriate diagnosis [89,90,91].

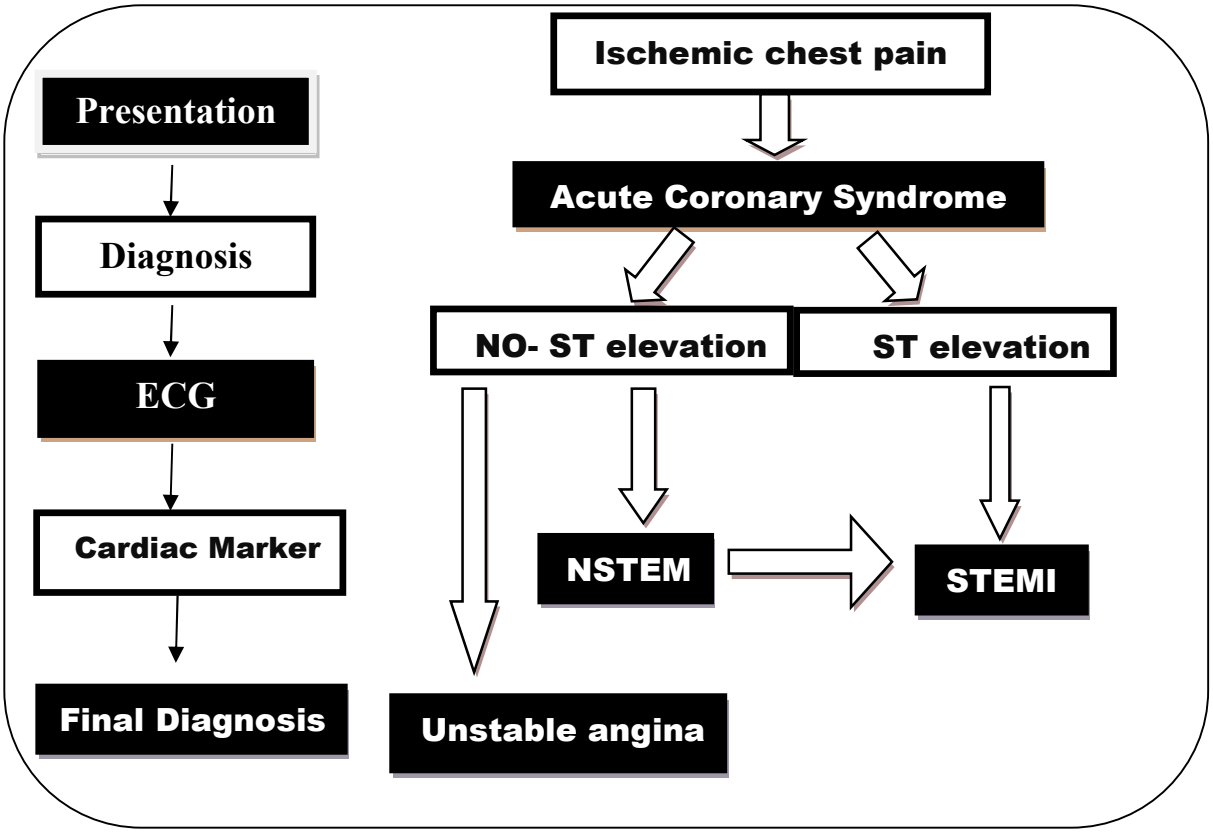

Figure (1-4) Steps in diagnosis of patients with ischemic pain [92].

1.1.8 Management of Acute Myocardial Infarction

Early risk classifications of patients with myocardial infarction permits for prophecy and triage through performance one of various necessary treatment sort, the TIMI risk score is simple to use, whilst GRACE is more precise, comprehensive, and viable to both of NSTEMI and STEMI myocardial infarction [93].

In NSTEMI, anti-thrombotic treatment is may settle down the vulnerable plaque and permit endogenous fibrinolysis to establish patency [94, 95].

The aim is to prevent advancement of the thrombus and cause a whole occlusion, PCI often done to recover blood flow and prevent frequent ischemia. PCI must have done within 24 h of NSTEMI if possible, yet several studies propose that PCI would be done in patients have minimum risk up to 48−72 h without clinical consequence while, doing PCI after 24 h has been correlating by long-term hospitalization [96,97]

In STEMI, the immediate procedure is to immediate reperfusion to restrict the infarction size, also antithrombotic therapy is used together, so patients commonly when had total arterial occlusion, reperfusion should have applied speedily for may be within one hour or ninety minute to restore patency [98].

Patients who subjected to fibrinolysis usually have residual stenosis, so reducing of stenosis through following angioplasty or stenting, or both, makes efficient perfusion and prevents acute re-occlusion [99].

1.2 Biochemical Parameters

1.2.1 Arginase

1.2.1.1 Definition

Arginase (EC 3531) is an important enzyme that have role in urea cycle through using arginine as substrate, so it's important for metabolism of excess nitrogen in the body, arginase in the body have two types Arginase1 and Arginase2 both of them are expressed by different gene [100].

Arginase1 are predominately exist in the liver, also exist in the bone marrow and endothelial cells, arginase1 that exist in the cytoplasm of liver

cell have a major function of urea syntheses also in the formation of ornithine, that often recycled during the urea cycle [101].

Arginase 2 are predominately existing in the mitochondria of all tissues but prevalence in the renal, prostate, GIT, most of muscles, and endocrine cells [102].

Arginase is metallo-enzyme that Arginase 1 is coded by 322 amino acids while Arginase 2 coded by 354 amino acids, in spite of coding by various gene there are 61% of amino acid are similar because of the active site of the two isoform are similar so they both need Mn2+ as activator [103,104].

Arginase 1 has genomic extent of 11500 base pairs however various single nucleotide polymorphisms (SNPs) may exist in the gene, these genetic defect of the arginase1 gene have role in human diseases incidence [105].

Some study in the world reveal that The rs2781666 Guanine / Thymine single nucleotide polymorphisms, that situated in promoter sequence, are associated with MI, coronary artery disease, diabetic Retinopathy in Type 2 Diabetes Mellitus, patients hypertension and erectile dysfunction [106-110].

1.2.1.2 Physiology

Role of arginase 1 in hepatic cell is stimulate the final stage of urea cycle, by which get rid of ammonia that generate through protein catabolism, ornithine is transformed to citrulline through the activity of ornithine-transcarbamylase and carbamoyl-phosphate synthase 1 (CPS1), also transform to polyamines through ornithine decarboxylase (ODC) and to proline through the activation of ornithine amino transferase (OAT), as clarify in figure (1-5) [111,112].

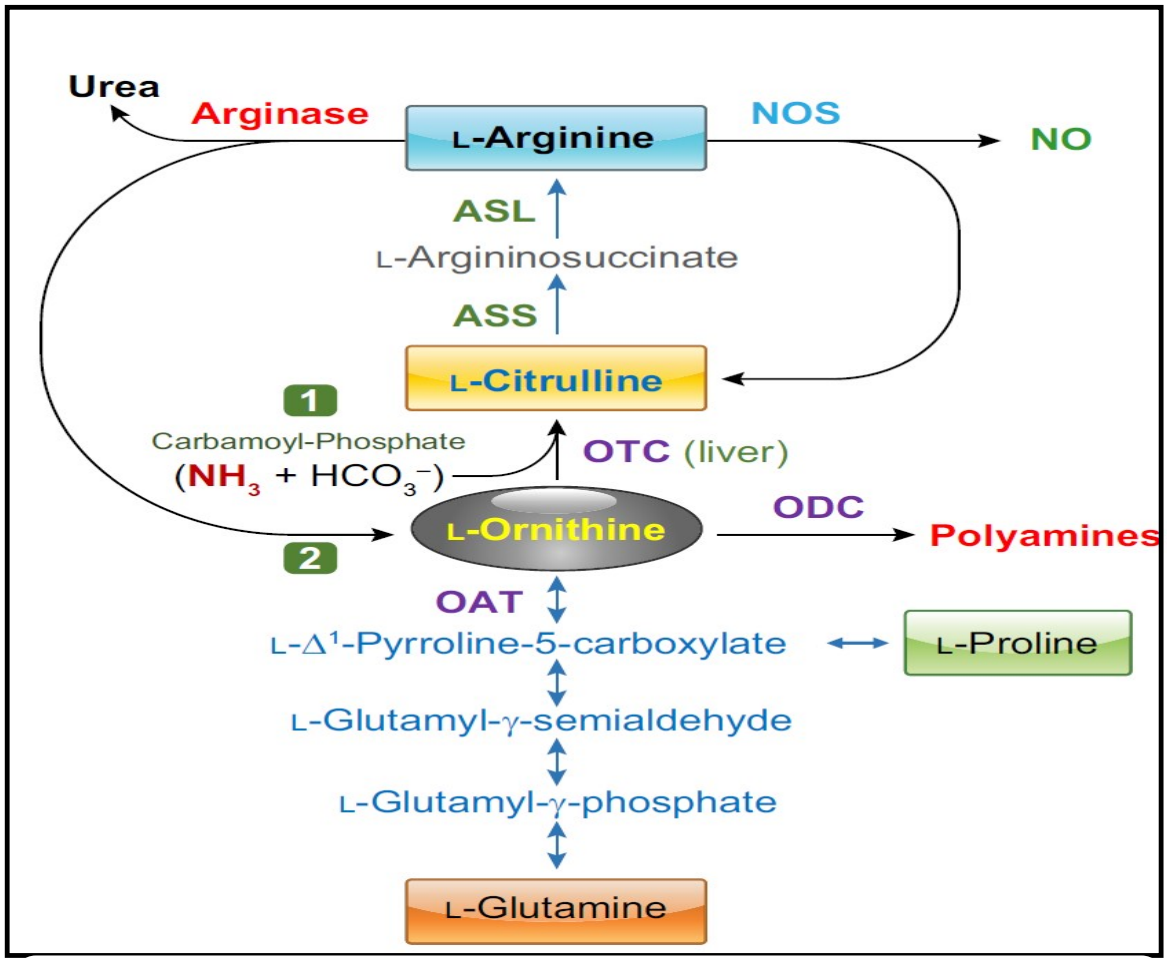

Figure (1-5) elucidate the arginase catabolism of arginine to ornithine and urea and other product [113].

remarkable function for injury healing, tissue restoration, and in nervous system development while proline is necessary for collagen synthesis [114,115].

Usually citrulline is converting back to L-arginine through argininosuccinate synthase enzyme and argininosuccinate lyase which consider a part of the ornithine cycle while generality of other tissues reduction the carbamoyl phosphate synthase 1 or ornithine transcarbamylase, so unable to finish the urea cycle [116,117].

The reason behind numerous researcher intense studies on the function of arginase enzyme in modifying nitric oxide synthesis concentration because the arginase and nitric oxide synthase (NOS) together use arginine as their usual substrate, so that over activity of arginase enzyme cause a lack in arginine for nitric oxide synthase, that developed to uncoupling of nitric oxide synthase, and decrement of nitric oxide syntheses, and increment syntheses of free radical like superoxide (O_2•–) and peroxynitrite (NO_3−)[118,119].

Arginine not only has important function in urea cycle and syntheses arginine is also substrate for nitric oxide synthase enzyme and 3 type of nitric oxide synthase are existing the first one in the nervous cells called neuronal nitric oxide synthase (NOS1), inducible nitric oxide synthase (NOS2), and the endothelial nitric oxide synthase (NOS3) [120].

The type 1 and type 3 are transcribed at relatively constant levels (constitutive gene) that need activation while the inducible nitric oxide synthase (NOS2) mostly regulated through transcriptional and posttranscriptional regulation [121].

Typical genetic expression and action of nitric oxide synthase 3 (eNOS) in vascular endothelial cells preserves appropriate blood influx through stimulate the reaction of arginine to nitric oxide and citrulline, so, eNOS have important protection function against vascular defect [122].

In the reaction the eNOS enzyme links heme and tetrahydrobiopterin through the N-terminal site and links the (calcium-calmodulin), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and nicotinamide adenine dinucleotide phosphate (NADP) by the C- terminal part [123].

Endothelial nitric oxide synthase need to be modified after translation because the enzyme fixed in the plasma membrane, while the co-enzymes tetrahydrobiopterin and heme are necessary for nitric oxide produce [124].

The site of eNOS in caveolae is appropirate for its action because the vicinity of the arginine transporter cationic amino acid transporter 1 and arginine reproduction enzymes argininosuccinate synthase enzyme, argininosuccinate lyase and ornithine transcarbamylase which assist nitric oxide syntheses, while endothelial nitric oxide suppression by caveolin [125].

Syntheses of nitric oxide through eNOS or neuronal nitric oxide synthase (nNOS) happen at minimum to mild average, while iNOS syntheses nitric oxide at rising average [126].

The nitric oxide synthesis by iNOS is requiring for alternate signal mechanisms of immune system and for body defense against bacterial [127].

Nitric oxide that syntheses by eNOS is spread and linked to vascular smooth muscle cells and stimulate soluble guanylate cyclase (sGC), which induce cGMP syntheses, potassium outflow, and relaxing of smooth muscle cell , consequently nitric oxide that syntheses by eNOS controls the vascular tone, and preserve suitable blood flux through prohibition platlets adherence at vascular endothelial cells also prevent platelets accumulation as seen in figure (1-6), another function of nitric oxide contain body protection versus microorganism and malignant, signaling through nervous cells, remodel the nerve synapses [128,129].

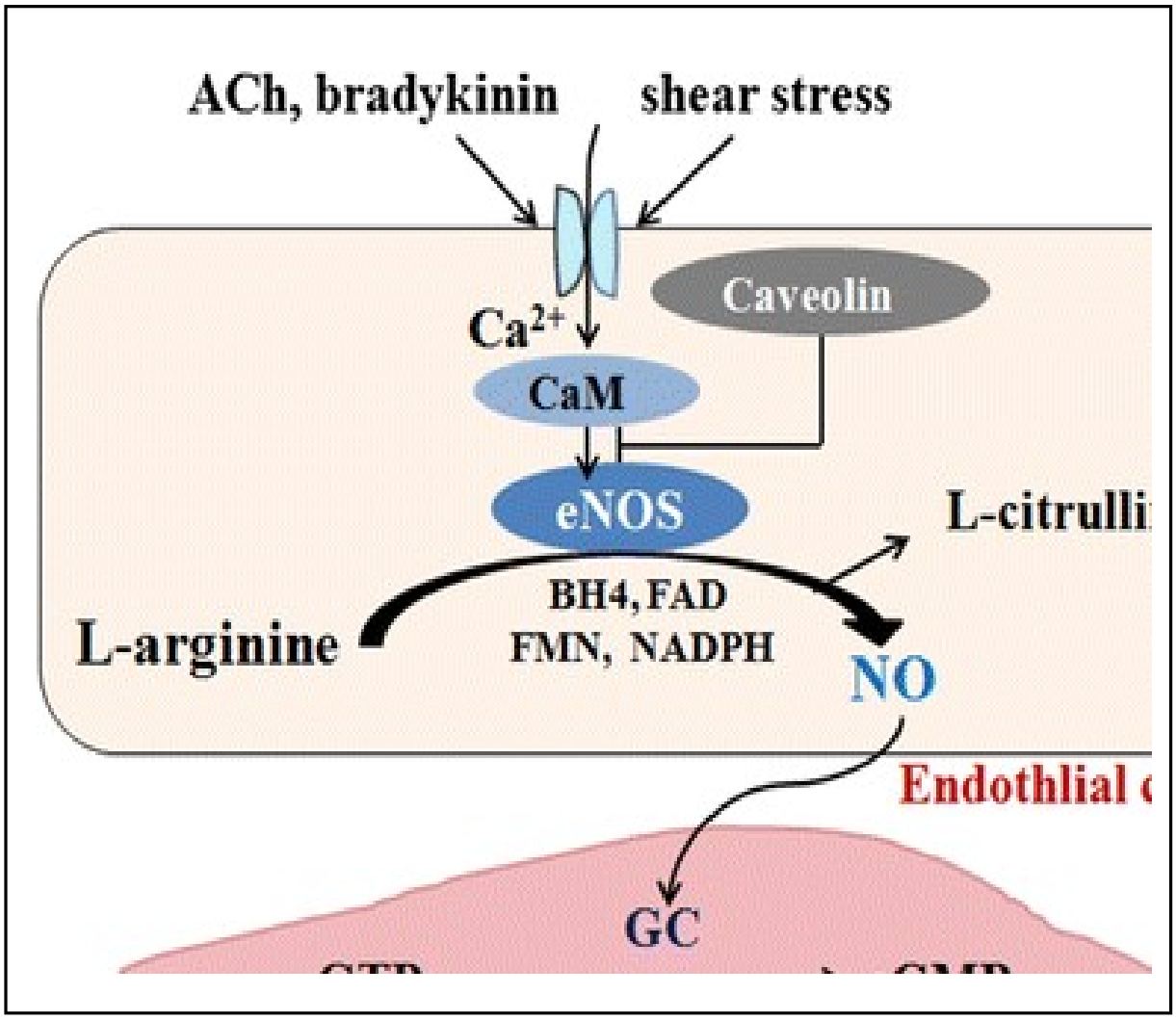

Figure (1-6) elucidate the regulatory function of endothelial NO synthase in cerebral blood flow [130].

1.2.1.3 Variation of arginase enzyme

Polymorphisms of arginase enzyme result in alteration cause raise in arginase level that outcome to: -

a- the alteration in L-arginine accessible cause a variance of L-ornithine syntheses and its metabolites (polyamines and proline), that may

lead to multiple systemic defect like, cells fibrosis and cell proliferation [131].

b- Alteration in nitric oxide concentration that related because arginine (when not involve in the urea cycle) is the exclusive substrate for all three types of NOS, and stimulates the syntheses of nitric oxide and the by-product L-citrulline [132].

Defect in arginase 1 cause dysfunction in urea cycle that outcomes a toxic buildup of ammonia If not treated, defect of the urea cycle result in mental disorders, seizures, and death, the treatment consist of lessening of patient's protein ingestion and supplementation with essential amino acids but serious cases require liver transplantation, that, Arginase 1 insufficient mice exhibit a high mortality rate shortly after birth [133,134].

While defects in A2 activity linked to a potency of lowering in A2 expression and decrease the risk of Alzheimer's disease [135].

1.2.1.4 The L-Arginine Paradox

Researchers in many studies noticed that treating by supplementation of arginine promote nitric oxide mediate biological impacts in vascular endothelial cells in spite of the fact endothelial nitric oxide synthase is apparently saturated by arginine [136].

The intracellular level of arginine in cells is extremely higher than the concentration required to keep up the extreme activity of endothelial nitric oxide synthase which Km is only 3uM, so, endothelial nitric oxide synthase should be saturated by arginine in normal situations [137].

Moreover, confusing the matter is the influence of arginase on nitric oxide function. The arginine Km of arginase is 2 mM, about one thousand

fold that for endothelial nitric oxide synthase, so, arginase enzyme should not be capable to compete with endothelial nitric oxide synthase for substrate [138].

Nevertheless, the V-max of endothelial nitric oxide synthase is approximately one thousand fold less than that of arginase enzyme that almost balances their ability to metabolize arginine [139].

So this clarify arginase strength to compete for the substrate, despite that, stated the high affinity of endothelial nitric oxide synthase for arginine, it is not likely that the arginine levels may decline to a scale that stated enzyme in a condition of substrate reduction, but, adding of extracellular arginine has demonstrated to promote endothelial relaxation in a types of disease cases recognized by vascular defect [140,141,142].

1.2.1.5 Reciprocal role between arginase and NOS in wound healing

Wound healing involves two phases. The initial acute phase, triggered immediately upon injury, generates oxidative stress as activated macrophages express elevated inducible nitric oxide synthase (iNOS), leading to significant nitric oxide (NO) production crucial for pathogen elimination. Subsequently, the repair stage begins within three to five days post-injury [143,144].

At this stage, arginase expression is raised that arginase catalyzed the conversion of arginine to ornithine that is metabolized through ornithine amino transferase to make proline that utilize for collagen production and ornithine decarboxylase to make the polyamines, promote the cells proliferation [145].

The equilibrium between the exhaustion of arginine from arginase and inducible nitric oxide synthase controls the consequence of injury heal, by arginase controlling of the recovery process and inducible nitric oxide synthase controlling the acute toxic stage [146].

1.2.1.6 Arginase role in cardiovascular disease

high expression and elevated concentration of arginase would compete with nitric oxide synthase for arginine so resulting in a reduction in the availability of nitric oxide that have given rise to the expansion of studies about the function of arginase in cases presented with vascular endothelial defect [147].

A reduction in the production of nitric oxide by vascular endothelial cells leads to impaired vasorelaxation and promotes the adherence of platelets to the endothelial lining [148].

Elevated concentration in the arginase rise the syntheses of ornithine which would involve in deleterious effect like cause hyperplasia, stiff and fibrosis in vascular cells [149].

In hypoxia, erythrocyte output nitric oxide sometimes with nitric oxide metabolites, subsequent to hypoxic vasodilation, which raise tissue perfusion and O2 delivery for preserving tissue O2 consumption also preventing of platelets accumulation [150].

Arginase-1 enzyme's crucial role lies in finely controlling the release of nitric oxide bioactivity within red blood cells. This regulation is intricately linked to the presence of endothelial nitric oxide synthase (eNOS) in erythrocytes. Notably, eNOS is typically active within these cells. However, in ischemic myocardiocytes, arginase-1 exerts its influence by

suppressing the synthesis of nitric oxide from erythrocytes. This intricate interplay underscores the significance of arginase-1 in modulating nitric oxide dynamics in the context of ischemic conditions. [151,152].

1.2.2 Fibronectin

1.2.2.1 Definition and structure

Fibronectin (FN) is a large glycoprotein consists from approximately 2300 amino acids and exists virtually in all types of tissue also is fundamental in numerous various cell and matrix interactions [153].

The FN protein is consisting from pieces of repeated, homologous chain that is found in three types the first type consists approximately 45 amino acids the second type consist approximately 60 amino acids while type three consist approximately 90 amino acids, type-1 and type-2 contain disulfide bonded loops while type-3 doesn't include disulfide bonded [154].

FN sequences are arranged in a style that composed an appearance of 2 arms that involve FN types, type-1 role is to link with one of fibrin, heparin or collagen while, the type-2 exist in the domain that responsible for linking collagen [155].

Type-3 exist in the domain that responsible for linking to various cells, as it has the ability of alternative RNA-splicing, FN can also bind to fibrin, a protein involved in blood clotting, this interaction is important for processes such as wound healing, some regions of FN have collagen-binding properties, allowing it to interact with collagen fibers in the extracellular matrix, FN contains heparin-binding domains that interact with heparin sulfate proteoglycans, another component of the extracellular matrix. [156,157]

Domains with various particular roles are the reason for the ability of the FN to associate with various kinds of cell, cytokines and extra cellular membrane [158].

The ability of alternative RNA-splicing makes Type-3 of FN to involve extra domain that called (EDA and EDB) fibronectin which have a particular function in vascular-development, cell-migration, differentiation, and the level of these domain is low in the blood of healthy but it's related to several medical problems like (hepatocellular, pulmonary and bone marrow fibrosis, malignant disease, diabetic nephropathy and atherosclerosis) [159-164].

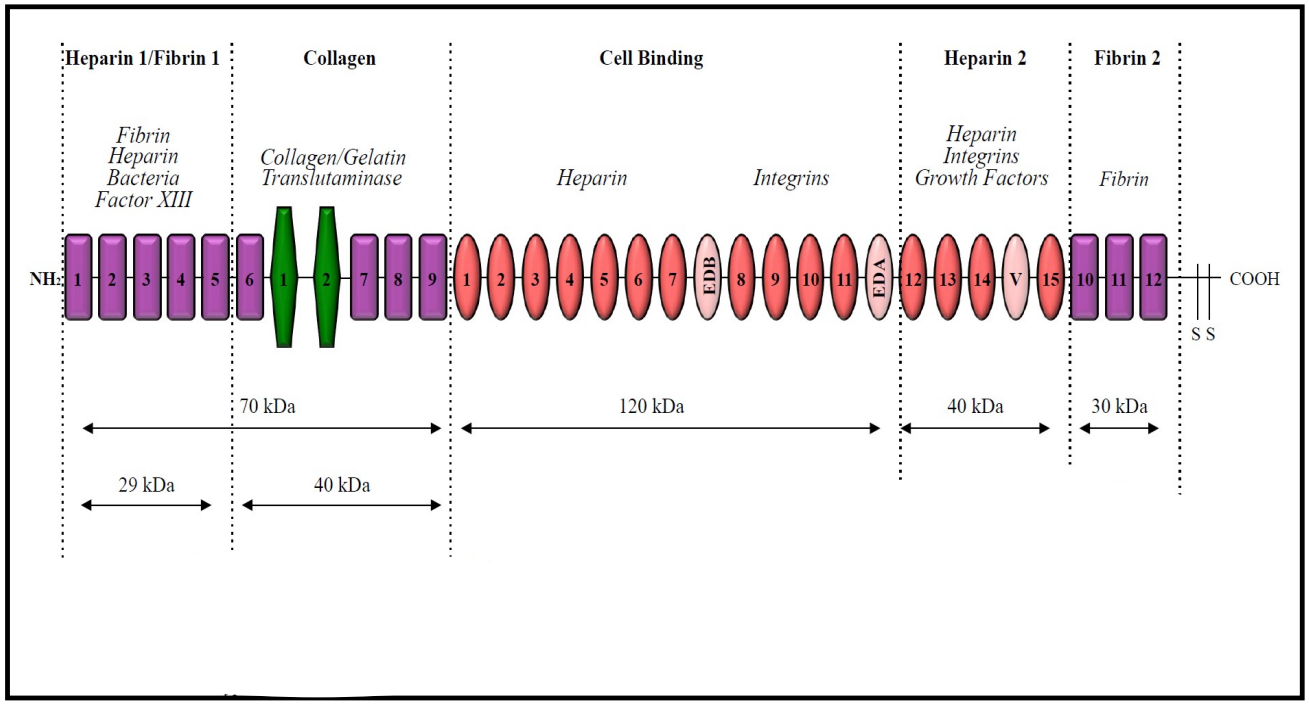

Figure (1-7) elucidate the fibronectin structure [165].

