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Arginase-I Polymorphisms and Activity in Patients with Myocardial Infarction in Babylon Province

A Thesis

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ

أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ

خَبِيرٌ﴾

صدق الله العلي العظيم

سورة المجادلة: (الآية 11)

Supervisor Certification

We certify that this thesis was prepared under our supervision at the College of Medicine, University of Babylon, as partial fulfillment of the requirement for the master degree of science (M.Sc.) in Clinical Biochemistry.

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In review of the available recommendation, I forward this thesis for debate by the examining committee.

Prof. Dr.

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In the Name of Allah Most Gracious Most Merciful

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I would like to thank the all patients for their cooperation, participate in the study.

Ali

Dedication

To my lovely father and mother

To my dear wife

To my children Malak, Mustafa and Tim

To all members of my family

To all friends and lovers

Ali

Summary

Myocardial infarction is a damage of myocardial cells secondary to interruption of coronary blood supply due to the formation of occlusive thrombus the site of the rupture or erosion of an atheromatous plaque in coronary artery causing heart cells to die. Myocardial ischemia occurs when the oxygen supply to the heart is not sufficient to meet metabolic needs. The most common underlying cause of myocardial ischemia is obstruction of the coronary arteries by atherosclerosis.

Increased arginase-I activity leads to reduced nitric oxide (NO) production and increased formation of reactive oxygen species due to uncoupling of the NO-producing enzyme endothelial NO synthase (eNOS), This contributes to endothelial dysfunction, plaque instability and increased susceptibility to myocardial infarction.

The aim of the present study was to investigate the potential association between the arginase-I activity and rs2781666 G/T polymorphism of arginase-I gene and myocardial infarction in the Babylon province /Iraq.

This study was designed as a case-control study and was constructed to study the role of arginase-I polymorphism and activity in patients with myocardial infarction. To achieve this aim, 45 patients with myocardial infarction ageing between (42-73years), 34 of them male and 11 of them female, and with 45 apparently healthy as control group, 33 of them male and 12 of them female.

All patients samples were collected from Shaheed Al-Mehrab Center, in Hilla city of Babylon province from the November 2021 to June 2022, while The control subject samples were collected from out of the hospitals and this

study was performed at the laboratory of Department of Biochemistry in College of Medicine / University of Babylon.

The sera obtained from the blood of all groups were used to measure the arginase-I activity and lipid profile, while whole blood samples from study subjects were used to extract DNA for the study of polymorphisms in the arginase-I gene.

Serum of arginase-I activity was measured by Enzyme linked immune sorbent assay (ELISA) (Bioassay china) and lipid profile was measured by spectrophotometeric method, whereas polymerase chain reaction-sequencing protocol was used to detect arginase-I present genotype and null genotype simultaneously.

The results showed that there was a significant increase in arginase-I activity between patients and control group ($p = 0.001$); the human arginase-I activity being higher in patients group with mean ± standard deviation (33.5 ± 11.08) as compared to the control group with mean ± standard deviation (24.9±6.56).

There were significant increases ($P<0.05$) in total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in patients group with mean ± standard deviation (205.1 ± 53.64, 226.1 ± 68.03, 131.9 ± 30.22 and 45.2± 13.62) respectively as compared control group with mean ± standard deviation (181.5 ± 52.46, 184.6 ± 49.26, 92.3 ± 31.23 and 36.9 ± 9.85) respectively, while serum high density lipoprotein (HDL) concentration was found to significantly decrease ($P = 0.001$) in patients group with mean ± standard deviation (28.4 ± 9.82) as compared control group with mean ± standard deviation (52.3 ± 12.48).

Regarding the genetic analysis, there was significant difference in genotype frequency between patients and control groups in modes, codominance, dominance ($p < 0.05$). and no significant difference in mode recessive ($p= 0.25$). In addition, there was significant difference in alleles frequency between patients and control group ($p = 0.006$).

Patients group showed a varying activity of arginase-I in rs2781666G/T. Subjects with the arginase-I variant genotype at rs2781666 showed higher activity of arginase-I than the cases with GG genotype. Carriers of the TT genotype showed maximal with mean ± standard deviation (49.4 ± 7.14), carriers of GT with mean ±standard deviation (30.7 ± 5.74) and GG with mean ±standard deviation (27.5 ± 5.33) genotype, respectively. There was significant difference in arginase-I activity among the carriers of different genotype of rs2781666G/T ($P< 0.001$) for GG vs GT; GG vs TT, and GT vs TT.

In conclusion, The high of arginase-I activity in the patients compared to control may give an impression it may play a role in the pathogenesis of myocardial infarction, this supported by the a significant association between rs2781666G/T polymorphism of arginase-I gene and myocardial infarction.

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List of Abbreviations

Abbreviation	Details
ARGI	Arginase-I
ATP	Adenosine triphosphate
ACS	Acute coronary syndrome
AUC	area under curve
BMI	Body Mass Index
bp	base pair
CK	Creatine Kinase
CHD	Coronary heart disease
CVD	Cardiovascular diseases
CI	Confidence interval
dNTPs	Deoxynucleoside triphosphates
DNA	Deoxyribonucleic acid
DKA	Diabetic ketoacidosis
EDTA	Ethylene diamine tetracetic acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
ECG	Electrocardiograph
eNOS	Endothelial Nitric oxide synthase
HDL-C	High Density Lipoprotein Cholesterol
HF	Heart failure
IHD	Ischemic heart disease
IDL	Intermediate Density Lipoprotein
LDL-C	Low Density Lipoprotein Cholesterol
LDH	lactate dehydrogenase
LPL	lipoprotein lipase
LCAT	Lecithin-cholesterol acyltransferase

MI	Myocardial Infarction
NO	Nitric oxide
NCBI	National Center for Biotechnology Information
NSTEMI	Non ST-segment elevation myocardial infarction
O-LDL	Oxidized Low Density Lipoprotein
OR	Odds ratio
PCR	Polymerase Chain Reaction
ROC	Receiver operating characteristic
rs