The FN exist in both soluble and insoluble manner that is found commonly as soluble in blood and syntheses by several cells but the mainly

from the liver, the soluble FN also found in various body fluid like synovial-fluid, CSF, amniotic fluid, and semen, soluble FN could produce and covering the mucus surfaces of the mouth [166].

Nearly on each kind of cells the insoluble FN is combined in the extracellular matrix, which play important role in cells adhesion, proliferation and migration [167].

Cellular adhesion to fibronectin occurs through integrin-mediated interactions, which are regarded as crucial receptors facilitating intermediate cell-matrix attachment, several integrin subtypes are ubiquitously expressed across various cell types, while others are selectively expressed in specific tissues. [168].

Integrin's are heterodimers comprise from eighteen alphas and eight beta subunits, that link in specific manner to various extra cellular membrane molecules, the interaction between FN and alpha-5 beta-1 integrin relies on specific peptide sequences within FN, one crucial sequence is the tripeptide motif "arginine, glycine, aspartate" (RGD), which is found in the tenth FN-type3 repeat. Alpha-5 beta-1 integrin recognizes and binds to this RGD sequence [169].

In addition to RGD, there is a secondary sequence near the ninth FN-type3 repeat of FN that plays a coordinating role, this sequence typically contains amino acids like "proline, histidine, serine, arginine, asparagine" (PHSRN). It doesn't bind directly to the integrin but contributes to the overall binding and signaling process [170].

The binding of integrins like alpha-5 beta-1 to FN is crucial for various cellular processes, including cell adhesion, migration, and signaling depending on FN's conformation, these processes can be modulated for

example, if FN is in a conformation that promotes strong integrin binding, it may enhance cell adhesion and migration, influencing tissue repair or development [171].

1.2.2.2 fibronectin functions

The FN enhances cells adhesion also evolve the interaction between cells and the matrix so have important function in tissue building and restoration

While FN is considering as an adhesive protein also have opsonic function so, FN consider very important in hemostasis and tissue reform during vascular damage [172].

Moreover, FN is covalently cross-linked with fibrin when thrombus has been built that's enhanced by the factor XIII-a, the binding of FN promotes the covalent cross-linked further that fibronectin enhances the clot stabilization, several studies show that clot with rise content of FN and factor XIII-a would be more proof against fibrinolysis also more powerful binding to the sub-endothelial frames [173].

When FN link to fibrin in the clot it will stimulate the fibroblast adherence so FN binding have important roles in facilitate the migration of fibroblasts cells to the clot that made in the body [174].

FN have role in linked to the collagen, this linking is very important for subsequent collagen fibrillogenesis, also FN is capable to bind with platelet-integrin's, that is important in platelet accumulation and adherence [175].

1.2.2.3 fibronectin role in myocardial infarction disease

The FN enhances cells adhesion and evolve the interaction between cells so have important function in tissue building and restoration MI is a

main instance of the fibronectin value in the tissue repairs operation so there will upregulation of fibronectin in heart tissue, moreover fibronectin has role in atherosclerosis incidence that increase fibronectin concentration play role in the conversion of vascular smooth-muscle phenotype from the contractile phenotype to synthetic phenotype so increase of atherosclerosis [172] [176.].

The presence of heart damage in individuals with hypertension can be detected by an observed increase in fibronectin expression, additionally, the characteristic left ventricular hypertrophy seen in hypertensive patients is associated with an unusual buildup of insoluble fibrillar collagen in the extracellular matrix (ECM), this collagen accumulation is responsible for the myocardial stiffness resulting from the action of angiotensin-2, which upregulates fibronectin expression necessary for cellular healing, this explains the various beneficial effects of antihypertensive drugs like ACE inhibitors on cardiac hypertrophy, as they inhibit the accumulation of fibronectin [177].

1.2.3 Lipid peroxidation

1.2.3.1 Definition

Lipid peroxidation (LPO) is considered a primary mechanism in causing oxidative damage to cellular structures, and its toxic effects ultimately lead to cell death [178].

At the beginning researchers focused on lipid peroxidation (LPO) in the context of food science due to its involvement in the deterioration of food oils and fats, however, other scientists have noted that LPO is the outcome of the production of highly reactive compounds by virulent

metabolites, these reactive species disrupt intra-cellular membranes, leading to cell death [179].

Lipid peroxidation (LPO) includes the syntheses and propagation of lipid radicals, a disarrangement of the double-bonds in unsaturated-lipids then finally damaging of lipids content of the outer membrane, this process leads to the production of a diverse range of end products, including alcohols, ketones, aldehydes, and numerous other compounds [180].

In pathological situations, excessive levels of reactive oxygen species (ROS) and nitrogen species (NO) are generated, leading to lipid peroxidation, this process is exacerbated by the high concentrations of polyunsaturated fatty acids (PUFA) and the presence of transition metals, resulting in the disruption of biological membranes and subsequently affecting organelles, causing various forms of damage [181].

The technique of harm and toxic action of these ROS species on cells are presently clarified through the consecutive steps of reversible and non-reversible oxidative stress defect [182].

Oxidative stress (OS) is considered as an imbalance condition by elevated of oxidants or reduction of the antioxidants, OS term identifying the syntheses of RO species also the presence of anti-oxidant for defenses [183].

Lipid oxidation (LO) is a series of reaction started through the hydrogen removal or from adding of an ROS that will cause in the oxidative destruction of PUFA [184].

However, PUFA are extremely prone for oxidation more than other fatty acid because the activated ($-CH_2-$) bridge appears as a major location for free radical attacks [185].

The existence of a double-bond neighboring to ($-CH_2-$) makes the carbon-hydrogen bond weakly so, (H) appear highly prone for elimination [186].

1.2.3.2 Reactive oxygen species initiation

Free radical syntheses continually through physiological oxidation of food, that result from the leakage of the respiratory chain in mitochondria that's approximately (1%- 4%) of O_2 that body get in respiration is change to ROS [187].

The mitochondria are considered the major sites for production of ($O_2\bullet^-$) by the reaction of Coenzyme-Q and O_2 in the respiratory chain, so its contain rise amount of superoxide dismutase (SOD) and glutathione to prohibit lipid peroxidation [188].

Several enzymes could generate free radical like xanthine oxidase and aldehyde oxidase form ($O_2\bullet^-$) or (H_2O_2), NADPH oxidase in leukocyte and macrophages syntheses ($O_2\bullet^-$) through respiratory burst that ($O_2\bullet^-$) is converted to H_2O_2 [189].

Macrophages could also syntheses nitric oxide radical by the inducible NOS that play important role in bactericidal processes enzymatic peroxidation also generate through lipooxygenase enzymes in platelets and WBCs [190].

Another source is the lonized ray that harm tissues through syntheses of OH•, H_2O_2 and $O_2\bullet^-$ [191].

$$H_2O \text{ ------- [gamma, UV radiation] ---- } \rightarrow H\bullet + OH\bullet$$

Another source of free radical is tobacco that contains rise scale of

ROS also inspiration of pollutants could elevate the syntheses of ROS [192].

The ROS which seems to be in charge of (O) toxicity involve the molecules that mediates of the partial reduction of (O) which involves, hydroxyl radicals (HO•), superoxide radical (O_2•$^{-}$), hydrogen-peroxide (H_2O_2), also there are more molecule like, peroxyl radical, (NO), peroxo-nitrite ($ONOO^{-}$) and singlet oxygen ($1O_2$) [193].

The HO• radical, produce mostly through a reaction known as Haber Weiss reaction, that in charge of harmful effect [194].

$$O_2^{-} + H_2O_2 \longrightarrow O_2 + HO\bullet + HO^{-}$$

Also it may produce from other reaction that's syntheses by the hydrogen-peroxide and ferrous iron through the fenton reactions, this reaction clarifies the harmful action of active redox-metal [195].

$$Fe_2^{+} + H_2O_2 \rightarrow [Fe\ [II]\ H_2O_2] \rightarrow Fe_3^{+} + HO^{-} + HO$$

Commonly Fe^{+2} interact quickly with hydrogen-peroxide than do Fe^{+3}, thus reducing factors like Vitamin C (ascorbic acid) catalyze fenton reactions converting Fe^{3+} to Fe^{2+} [196].

$$Fe_3 + \text{ascorbate} \rightarrow Fe_2 + \text{semi-dehydroascorbate}$$

In consequence, presents of iron, vitamin C and hydrogen-peroxide consider perfect origins of HO• radicals, other free radicals from the ROS involve superoxide anion radical (O_2-) and hydrogen peroxide radical, normally syntheses as intermediate of respiratory chain, reaction [197,198].

Syntheses of superoxide anion radical are resulted by the auto-oxidation of the ubiquinone (in complexes one and three) while the

syntheses of hydrogen peroxide radical happen through manganese superoxide dismutase [199].

When the respiratory chain operation is blocked on the complexes one and three, it will catalyze the superoxide anion radical syntheses at the NADH oxidizing flavin group and/or at the Co Enzyme-Q binding complex so mitochondria are thought is the major intra-cellular origin of ROS [200].

While (No) produce through the (beckman radi freeman) pathway, NO derived radicals have specific character that it's could diffuse through membranes, presence in the non-hydrophilic part of membranes and presence in the lipoproteins result in quickly action with the fatty acids and lipid peroxyl radicals [201].

The NO radical attack lipid in various technique that involve nitric oxide auto-oxidation to syntheses nitrate, electrophilic addition of nitric oxide relates species to unsaturated fatty acids, radical actions of lipid peroxyl with nitric oxide, also other radical is peroxynitrite [ONOO] has role in oxidation and nitration, above radicals are also play important role on adaptative-inflammatory reaction [202,203].

1.2.3.3 Cytotoxic effects at the molecular level include

Cytotoxic effects at the molecular level encompass various mechanisms that lead to cell damage or death. Some of the key molecular-level cytotoxic effects include:

a) Constitutional disturbance of the membrane double layer and change its fluidity [204].

b) Raised the permeability of cellular component [205].

c) Secrete of lysosomes enzymes [206].

d) Inactive of intracellular enzymes and transporters like the defect result in sodium potassium ATPase [207].

e) Separation in the polypeptide chain [208].

f) Destroy of DNA strand and resulting in a gene mutation [209].

g) Activation of antioxidant because of the presence of free radical cause exhaustion of NADPH results from the use of coenzyme by the glutathione peroxidase/glutathione reductase [210].

1.2.3.4 The benefit of free radical involve

While free radicals are often associated with damage to cells and tissues, it's important to note that they also play essential roles in normal physiological processes. Here are some benefits of free radicals:

a) Prostaglandins and thromboxane A2 formation through cyclooxgynase activity which is a functional enzyme that perform a complex free radical reaction [211].

b) In Bactericidal function by Neutrophils that utilize oxygen to syntheses of lethal ROS by inducible nitric oxide synthase like H2O2, O2- and HO• that destroy the ingested bacteria [212].

1.2.3.5 Mechanisms of lipid peroxidation

Lipid peroxidation processes can be classified in three stages

A. Initiation stage

The (PUFA) exist in the cellular membranes are simply damaged through (LO) over the initiation stage because of weakly methylene beside the double bond, the major incident is the syntheses of R• radical (polyunsaturated fatty acid radical) [213].

$$RH + OH\bullet \rightarrow R\bullet + H_2O \qquad [1]$$

B. Propagation stage

The (R•) quickly interacts with O_2 and syntheses of a (ROO•) that capable of attacking another (PUFA).

$$R\bullet + O_2 \rightarrow ROO\bullet \quad [2]$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet \quad [3]$$

The outcomes of 2 and 3 are the syntheses of R' to ROOH a hydro-peroxide, yet R• will quickly interact with O_2 so another hydro-peroxide will have formed, this would lead to continual making of hydro-peroxide with exhausting of amounts of polyunsaturated fatty acid, so ROS continual produce in the cells adjacent so propagation reaction is beginning, moreover aggregation of this injured lipid result in the ruin of the fine structure, of cellular membrane [214].

C. Termination stage

The peroxidation chain will be continuous until a ROO interact with other one to make passive outputs [215].

$$ROO\bullet + ROO\bullet \rightarrow RO\text{--}OR + O_2 \quad [4]$$

$$R\bullet + R\bullet \rightarrow R—R \quad [5]$$

$$ROO\bullet + R\bullet \rightarrow RO—OR \quad [6]$$

1.2.3.6 Lipid peroxidation role in cardiovascular disease

Oxidative stress initiate by non-controlled syntheses of ROS and defect in anti-oxidant is considered one of the main reason of cardio artery disease and heart failure, moreover, the heart is considered a highly

oxidative organ in order of its highly energy demand and its contain rise amount of mitochondria and minimum levels of anti-oxidants compared to other organs so heart is vulnerable to accumulation of LPO [216].

LPO production are highly activity molecules which capable to modified cells content and result in protein misfolding and accumulation then cell destruction LPO production include: -

A. Oxysterols block the reverse cholesterol transportation from which is important to eject cholesterol from tissue and foam-LDL cells so its result in atherogenic effect [217].

B. 4-hydroxy-2-nonenal (4-HNE) syntheses from the oxidation of phospholipid and has deleterious effect on heart tissue that elevated reactive oxygen species production, apoptosis, destruction to cellular organelles and defect the cell signal, modified cell proliferation, fibrosis, defect in muscle contraction function so its considered one of the oxidative-stress marker and related to CVD [218].

C. Malondialdehyde is one of the main LPO products that have deleterious effect in cardiac tissue like endothelial injury, vascular inflammatory effect and cellular membrane defect, also oxidized important protein in mitochondria resulting in misfolding and dysfunction of respiratory chain reaction so increase free radical the main protein oxidized involve the beta unit of ATP-synthase NADH-oxidoreductase, Cytochrome-b and c-1 complex [219].

Aims of Study

1) Study the gene polymorphism of arginase 1 gene and its correlation with STEMI and NSTEMI acute myocardial infarction in Babylon province.
2) Detect the levels of fibronectin, arginase activity and lipid peroxidation and its relationships with STEMI and NSTEMI acute myocardial infarction in Babylon province.
3) Relation of lipid peroxidation with fibronectin levels and arginase activity in STEMI and NSTEMI acute myocardial infarction in Babylon province
4) Study the relation of biochemical and genetic parameters with study groups.

CHAPTER TWO

Materials & Methods

2.1 Materials :

2.1.1. Chemicals:

Chemicals and kits utilized in this study were mention in table (2.1).

Table (2.1): Chemical Substances Used in This Study

No.	Chemical substance	Origin
1	Loading dye	Intron / Korea
2	DNA extraction kit (blood)	Geneaid / Korea
3	Premix PCR	Intron / Korea
4	TBE buffer	Conda / USA
5	Agarose	Conda / USA
6	100 bp DNA ladder	Intron / Korea
7	50 bp DNA ladder	Intron / Korea
8	Primer	Alph DNA/Canada
9	Red safe staining solution	Intron / Korea
10	Absolute ethanol	Fluka / Germany
11	Arginase Kit	BioAssay Systems (USA)
12	MDA(Malondialdehyde)	Elabscience (USA)
13	Fibronectin kit	Mybiosource (USA)
14	Troponin I (Chemiluminescence) Roche	(Switzerland)

2.1.2. Instruments and Types of Equipment:

Instruments utilized in the study mention in table (2.2).

Table (2.2) Instruments and equipment's used in this study.

No.	Instruments and Materials	Origin
1	Elisa reader and washer	Biotek/USA
2	Printer	Epson / Indonesia
3	Vortex (Electronic)	Kunkel / Germany
4	Centrifuge	Hettich / Germany
5	UV transilluminator	Cleaver Scientific / UK
6	Distiller	GFL / Germany
7	Incubator	Thermo Fisher Scientific / Germany
8	Sensitive balance	MettlerTdedo / USA
9	Water bath	Grant / England
10	T100-thermo cycler	Bio Rad / USA
11	Autoclave	Haramaya / Japan
12	Deep Freeze	GFL / Germany

13	Micropipettes (5-50ul) , (2-20ul) , (20-200ul) , (100-1000ul)	Slamed / Germany
14	Water bath	Grant / England
15	Gel electrophoresis	Bioneer / Korea
16	Disposable syringe (5ml)	Qatar
17	1 ml pipette tips	China
18	0.1 ml pipette tips	China
19	0.01 ml pipette tips	China
20	EDTA tube (5ml)	AFCO / Jordan
21	Eppendorf tube (1.5ml)	China
22	Test tube with Separating gel	AFCO / Jordan
23	Centrifuge Microtubes	Hermle
24	UV Sterilization Cabinet	Cleaver Scientific / UK

2.1.3. Subjects:

2.1.3.1. Patient Group:

The study had been done at the laboratory of Biochemistry and Chemistry Department, College of Medicine, the collection of samples was conducted in the time from 1st of February 2022 to 30th of April 2022, ninety persons were taken and classified as two groups 45 patients with STEMI and 45 presented as NSTEMI of age ranging from 46 - 74 years all of those patients admitted to merjan medical city in Hilla city and all patients of these groups were diagnosed depending in ECG, cardiac troponine I, sign and symptoms by cardiologist doctors.

2.1.3.1.1 Inclusion Criteria

Patients with myocardial infraction were included in this study.

2.1.3.1.2 Exclusion Criteria

A. Congenital Heart Disease.
B. Other ischemic heart disease such as:- Angina Pectoris and Unstable Angina
C. Hypertension
D. diabetes mellitus
E. liver disease
F. cancer
G. renal disease

2.1.3.2. Control Group

Ninety persons were taken from the outpatient clinic of merjan medical city in Hilla city as a control group of the age ranging from 45 - 72 years.

Permission had been taking from all participants to this study after they informed about the aim and advantages of this study, each person

who involved to the control group underwent full history and physical examination including: age, gender, address, smoking, family history of myocardial infraction.

2.2. Methods:

2.2.1. Collection of the Blood:

Venous blood samples had been drawn from patient and control subjects through the usage of disposable syringes, 5 ml of blood obtained from every subject through vein-puncture, 1 ml place into EDTA tubes and the residual amount pushed slowly into disposable tubes containing separating gel.

Blood exist in EDTA tubes was stored in ⁻40°C (deep freeze) in order to utilize later in genetic part of the study, blood in the gel tubes remained to be clot at room temperature (RT) (20-25) °C for 12-16 minutes and then centrifuged at 2000 × g for approximately 15-20 minutes then the serum was obtained and stored at -20˚C until analysis.

2.2.2 Biochemical study

2.2.2.1 Measurement of Lipid Peroxidation

LPO is non-stable so when oxidase a PUFA it will break and yield a mixture of compounds which involve malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) so estimation of MDA considers a method for lipid peroxidation detection [220].

A. Principle

The MDA test kit relied on Competitive ELISA. The ELISA-microplate had been precoated with malondialdehyde. Throughout the reaction, malondialdehyde present in the serum or standard competed with a pre-fixed malondialdehyde for binding to the Ab specific to

malondialdehyde. Any excess conjugate and serum or standard that didn't bind were washed away. Afterward, avidin-horseradish peroxidase (HRP) was applied, followed by the placement of TMB. The reaction was halted using a stop-solution, and the optical density (OD) was measured at a wavelength of 450 nanometers using an ELISA reader [221].

Table (2.3) Components of the MDA ELISA kit:

No.	Description	Quantity
1	ELISA Plate	96 well
2	Reference Standard	2 vial
3	Sample and Standard dilution buffer	20 ml
4	Biotin- detection antibody (Concentrated)	120 ul
5	Antibody dilution buffer	14 ml
6	Horseradish peroxidase reagent (Concentrated)	120 ul
7	HRP Conjugate dilution	14 ml
8	Substrate reagent	10 ml
9	stop solution	10 ml
10	Washing buffer solution (25x)	30 ml

B. Preparation of Washing Buffer:

The 30 ml of washing buffer was diluted by the addition of 720 ml of distilled or deionized water.

C. Preparation of Standard:

The reference standard was centrifuged at 10,000 ×g for one minute, and then 1 ml of both a reference standard and sample diluent (SD) was left for ten minutes and then mixed for a short duration, after which it was allowed to resolve completely. The working solution (WS) with 2000 ng per mL was prepared.

Seven tubes were chosen and labeled from (A to G). In each tube, 0.5ml of Standard and sample diluent was added, and 0.5ml of the (WS) was added to the A-tube, resulting in the creation of a 1000 ng/mL solution (WS). From the A-tube, 0.5ml was transferred to the subsequent one (B-tube), and this process was repeated as indicated in figure (2.1) until the G tube was reached, which should contain only standard-WS and be considered as a blank tube.

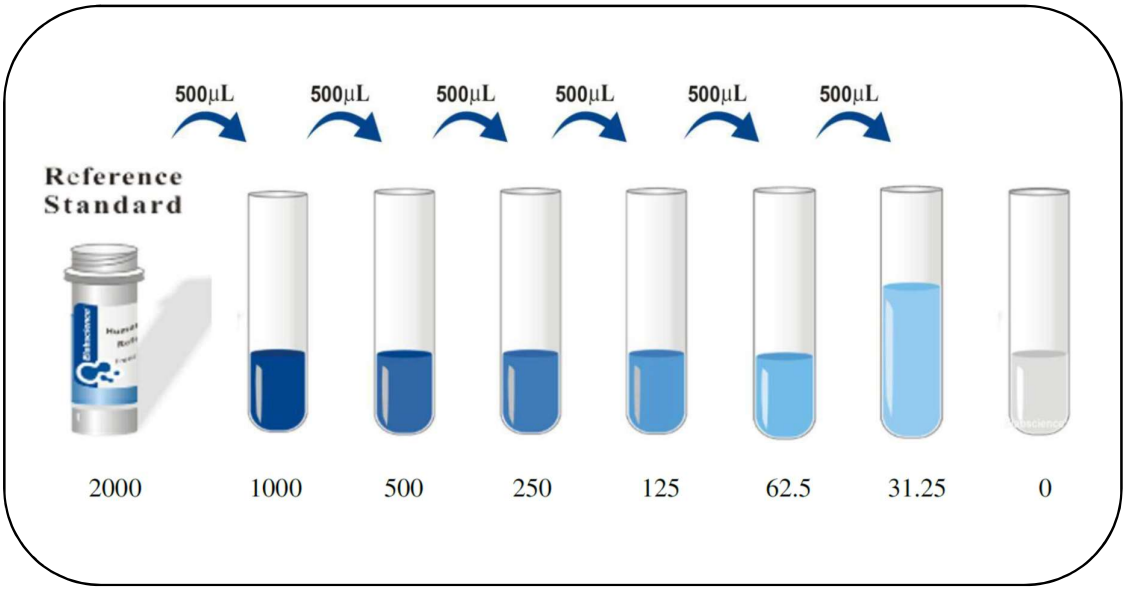

Figure (2.1) MDA standard preparation

D. Preparation of Sample and Storage:

The blood that was drawn into the gel-containing tubes was allowed to clot at room temperature for 10-15 minutes, after which it was centrifuged at 3000 × g for approximately 30 minutes, resulting in the separation of the serum.

When plasma samples collected by EDTA or Citrate tubes were used, they were mixed for a few minutes and then centrifuged for about 30 minutes at 3000 × g.

Assessment samples were reserved within 24 hours in a refrigerator at 2-8 °C, and when there was a need to reserve them for an extended period, they were reserved at -20°C.

E. Preparation of Biotinylated-Antibody

The biotinylated-antibody was centrifuged for one minute, and then the antibody diluent was added at a rate of 10 ml for each 0.1 ml of concentrated antibody solution.

F. Preparation of Horseradish Peroxidase Working-Solution:

First, the concentrated-horseradish peroxidase conjugate was centrifuged at 1000×g for one minute. After that, 0.1 ml of concentrated-horseradish peroxidase was placed into 10 ml of horseradish peroxidase-diluent.

G. Assay Procedure

1- The position for diluted-standard, blank, and serum was located, and then 50 µL from the standard, serum, and blank was placed in the located well. Subsequently, 50 µL of biotinylated-detection antibody-WS was directly placed in all the wells. A sealer was put on the top of the plate, and then it was incubated for 45 minutes at 37 ℃.

2- The plate was washed three times by an ELISA washer to remove liquid from the plate. then It was soaked in 0.35 ml of washing buffer for one minute, and as part of the washing procedure, the solution was aspirated. This process was repeated three times, and the microplate was inverted and dried by blotting it with a clean paper towel.

3- 0.1 ml of WS of Horseradish peroxidase-conjugate was placed in every well in the microplate. A sealer was put on the top of the plate, and then it was incubated for 30 minutes at 37 ℃.

4- The plate was washed five times by an ELISA washer.

5- 90 µl of Substrate was placed in every well, and then a sealer was put on the top of the plate. It was incubated for 15 minutes at 37 ℃, with precautions taken to avoid light exposure to the microplate.

6- The ELISA reader was operated around 15 minutes before reading the microplate.

7- 50 µL of Stop-solution was placed in every well in the microplate.

8- The optical density of the microplate was read by a microplate reader at 450 nanometers.

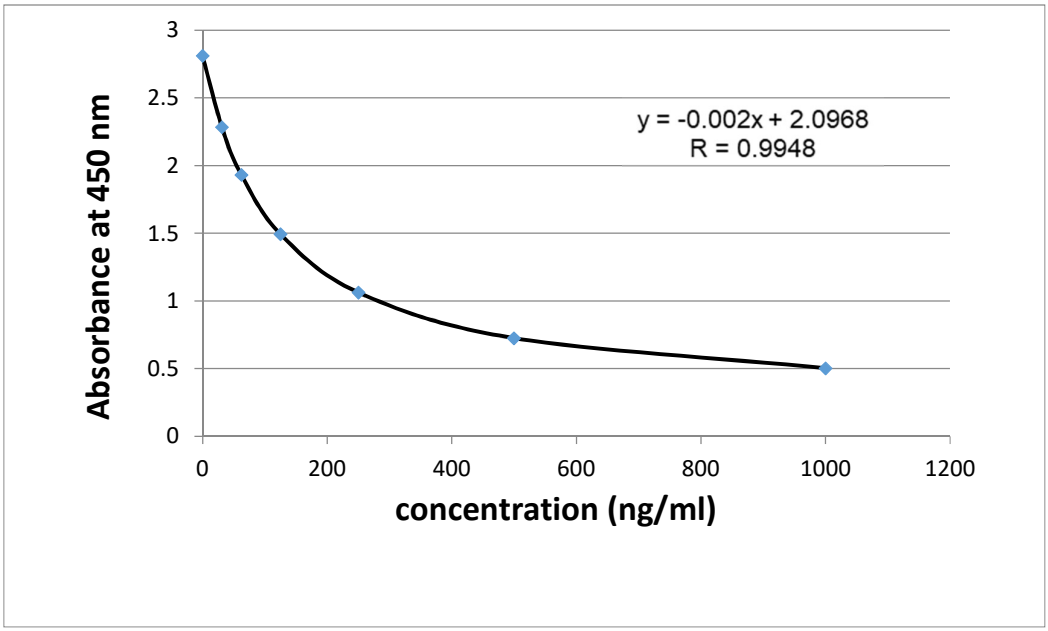

Figure (2.2) standard curve of MDA

2.2.2.2 Measurement of arginase activity

A. Principle

The principle of the ARG-activity determination procedure in the arginase kit was based on the use of a chromogen that resulted in the synthesis of a colored compound during the ARG reaction, with the

strength of the color being directly proportional to the ARG-activity in the serum of the study.

Table (2.4) Components of the arginase ELISA kit:

No.	Description	Quantity
1	Arginine Buffer	1.5 ml
2	Reagent-A	12 ml
3	Reagent-B	12 ml
4	Urea standard (1mM)	0.5 ml
5	Mn Solution	300 ul

B. Assay Procedure

1- The urea standard (WS) was prepared in the first step by mixing 24 ul of standard with 176 uL of distilled water. Then, 50uL of this mixture was placed with 50 µL of distilled water in the standard well of the plate.

2- The substrate-buffer was prepared by mixing four volumes of ARG-buffer with one volume of manganese vial, with the consideration that every test required 10ul of the buffer.

3- 40 ul of each serum sample was placed in two plate wells. Then, 10 ul of the buffer was added to one of each sample well to create a sample-blank. Incubation was carried out at 37°C for two hours.

5- Reagent A and B were mixed, and then 0.2 ml was added to all wells to terminate the reaction.

6- 10 ul of the buffer was added to a well for the sample blank, mixed, and incubated for one hour at room temperature. The reading was taken at 430 nanometers, and the activity of ARG was calculated using the equation below: -

$$\text{ARG activity(IU/L)} = \frac{\text{OD Sample} - \text{OD Blank}}{\text{OD Standard}} \times [\textit{Urea Standard}] \times 50\,x\,10^3 / (40 \times t)$$

$$\text{ARG activity(IU/L)} = \frac{\text{OD Sample} - \text{OD Blank}}{\text{OD Standard}} \times 10.4$$

2.2.2.3 Measurement of fibronectin

A. Principle

The detection principle of the fibronectin ELISA kit relied on a mechanism known as quantitative-sandwich immunoassay. The microplate had been previously coated with a monoclonal antibody specific to fibronectin. Subsequently, when serum was applied to the microplate, fibronectin would bind to the antibody that had been pre-coated onto the microplate.

To determine the level of fibronectin present in the serum, detection-A and detection-B solutions, specific to fibronectin, were added to every well in the microplate to sandwich the fibronectin immobilized on the plate.

The microplate was then subjected to incubation. Following that, the microplate was washed by an ELISA washer to separate the non-bound components. Subsequently, substrate was placed in every well of the microplate, and a stop solution was used to halt the reaction.

Table (2.5) Components of the fibronectin ELISA kit:

No.	Description	Quantity
1	ELISA Plate	96 well
2	Standard	2 vial
3	Detection-A	120ul
4	Detection-B	120ul
5	TMB- Substrate	9 ml
6	Washing buffer solution (30x)	20 ml
7	Standard-diluent	20 ml
8	Assay diluent-A	12 ml
9	Assay diluent-B	12 ml
10	Stop solution	6 ml

B. Preparation of Standard:

Standard-WS was prepared by placing 1 ml of standard-diluent into the standard vial and allowing it to stand for 10 minutes at room temperature. Then it was mixed gently. Seven tubes were selected and numbered from (A to G). Then, 500 ul of standard-diluent was added to every tube. Next, 0.5 ml of the (Standard-WS) was added to the A-tube and mixed to create a 100 ug/mL of (Standard-WS). 0.5 ml was taken from the A-tube into the subsequent one, and this process was repeated as indicated in figure (2.4) until the G tube was reached, which should only contain standard-WS and be considered a blank tube.

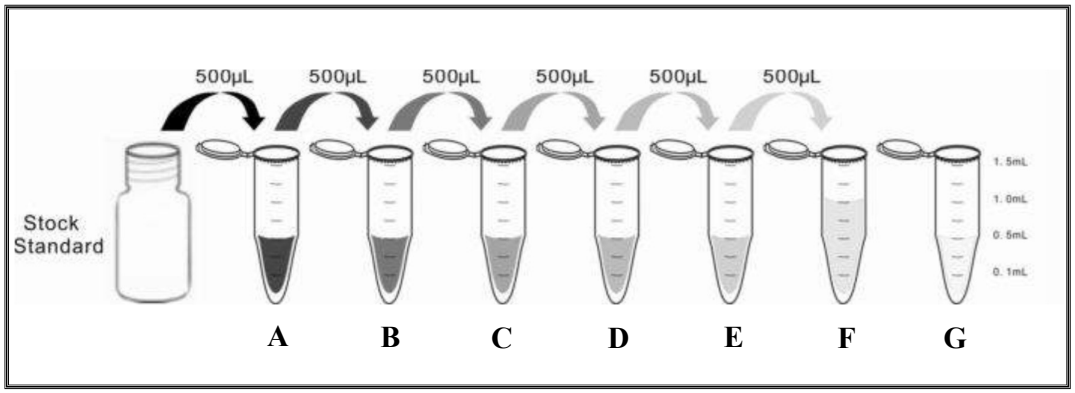

Figure (2.3) Fibronectin standard preparation

C. Preparation of Detection Reagent:

Firstly centrefigation of stock-reagent then place 10 ml of diluent to each 100ul from concentrated reagent to obtain the WS.

D. Preparation of Wash Solution:

20 ml of washing buffer was added to 580 ml of distilled or deionized water. The washing buffer could be reused, but if crystals appeared, it had to be warmed to 25°C and mixed gently until they disappeared.

E. Assay Procedure

1. The position for the diluted-standard, blank, and serum was located, and 100 µL of standard, serum, and blank were placed in the respective wells. The plate was sealed, and then it was incubated for 60 minutes at 37°C.
2. Afterward, the solution was eliminated from the microplate without washing.
3. 0.1 ml of detection reagent-A was placed in every well of the microplate. It was then gently mixed, and the plate was sealed, and incubated for 60 minutes at 37°C.
4. The plate was washed three times by an ELISA washer, removing the liquid from the plate and adding 0.35 ml of washing buffer each time. After each addition, fluid aspiration was performed. This process was repeated three times, and the plate was dried by blotting it with clean paper towels.
5. 0.1 ml of detection reagent-B was placed in every well of the microplate. It was gently mixed, and the plate was sealed. Then incubated for 30 minutes at 37°C.
6. The plate was washed five times by an ELISA washer, removing the liquid from the plate and adding 0.35 ml of washing buffer each time. After each addition, fluid aspiration was performed. This process was repeated five times, and the plate was dried by blotting it with clean paper towels.
7. 90 µl of substrate was placed in every well. The plate was sealed and placed in a 37°C environment for 15 minutes while avoiding exposure to light.
8. 50 µl of stop-solution was placed in every well.

9. The optical density of the microplate was read using a microplate reader at 450 nanometers.

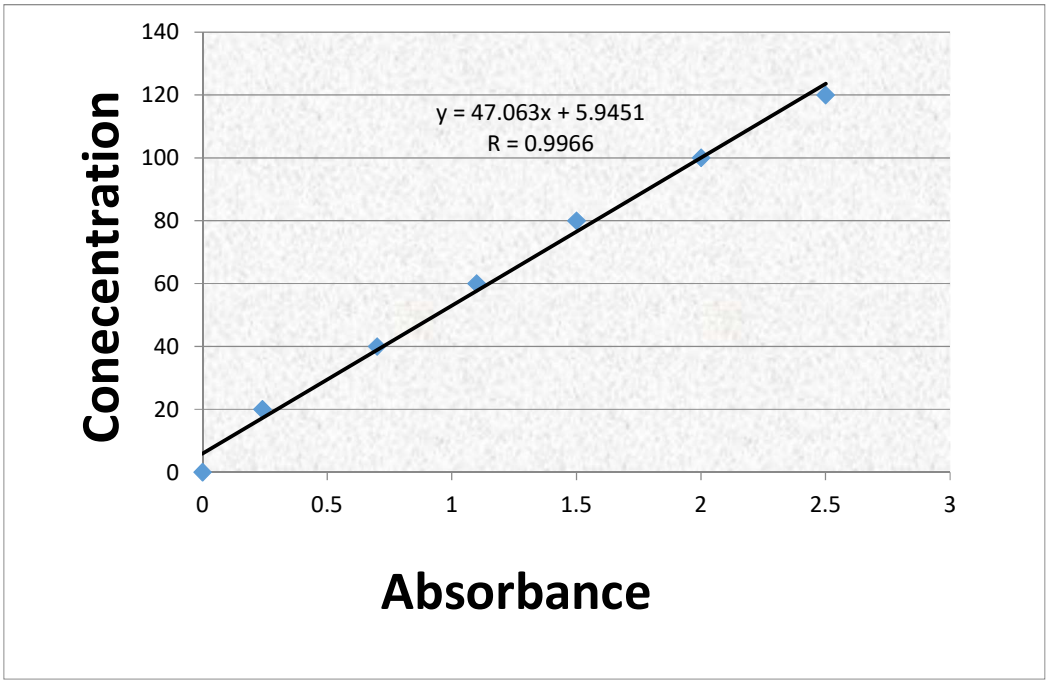

Figure (2.4) Standard curve of Fibronectin

2.2.2.4 Determination of Troponin I titer

A. Principle

The test was conducted using cobas-e411, whose principle depended on the competition of analyte in serum with a ruthenium labeled, similar to the ELISA-Sandwich method. A voltage was applied, and subsequently, the electro-chemiluminescence signal was determined as per the manufacturer's instructions.

The troponin titer that was produced was standardized through a two-point calibration performed using calibrators supplied with the kit.

Table (2.6) Components of the troponin kit:

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

B. Procedure

The troponin determination was fully automated using cobas-e4 and consisted of two stages:

In stage 1, 0.03 ml of serum was auto-mixed with 0.06 ml of monoclonal antibodies and 0.06 ml of monoclonal antibodies labeled by a ruthenium complex. This was followed by incubation for five minutes, which was sufficient for the sandwich reaction.

In stage 2, there was an auto-addition of streptavidin-coated micro-particles, ensuring that all mixtures were linked by the solid phase. This mixture was incubated for 4 minutes. Then, the compounds in the reaction mixture were transferred to the measurement chamber, where the micro-particles were magnetically captured on the top of the electrode. Non-binding materials were then washed out using washing buffer. The application of a voltage to the electrode resulted in the production of

chemiluminescent emission, which was determined through a photomultiplier. The results were defined using the calibration curve produced through the utilization of a two-point calibration.

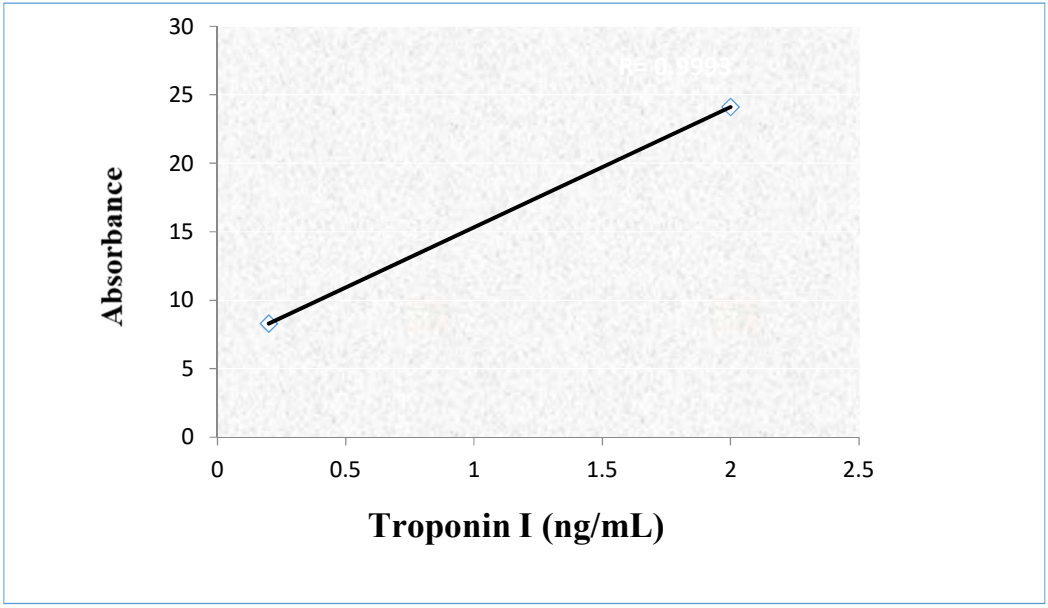

Figure (2.5) Standard curve of troponin

2.2.3 Genetic study

2.2.3.1 DNA Extraction

A. Principle

The kit for DNA extraction was utilized to purify the DNA from whole blood. Several reagents, such as the chaotropic salt with proteinase-k reagent, were used, and they played a role in the destruction of proteins and cell lysis. This process caused the DNA to bind to the fiber matrix site in the spin column. The wash-buffer reagent had an important role in expelling contaminants, and the purified genomic DNA was eluted by applying a minimum salt elution buffer or Tris EDTA buffer (TE buffer).

Table (2.7) Components of DNA Extraction Kit

Item	Quantity
GST Buffer	30 ml
GSB Buffer	40 ml
W1 Buffer	45 ml
Wash Buffer	25 ml
Elution Buffer	30 ml
Proteinase-K	2 vial
GS Column	100 pieces
Two-ml collection tube	200 pieces

B. Reagent Preparation

A. The wash buffer was prepared by placing absolute ethanol with 100% concentration into the wash buffer and mixing it.

B. The proteinase-K was prepared by adding distilled water to the proteinase-K and mixing it using a vortex.

C. Procedures for DNA Extraction

Step-1: Preparation of the Blood Sample

Firstly, 0.2 ml of whole blood was placed in a 1.5 ml microcentrifuge tube. Afterward, 20 µl of Proteinase-K was added, mixed slowly, and then it was placed in an incubator at 60°C for five minutes.

Step-2: Cell-Lysis

0.2 ml of the GSB-buffer was added to the microcentrifuge tube and mixed vigorously. It was then placed in an incubator at 60°C for five minutes. During incubation, the tube was overturned every two minutes.

At this time, the determined amount of elution-buffer (0.2 ml/sample) was transferred to a microcentrifuge tube for incubation at 60°C (for stage-E, the DNA-elution).

Step-3: DNA Binding

0.2 ml of absolute ethanol was added to the lysate-sample, then mixed vigorously for ten seconds. Any precipitate observed was removed using a tip. Afterward, the GC-column was placed in a two-ml collection tube, and the combination was added to the GC-column. The mixture was centrifuged for one minute at 15,000 × g, and then the collection tube was removed, and the GC-column was placed in a new collection tube.

Step-4:

Wash After adding 0.4 ml of wash-buffer to the GC-column, it was centrifuged at 15,000 × g for thirty seconds. The residual liquid was then removed, and the GC column was placed in the same tube.

0.6 ml of wash-buffer was added to the GC-column, and it was centrifuged at 15,000 × g for 30 seconds. The residual liquid was removed, and the GC column was placed in the same tube. This centrifugation step was repeated at 15,000 × g for three minutes.

Step-5: DNA Elution

The GS-column, after washing, was transferred to a new microcentrifuge tube. Then, 0.1 ml of the previously heated elution-buffer was placed in the center of the column matrix and allowed to stand for three minutes. Afterward, it was centrifuged at 15,000 × g for 30 seconds.

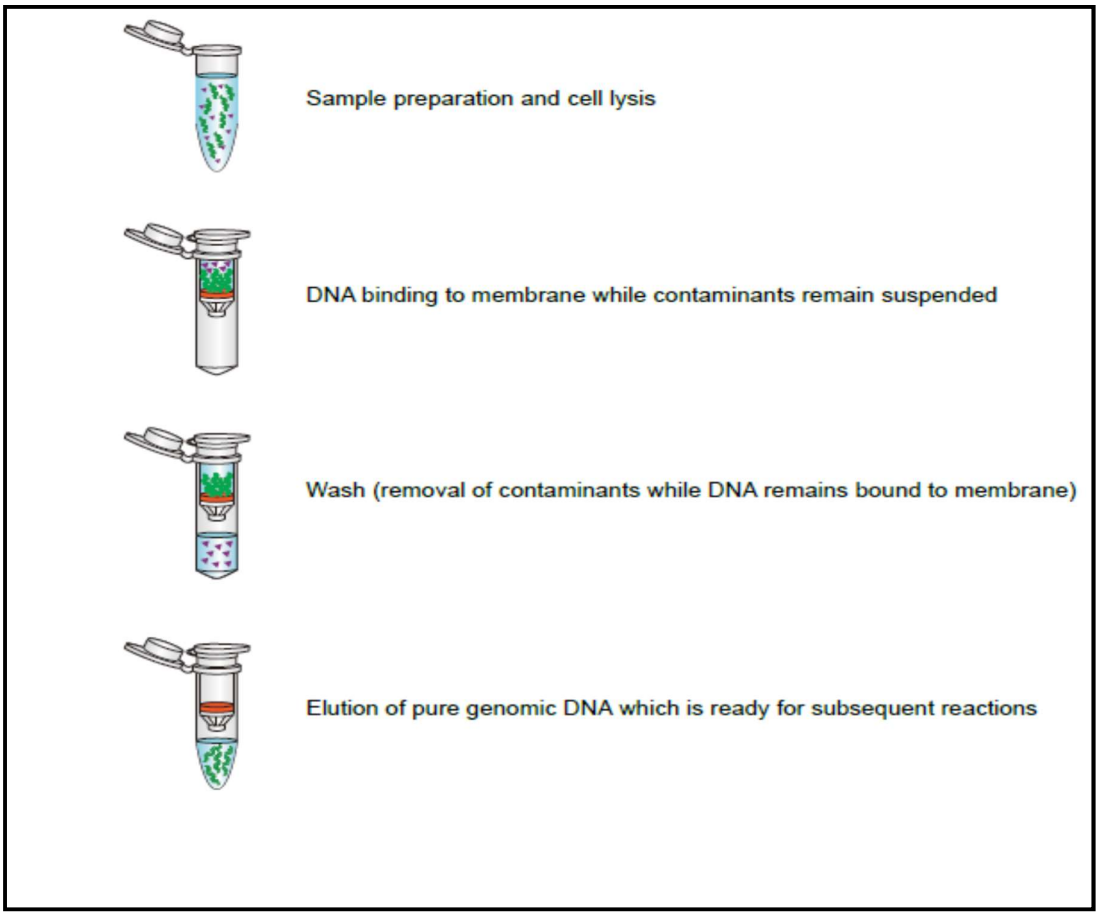

Figure (2.6) Extraction protocol diagram

2.2.3.2 Estimation of DNA Concentrations and Purity by Absorbance

After the completion of DNA extraction, a necessary procedure was carried out to determine the DNA concentration and assess the DNA's purity using a nano-drop. Absorbance readings were taken at 260 nm., as shown in figure (2-8).

2.2.3.3 Agarose Gel Electrophoresis

Electrophoresis is a crucial step following DNA extraction. It allows us to visualize DNA bands and evaluate the results of PCR operations.

Gel Preparation:

1. To prepare 50 ml of a 2% agarose solution, 1 g of agarose powder was measured and placed into a conical flask, and then 50 ml of prepared TBE (Trisbase pH 6.8, Borate, and Disodium EDTA) was added.
2. The mixture was heated on a hot plate until the agarose dissolved, and the solution became clear. The gel was allowed to cool to (45-50°C), after which 2.5 ul of red safe staining was added.
3. The resulting solution was poured into the gel tray, and any air bubbles were removed. A comb was inserted into the cast to create wells for loading the samples, and the agarose was allowed to solidify at room temperature for 30-60 minutes.
4. The comb was removed, and then the gel tray was placed in an electrophoresis tank, ensuring that the gel was completely submerged by the electrophoresis buffer.

Loading the Samples:

Initially, a mixture of 3 ul of loading buffer and 5 ul of the presumed DNA was carefully deposited into the well created after removing the comb.

Gel screening was accomplished by adding 10 ul of DNA ladder as a size marker, positioning it in parallel with the sample well formed after comb removal.

Electrophoresis was carried out at 70 volts and 65 amperes for one hour, allowing the stain to migrate to the opposite side of the gel.

2.2.3.4 Agarose Gel Photo Documentation:

The agarose gel was visualized under the UV-transilluminator provided by the gel documentation unit. The gel was positioned above the

device and exposed to UV light at 336 nm, with photos captured by a camera, as illustrated in the figure (2.10).

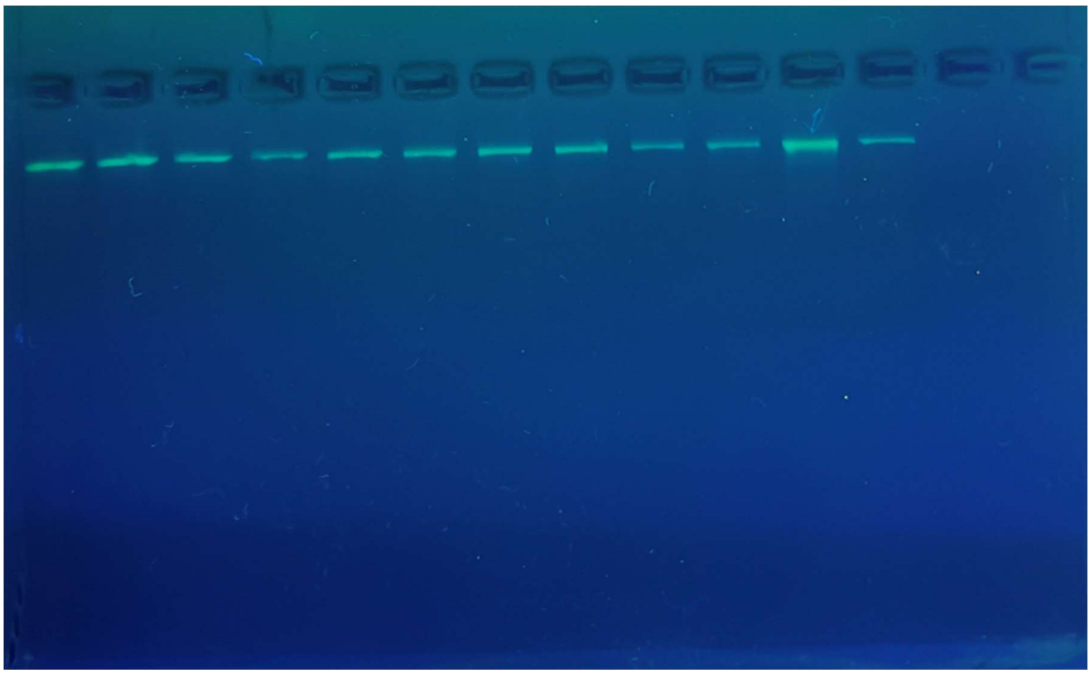

Figure (2.7): Gel electrophoresis of genomic DNA extraction from Blood, 1% agarose gel at 30 minute.

2.2.3.5 Preparation of Primers

The primers were initially lyophilized and needed to be thawed in free dd-H_2O to achieve a required concentration of 100 pmol/µl, which was considered a stock solution and stored at -20°C. Subsequently, a working solution (WS) with a concentration of 10 pmol/µl was prepared by combining 10 ul of the stock with 90 ul of free dd-H_2O to reach the desired volume.

The procedure for primer preparation included the following steps:

A. Invert the tube upside down before opening the cap.

B. Add the specified quantity of ddH_2O as indicated in the leaflet to obtain a concentration of 100 pmoles/µl.

C. Vortex the tube for 30 seconds.

D. Create the WS by mixing 10 ul of the stock primer with 90 µl of free nuclease water.

E. Both the stock primer and WS were stored at -20°C.

F. Before using the WS in PCR, it was recommended to vortex it, and then it should be stored at -20°C.

When preparing the primers, it was advisable to divide them into smaller portions using Eppendorf tubes for individual use.

2.2.3.6 PCR Amplification

Determination of Arginase-1 (rs2781666) genotyping

The primer sequences for which amplify the polymorphic region of Arginase-1 gene (rs2781666), primers were used according to *Muhammad Jadoon, et al.*[222] *Monika Buraczynska, et al.* [223], *Syed Fawad Ali Shah, and Tahir Iqbal, et al* [224], a set of primers are shown in Table (2.8).

Table (2.8) set of primers

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- CGGAAGGATCTTTAAGGTGCC- 3'	56.9	50	294 b.p
Reverse	5'- CCATGTGTCCGATGCAGTTCTG- 3'	58.9	50	

The component of master premix that's used for gene amplification are shown in Table (2.9).

Table (2.9) master premix contents

Material	Concentration
i- Taq DNA polymerase	5 U/ul
DNTPs	2.5mM
Reaction buffer	1x
Gel loading buffer	1x

optimization of PCR-condition was done through made a several trial with various temperature ranging from 56ºC to 62ºC, this done by Gradient-PCR then fixing of most favorable annealing temperature (61ºC), also altered the addition of DNA template from 2ul to1.5ul, the alteration in these 2 factors result in optimizing the PCR-condition, the ingredients of PCR reaction-tube are elucidate in Table (2.10).

Table: (2.10) PCR reaction tube component.

Component	Concentration
PCR premix	5ul
Templet DNA	1.5ul
Forward primers (10 pmol/µl)	1ul
Reverse primers (10 pmol/µl)	1ul
Dd water	16.5ul

So the step of detection of favorable temperature is followed by DNA-amplification which operated by T100-thermocycler apparatus, the setting of thermocycler that utilize to amplify the precise DNA is elucidate in table (2-11).

Table (2.11) the program of PCR cycle

PCR-Cycle		Temperature	Time
1 Cycle	Initial Denaturation	94°C	5 minute
35 Cycle	Denaturation	94°C	45second
	Annealing	61°C	45second
	Extension	72°C	45second
1 Cycle	Final Extension	72°C	75minute

2.2.3.7 Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

restriction fragment length polymorphism (RFLP) was utilized for genotyping relying on tail-restriction enzyme; procedure which utilized for single nucleotide polymorphism method is elucidating in Table (2.12).

Table (2.12) restriction fragment length polymorphism procedure

Protocol	Volume
Product PCR	5 ul
Restriction enzyme	2 ul
Buffer	3 ul
Temperature / time	65 °C / 4 hours

After (RFLP) by tail-enzyme the genotyping was detected by electrophoresis on 2.5% agarose, the bands are staining by red-safe and seen n under ultra-violet light.

2.3 Statistical Analysis

Information's were examined by (SPSS-26), and determined as a mean and standard-deviation (SD), continued variables were analyzed through using (T test) which has been utilized to detected the significant difference between the groups also Chi-square (x2) test had been used, genetic determination was done utilizing Chi-square (x2) test, while P values <0.05 was counted as significant and <0.001 counted as highly significant.

CHAPTER THREE

Results & Discussion

3. Result and discussion

This study involved 90 patients with MI (45 STEMI and 45 NSTEMI) and 90 healthy subjects (control group) in different areas of Babylon province in Iraq.

3.1. Demographic characteristics of patients and control subjects enrolled in this study

3.1.1. Age

in this study there was no significant difference between the mean-age of patients and control group as shown in table (3-1), The mean ± SD age of STEMI group was (60.6 ± 10.37) years and NSTEMI was (59.93 ± 9.73) while that of control group was (58.55 ± 9.58) years.

Table (3-1): Age distribution in patients and control groups.

	Group	No.	Mean & Standard Deviation
Age (years)	STEMI	45	**60.6 ± 10.37**
	Non-STEMI	45	**59.93 ± 9.73**
	Control	90	**58.55 ± 9.58**
P-value	**STEMI versus Control group (P >0.05)**		
	non-STEMI versus Control group (P >0.05)		

*** Significant difference (P < 0.05), **highly significant (P < 0.001)**

In the present study the age is classified in different groups to elucidate the disease incidence in different age stage as shown in figure (3-1),

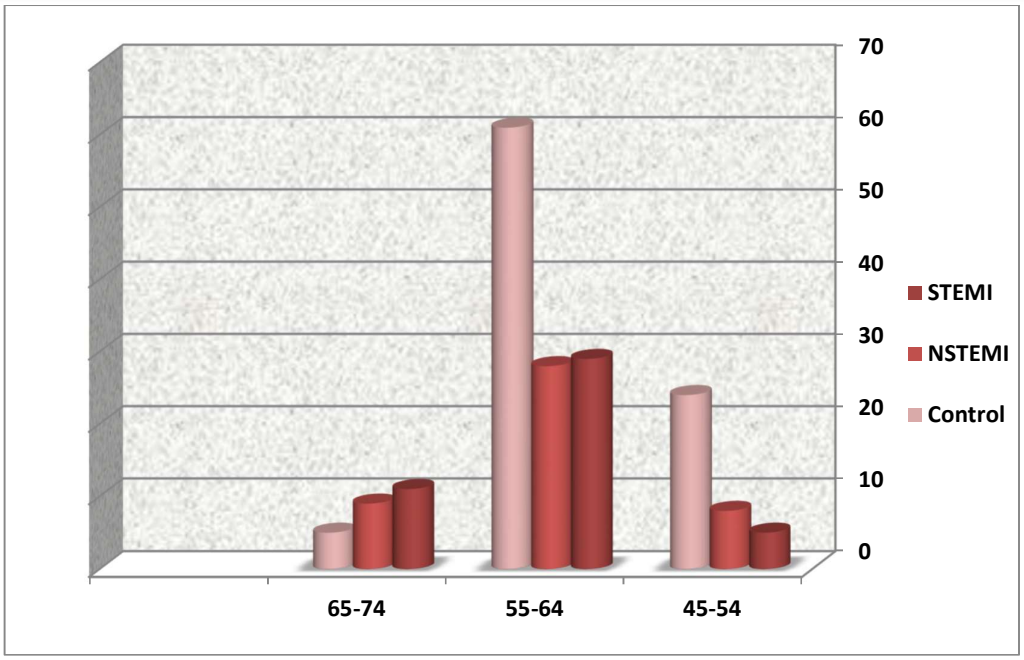

Figure (3.1): Age distribution of study groups.

The figure above observed that disease incidence in this study is increase in the age between 55 and 64 years, this agree with a study in karballa university by Muna Abdul Kadhum Zeidan *et al*, which study the risk factors for myocardial infraction in AL-Zahraa hospital and found that the high number of patients in their study were aged between 55 to 61 years [225].

3.1.2 Sex

In the present study, the number of males in the myocardial infraction groups was 69 (76.6%) that 35 (77.8%) in STEMI group and 34 (75.6%) in NSTEMI group while the females were 21 (23.3%) that 10 (22.2%) in STEMI group and 11 (24.4%) in NSTEMI group as elucidate in figure (3-2) thus, there were a significant association between male in STEMI incidence versus control group with a p-value (< 0.05) and also association appeared between male in NSTEMI incidence versus control group with a p-value (< 0.05) as shown in table (3.3), that resulted from the protective role of sex-hormones mainly estrogen against MI so delay the incidence of AMI in women for about ten

years contrasted with men moreover, rise testosterone scales in men promote AMI [226].

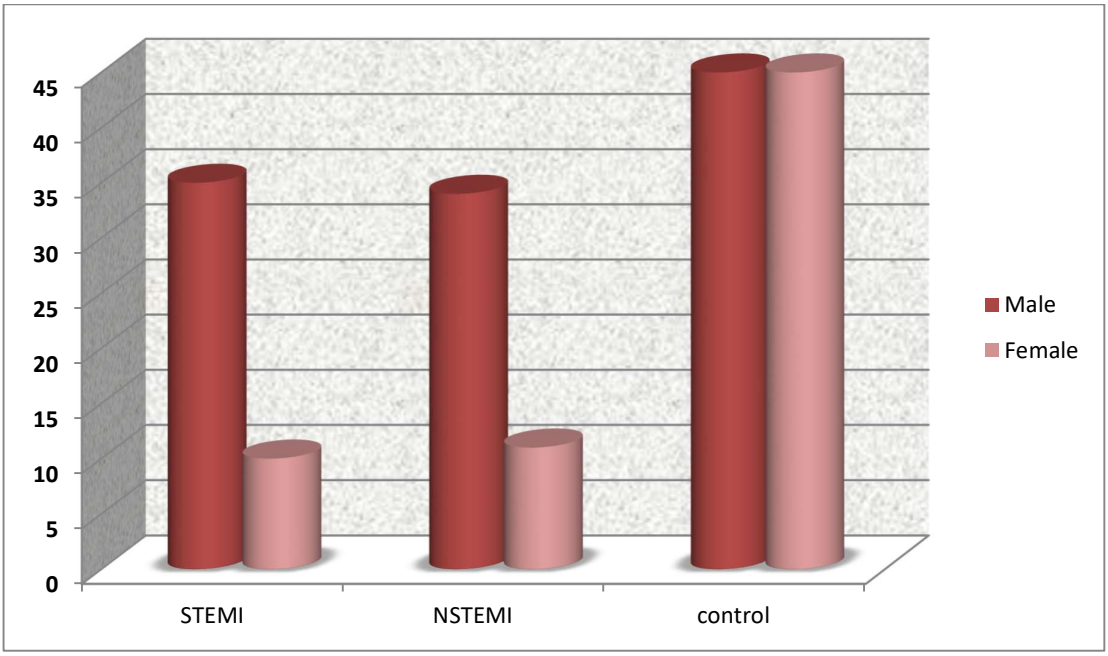

Figure (3.2): sex distribution in patients group.

Several studies discussed the relation between sex as a risk factors with MI incidence, a study that goes with this study results are the study by Mohamad Alkhouli *et al*, about sex-linked variation in the incidence of AMI and conclude that females have lower incidence of AMI and minimal probability of submitting invasive treatment contrasted with male [227].

Other study agree with present study results is that conducted in college of health sciences, Howler medical university by Shwan Othman Amen, Ahmed Himdad Hawez *et al*, about vitamin D deficiency in Patients with AMI found a statically significant association between male and AMI incidence [228].

3.1.3. Residency

The distribution of AMI group and controls group according to residency is seen in figure (3-3), patients' group included 66 (73.3%) patients from urban regions that 32 (71.1%) in STEMI group and 34 (75.6%) in NSTEMI group

while there were 24 (26.6 %) patients from rural regions that 13 (28.9%) in STEMI group and 11 (24.4%) in NSTEMI group as seen in figure (3-3), whereas, control group included 72 (80.0%) participants from urban region while 18 (20.0%) participants were from rural regions, so there were no statically significant association between residency and the two myocardial infraction groups as seen in table (3-3)

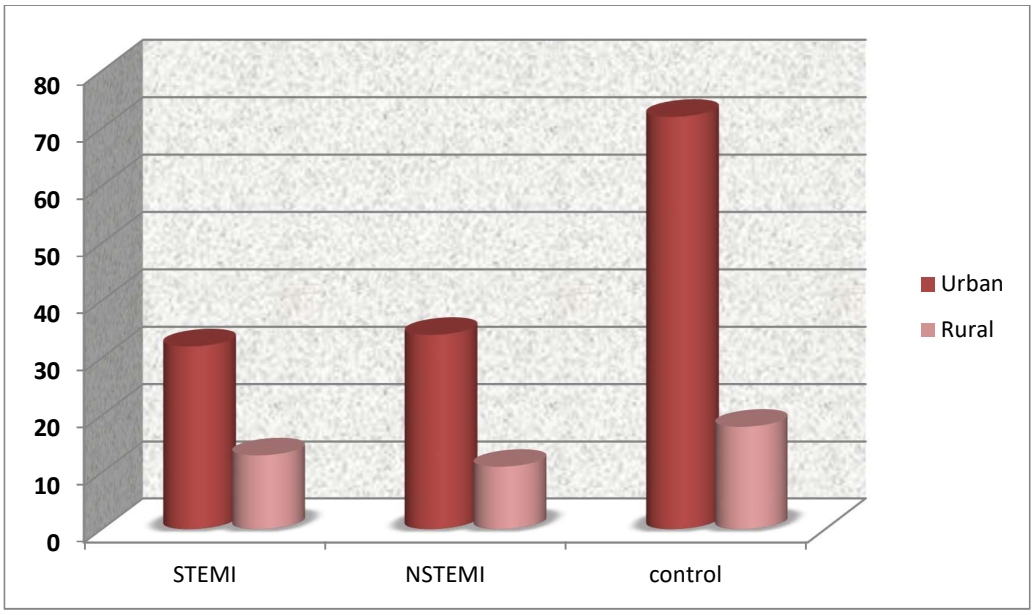

Figure (3-3): Residency distribution in patients group.

3.1.4. Body Mass Index (BMI)

The distribution of mean ± SD of BMI for STEMI group was (29.8 ± 4.39) kg/m^2 while that for NSTEMI group was (29.08 ± 4.34) kg/m^2 and for controls group was (27.20 ± 4.30) kg/m^2, so there was statically significant relation between STEMI group and control groups with (p-value = 0.032), also a significant relation between NSTEMI group and control groups (p-value = .0041) as seen in table (3-2), that adiposopathy which is resulted by positive caloric balance and sedentary lifestyles is the main causes of decrement adipose-tissue blood flux, modified O2 levels in the tissue, and defect in fat metabolism

that developed to atherosclerosis and myocardial infraction, the result is goes with study in Iraq by Hind Shakir Ahmed *et al,* about apo-a assessment that shown a statically significant association of BMI and myocardial infraction [229].

Table (3-2): BMI distribution in patients and control groups.

Variable	Subjects	No.	Mean & Standard Deviation
BMI (kg/m^2)	STEMI	45	**29.8± 4.39**
	Non-STEMI	45	**29.0 ± 4.34**
	Control	90	**27.20 ± 4.3**
P-value	**STEMI versus Control group (P < 0.05) *** **non-STEMI versus Control group (P < 0.05)***		

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

A recently article about the risk factors profile in group of MI patients by Morios Sugris, Alexios *et al*, mention that "when obesity levels increasing, the risk of premature type 1 MI is continue to increasing"[230].

Another study by Tripti Rastogi, Frederick K *et al,* about risk factors for heart failure in patients with and without history of MI, observed a statically significant association between BMI and myocardial infraction [231].

3.1.5. Smoking

In the present study the number of smoking participant among AMI group was 47 (52%) that 25 (55.6%) in STEMI group and 22 (48.9%) in NSTEMI group as seen in figure (3-4), while of controls group was 28 (31.1%),

so there is a significant association between male in STEMI incidence versus control group with a p-value (< 0.05) and also association between male in NSTEMI incidence versus control group with a p-value (< 0.05) as shown in table (3.3), cigarettes elevate both of blood pressure and heart rate which consider the main risk factor for atherosclerosis and other defect include the cardiovascular dysfunction by elevate vascular resistance, reduce vasodilation so tissue blood-flux this not only influence the peripheral vasculature yet also consist a narrowing of the coronary-arteries, that reduce coronary blood flow and increment coronary resistance, in spite of rise the myocardiocyte O2 demand, moreover tobacco impaired systolic left and right ventricles function through decreasing ejection fraction [232,233].

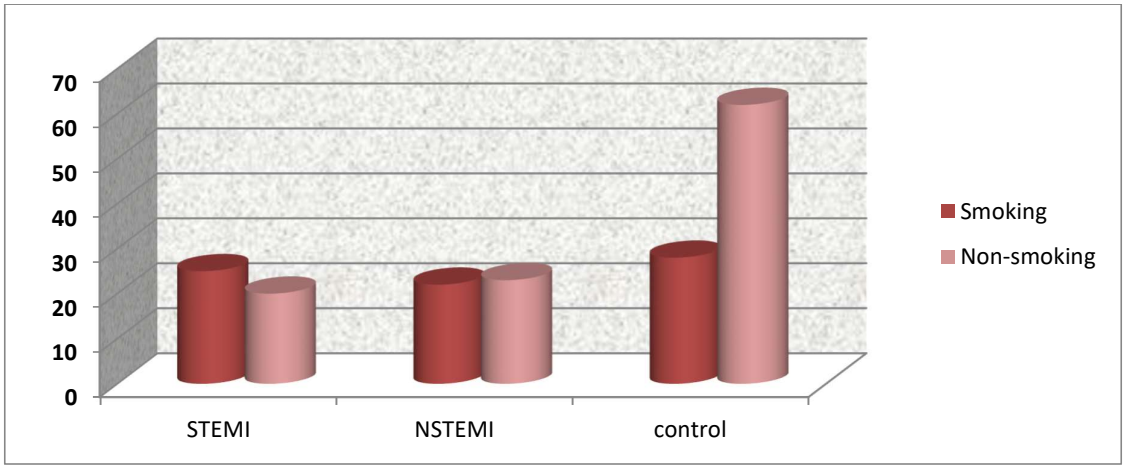

Figure (3-4): Smokers distribution in patient's groups.

This result agrees with previous studies by Sulaiman Yaseen *et al.* in Azadie teaching-hospital in Duhuk, about the elevation level of uric acid in MI patients that consider the smoking is one of the more prevalent risk factor of MI in their study [234].

Another study by Amir Aker,Walid Saliba *et al*, about the effect of smoking in one year After STEMI on cardiovascular events and conclude that smoking is relation with raised risk for acute STEMI at a young age [235].

Table (3-3): Represents general characteristic of control and STEMI groups.

Study Variables		STEMI	Control	P-value
Sex	Male	35 (77.7%)	45 (50.0%)	< 0.05
	Female	10 (22.2%)	45 (50.0%)	
Residency	Urban	32 (71.1%)	72 (80.0%)	> 0.05
	Rural	13 (28.8%)	18 (20.0%)	
Smoking	Smoking	25 (55.5%)	28 (31.1%)	< 0.05
	Non- Smoking	20 (44.4%)	62 (68.8%)	

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

Table (3-4): Represents general characteristic of control and NSTEMI groups.

Study Variables		NSTEMI	Control	P-value
Sex	Male	34 (75.5%)	45 (50.0%)	< 0.05
	Female	11 (24.4%)	45 (50.0%)	
Residency	Urban	34 (75.5%)	72 (80.0%)	> 0.05
	Rural	11 (24.4%)	18 (20.0%)	
Smoking	Smoking	22 (48.8%)	28 (31.1%)	< 0.05
	Non- Smoking	23 (51.1%)	62 (68.8%)	

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

3.2. Biochemical parameters:

3.2.1. Serum Arg activity among AMI groups and Control group.

The distribution of mean ± SD of Arg was statically significant increase in STEMI and NSTEMI groups from control group with p-value of less than 0.001 that the mean ± SD of Arg activity in STEMI group was (45.42±7.88) IU/L and

NSTEMI was (39.51±8.65) IU/L while for controls group was (18.32±4.39) IU/L, so there was statically significant relation between increased arginase activity in STEMI groups versus control group with p-value of less than (0.001) also the arginase activity is statically increased in NSTEMI groups versus control group with p-value of less than (0.001) as seen in table (3-5).

Elevated activity of arginase would compete with NOS for arginine so lower syntheses of nitric oxide by vascular endothelial cell result in defect the vaso-relaxation and stimulate adhesion of platelets to the endothelium and increase the syntheses of ornithine which would involve in deleterious effect like cause hyperplasia, stiff and fibrosis in vascular cells [148,149].

Table (3-5): Arginase activity(IU/L) among patients and control groups.

Parameter	Subjects	No.	Mean ± Standard Deviation	STEMI versus Control group P-value	NSTEMI versus Control group P-value	STEMI versus non-STEMI group P-value
Arginase activity (IU/L)	STEMI	45	45.42±7.88	(P < 0.001)**	(P < 0.001)**	(P < 0.05) *
	Non-STEMI	45	39.51±8.65			
	Control	90	18.32±4.39			

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

The results of our study align with previous research conducted by Syed Fawad Ali *et al*., who observed a statistically significant relationship between arginase and myocardial infarction group in their study, indicated by a p-value of less than 0.05 [223].

Other study conducted by Rui Zhanga, Zhenjun Ji *et al*. at Southeast University, China which pointed that arginase-1 may be a possible cardiac marker for acute myocardial infraction, that may be utilized as a supporter indicator in the detection of MI [236].

Similarly, a study conducted in Department of Clinical Science and Education in Stockholm, Sweden by Tengbom J *et al.* found a statistically significant association between ARG1 and AMI. Their conclusion indicated that ARG1 levels are elevated in patients admitted to the emergency department with acute myocardial infarction, proposing a potential role for ARG1 in the evolution of myocardial infarction [237].

The present study findings are consistent with the research conducted by Pernow, John, Jung, Christian *et al*. at the University of Düsseldorf, Germany. Their study focused on the function of ARG in cardiovascular disease and concluded that elevated ARG activity is linked to atherosclerosis and hypoxia, with ARG participating in endothelial dysfunction [238].

In the present study there was statically significant relation between arginase activity in STEMI groups versus NSTEMI group with p-value of (0.001) as seen in table (3-4), that in STEMI there were notable increase in ADMA cause inhibition in nitric oxide synthase and increase of arginase activity that agreed with study by Zsófia Lenkey Endre Sulyok *et al.* in University of Pécs, in Hungary about Arginine, NO, ADMA Pathway and the Coronary Circulation [239]

3.2.2 Serum fibronectin concentration among AMI groups and Control group.

The distribution of mean ± SD of fibronectin was statically significant increase in STEMI and NSTEMI groups from control group with p-value of less than 0.001 that mean ± SD of fibronectin in STEMI group was (31.03±4.82) ug/ml and NSTEMI was (27.55±3.90) ug/ml while for controls group was (8.02±2.42) ug/ml as seen in table (3-6).

Table (3-6): Fibronectin levels (ug/ml) among patients and control groups.

Parameter	Subjects	No.	Mean ± Standard Deviation	STEMI versus Control group P-value	NSTEMI versus Control group P-value	STEMI versus non-STEMI group P-value
Fibronectin (ug/ml)	STEMI	45	31.03±4.82	(P < 0.001)**	(P < 0.001)**	(P < 0.001)**
	Non-STEMI	45	27.55±3.9			
	Control	90	8.02 ±2.42			

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

A number of clinical researches present an immune-histochemical proof that the FN elevated after AMI resulted secondary to cardiac-tissue repair operation, also elevation of FN-receptor expression (integrin-a5b1) happen in the time of recovery in both the infarcted and not-infarcted sites also in atherosclerosis incidence that increase fibronectin concentration play role in the conversion of vascular smooth-muscle phenotype from the contractile phenotype to synthetic phenotype so increase of atherosclerosis[172] [240].

The result of present study is agreeing with study by Tamarah Mustafa Nadir, Abdulsalam Tawfeeq Salih Alsamarai *et al.* in Samarra city which study the level of FN in serum of MI patient and conclude that level of FN was significantly increase in MI patient [241].

Other study by Silvia Damiana Viconà, Donatella Binti *et al*. in University of Pavvia, Italy which found in their study that FN was significantly elevated in the extracellular matrix of the AMI patient contrasted healthy peoples [242].

Recent study by A Boğra, T Daaş *et al.* conclude that FN assemble in the myocardiocyte after tissue injury of the cell membrane, and the elevated FN levels in plasma resulted from injury in plasma membrane integrity in the time

of MI, also the time in which the FN start to elevated was from 8-24 hours [243].

From the present study there was astatically significant increase in fibronectin concentration in STEMI group from NSTEMI group with p-value (< 0.001) that's may be resulted from the up regulation of transforming growth factor β that increase in STEMI and induce the fibronectin syntheses [244]

3.2.3 Serum Malondialdehyde (MDA) concentration among AMI groups and Control group

The distribution of mean ± SD of malondialdehyde was statically significant increase in STEMI and NSTEMI groups versus control group with p-value of less than 0.001 that the mean ± SD of malondialdehyde in STEMI group was (70.22±15.69) ng/ml and NSTEMI was (66.06±7.12) ng/ml while for controls group was (17.71±4.98) ng/ml while there was no statically significant relation of MDA between STEMI and NSTEMI patients as seen in table (3-7).

Table (3-7): MDA levels (ng/ml) among patients and control groups.

Parameter	Subjects	No.	Mean ± Standard Deviation	STEMI versus Control group P-value	NSTEMI versus Control group P-value	STEMI versus non-STEMI group P-value
MDA (ng/ml)	STEMI	45	70.22±15.69	(P < 0.001)**	(P < 0.001)**	(P > 0.05)
	Non-STEMI	45	66.06±7.12			
	Control	90	17.71±4.98			

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

Lipid peroroxidation production are highly active and dangerous molecules that accumulate and modified cells content and result in protein misfolding and cell destruction that MDA oxidized important protein in myocardiocyte mitochondria like ATP synthase NADH oxidoreductase, Cytochrome b and c1 complex resulting in misfolding and dysfunction of respiratory chain reaction so increase free radical production.

The result of present study agreed with a study in College of Dentistry, Tikrit University by Intesar J. Mohammed *et al*. they when they evaluate the lipid peroxidation in AMI patient found a statically significant increase of MDA level in AMI patient when compared to control levels [245].

Recently Europe study by Paula da Silva MV *et al.* also found in their study that malondialdehyde was significantly elevated among the patients with acute coronary syndrome according to their dividing as three groups unstable angina, NSTEMI, and STEMI [246].

other study by YuYina, WeiHan *et al.* study the relation between activities MDA in peripheral blood of patients with AMI and the complication of arrhythmia conclude that ambulatory electrocardiography associate with the peripheral blood malondialdehyde in AMI patients complicated with arrhythmia, which may prophesy AMI condition [247].

Malondialdehyde found to be statistically significant in AMI patients with p-value < 0.01 when compared to the control group in the study by Mriganka Baruah *et al.* about Markers of Oxidative Stress in AMI [248].

3.3 Molecular Genetics

3.3.1 Arginase-1 genotyping

Conventional PCR had been utilized for amplifying a require DNA through operating by a specified primer pairs, after amplified The outcome, which was operated to locate the arginase 1 polymorphic site resulted in one band that visualized by using agarose-gel electrophoresis, these band was 294 base pair (bp) representing the existence of arginase 1 genotyping, as seen in figure (3-5).

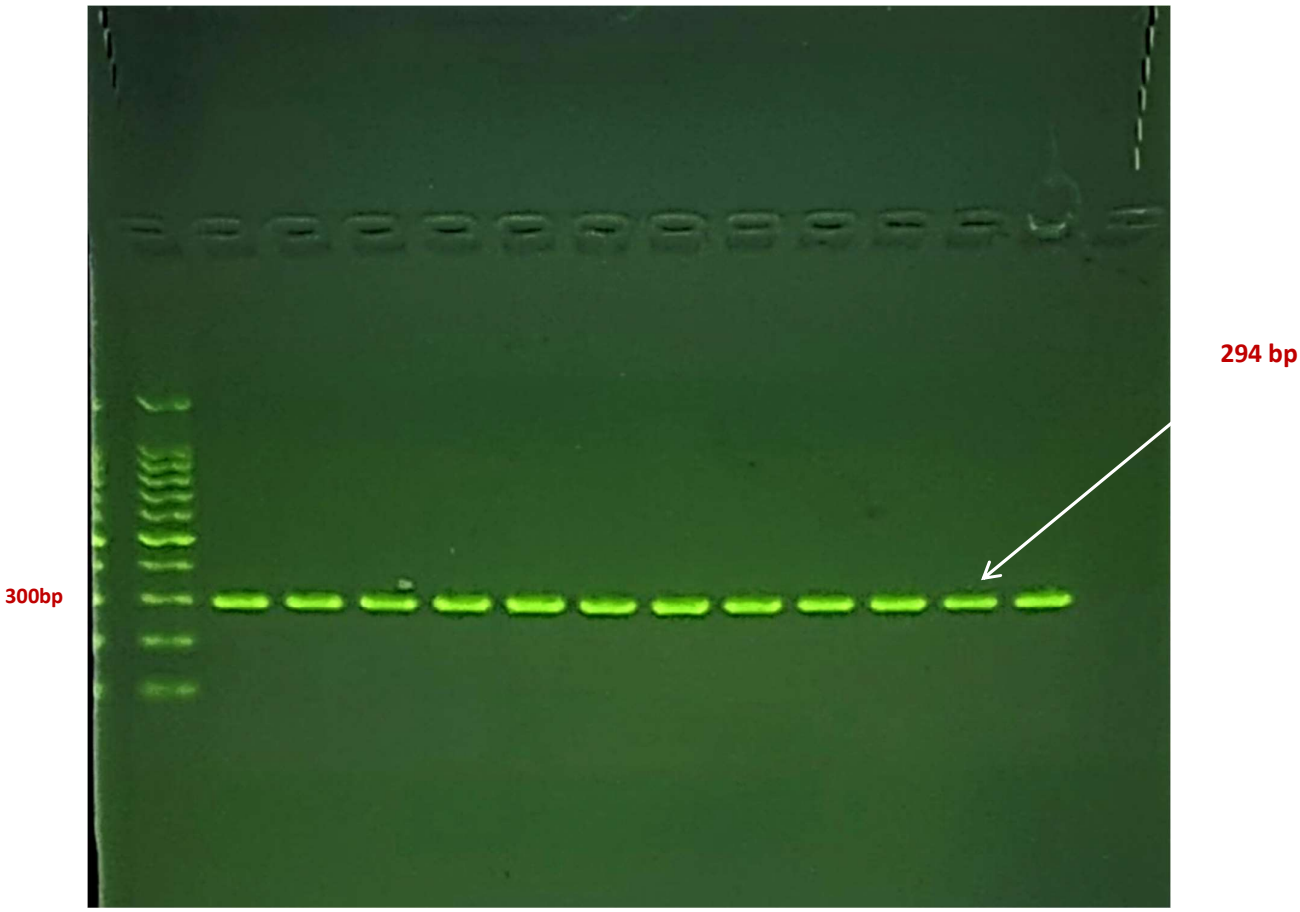

Figure (3.5): PCR product, the band 294 bp for gene. The product was electrophoresis on 2% agarose at 5 volt/cm. 1x TBE buffer for 1 hour. (Ladder 100)

the outcomes fragment of arginase1 294 bp was digested with tail restriction enzyme at 65 °C for 4 hours, then fragments were determined on a 2.5% agarose gel, the G allele was not cleaved and remained with the 294 bp fragment while the appears of the restriction location produce 2 fragments of 178bp and 116 bp for T allele, as seen in Figure (3-6).

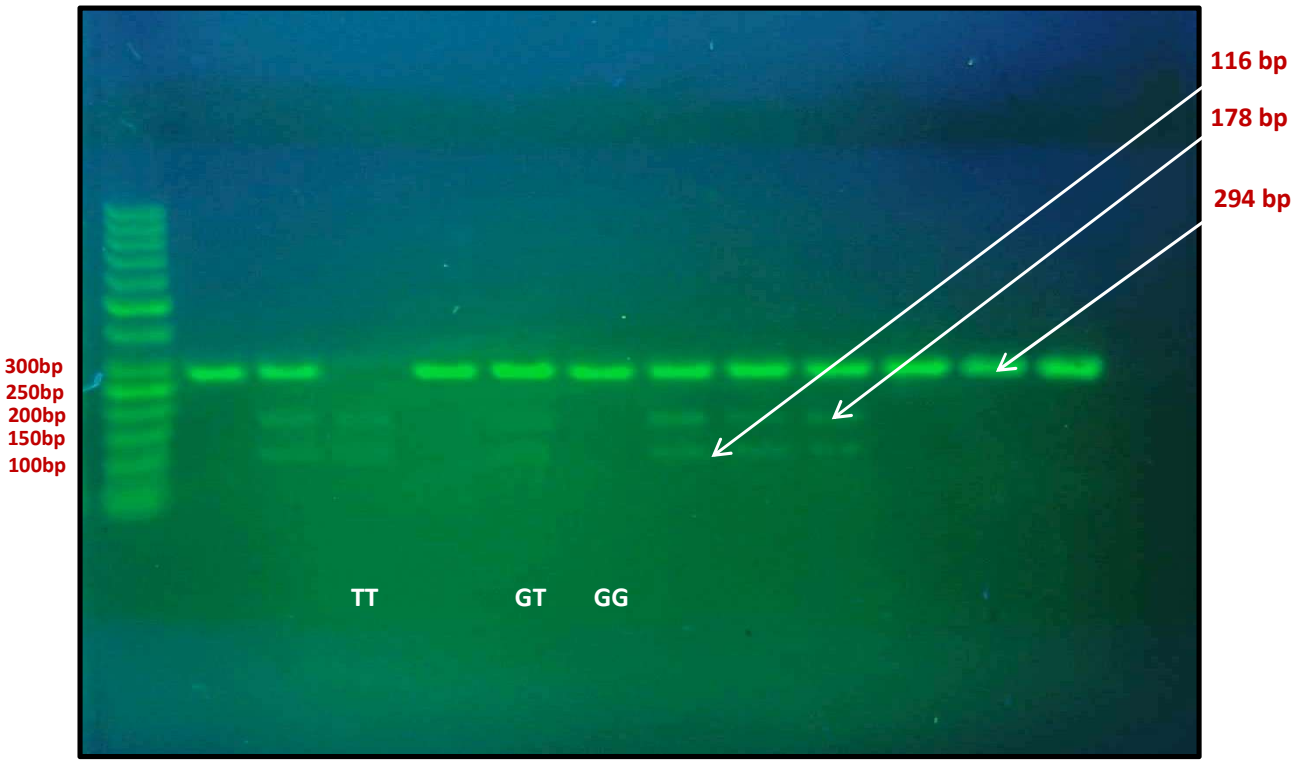

Figure (3-6): Electrophoresis pattern of PCR product digested with *TaiI* restriction enzyme (2.5% agarose gel) DNA molecular marker 50 plus bp size by Red stain stained bands in the gel.

3.3.2 Genotype and Allele Frequency

The genotype and allele frequencies of the arginase1(G→T) variant in the study groups are seen in the table (3-8)

Table (3-8): genotype and allele frequencies of the case and control groups

Mode	Arginase-1 genotype or allele	control	STEMI	NSTEMI	Chi square (p value) of STEMI versus control	Chi square (p value) of NSTEMI versus control
Codominant	GG	68 (75.6%)	17 (37.8%)	19 (42.2%)	$\chi2$= 18.541 P <0.001**	$\chi2$= 14.58 P=0.001*
	GT	19 (21.1%)	23 (51.1%)	22 (48.9%)		
	TT	3 (3.3%)	5 (11.1%)	4 (8.9%)		
dominant	GG	68 (75.6%)	17 (37.8%)	19 (42.2%)	$\chi2$= 18.36 P <0.001**	$\chi2$= 14.54 P<0.001**
	GT + TT	22 (24.4%)	28 (62.2%)	26 (57.8%)		
Recessive	GG +GT	87 (96.7%)	40 (88.9%)	41 (91.1%)	$\chi2$= 3.255 P > 0.05	$\chi2$= 1.883 P> 0.05
	TT	3 (3.3%)	5 (11.1%)	4 (8.9%)		
Allele frequency	G	155 (86.1%)	57 (63.3%)	60 (66.7%)	$\chi2$= 18.456 P<0.001**	$\chi2$= 13.985 P<0.001**
	T	25 (13.9%)	33 (36.7%)	30 (33.3%)		

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

Results of genotyping observed that arginase-1 polymorphism was obtained as homologous (GG) genotype in 17 samples (37.8%) in the STEMI group and in 19 samples (42.2%) in the MI NSTEMI group while present in 68 samples (75.6%) of the control group.

Moreover, the heterozygous genotype (GT) observed in 23 samples (51.1%) in the MI STEMI group and the (GT) genotype present in 22 samples (48.9%) in the MI NSTEMI group while present only in 19 samples (21.1%) of the control group.

The homologous genotype (TT) of the rare allele is obtained in 5 samples (11.1%) in the MI STEMI group and present in 4 samples (8.9%) in the MI NSTEMI group while present only in 3 samples (3.3%) of the control group, as seen in figure (3-7).

So to determine the significance of these results, the study genotypes are distributed in the study groups and the chi-square test was used to investigate the p-value, as seen in Table (3.8).

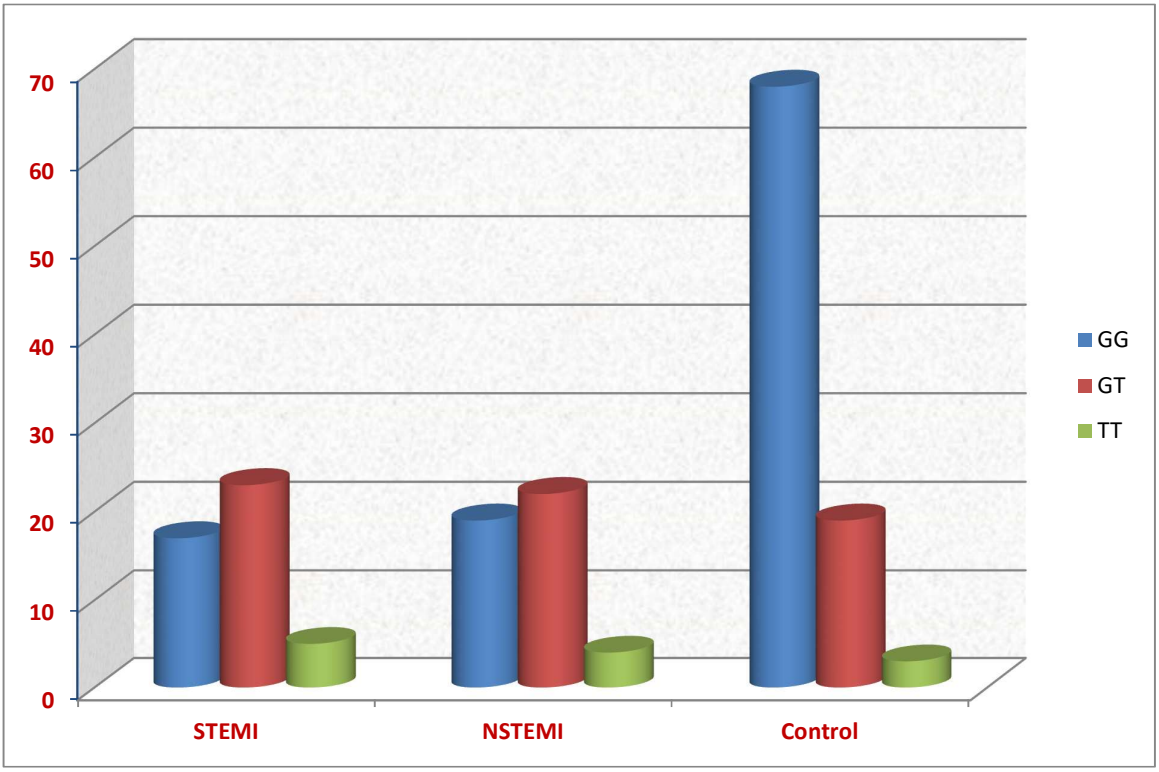

Figure (3-7): genotype and allele frequencies of the control and case groups

From Table (3-8), the present study, explored a statically significant association of the rs2781666 SNPs in the arginase1 gene with STEMI by a p-value of less than (0.001) and with NSTEMI with a p-value of (0.001), moreover

the STEMI and NSTEMI groups showed higher frequency of T-allele compared to the healthy group, that 33 (36.7%) of STEMI samples and 30 (33.3%) of NSTEMI samples had T genotype compared to 25 (13.9%) of healthy samples with T genotype with p-value less than 0.001.

Polymorphism in the arginase 1 gene resulted in high expression of arginase enzyme that would compete with NOS for arginine so diminish nitric oxide presence that result in corrupted of the vasodilation and stimulate adhesion of leukocytes to the endothelium and vascular endothelial defect, moreover elevated concentration in the arginase rise the syntheses of ornithine which would involve in deleterious effect like cause hyperplasia, stiff and fibrosis in vascular cells [147,149].

This result agrees with a study by sumaira Akram, Sadia Nawaz *et al.* at University Islamabad that conclude that statically significant relation of rs2781666 SNPs in the arginase1 gene with CAD in patients group and proposed that inherited SNPs in the arginase1 gene is related with coronary atherosclerosis incidence [222].

The ARG1 rs2781666 polymorphism was associated with AMI, in other study in Hospital la Rabta, Cardiology, Tunis, by Mohamed Sami Mouralib Monia Elasmi *et al*. also obtained statically significant association between rs2781666 G/T polymorphism of arginase 1 gene and AMI in Tunisian male [249].

Other recent study obtained a significant association between up-regulation of arginase and MI was a study by Nawzad Saleh1, Magnus Settergren *et al.* about arginase up-regulation in STEMI patient conclude that the noticeable elevated of gene and protein expression of ARG1 at the time of hospital admission shows a role of ARG1 in the development of STEMI [250].

J Tengbom, D Verouhis *et al*. at their study about up-regulation of protein and gene expression of ARG1in STEMI patients demonstrate elevated

gene expression and serum scale of ARG1 in the acute setting of STEMI patients [237].

3.4 Effect of Arg-1 Polymorphism on chemical parameters

The effect of Arg-1 polymorphism on chemical parameters in study groups, are elucidate in table (3-9).

Table (3-9): Mean value of chemical parameters by Arg-1 genotype in study groups

Parameters	GG	GT+TT	P-value
Arginase (IU/L)	**33.74±14.19**	**38.61±11.8**	**< 0.05***
Fibronecten (ug/ml)	**20.71±11.29**	**23.76±10.2**	**> 0.05**
MDA (ng/ml)	**46.1±30.1**	**56.18±20.93**	**< 0.05***

*** Significant difference (P < 0.05) **highly significant (P < 0.001)**

From table (3-9), the present study, explored astatically significant association between the rs2781666 SNPs in the arginase1 gene and mean of arginase activity in the study groups with a p-value of less than (0.05), that polymorphisms of arginase enzyme result in alteration cause raise in arginase activity.

The result of the present study agree with study by Muhammad Arshad Raffiq, Sabir Hussin *et al*. at Shifa hospital, Islamabad, about the relation of arginase SNPs with essential-hypertension they found a highly significant relation between arginase activity and arginase SNPs [251].

Other recent study obtained a significant association between arginase SNPs and arginase activity is done by Humayoon Shafique Satti, Muhmmad Arshad Rafiq *et al.* when they found a statically significant increase in arginase activity in GT+TT genotypes when it compared to GG genotypes in their study groups [222].

The present study appeared astatically significant relation between the rs2781666 SNPs in the arginase1 gene and mean of MDA concentration in the study groups with a p-value of less than (0.05), that polymorphisms of arginase enzyme result in increased arginase activity that modifying nitric oxide synthesis levels because the arginase and nitric oxide synthase (NOS) together use arginine as their usual substrate, so that over activity of arginase enzyme cause a lack in arginine for nitric oxide synthase, that developed to uncoupling of nitric oxide synthase, and decrement of nitric oxide syntheses, and increment syntheses of free radical like superoxide (O_2^-) and peroxynitrite (NO_3^-) which resulted in lipid peroxidation [118,119].

While no statically significant relation in this study appeared between the rs2781666 SNPs in the arginase1 gene and mean of fibronectin in the study groups with a p-value of more than (0.05)

3.5 Correlation of lipid peroxidation with study parameters

3.5.1 Correlation of lipid peroxidation with arginase activity in AMI patients

In the present study there were a strong positive correlation between MDA and arginase activity in STEMI patients with (R=0.774) when studied statically by pearson correlation coefficient, also strong positive correlation were determined between MDA and arginase activity in NSTEMI patients with (R=0.741) as seen in figure (3-8) and (3-9).

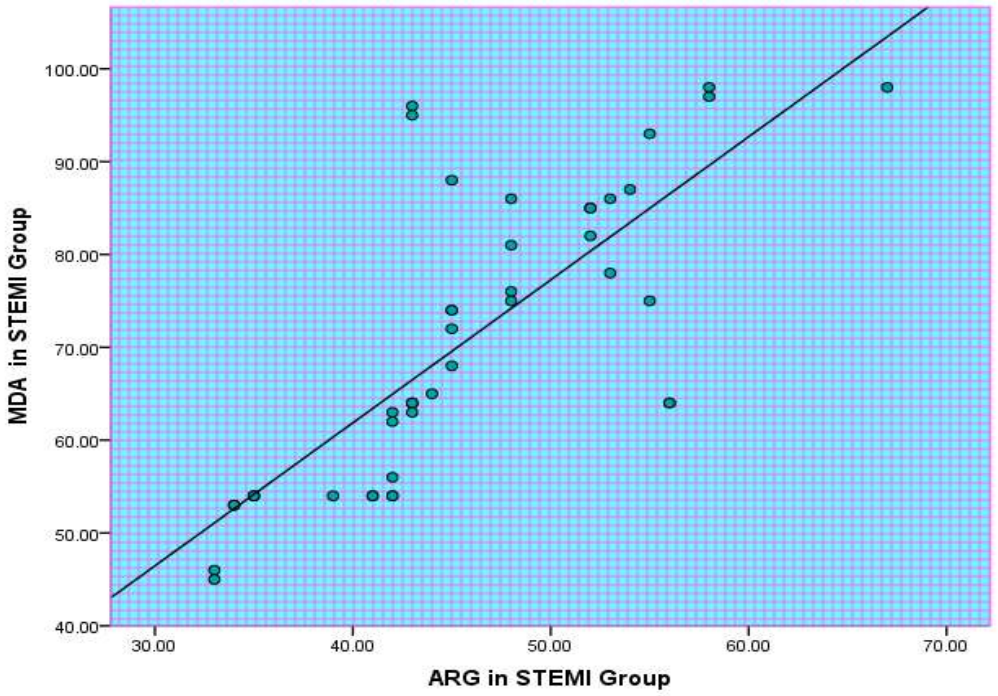

Figure (3-8): Correlation Between Arginase activity and MDA in STEMI patients (R=0.774, P value < 0.05)

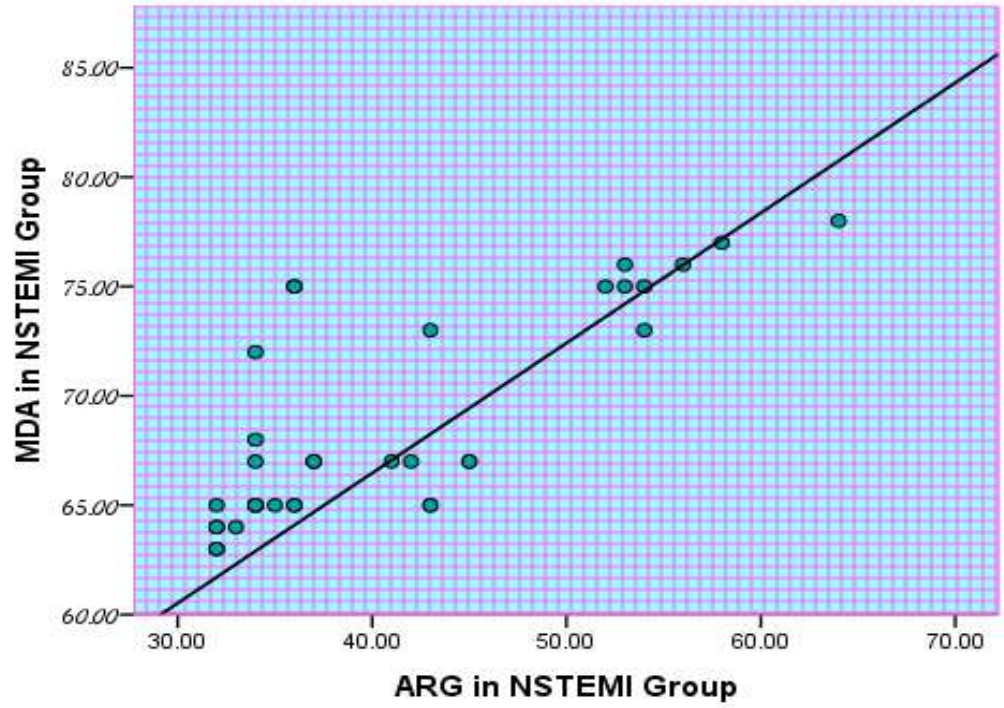

Figure (3-9): Correlation Between Arginase activity and MDA in NSTEMI patients (R=0.741, P value < 0.05)

Increase of arginase activity result in reduce of arginine that required for NOS so uncoupling of nitric oxide synthase, that followed by decrement of nitric oxide production, and increment of free radical production like superoxide (O_2^-) and peroxynitrite (NO_3^-) which considered as main causes of lipid peroxidation [118,119].

3.5.2 Correlation of lipid peroxidation with fibronectin in AMI patients

In the present study there were a weak positive correlation between MDA and fibronectin in STEMI patients with (R=0.225) when studied statically by pearson correlation coefficient, also weak positive correlation were determined between MDA and fibronectin in NSTEMI patients with (R=0.258) as seen in figure (3-10) and (3-11), That Fibronectin type III domain-containing 5 overexpression stimulate lipid oxidation and energy expenditure and decrease circulating free fatty acid concentrations at baseline [252].

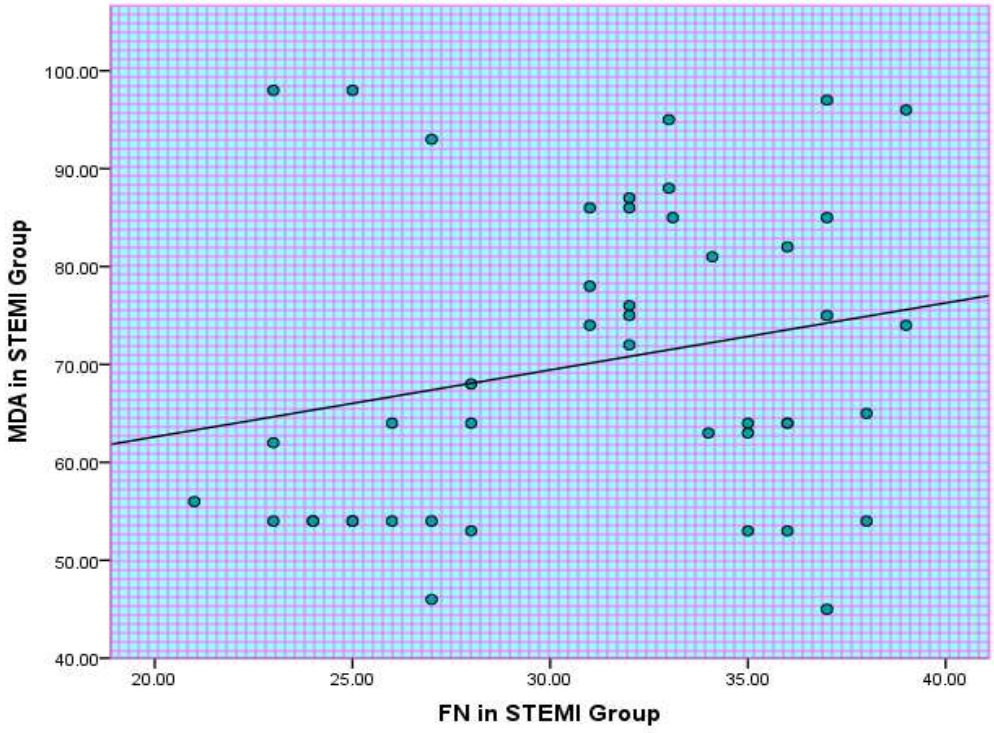

Figure (3-10): Correlation Between Fibronectin and MDA in STEMI Patients (R=0.225, P value =0.137)

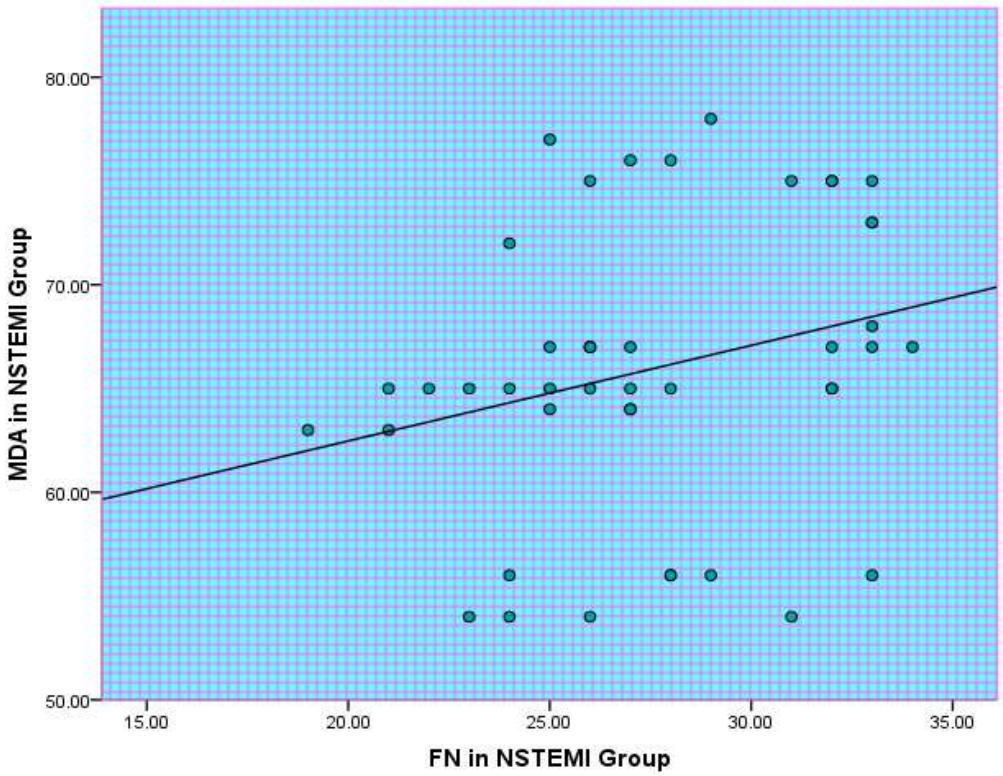

Figure (3-11): Correlation Between Fibronectin and MDA in NSTEMI Patients (R=0.258, P value =0.87)

CHAPTER FOUR

Conclusions & Recommendations

Conclusions

1. The presence of the Arginase-1 rs2781666 gene polymorphism was associated with both STEMI and NSTEMI groups, suggesting a genetic predisposition to AMI in this population.
2. Serum fibronectin levels, arginase activity and MDA levels exhibited a statistically significant increase in both STEMI and NSTEMI groups of AMI patients, underscoring its potential as a diagnostic marker or contributor to AMI.
3. The study revealed a strong positive correlation between lipid peroxidation and arginase activity, suggesting potential crosstalk between oxidative stress and arginine metabolism in AMI. Additionally, a weak correlation was observed between lipid peroxidation and fibronectin levels.
4. Specifically, the rs2781666 SNPs in the arginase1 gene were significantly associated with arginase activity and lipid peroxidation but not with fibronectin levels in study groups, highlighting complex interactions between genetic factors and biochemical markers.

Recommendations

1. Further Genetic Investigations: Given the association between the Arginase-1 rs2781666 gene polymorphism and AMI, it is essential to conduct more in-depth genetic studies. Research should focus on elucidating other candidate genes polymorphism for Arginase-1 like rs2781667; rs2781668; rs17599586 gene and its association with acute myocardial infarction.
2. Clinical Biomarker Utilization: Serum arginase activity, fibronectin levels, and MDA levels have shown significant associations with AMI in this study. Clinicians should consider incorporating these biomarkers into routine diagnostic and risk assessment protocols for AMI patients in Babylon province. This may aid in early detection and monitoring of AMI cases.
3. Oxidative Stress Management: The strong correlation between lipid peroxidation and arginase activity highlights the importance of addressing oxidative stress in AMI prevention and management. Lifestyle modifications, including dietary changes and antioxidant-rich diets, should be promoted to mitigate oxidative damage in at-risk populations.
4. Longitudinal Studies: Long-term, prospective studies are needed to track the progression of AMI and its associated biomarkers over time. Such studies can provide valuable insights into disease development, prognosis, and the effectiveness of preventive measures.

References

References

1. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Fourth universal definition of myocardial infarction (2018). Journal of the American College of Cardiology. 2018 Oct 30;72(18):2231-64.

2. Heusch G. Myocardial ischaemia–reperfusion injury and cardioprotection in perspective. Nature Reviews Cardiology. 2020 Dec;17(12):773-89.

3. Feng K, Pi X, Liu H, Sun K. Myocardial infarction classification based on convolutional neural network and recurrent neural network. Applied Sciences. 2019 Jan;9(9):1879.

4. Davidson SM, Adameová A, Barile L, Cabrera-Fuentes HA, Lazou A, Pagliaro P, Stensløkken KO, Garcia-Dorado D, EU-CARDIOPROTECTION COST Action (CA16225). Mitochondrial and mitochondrial-independent pathways of myocardial cell death during ischaemia and reperfusion injury. Journal of Cellular and Molecular Medicine. 2020 Apr;24(7):3795-806.

5. Helwani MA, Amin A, Lavigne P, Rao S, Oesterreich S, Samaha E, Brown JC, Nagele P. Etiology of acute coronary syndrome after noncardiac surgery. Anesthesiology. 2018 Jun;128(6):1084-91.

6. Michaud K, Basso C, d'Amati G, Giordano C, Kholová I, Preston SD, Rizzo S, Sabatasso S, Sheppard MN, Vink A, van der Wal AC. Diagnosis of myocardial infarction at autopsy: AECVP reappraisal in the light of the current clinical classification. Virchows Archiv. 2020 Feb;476(2):179-94.

7. Sharma M, San Tan R, Acharya UR. A novel automated diagnostic system for classification of myocardial infarction ECG signals using an optimal biorthogonal filter bank. Computers in biology and medicine. 2018 Nov 1;102:341-56.

8. Mohamed MO, Contractor T, Abramov D, Parwani P, Michos ED, Fischman D, Alraies MC, Bagur R, Mamas MA. Sex-based differences in prevalence and outcomes of common acute conditions associated with type 2 myocardial infarction. The American Journal of Cardiology. 2021 May 15;147:8-15.

9. Ciliberti G, Compagnucci P, Urbinati A, Bianco F, Stronati G, Lattanzi S, Russo AD, Guerra F. Myocardial infarction without obstructive coronary artery disease (MINOCA): a practical guide for clinicians. Current Problems in Cardiology. 2021 Mar 1;46(3):100761.

10. Lozano I, Rondán J, Vegas JM, Segovia E. Definition of myocardial infarction type 4a: can we define its diagnosis and systematize clinical practice?. Revista Espanola De Cardiologia (English Ed.). 2019 Apr 7;72(8):695-6.

11. White HD, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, Edelberg JM, Erglis A, Goodman SG, Hanotin C, Harrington RA. Effects of alirocumab on types of myocardial infarction: insights from the ODYSSEY OUTCOMES trial. European Heart Journal. 2019 Sep 1;40(33):2801-9.

12. Denessen EJ, Heuts S, Daemen JH, Vroemen WH, Sels JW, Segers P, Van'T Hof AW, Maessen JG, Bekers O, Van Der Horst IC, Mingels AM. High-sensitivity cardiac troponin I and T kinetics after coronary artery bypass grafting in relation to current definitions of myocardial infarction: a systematic review and meta-anal. European Heart Journal. 2021 Oct;42(Supplement_1):ehab724-2251.

13. Melly L, Torregrossa G, Lee T, Jansens JL, Puskas JD. Fifty years of coronary artery bypass grafting. Journal of thoracic disease. 2018 Mar;10(3):1960.

14. De Lemos JA, Newby LK, Mills NL. A proposal for modest revision of the definition of type 1 and type 2 myocardial infarction. Circulation. 2019 Nov 26;140(22):1773-5.

15. Dazhi DE, Ling LI, Guangma XU, Jianting GA, Yin SH, Ying SH, Ruikai ZH, Yingzhong LI. Epidemiology and serum metabolic characteristics of acute myocardial infarction patients in chest pain centers. Iranian journal of public health. 2018 Jul;47(7):1017.

16. Dugani SB, Murad W, Damilig K, Atos J, Mohamed E, Callachan E, Farukhi Z, Shaikh A, Elfatih A, Yusef S, Hydoub YM. Premature myocardial infarction in the Middle East and North Africa: rationale for the Gulf PREVENT study. Angiology. 2020 Jan;71(1):17-26.

17. Vallabhajosyula S, Vallabhajosyula S, Burstein B, Ternus BW, Sundaragiri PR, White RD, Barsness GW, Jentzer JC. Epidemiology of in-hospital cardiac arrest complicating non–ST-segment elevation myocardial infarction receiving early coronary angiography. American heart journal. 2020 May 1;223:59-64.

18. Gabriel-Costa D. The pathophysiology of myocardial infarction-induced heart failure. Pathophysiology. 2018 Dec 1;25(4):277-84.

19. Curley D, Lavin Plaza B, Shah AM, Botnar RM. Molecular imaging of cardiac remodelling after myocardial infarction. Basic research in cardiology. 2018 Mar;113(2):1-8.

20. Saleh M, Ambrose JA. Understanding myocardial infarction. F1000Research. 2018;7

21. Ek A, Ekblom Ö, Hambraeus K, Cider Å, Kallings LV, Börjesson M. Physical inactivity and smoking after myocardial infarction as

predictors for readmission and survival: results from the SWEDEHEART-registry. Clinical Research in Cardiology. 2019 Mar;108(3):324-32.

22. Wang C, Pei YY, Ma YH, Ma XL, Liu ZW, Zhu JH, Li CS. Risk factors for acute kidney injury in patients with acute myocardial infarction. Chinese medical journal. 2019 Jul 20;132(14):1660-5.

23. Lu Y, Li SX, Liu Y, Rodriguez F, Watson KE, Dreyer RP, Khera R, Murugiah K, D'Onofrio G, Spatz ES, Nasir K. Sex-Specific Risk Factors Associated With First Acute Myocardial Infarction in Young Adults. JAMA network open. 2022 May 2;5(5):e229953-.

24. Sinha VP, Kumar A, Singh UN. A Retrospective Research Assessing the Rising Risk Factors of Acute Myocardial Infarction.

25. Yandrapalli S, Nabors C, Goyal A, Aronow WS, Frishman WH. Modifiable risk factors in young adults with first myocardial infarction. Journal of the American College of Cardiology. 2019 Feb 12;73(5):573-84.

26. Sagris M, Antonopoulos AS, Theofilis P, Oikonomou E, Siasos G, Tsalamandris S, Antoniades C, Brilakis ES, Kaski JC, Tousoulis D. Risk factors profile of young and older patients with Myocardial Infarction. Cardiovascular Research. 2022 Jun;118(10):2281-92.

27. Biery DW, Berman AN, Singh A, Divakaran S, DeFilippis EM, Collins BL, Gupta A, Fatima A, Qamar A, Klein J, Hainer J. Association of smoking cessation and survival among young adults with myocardial infarction in the partners YOUNG-MI registry. JAMA network open. 2020 Jul 1;3(7):e209649-.

28. Höpner J, Junge U, Schmidt-Pokrzywniak A, Fischer C, Mikolajczyk R. Determinants of persistent smoking after acute

myocardial infarction: an observational study. BMC Cardiovascular Disorders. 2020 Dec;20(1):1-7.

29. Bhatta DN, Glantz SA. Electronic cigarette use and myocardial infarction among adults in the US population assessment of tobacco and health. Journal of the American Heart Association. 2019 Jun 18;8(12):e012317.

30. Attard, R., Dingli, P., Doggen, C.J., Cassar, K., Farrugia, R. and Bezzina Wettinger, S., 2021. The impact of frequency, pattern, intensity, and type of alcohol consumption, and its combined effect with smoking on inflammation, lipid profile, and the risk of myocardial infarction. Journal of Public Health, 29, pp.611-624.

31. Piano MR, Thur LA, Hwang CL, Phillips SA. Effects of alcohol on the cardiovascular system in women. Alcohol Research: Current Reviews. 2020;40(2).

32. Kadri AN, Khodor S, Ali A, Nusairat L, Mahmood A, Nahhas G, Dabbous S, Spears J, Jafri S, Werns S. National Trends of Tobacco, Alcohol, and Drug Use in Patients Admitted With Acute Myocardial Infarction. Cardiovascular Revascularization Medicine. 2021 May 1;26:26-31.

33. Ding C, O'Neill D, Bell S, Stamatakis E, Britton A. Association of alcohol consumption with morbidity and mortality in patients with cardiovascular disease: original data and meta-analysis of 48,423 men and women. BMC medicine. 2021 Dec;19(1):1-4.

34. Gao Y, Xu HY, Guo YK, Wen XL, Shi R, Li Y, Yang ZG. Impact of myocardial scars on left ventricular deformation in type 2 diabetes mellitus after myocardial infarction by contrast-enhanced cardiac magnetic resonance. Cardiovascular Diabetology. 2021 Dec;20(1):1-2.

35. Furtado RH, Bonaca MP, Raz I, Zelniker TA, Mosenzon O, Cahn A, Kuder J, Murphy SA, Bhatt DL, Leiter LA, McGuire DK. Dapagliflozin and cardiovascular outcomes in patients with type 2 diabetes mellitus and previous myocardial infarction: subanalysis from the DECLARE-TIMI 58 Trial. Circulation. 2019 May 28;139(22):2516-27.

36. Penna C, Andreadou I, Aragno M, Beauloye C, Bertrand L, Lazou A, Falcão-Pires I, Bell R, Zuurbier CJ, Pagliaro P, Hausenloy DJ. Effect of hyperglycaemia and diabetes on acute myocardial ischaemia–reperfusion injury and cardioprotection by ischaemic conditioning protocols. British journal of pharmacology. 2020 Dec;177(23):5312-35.

37. Schmitz T, Thilo C, Linseisen J, Heier M, Peters A, Kuch B, Meisinger C. Admission ECG changes predict short term-mortality after acute myocardial infarction less reliable in patients with diabetes. Scientific reports. 2021 Mar 18;11(1):1-0.

38. Istanbuly S, Matetic A, Roberts DJ, Myint PK, Alraies MC, Van Spall HG, Mohamed MO, Bharadwaj A, Mamas MA. Relation of Extracardiac Vascular Disease and Outcomes in Patients With Diabetes (1.1 Million) Hospitalized for Acute Myocardial Infarction. The American Journal of Cardiology. 2022 Jul 15;175:8-18.

39. Hedayatnia M, Asadi Z, Zare-Feyzabadi R, Yaghooti-Khorasani M, Ghazizadeh H, Ghaffarian-Zirak R, Nosrati-Tirkani A, Mohammadi-Bajgiran M, Rohban M, Sadabadi F, Rahimi HR. Dyslipidemia and cardiovascular disease risk among the MASHAD study population. Lipids in health and disease. 2020 Dec;19(1):1-1.

40. Vekic J, Zeljkovic A, Cicero AF, Janez A, Stoian AP, Sonmez A, Rizzo M. Atherosclerosis development and progression: the role of atherogenic small, dense LDL. Medicina. 2022 Feb 16;58(2):299.

41. Tuan PM, Vu DT. ST-elevation myocardial infarction complicated by cardiac arrest in a young patient with familiar dyslipidemia. Tạp chí Nghiên cứu Y học. 2021 Dec 31;148(12):141-5.

42. Keirns BH, Sciarrillo CM, Koemel NA, Emerson SR. Fasting, non-fasting and postprandial triglycerides for screening cardiometabolic risk. Journal of Nutritional Science. 2021;10.

43. Rodrigo R, González-Montero J, Sotomayor CG. Novel combined antioxidant strategy against hypertension, acute myocardial infarction and postoperative atrial fibrillation. Biomedicines. 2021 May 30;9(6):620.

44. Auda MA, Majid A, Al-Fartosi KG. Protective role of polyphenolic compounds extracted from cyperus rotundus rhizomes and taurine on troponin-I and some oxidant/antioxidants parameters of female rats treated with isoproterenol–induced myocardial infraction. University of Thi-Qar Journal. 2018;13(2):61-76.

45. Omer RA, Jamal MY. Self-Reported Sleep Disorder, Anxiety and Depression in Iraqi Patients Post-Myocardial Infraction. Al-Rafidain Journal of Medical Sciences (ISSN: 2789-3219). 2022 Jun 27;2:122-6.

46. Bandyopadhyay D, Chakraborty S, Amgai B, Patel N, Hajra A, Heise L, Sud K, Ghosh RK, Herzog E, Aronow WS, Fonarow GC. Acute myocardial infarction in the young-National Trend Analysis with gender-based difference in outcomes. International Journal of Cardiology. 2020 Feb 15;301:21-8.

47. Ueda K, Adachi Y, Liu P, Fukuma N, Takimoto E. Regulatory actions of estrogen receptor signaling in the cardiovascular system. Frontiers in Endocrinology. 2020 Jan 10;10:909.

48. Alsawas M, Wang Z, Murad MH, Yousufuddin M. Gender disparities among hospitalised patients with acute myocardial infarction,

acute decompensated heart failure or pneumonia: Retrospective cohort study. BMJ open. 2019 Jan 1;9(1):e022782.

49. Said M, Wahrenberg A, Warnqvist A, Jernberg T, Witt N, Svensson P. Association between family history and severity of coronary artery disease in a nationwide sample of patients with a first time myocardial infarction. European Heart Journal. 2021 Oct;42(Supplement_1):ehab724-1276.

50. Kwak JH, Ha EK, Kim JH, Cha HR, Lee SW, Han MY. Association of Familial History of Diabetes, Hypertension, Dyslipidemia, Stroke, or Myocardial Infarction With Risk of Kawasaki Disease. Journal of the American Heart Association. 2022 Jun 14:e023840.

51. Tedeschi D, Rizzi A, Biscaglia S, Tumscitz C. Acute myocardial infarction and large coronary thrombosis in a patient with COVID-19. Catheterization and Cardiovascular Interventions. 2021 Feb 1;97(2):272-7.

52. Patel H, Rosengren A, Ekman I. Symptoms in acute coronary syndromes: does sex make a difference?. American heart journal. 2004 Jul 1;148(1):27-33.

53. Aye YN, Mai AS, Zhang A, Lim OZ, Lin N, Ng CH, Chan MY, Yip J, Loh PH, Chew NW. Acute myocardial infarction and myocarditis following COVID-19 vaccination. QJM: An International Journal of Medicine. 2021 Sep 29.

54. Khan H, Rasmussen D, Gittner L, Rafiq A, Blakely S, Shabaneh O, Reddy PH. Gender differences in prevalence of myocardial infarction in rural West Texans. Journal of Public Health. 2020 May 18:1-3.

55. Moore A, Goerne H, Rajiah P, Tanabe Y, Saboo S, Abbara S. Acute myocardial infarct. Radiologic Clinics. 2019 Jan 1;57(1):45-55.

56. Sadhukhan D, Pal S, Mitra M. Automated identification of myocardial infarction using harmonic phase distribution pattern of ECG data. IEEE Transactions on Instrumentation and Measurement. 2018 Apr 5;67(10):2303-13.

57. Ralston SH, Penman ID, Strachan MW, Hobson R, editors. Davidson's Principles and Practice of Medicine E-Book. Elsevier Health Sciences; 2018 Feb 2..

58. Kayikcioglu I, Akdeniz F, Köse C, Kayikcioglu T. Time-frequency approach to ECG classification of myocardial infarction. Computers & Electrical Engineering. 2020 Jun 1;84:106621.

59. Meyers HP, Bracey A, Lee D, Lichtenheld A, Li WJ, Singer DD, Kane JA, Dodd KW, Meyers KE, Thode HC, Shroff GR. Comparison of the ST-elevation myocardial infarction (STEMI) vs. NSTEMI and occlusion MI (OMI) vs. NOMI paradigms of acute MI. The Journal of emergency medicine. 2021 Mar 1;60(3):273-84.

60. Wiśniewski P, Rostoff P, Gajos G, Nessler J, Kruszelnicka O. Predictive value of electrocardiographic ST-segment elevation myocardial infarction equivalents for detecting acute coronary artery occlusion in patients with non–ST-segment elevation myocardial infarction. Kardiologia Polska (Polish Heart Journal). 2019;77(6):624-31.

61. Sridhar C, Lih OS, Jahmunah V, Koh JE, Ciaccio EJ, San TR, Arunkumar N, Kadry S, Rajendra Acharya U. Accurate detection of myocardial infarction using non linear features with ECG signals. Journal of Ambient Intelligence and Humanized Computing. 2021 Mar;12(3):3227-44

62. Aydin S, Ugur K, Aydin S, Sahin İ, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. Vascular health and risk management. 2019;15:1.

63. Baloglu UB, Talo M, Yildirim O, San Tan R, Acharya UR. Classification of myocardial infarction with multi-lead ECG signals and deep CNN. Pattern Recognition Letters. 2019 May 1;122:23-30.

64. Barberi C, van den Hondel KE. The use of cardiac troponin T (cTnT) in the postmortem diagnosis of acute myocardial infarction and sudden cardiac death: A systematic review. Forensic science international. 2018 Nov 1;292:27-38.

65. Ndrepepa G. Aspartate aminotransferase and cardiovascular disease—a narrative review. J. Lab. Precis. Med. 2021 Jan;6(6).

66. Li L, Zhou Q, Xu J. Changes of laboratory cardiac markers and mechanisms of cardiac injury in coronavirus disease 2019. BioMed Research International. 2020 May 27;2020.

67. Saleem FR, Chandru S, Biswas M. Evaluation of total LDH and its isoenzymes as markers in preeclampsia. Journal of Medical Biochemistry. 2020 Sep 9;39(3):392.

68. Cao F, Zervou S, Lygate CA. The creatine kinase system as a therapeutic target for myocardial ischaemia–reperfusion injury. Biochemical Society Transactions. 2018 Oct 19;46(5):1119-27.

69. Brewster LM. Creatine kinase, energy reserve, and hypertension: from bench to bedside. Annals of Translational Medicine. 2018 Aug;6(15).

70. Rayner JJ, Peterzan MA, Watson WD, Clarke WT, Neubauer S, Rodgers CT, Rider OJ. Myocardial energetics in obesity: enhanced ATP delivery through creatine kinase with blunted stress response. Circulation. 2020 Apr 7;141(14):1152-63.

71. Sawalha K, Vedala K, Liu E. CK-MB Elevation in Mild Hypothermia: What We Did and We Should Have Done. Journal of

Investigative Medicine High Impact Case Reports. 2021 Feb;9:2324709621995335.

72. Kachooei E, Cordina NM, Potluri PR, Guse JA, McCamey D, Brown LJ. Phosphorylation of Troponin I finely controls the positioning of Troponin for the optimal regulation of cardiac muscle contraction. Journal of Molecular and Cellular Cardiology. 2021 Jan 1;150:44-53.

73. Packer M, Januzzi JL, Ferreira JP, Anker SD, Butler J, Filippatos G, Pocock SJ, Brueckmann M, Jamal W, Cotton D, Iwata T. Concentration-dependent clinical and prognostic importance of high-sensitivity cardiac troponin T in heart failure and a reduced ejection fraction and the influence of empagliflozin: the EMPEROR-Reduced trial. European journal of heart failure. 2021 Sep;23(9):1529-38.

74. Schmid J, Liesinger L, Birner-Gruenberger R, Stojakovic T, Scharnagl H, Dieplinger B, Asslaber M, Radl R, Beer M, Polacin M, Mair J. Elevated cardiac troponin T in patients with skeletal myopathies. Journal of the American college of cardiology. 2018 Apr 10;71(14):1540-9.

75. Rovina RL, Pinto AP, Muñoz VR, da Rocha AL, Rebelo MA, Teixeira GR, Tavares ME, Pauli JR, de Moura LP, Cintra DE, Ropelle ER. Genetic deletion of IL-6 increases CK-MB, a classic cardiac damage marker, and decreases UPRmt genes after exhaustive exercise. Cell Biochemistry and Function. 2022 Apr 12.

76. Parsanathan R, Jain SK. Novel invasive and noninvasive cardiac-specific biomarkers in obesity and cardiovascular diseases. Metabolic syndrome and related disorders. 2020 Feb 1;18(1):10-30.

77. Bjørkavoll-Bergseth M, Erevik CB, Kleiven Ø, Eijsvogels TM, Skadberg Ø, Frøysa V, Wiktorski T, Auestad B, Edvardsen T, Moberg Aakre K, Ørn S. Determinants of Interindividual Variation in

Exercise-Induced Cardiac Troponin I Levels. Journal of the American Heart Association. 2021 Sep 7;10(17):e021710.

78. Chaulin AM. Elevation mechanisms and diagnostic consideration of cardiac troponins under conditions not associated with myocardial infarction. Part 1. Life. 2021 Sep 2;11(9):914.

79. Airaksinen KJ. Cardiac troponin release after endurance exercise: still much to learn. Journal of the American Heart Association. 2020 Feb 18;9(4):e015912.

80. Shahsavarinia K, Niaki NM, Taghizadieh A, Habibi P, Separham A, Gilani N, Dolatkhah N. Serum Concentrations of Cardiac Troponin I Is Correlated With the Outcomes of Patients With Chronic Obstructive Pulmonary Disease Exacerbation Referred to Emergency Department. Acta Medica Iranica. 2022;60(5):275.

81. Nguyen MT, Kung WM, Lehman S, Chew DP. High-sensitivity troponin in chronic kidney disease: Considerations in myocardial infarction and beyond. Reviews in Cardiovascular Medicine. 2020 Jun 30;21(2):191-203.

82. Ghersin I, Zahran M, Azzam ZS, Suleiman M, Bahouth F. Prognostic value of cardiac troponin levels in patients presenting with supraventricular tachycardias. Journal of Electrocardiology. 2020 Sep 1;62:200-3.

83. Chauin A. The main causes and mechanisms of increase in cardiac troponin concentrations other than acute myocardial infarction (part 1): physical exertion, inflammatory heart disease, pulmonary embolism, renal failure, sepsis. Vascular Health and Risk Management. 2021;17:601.

84. Ebrahimi M, Arab MM, Moghadam HZ, Yazdi MJ, Foroughian M. Risk Stratification of Pulmonary Thromboembolism using Brain

Natriuretic Peptide and Troponin I; a Brief Report. Archives of Academic Emergency Medicine. 2022;10(1).

85. Zhang Y, Luo Y, Nijiatijiang G, Balati K, Tuerdi Y, Liu L. Correlations of changes in brain natriuretic peptide (BNP) and cardiac troponin I (cTnI) with levels of C-reactive protein (CRP) and TNF-α in pediatric patients with sepsis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2019;25:2561.

86. Goel H, Melot J, Krinock MD, Kumar A, Nadar SK, Lip GY. Heart-type fatty acid-binding protein: An overlooked cardiac biomarker. Annals of Medicine. 2020 Nov 16;52(8):444-61.

87. Vera MA, Koch CD, Kavsak PA, El-Khoury JM. Determination of 97.5 th and 99th percentile upper reference limits for heart-type fatty acid-binding protein (H-FABP) in a US population. Clinica Chimica Acta. 2021 Dec 1;523:397-401.

88. Navarro C, Kurth MJ, Ruddock M, Fishlock S, McLaughlin J. An Algorithm Based on Combining hs-cTnT and H-FABP for Ruling Out Acute Myocardial Infarction. In2019 Computing in Cardiology (CinC) 2019 Sep 8 (pp. Page-1). IEEE.

89. Ye XD, He Y, Wang S, Wong GT, Irwin MG, Xia Z. Heart-type fatty acid binding protein (H-FABP) as a biomarker for acute myocardial injury and long-term post-ischemic prognosis. Acta pharmacologica sinica. 2018 Jul;39(7):1155-63.

90. Miao Y, Liu Y, Liu C, Yao L, Kang X, Lv M. Diagnostic Value of Echocardiography Combined with Serum h-FABP and cTnI in Myocardial Infarction and Its Evaluation Value in Left Ventricular Function. Evidence-Based Complementary and Alternative Medicine. 2022 May 24;2022.

91. Rezar R, Jirak P, Gschwandtner M, Derler R, Felder TK, Haslinger M, Kopp K, Seelmaier C, Granitz C, Hoppe UC, Lichtenauer M. Heart-type fatty acid-binding protein (H-FABP) and its role as a biomarker in heart failure: what do we know so far?. Journal of Clinical Medicine. 2020 Jan 7;9(1):164.

92. DeFilippis AP, Chapman AR, Mills NL, De Lemos JA, Arbab-Zadeh A, Newby LK, Morrow DA. Assessment and treatment of patients with type 2 myocardial infarction and acute nonischemic myocardial injury. Circulation. 2019 Nov 12;140(20):1661-78.

93. Mahmud E, Dauerman HL, Welt FG, Messenger JC, Rao SV, Grines C, Mattu A, Kirtane AJ, Jauhar R, Meraj P, Rokos IC. Management of acute myocardial infarction during the COVID-19 pandemic: a position statement from the Society for Cardiovascular Angiography and Interventions (SCAI), the American College of Cardiology (ACC), and the American College of Emergency Physicians (ACEP). Journal of the American College of Cardiology. 2020 Sep 15;76(11):1375-84.

94. Jing ZC, Zhu HD, Yan XW, Chai WZ, Zhang S. Recommendations from the Peking Union Medical College Hospital for the management of acute myocardial infarction during the COVID-19 outbreak.

95. Gong FF, Vaitenas I, Malaisrie SC, Maganti K. Mechanical complications of acute myocardial infarction: a review. JAMA cardiology. 2021 Mar 1;6(3):341-9.

96. Puymirat E, Cayla G, Simon T, Steg PG, Montalescot G, Durand-Zaleski I, Le Bras A, Gallet R, Khalife K, Morelle JF, Motreff P. Multivessel PCI guided by FFR or angiography for myocardial infarction. New England Journal of Medicine. 2021 Jul 22;385(4):297-308.

97. Hemradj VV, Karami M, Sjauw KD, Engström AE, Ouweneel DM, de Brabander J, Vis MM, Wykrzykowska JJ, Beijk MA, Koch KT, Baan J. Pre-PCI versus immediate post-PCI Impella initiation in acute myocardial infarction complicated by cardiogenic shock. PLoS One. 2020 Jul 20;15(7):e0235762.

98. Gao N, Qi X, Dang Y, Li Y, Wang G, Liu X, Zhu N, Fu J. Establishment and validation of a risk model for prediction of in-hospital mortality in patients with acute ST-elevation myocardial infarction after primary PCI. BMC cardiovascular disorders. 2020 Dec;20(1):1-0.

99. Mathias W, Tsutsui JM, Tavares BG, Fava AM, Aguiar MO, Borges BC, Oliveira MT, Soeiro A, Nicolau JC, Ribeiro HB, Chiang HP. Sonothrombolysis in ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. Journal of the American College of Cardiology. 2019 Jun 11;73(22):2832-42.

100. Li M, Qin J, Xiong K, Jiang B, Zhang T. Review of arginase as a promising biocatalyst: characteristics, preparation, applications and future challenges. Critical Reviews in Biotechnology. 2022 Jul 4;42(5):651-67.

101. Azambuja JH, Ludwig N, Yerneni SS, Braganhol E, Whiteside TL. Arginase-1+ exosomes from reprogrammed macrophages promote glioblastoma progression. International journal of molecular sciences. 2020 Jun 2;21(11):3990.

102. Dowling JK, Afzal R, Gearing LJ, Cervantes-Silva MP, Annett S, Davis GM, De Santi C, Assmann N, Dettmer K, Gough DJ, Bantug GR. Mitochondrial arginase-2 is essential for IL-10 metabolic reprogramming of inflammatory macrophages. Nature communications. 2021 Mar 5;12(1):1-4.

103. Umar HI, Saliu TP, Josiah SS, Ajayi A, Danjuma JB. In silico studies of bioactive compounds from selected African plants with

inhibitory activity against nitric oxide synthase and arginase implicated in asthma. Egyptian Journal of Medical Human Genetics. 2021 Dec;22(1):1-16.

104. Oda K, Sakaguchi T, Matoba Y. Catalytic mechanism of DcsB: Arginase framework used for hydrolyzing its inhibitor. Protein Science. 2022 Jun;31(6):e4338.

105. Derakhshani A, Hemmat N, Asadzadeh Z, Ghaseminia M, Shadbad MA, Jadideslam G, Silvestris N, Racanelli V, Baradaran B. Arginase 1 (Arg1) as an up-regulated gene in COVID-19 patients: a promising marker in COVID-19 immunopathy. Journal of Clinical Medicine. 2021 Mar 4;10(5):1051.

106. Zhang N, Aiyasiding X, Li WJ, Liao HH, Tang QZ. Neutrophil degranulation and myocardial infarction. Cell Communication and Signaling. 2022 Dec;20(1):1-23.

107. Sarosh M, Nurulain SM, Shah ST, Khan MJ, Muneer Z, Bibi N, Shah SF, Hussain S. Association analysis of single nucleotide polymorphisms in autophagy related 7 (ATG7) gene in patients with coronary artery disease. Medicine. 2022 Jul 7;101(26).

108. Buraczynska M, Zakrocka I. Arginase Gene Polymorphism Increases Risk of Diabetic Retinopathy in Type 2 Diabetes Mellitus Patients. Journal of Clinical Medicine. 2021 Nov 19;10(22):5407.

109. Li Z, Wang L, Ren Y, Huang Y, Liu W, Lv Z, Qian L, Yu Y, Xiong Y. Arginase: shedding light on the mechanisms and opportunities in cardiovascular diseases. Cell Death Discovery. 2022 Oct 8;8(1):413.

110. Mostafa T, Taymour M. Gene polymorphisms affecting erectile dysfunction. Sexual Medicine Reviews. 2020 Oct 1;8(4):561-72.

111. Matos A, Carvalho M, Bicho M, Ribeiro R. Arginine and Arginases Modulate Metabolism, Tumor Microenvironment and Prostate Cancer Progression. Nutrients. 2021 Dec 16;13(12):4503.

112. Couchet M, Breuillard C, Corne C, Rendu J, Morio B, Schlattner U, Moinard C. Ornithine Transcarbamylase–From Structure to Metabolism: An Update. Frontiers in Physiology. 2021;12.

113. Caldwell RW, Rodriguez PC, Toque HA, Narayanan SP, Caldwell RB. Arginase: a multifaceted enzyme important in health and disease. Physiological reviews. 2018 Apr 1;98(2):641-65.

114. Sagar NA, Tarafdar S, Agarwal S, Tarafdar A, Sharma S. Polyamines: functions, metabolism, and role in human disease management. Medical Sciences. 2021 Jun;9(2):44.

115. Igarashi K, Kashiwagi K. The functional role of polyamines in eukaryotic cells. The international journal of biochemistry & cell biology. 2019 Feb 1;107:104-15.

116. Volino-Souza M, Oliveira GV, Conte-Junior CA, Figueroa A, Alvares TS. Current Evidence of Watermelon (Citrullus lanatus) Ingestion on Vascular Health: A Food Science and Technology Perspective. Nutrients. 2022 Jul 15;14(14):2913.

117. Matsumoto S, Häberle J, Kido J, Mitsubuchi H, Endo F, Nakamura K. Urea cycle disorders—update. Journal of human genetics. 2019 Sep;64(9):833-47.

118. Vural H, Tamer MN, Weiskirchen R. Evaluation of Arginine-Nitric Oxide Pathway in Patients with Hyperthyroidism. Electron J Gen Med. 2021; 18 (2): em278.

119. Agunloye OM, Oboh G, Ademiluyi AO, Ademosun AO, Akindahunsi AA, Oyagbemi AA, Omobowale TO, Ajibade TO, Adedapo AA. Cardio-protective and antioxidant properties of caffeic acid and

chlorogenic acid: Mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats. Biomedicine & Pharmacotherapy. 2019 Jan 1;109:450-8.

120. Kikuchi R, Ambe K, Kon H, Takada S, Watanabe H. Nitric oxide synthase (NOS) isoform expression after peripheral nerve transection in mice. The Bulletin of Tokyo Dental College. 2018;59(1):15-25.

121. Zheng H, Li J, Feng C. An isoform-specific pivot modulates the electron transfer between the flavin mononucleotide and heme centers in inducible nitric oxide synthase. JBIC Journal of Biological Inorganic Chemistry. 2020 Dec;25(8):1097-105.

122. Zuo D, Pi Q, Shi Y, Luo S, Xia Y. Dihydroxyeicosatrienoic acid, a metabolite of epoxyeicosatrienoic acids upregulates endothelial Nitric Oxide synthase expression through transcription: mechanism of vascular endothelial function protection. Cell Biochemistry and Biophysics. 2021 Jun;79(2):289-99.

123. Singh N, Geethika M, Eswarappa SM, Mugesh G. Manganese-Based Nanozymes: Multienzyme Redox Activity and Effect on the Nitric Oxide Produced by Endothelial Nitric Oxide Synthase. Chemistry–A European Journal. 2018 Jun 12;24(33):8393-403.

124. Yuyun MF, Ng LL, Ng GA. Endothelial dysfunction, endothelial nitric oxide bioavailability, tetrahydrobiopterin, and 5-methyltetrahydrofolate in cardiovascular disease. Where are we with therapy?. Microvascular research. 2018 Sep 1;119:7-12.

125. Nasoni MG, Benedetti S, Crinelli R, Palma F, Canonico B, Monittola F, Zerbinati C, Iuliano L, Luchetti F. 3β-Hydroxy-5β-hydroxy-B-norcholestane-6β-carboxaldehyde (SEC-B) Induces Proinflammatory Activation of Human Endothelial Cells Associated with Nitric Oxide

Production and Endothelial Nitric Oxide Synthase/Caveolin-1 Dysregulation. Antioxidants. 2022 Jun 10;11(6):1148.

126. Sarmah N, Nauli AM, Ally A, Nauli SM. Interactions among Endothelial Nitric Oxide Synthase, Cardiovascular System, and Nociception during Physiological and Pathophysiological States. Molecules. 2022 Apr 29;27(9):2835.

127. Cinelli MA, Do HT, Miley GP, Silverman RB. Inducible nitric oxide synthase: Regulation, structure, and inhibition. Medicinal research reviews. 2020 Jan;40(1):158-89.

128. Guerby P, Tasta O, Swiader A, Pont F, Bujold E, Parant O, Vayssiere C, Salvayre R, Negre-Salvayre A. Role of oxidative stress in the dysfunction of the placental endothelial nitric oxide synthase in preeclampsia. Redox biology. 2021 Apr 1;40:101861.

129. Pignatelli P, Fabietti G, Ricci A, Piattelli A, Curia MC. How periodontal disease and presence of nitric oxide reducing oral bacteria can affect blood pressure. International journal of molecular sciences. 2020 Oct 13;21(20):7538.

130. Krause BJ. Novel insights for the role of nitric oxide in placental vascular function during and beyond pregnancy. Journal of Cellular Physiology. 2021 Dec;236(12):7984-99.

131. Niu F, Yu Y, Li Z, Ren Y, Li Z, Ye Q, Liu P, Ji C, Qian L, Xiong Y. Arginase: An emerging and promising therapeutic target for cancer treatment. Biomedicine & Pharmacotherapy. 2022 May 1;149:112840.

132. James AS, Eteng OE, Dosumu OA, Moses CA, Ogbonna CU, Adeleye OA, Ugwor EI, Omilo BC, Fabunmi RF, Olakitan AM, Ugbaja RN. Morin Augmented Myocardial eNOS/cGMP/PKG Signaling Pathway and Abated Oxidative and Inflammo-apoptotic Responses in

Diethyl Phthalate and Bisphenol-S Co-Exposed Male Albino Rats. Inflammation. 2022 Jul 28:1-5.

133. Kido J, Matsumoto S, Takeshita E, Hayakawa C, Yamada K, Kagawa J, Nakajima Y, Ito T, Iijima H, Endo F, Nakamura K. Current status of surviving patients with arginase 1 deficiency in Japan. Molecular Genetics and Metabolism Reports. 2021 Dec 1;29:100805.

134. Angarita SA, Truong B, Khoja S, Nitzahn M, Rajbhandari AK, Zhuravka I, Duarte S, Lin MG, Lam AK, Cederbaum SD, Lipshutz GS. Human hepatocyte transplantation corrects the inherited metabolic liver disorder arginase deficiency in mice. Molecular genetics and metabolism. 2018 Jun 1;124(2):114-23.

135. Mill J, Patel V, Okonkwo O, Li L, Raife T. Erythrocyte sphingolipid species as biomarkers of Alzheimer's disease. Journal of Pharmaceutical Analysis. 2022 Feb 1;12(1):178-85.

136. Wei D, Wu D, Zeng W, Che L, Xu S, Fang Z, Feng B, Li J, Zhuo Y, Wu C, Zhang J. Arginine promotes testicular development in boars through nitric oxide and putrescine. Journal of Animal Physiology and Animal Nutrition. 2022 Mar;106(2):266-75.

137. Wu X, Sun X, Sharma S, Lu Q, Yegambaram M, Hou Y, Wang T, Fineman JR, Black SM. Arginine recycling in endothelial cells is regulated BY HSP90 and the ubiquitin proteasome system. Nitric Oxide. 2021 Mar 1;108:12-9.

138. Shosha E, Fouda AY, Narayanan SP, Caldwell RW, Caldwell RB. Is the arginase pathway a novel therapeutic avenue for diabetic retinopathy?. Journal of clinical medicine. 2020 Feb 5;9(2):425.

139. Gresele P, Momi S, Guglielmini G. Nitric oxide-enhancing or-releasing agents as antithrombotic drugs. Biochemical Pharmacology. 2019 Aug 1;166:300-12.

140. Costa G, Shushanof M, Bouskela E, Bottino D. Oral l-arginine (5 g/day) for 14 days improves microcirculatory function in healthy young women and healthy and type 2 diabetes mellitus elderly women. Journal of Vascular Research. 2022;59(1):24-33.

141. Galluccio E, Spadoni S, Fontana B, Bosi E, Piatti P, Monti LD. Long lasting protective effects of early l-arginine treatment on endothelium in an in vitro study. Clinical Nutrition. 2021 Apr 1;40(4):1519-29.

142. Khalaf D, Krüger M, Wehland M, Infanger M, Grimm D. The effects of oral l-arginine and l-citrulline supplementation on blood pressure. Nutrients. 2019 Jul 22;11(7):1679..

143. Arribas-López E, Zand N, Ojo O, Snowden MJ, Kochhar T. The effect of amino acids on wound healing: a systematic review and meta-analysis on arginine and glutamine. Nutrients. 2021 Jul 22;13(8):2498.

144. Zhou Y, Liu G, Huang H, Wu J. Advances and impact of arginine-based materials in wound healing. Journal of Materials Chemistry B. 2021;9(34):6738-50.

145. Abd El-Aleem SA, Mohammed HH, Saber EA, Embaby AS, Djouhri L. Mutual inter-regulation between iNOS and TGF-β1: Possible molecular and cellular mechanisms of iNOS in wound healing. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2020 Oct 1;1866(10):165850.

146. Liu T, Liu G, Zhang J, Ding Z, Li Y, Sigdel K, Wang X, Xie H. L-Arginine based polyester amide/hyaluronic acid hybrid hydrogel with dual anti-inflammation and antioxidant functions for accelerated wound healing. Chinese Chemical Letters. 2022 Apr 1;33(4):1880-4.

147. Shatanawi A, Momani MS, Al-Aqtash R, Hamdan MH, Gharaibeh MN. L-Citrulline supplementation increases plasma nitric oxide levels

and reduces arginase activity in patients with Type 2 Diabetes. Frontiers in pharmacology. 2020 Dec 22;11:584669.

148. Naimunisa S. Role of berries in treating hypertension. TNNMC Journal of Medical & Surgical Nursing. 2021;9(2):47-50.

149. S. Clemente G, van Waarde A, F. Antunes I, Dömling A, H. Elsinga P. Arginase as a potential biomarker of disease progression: a molecular imaging perspective. International Journal of Molecular Sciences. 2020 Jul 25;21(15):5291.

150. Premont RT, Reynolds JD, Zhang R, Stamler JS. Role of nitric oxide carried by hemoglobin in cardiovascular physiology: developments on a three-gas respiratory cycle. Circulation research. 2020 Jan 3;126(1):129-58.

151. Gajecki D, Gawryś J, Wiśniewski J, Fortuna P, Szahidewicz-Krupska E, Doroszko A. A Cross-Talk between the Erythrocyte L-Arginine/ADMA/Nitric Oxide Metabolic Pathway and the Endothelial Function in Subjects with Type 2 Diabetes Mellitus. Nutrients. 2021 Jul 4;13(7):2306.

152. Mahdi A, Cortese-Krott MM, Kelm M, Li N, Pernow J. Novel perspectives on redox signaling in red blood cells and platelets in cardiovascular disease. Free Radical Biology and Medicine. 2021 May 20;168:95-109.

153. Lin TC, Yang CH, Cheng LH, Chang WT, Lin YR, Cheng HC. Fibronectin in cancer: friend or foe. Cells. 2019 Dec 20;9(1):27.

154. Lemańska-Perek A, Adamik B. Fibronectin and its soluble EDA-FN isoform as biomarkers for inflammation and sepsis. Adv Clin Exp Med. 2019 Nov 1;28(11):1561-7.

155. Dalton CJ, Lemmon CA. Fibronectin: molecular structure, fibrillar structure and mechanochemical signaling. Cells. 2021 Sep 16;10(9):2443.

156. Zhang X, Hu C, Wu HM, Ma ZG, Tang QZ. Fibronectin type III domain-containing 5 in cardiovascular and metabolic diseases: a promising biomarker and therapeutic target. Acta Pharmacologica Sinica. 2021 Sep;42(9):1390-400.

157. Saw PE, Xu X, Kang BR, Lee J, Lee YS, Kim C, Kim H, Kang SH, Na YJ, Moon HJ, Kim JH. Extra-domain B of fibronectin as an alternative target for drug delivery and a cancer diagnostic and prognostic biomarker for malignant glioma. Theranostics. 2021;11(2):941.

158. Lieverse RI, Marcus D, van der Wiel AM, Van Limbergen EJ, Theys J, Yaromina A, Lambin P, Dubois LJ. Human fibronectin extra domain B as a biomarker for targeted therapy in cancer. Molecular oncology. 2020 Jul;14(7):1555-68.

159. Baglieri J, Brenner DA, Kisseleva T. The role of fibrosis and liver-associated fibroblasts in the pathogenesis of hepatocellular carcinoma. International journal of molecular sciences. 2019 Apr 7;20(7):1723.

160. Peng DH, Luo Y, Huang LJ, Liao FL, Liu YY, Tang P, Hu HN, Chen W. Correlation of Krebs von den Lungen-6 and fibronectin with pulmonary fibrosis in coronavirus disease 2019. Clinica Chimica Acta. 2021 Jun 1;517:48-53.

161. Malara A, Gruppi C, Massa M, Rosti V, Barosi G, Balduini A. Plasma EDA fibronectin in primary myelofibrosis correlates with bone marrow fibrosis and predicts splenomegaly. Blood. 2019 Nov 13;134:1688.

162. Li B, Shen W, Peng H, Li Y, Chen F, Zheng L, Xu J, Jia L. Fibronectin 1 promotes melanoma proliferation and metastasis by inhibiting apoptosis and regulating EMT. OncoTargets and therapy. 2019;12:3207.

163. Wang Y, Zhao M, Zhang Y. Identification of fibronectin 1 (FN1) and complement component 3 (C3) as immune infiltration-related biomarkers for diabetic nephropathy using integrated bioinformatic analysis. Bioengineered. 2021 Jan 1;12(1):5386-401.

164. Park JE, JebaMercy G, Pazhanchamy K, Guo X, Ngan SC, Liou KC, Lynn SE, Ng SS, Meng W, Lim SC, Leow MK. Aging-induced isoDGR-modified fibronectin activates monocytic and endothelial cells to promote atherosclerosis. Atherosclerosis. 2021 May 1;324:58-68.

165. Mezzenga R, Mitsi M. The molecular dance of fibronectin: conformational flexibility leads to functional versatility. Biomacromolecules. 2018 Nov 7;20(1):55-72.

166. Filla MS, Faralli JA, Desikan H, Peotter JL, Wannow AC, Peters DM. Activation of αvβ3 integrin alters fibronectin fibril formation in human trabecular meshwork cells in a ROCK-independent manner. Investigative ophthalmology & visual science. 2019 Sep 3;60(12):3897-913.

167. Saba TM. Fibronectin: role in phagocytic host defense and lung vascular integrity. Fibronectin in Health and Disease. 2018 Jan 18:49-68.

168. Zhang L, Yan H, Tai Y, Xue Y, Wei Y, Wang K, Zhao Q, Wang S, Kong D, Midgley AC. Design and evaluation of a polypeptide that mimics the integrin binding site for EDA fibronectin to block profibrotic cell activity. International journal of molecular sciences. 2021 Feb 4;22(4):1575.

169. Jain M, Chauhan AK. Role of integrins in modulating smooth muscle cell plasticity and vascular remodeling: from expression to therapeutic implications. Cells. 2022 Feb 13;11(4):646.

170. Klavert J, Van der Eerden BC. Fibronectin in Fracture Healing: Biological Mechanisms and Regenerative Avenues. Frontiers in Bioengineering and Biotechnology. 2021 Apr 16;9:663357.

171. Kennelly TM, Li Y, Cao Y, Qwarnstrom EE, Geoghegan M. Distinct binding interactions of α5β1-integrin and proteoglycans with fibronectin. Biophysical Journal. 2019 Aug 20;117(4):688-95.

172. Yu Q, Xue Y, Liu J, Xi Z, Li Z, Liu Y. Fibronectin promotes the malignancy of glioma stem-like cells via modulation of cell adhesion, differentiation, proliferation and chemoresistance. Frontiers in Molecular Neuroscience. 2018 Apr 13;11:130.

173. McDonagh J, Hada M, Kaminski M. Plasma fibronectin and fibrin formation. InPlasma Fibronectin 2020 Aug 18 (pp. 121-148). CRC Press.

174. Jara CP, Wang O, do Prado TP, Ismail A, Fabian FM, Li H, Velloso LA, Carlson MA, Burgess W, Lei Y, Velander WH. Novel fibrin-fibronectin matrix accelerates mice skin wound healing. Bioactive Materials. 2020 Dec 1;5(4):949-62.

175. Musiime M, Chang J, Hansen U, Kadler KE, Zeltz C, Gullberg D. Collagen assembly at the cell surface: dogmas revisited. Cells. 2021 Mar 16;10(3):662.

176. Wang C, Wu H, Xing Y, Ye Y, He F, Yin Q, Li Y, Shang F, Shyy JY, Yuan ZY. Endothelial-derived extracellular microRNA-92a promotes arterial stiffness by regulating phenotype changes of vascular smooth muscle cells. Scientific reports. 2022 Jan 10;12(1):1-2

177. Alsamarai AT, Husain AA, Al OR. Correlation between salivary and serum fibronectin in hypertensive patients. EurAsian Journal of BioSciences. 2020 Jan 1;14:1763-5.

178. Angelova PR, Esteras N, Abramov AY. Mitochondria and lipid peroxidation in the mechanism of neurodegeneration: Finding ways for prevention. Medicinal Research Reviews. 2021 Mar;41(2):770-84.

179. Ursini F, Maiorino M. Lipid peroxidation and ferroptosis: the role of GSH and GPx4. Free Radical Biology and Medicine. 2020 May 20;152:175-85.

180. Konings AW. Lipid peroxidation in liposomes. InLiposome technology 2019 Jul 23 (pp. 139-161). CRC Press.

181. Recknagel RO, Glende EA, Britton RS. Free radical damage and lipid peroxidation. InHepatotoxicology 2020 Jan 16 (pp. 401-436). CRC press.

182. Kehm R, Baldensperger T, Raupbach J, Höhn A. Protein oxidation-formation mechanisms, detection and relevance as biomarkers in human diseases. Redox Biology. 2021 Jun 1;42:101901.

183. Li Y, Zhao T, Li J, Xia M, Li Y, Wang X, Liu C, Zheng T, Chen R, Kan D, Xie Y. Oxidative Stress and 4-hydroxy-2-nonenal (4-HNE): Implications in the Pathogenesis and Treatment of Aging-related Diseases. Journal of Immunology Research. 2022 Mar 23;2022.

184. Ogueji E, Nwani C, Mbah C, Iheanacho S, Nweke F. Oxidative stress, biochemical, lipid peroxidation, and antioxidant responses in Clarias gariepinus exposed to acute concentrations of ivermectin. Environmental Science and Pollution Research. 2020 May;27(14):16806-15.

185. Hematyar N, Rustad T, Sampels S, Kastrup Dalsgaard T. Relationship between lipid and protein oxidation in fish. Aquaculture Research. 2019 May;50(5):1393-403.

186. Yaman SO, Ayhanci A. Lipid peroxidation. Lipid Peroxidation. IntechOpen. 2021 Feb 12:1-1.

187. Huang X, Ahn DU. Lipid oxidation and its implications to meat quality and human health. Food science and biotechnology. 2019 Oct;28(5):1275-85.

188. Jamshidi-Kia F, Wibowo JP, Elachouri M, Masumi R, Salehifard-Jouneghani A, Abolhasanzadeh Z, Lorigooini Z. Battle between plants as antioxidants with free radicals in human body. Journal of Herbmed Pharmacology. 2020;9(3):191-9.

189. Ratajczak E, Małecka A, Ciereszko I, Staszak AM. Mitochondria are important determinants of the aging of seeds. International Journal of Molecular Sciences. 2019 Mar 28;20(7):1568.

190. Romão MJ. Structural insights on aldehyde oxidase and xanthine oxidoreductase and their roles in xenobiotic metabolism. Free Radical Biology and Medicine. 2018 May 20;120:S23.

191. Somasundaram V, Basudhar D, Bharadwaj G, No JH, Ridnour LA, Cheng RY, Fujita M, Thomas DD, Anderson SK, McVicar DW, Wink DA. Molecular mechanisms of nitric oxide in cancer progression, signal transduction, and metabolism. Antioxidants & Redox Signaling. 2019 Mar 1;30(8):1124-43.

192. Ashfaq A, Clochard MC, Coqueret X, Dispenza C, Driscoll MS, Ulański P, Al-Sheikhly M. Polymerization reactions and modifications of polymers by ionizing radiation. Polymers. 2020 Nov 30;12(12):2877.

193. Goel R, Bitzer ZT, Reilly SM, Foulds J, Muscat J, Elias RJ, Richie Jr JP. Influence of smoking puff parameters and tobacco varieties on free Radicals yields in cigarette mainstream smoke. Chemical research in toxicology. 2018 Apr 27;31(5):325-31.

194. Foret MK, Do Carmo S, Lincoln R, Greene LE, Zhang W, Cuello AC, Cosa G. Effect of antioxidant supplements on lipid peroxidation

levels in primary cortical neuron cultures. Free Radical Biology and Medicine. 2019 Jan 1;130:471-7.

195. Perona Martínez F, Nusantara AC, Chipaux M, Padamati SK, Schirhagl R. Nanodiamond relaxometry-based detection of free-radical species when produced in chemical reactions in biologically relevant conditions. ACS sensors. 2020 Dec 3;5(12):3862-9.

196. Xin H, Wang F, Luo R, Lei J. Parallel Lipid Peroxide Accumulation Strategy Based on Bimetal–Organic Frameworks for Enhanced Ferrotherapy. Chemistry–A European Journal. 2021 Mar 1;27(13):4307-11.

197. Selyutina OY, Kononova PA, Koshman VE, Fedenok LG, Polyakov NE. The Interplay of Ascorbic Acid with Quinones-Chelators—Influence on Lipid Peroxidation: Insight into Anticancer Activity. Antioxidants. 2022 Feb 13;11(2):376.

198. Costa TJ, Barros PR, Arce C, Santos JD, da Silva-Neto J, Egea G, Dantas AP, Tostes RC, Jimenez-Altayo F. The homeostatic role of hydrogen peroxide, superoxide anion and nitric oxide in the vasculature. Free Radical Biology and Medicine. 2021 Jan 1;162:615-35.

199. Piccirillo S, Magi S, Preziuso A, Serfilippi T, Cerqueni G, Orciani M, Amoroso S, Lariccia V. The Hidden Notes of Redox Balance in Neurodegenerative Diseases. Antioxidants. 2022 Aug;11(8):1456.

200. Raimondi V, Ciccarese F, Ciminale V. Oncogenic pathways and the electron transport chain: a dangeROS liaison. British journal of cancer. 2020 Jan;122(2):168-81.

201. Lancaster Jr JR. Historical origins of the discovery of mammalian nitric oxide (nitrogen monoxide) production/physiology/pathophysiology. Biochemical Pharmacology. 2020 Jun 1;176:113793.

202. Zhang D, Gong C, Wang J, Mu C, Wang W, Zhang X. Beyond lipid peroxidation: Distinct mechanisms observed for POPC and POPG oxidation initiated by UV-enhanced Fenton reactions at the air–water interface. Journal of Mass Spectrometry. 2021 Apr;56(4):e4626.

203. Spickett CM. Formation of oxidatively modified lipids as the basis for a cellular epilipidome. Frontiers in Endocrinology. 2020 Dec 21;11:602771.

204. Lopes R, Costa M, Ferreira M, Gameiro P, Fernandes S, Catarino C, Santos-Silva A, Paiva-Martins F. Caffeic acid phenolipids in the protection of cell membranes from oxidative injuries. Interaction with the membrane phospholipid bilayer. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2021 Dec 1;1863(12):183727.

205. Wan Q, Zhang R, Zhuang Z, Li Y, Huang Y, Wang Z, Zhang W, Hou J, Tang BZ. Molecular engineering to boost AIE-active free radical photogenerators and enable high-performance photodynamic therapy under hypoxia. Advanced Functional Materials. 2020 Sep;30(39):2002057.

206. Borys J, Maciejczyk M, Antonowicz B, Sidun J, Świderska M, Zalewska A. Free radical production, inflammation and apoptosis in patients treated with titanium mandibular fixations—an observational study. Frontiers in immunology. 2019 Nov 8;10:2662.

207. Xie H, Huang J, Woo MW, Hu J, Xiong H, Zhao Q. Effect of cold and hot enzyme deactivation on the structural and functional properties of rice dreg protein hydrolysates. Food Chemistry. 2021 May 30;345:128784.

208. Bessalah S, Jebahi S, Faraz A, Raoufi A, Tırınk C, Dridi W, Daouad M, Keskes H, Khorchani T, Farah K, andMohamed Hammadi

AW. Effect of Gamma Radiation on Novel Gelatin Extracted from Camel Skin for Pharmaceutical Application.

209. Taffe BG, Kensler TW. Free radicals and signal transduction in tumor promotion. InGenes and Signal Transduction in Multistage Carcinogenesis 2020 Nov 25 (pp. 391-413). CRC Press.

210. El-Mahdy MA, Ewees MG, Eid MS, Mahgoup EM, Khaleel SA, Zweier JL. Electronic cigarette exposure causes vascular endothelial dysfunction due to NADPH oxidase activation and eNOS uncoupling. American Journal of Physiology-Heart and Circulatory Physiology. 2022 Apr 1;322(4):H549-67.

211. Wood I, Trostchansky A, Xu Y, Qian S, Radi R, Rubbo H. Free radical-dependent inhibition of prostaglandin endoperoxide H Synthase-2 by nitro-arachidonic acid. Free Radical Biology and Medicine. 2019 Nov 20;144:176-82.

212. Dunham-Snary KJ, Surewaard BG, Mewburn JD, Bentley RE, Martin AY, Jones O, Al-Qazazi R, Lima PA, Kubes P, Archer SL. Mitochondria in human neutrophils mediate killing of Staphylococcus aureus. Redox biology. 2022 Feb 1;49:102225.

213. Sadžak A, Mravljak J, Maltar-Strmečki N, Arsov Z, Baranović G, Erceg I, Kriechbaum M, Strasser V, Přibyl J, Šegota S. The structural integrity of the model lipid membrane during induced lipid peroxidation: The role of flavonols in the inhibition of lipid peroxidation. Antioxidants. 2020 May 15;9(5):430.

214. Farhoosh R. Critical kinetic parameters and rate constants representing lipid peroxidation as affected by temperature. Food Chemistry. 2021 Mar 15;340:128137.

215. Fadly D, Kusharto CM, Kustiyah L, Suptijah P, Muttalib YS. In Vitro Study of Antioxidant Activity of Carboxymethyl Chitosan derived

from Silkworm (Bombyx mori L.) Pupa against Human Plasma Lipid Peroxidation. Systematic Reviews in Pharmacy. 2020 Jul 1;11(7).

216. Piquereau J, Ventura-Clapier R. Maturation of cardiac energy metabolism during perinatal development. Frontiers in Physiology. 2018 Jul 19;9:959.

217. Afonso MS, Machado RM, Lavrador MS, Quintao EC, Moore KJ, Lottenberg AM. Molecular pathways underlying cholesterol homeostasis. Nutrients. 2018 Jun 13;10(6):760.

218. Gianazza E, Brioschi M, Fernandez AM, Banfi C. Lipoxidation in cardiovascular diseases. Redox biology. 2019 May 1;23:101119.

219. Scioli MG, Storti G, D'Amico F, Rodríguez Guzmán R, Centofanti F, Doldo E, Céspedes Miranda EM, Orlandi A. Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: potential diagnostic biomarkers and therapeutic targets. Journal of Clinical Medicine. 2020 Jun 25;9(6):1995.

220. Sutaria SR, Gori SS, Morris JD, Xie Z, Fu XA, Nantz MH. Lipid Peroxidation Produces a Diverse Mixture of Saturated and Unsaturated Aldehydes in Exhaled Breath That Can Serve as Biomarkers of Lung Cancer—A Review. Metabolites. 2022 Jun;12(6):561.

221. Kumar V, Gill KD. To Perform Enzyme-Linked Immunosorbent Assay. InBasic Concepts in Clinical Biochemistry: A Practical Guide 2018 (pp. 153-155). Springer, Singapore.

222. Shah SF, Khan MJ, Iqbal T, Akram S, Waheed F, Satti HS, Rafiq MA, Hussain S. Arginase-1 variants and the risk of familial coronary artery disease in subjects originating from Pakistan. Genetic Testing and Molecular Biomarkers. 2019 Jan 1;23(1):32-8.

223. Buraczynska M, Zakrocka I. Arginase Gene Polymorphism Increases Risk of Diabetic Retinopathy in Type 2 Diabetes Mellitus Patients. Journal of Clinical Medicine. 2021 Nov 19;10(22):5407.

224. Shah SF, Akram S, Iqbal T, Nawaz S, Rafiq MA, Hussain S. Association analysis between ARG1 gene polymorphisms and idiopathic dilated cardiomyopathy. Medicine. 2019 Nov;98(47).

225. Zeidan MA, Ahmed S, Aldeen al-khateeb D. Assessment of Risk factors For myocardial Infraction in Sample of Patients Attending in Coronary Care Unit Ward in AL-Zahra hospital-Karbala City. Ibn AL-Haitham Journal For Pure and Applied Sciences. 2017 Sep 25;30(2):1-9.

226. Shih JY, Chen ZC, Chang HY, Liu YW, Ho CH, Chang WT. Risks of age and sex on clinical outcomes post myocardial infarction. IJC Heart & Vasculature. 2019 Jun 1;23:100350.

227. Alkhouli M, Alqahtani F, Jneid H, Al Hajji M, Boubas W, Lerman A. Age-stratified sex-related differences in the incidence, management, and outcomes of acute myocardial infarction. InMayo Clinic Proceedings 2021 Feb 1 (Vol. 96, No. 2, pp. 332-341). Elsevier.

228. Amen SO, Baban ST, Yousif SH, Baban ZT, Hawez AH, Jalal DM. High prevalence of vitamin D deficiency in patients with acute myocardial infarction: An Iraqi single-center study. Medical Journal of Babylon. 2020 Oct 1;17(4):358.

229. Ahmed HS. Assessment of ApolipoproteinsA1, E, and Insulin Resistance in Iraqi Male Patients with Acute Myocardial Infarction. Iraqi Journal of Community Medicine. 2020 Jul;33(2).

230. Sagris M, Antonopoulos AS, Theofilis P, Oikonomou E, Siasos G, Tsalamandris S, Antoniades C, Brilakis ES, Kaski JC, Tousoulis D. Risk

factors profile of young and older patients with Myocardial Infarction. Cardiovascular Research. 2022 Jun;118(10):2281-92.

231. Rastogi T, Ho FK, Rossignol P, Merkling T, Butler J, Clark A, Collier T, Delles C, Jukema JW, Heymans S, Latini R. Comparing and contrasting risk factors for Heart failure in patients with and without history of myocardial infarction: Data from HOMAGE and the UK Biobank. European Journal of Heart Failure. 2022 Apr 1.

232. Darabseh MZ, Selfe J, Morse CI, Degens H. Is vaping better than smoking for cardiorespiratory and muscle function?. Multidisciplinary respiratory medicine. 2020 Jan 1;15(1).

233. Hendriks T, van Dijk R, Alsabaan NA, van der Harst P. Active tobacco smoking impairs cardiac systolic function. Scientific Reports. 2020 Apr 20;10(1):1-6.

234. Mohammed SY, Rasool MT. The prognostic value of serum uric acid in ST-segment elevation myocardial infarction. Medical Journal of Babylon. 2021 Jul 1;18(3):230.

235. Aker A, Saliba W, Schnaider S, Eitan A, Jaffe R, Zafrir B. The Impact of Smoking Status 1 Year After ST-Segment Elevation Myocardial Infarction on Cardiovascular Events and Mortality in Patients Aged≤ 60 Years. The American Journal of Cardiology. 2022 May 22.

236. Zhang R, Ji Z, Qu Y, Yang M, Su Y, Zuo W, Zhao Q, Ma G, Li Y. Clinical value of ARG1 in acute myocardial infarction patients: Bioinformatics-based approach. Biomedicine & Pharmacotherapy. 2020 Jan 1;121:109590.

237. Tengbom J, Cederstrom S, Verouhis D, Sorensson P, Bohm F, Saleh N, Jernberg T, Lundman P, Eriksson P, Gabrielsen A, Caidahl K. P6599 Upregulation of protein and gene expression of arginase-1 in

patients with ST elevation myocardial infarction. European Heart Journal. 2019 Oct 1;40(Supplement_1):ehz746-1187.

238. Buğra A, Daş T. The Role of Immunohistochemical Markers in the Diagnosis of Early Myocardial Infarction. Cureus. 2022 Feb 19;14(2).

239. Cziráki A, Lenkey Z, Sulyok E, Szokodi I, Koller A. L-arginine-nitric oxide-asymmetric dimethylarginine pathway and the coronary circulation: translation of basic science results to clinical practice. Frontiers in pharmacology. 2020 Sep 29;11:569914.

240. Awasthi V, Gali H, Hedrick AF, Da H, Eeda V, Jain D. Positron Emission Tomography (PET) with 18F-FGA for Diagnosis of Myocardial Infarction in a Coronary Artery Ligation Model. Molecular imaging. 2022 Feb 9;2022.

241. Nadir TM, Alsamarai AT. Evaluation of correlation between serum and salivary fibronectin in myocardial infarction patients in Samarra city/Iraq. Samarra Journal of Pure and Applied Science. 2021 Sep 24;3(1):46-55.

242. Visonà SD, Benati D, Monti MC, Galiè M, Andrello L, Frontini A, Osculati A. Diagnosis of sudden cardiac death due to early myocardial ischemia: An ultrastructural and immunohistochemical study. European Journal of Histochemistry: EJH. 2018 Apr 4;62(2).

243. Buğra A, Daş T. The Role of Immunohistochemical Markers in the Diagnosis of Early Myocardial Infarction. Cureus. 2022 Feb 19;14(2).

244. Liu Z, Zhang K, Wang Q, Li Y, Zhang L. Serum TGF-β1 in patients with acute myocardial infarction. British Journal of Biomedical Science. 2016 Jul 4;73(2):90-3.

245. Sarhat ER, Mohammed IJ, Mohammed NY, Khairy BS, Hassan GF. Evaluation of salivary oxidative stress marker (lipid peroxidation),

and non-enzymatic antioxidants (vitamin c and vitamin e) in patients with acute myocardial infarction. Tikrit Journal for Dental Sciences. 2019;7(1).

246. Paula da Silva MV, Villar-Delfino PH, Nogueira-Machado JA, Volpe O. IL-6, IL-1β and MDA correlate with Thrombolysis in Myocardial Infarction (TIMI) risk score in patients with Acute Coronary Syndrome. Recent Advances in Inflammation & Allergy Drug Discovery. 2022 Feb 11.

247. Yin Y, Han W, Cao Y. Association between activities of SOD, MDA and Na+-K+-ATPase in peripheral blood of patients with acute myocardial infarction and the complication of varying degrees of arrhythmia. Hellenic Journal of Cardiology. 2019 Nov 1;60(6):366-71.

248. Baruah M. C-reactive protein (crp) and markers of oxidative stress in acute myocardial infarction. InClinical Significance of C-reactive Protein 2020 (pp. 95-115). Springer, Singapore.

249. Sediri Y, Kallel A, Ali SB, Omar S, Mourali MS, Elasmi M, Taieb SH, Sanhaji H, Feki M, Mechmeche R, Jemaa R. Association of rs2781666 G/T polymorphism of arginase I gene with myocardial infarction in Tunisian male population. Clinical biochemistry. 2010 Jan 1;43(1-2):106-9.

250. Tengbom J, Cederström S, Verouhis D, Böhm F, Eriksson P, Folkersen L, Gabrielsen A, Jernberg T, Lundman P, Persson J, Saleh N. Arginase 1 is upregulated at admission in patients with ST-elevation myocardial infarction. Journal of Internal Medicine. 2021 Nov;290(5):1061-70.

251. Shah SF, Iqbal T, Qamar R, Rafiq MA, Hussain S. ARG1 gene polymorphisms and their association in individuals with essential

hypertension: a case–control study. DNA and Cell Biology. 2018 Jul 1;37(7):609-16.

252. Zhang X, Hu C, Wu HM, Ma ZG, Tang QZ. Fibronectin type III domain-containing 5 in cardiovascular and metabolic diseases: a promising biomarker and therapeutic target. Acta Pharmacologica Sinica. 2021 Sep;42(9):1390-400.

Appendix

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:

الخلاصة

الإصابة القلبية الحادة المعروفة شائعًا باسم النوبة القلبية، تُعد واحدة من أخطر أمراض القلب والأوعية الدموية. إنها حالة تهدد الحياة تحدث عندما يحدث انسداد في تدفق الدم إلى عضلة القلب، مما يؤدي إلى تلف الأنسجة القلبية. من الناحية الطبية، تتميز حالة الإصابة القلبية الحادة بموت الخلايا القلبية نتيجة نقص التروية الدموية والتي تحدث نتيجة لعدم توازن بين الإمداد والطلب على الأوكسجين، إما بسبب انخفاض إمداد الأوكسجين أو طلب زائد. في بعض الحالات، يمكن أن يحدث تلف في عضلة القلب حتى في حالة عدم تكوين جلطة دموية، مما يسبب تلفًا دائمً

احتشاء العضلة القلبية الحاد أحد أكثر أمراض القلب والأوعية الدموية تهديدًا، وتسمى أيضًا النوبة القلبية، وهي حالة تهدد الحياة تحدث عندما يكون هناك انسداد في الدم الذي يتدفق إلى عضلة القلب، وبالتالي يؤدي الى تلف الأنسجة، ويحدث نتيجة موت خلايا عضلة القلب بسبب نقص التروية في بعض الأحيان، قد يحدث نتيجة عدم التوازن في نسبة الأكسجين (قلة التجهيز او زيادة الاحتياج) أو بسبب انخفاض تدفق الدم الى خلايا عضلة القلب مما يسبب تلف لعضلة القلب بدون تكوين جلطة دموية والذي يسبب ضررًا دائمًا.

أجريت هذه الدراسة في مدينة الحلة من الأول من شباط ٢٠٢٢ حتى نيسان ٢٠٢٣ وتم جمع العينات من مدينة المرجان الطبية في مدينة الحلة بمحافظة بابل حيث تم تشخيص جميع مرضى هذه المجموعات من قبل أطباء القلب.

في هذه الدراسة، تم أخذ ٩٠ شخصًا كمجموعة سيطرة و٩٠ مريضًا تم أخذهم كمجموعة المرضى تم تقسيمهم الى مجموعتين مجموعة تتضمن ٤٥ مريض مصاب باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي و٤٥ مريض مصاب باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي.

تم استخدام الأمصال التي تم الحصول عليها من دم الأشخاص الخاضعين للدراسة لقياس نشاط الأرجيناز بطريقة القياس اللوني وقياس تراكيز كل من المالونالديهايد والفيبرونكتين باستخدام تقنية الاليزا، بينما تم استخدام عينات الدم الكامل من الأشخاص الخاضعين للدراسة لاستخراج الحمض النووي من أجل دراسة وراثية لتعدد الأشكال الجينية لانزيم الارجنيز.

في هذه الدراسة لوحظ زيادة في نشاط الأرجيناز بشكل كبير ($p< ٠,٠٠١$) في مجموعتي المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي والمرضى المصابين باحتشاء العضلة

القلبية غير المصاحب لارتفاع قطعت أس- تي بالمقارنة مع مجموعة السيطرة، كما لوحظ وجود زيادة معنوية لفعالية انزيم الارجنيز (p= ٠,٠٠١) في مجموعة المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي عند المقارنة بالمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي.

ولوحظ زيادة في تركيز الفيبرونكتين بشكل كبير (p< ٠,٠٠١) في مجموعتي المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي والمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي بالمقارنة مع مجموعة السيطرة، كما لوحظ وجود زيادة معنوية لفعالية انزيم الارجنيز (p<٠,٠٠١) في مجموعة المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي عند المقارنة بالمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي.

ولوحظ زيادة في تركيز المالونالديهايد بشكل كبير (p< ٠,٠٠١) في مجموعتي المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي والمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي بالمقارنة مع مجموعة السيطرة، كما لوحظ عدم وجود علاقة معنوية لتركيز المالونالديهايد عند المقارنة بين مجموعة المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي والمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي.

في هذه الدراسة ، كان هناك ارتباط ذو دلالة إحصائية (p< ٠,٠٠١) بين تعدد أشكال النوكليوتيد المفردة في جين الارجنيز-١ (rs-٢٧٨١٦٦) في مجموعتي المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي والمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي بالمقارنة مع مجموعة السيطرة ، علاوة على ذلك أظهرت مجموعتي المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي والمرضى المصابين باحتشاء العضلة القلبية غير المصاحب لارتفاع قطعت أس- تي ظهور اعلى للأليل ـ T مقارنة بمجموعة السيطرة.

في مجوعة المرضى المصابين باحتشاء العضلة القلبية المصاحب بارتفاع قطعت أس- تي كان هناك ارتباط إيجابي قوي بين المالونالديهايد ونشاط أرجيناز (R=٠,٧٧٤)، وكان هناك ارتباط إيجابي ضعيف بين المالونالديهايد والفيبرونيكتين (R=٠,٢٢٥) بينما في مجوعة المرضى المصابين باحتشاء

العضلة القلبية غير المصاحب بارتفاع قطعت أس- تي كان هناك ارتباط إيجابي قوي بين المالونالديهايد ونشاط أرجيناز (R =٠,٧٤١) وكان هناك ارتباط إيجابي ضعيف بين المالونالديهايد والفيبرونيكتين (R =٠,٢٥٨).

استنتجت هذه الدراسة بعض العوامل الخطورة لمرض احتشاء العضلة القلبية الحاد حيث أن الذكور أكثر عرضة للإصابة، وكذلك الشخص الذي لديه مؤشر كتلة جسم مرتفع والمدخنين هم أكثر عرضة لمرض احتشاء العضلة القلبية الحاد.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل /كلية الطب
فرع الكيمياء والكيمياء الحياتية

ارتباط تعدد الانماط الجينية للارجنيز-١ ومستويات الفايبرونكتين مع تأكسد الدهون لدى مرضى احتشاء العضلة القلبية الحاد في محافظة بابل

رسالة

مقدمة الى مجلس كلية الطب في جامعة بابل

كجزء من متطلبات نيل شهادة دكتوراه الفلسفة في العلوم / الكيمياء الحياتية السريرية

من قبل

ناصـــر زيـــاد ناصـــر حسيـــن

بكالوريوس تقنيات التحليلات المرضية/ كلية التقنيات الصحية والطبية – بغداد

(٢٠١٠)

ماجستير كيمياء حياتية سريرية /جامعة بابل / كلية الطب

(٢٠١٨)

اشـــــــــــراف

الأســـــــتاذ الدكتور
مؤيـــــد عمران الغزالـــي

الأســـــتاذ الدكتور
حســـن سالم الجميلي

٢٠٢٣ م

١٤٤٥ هـ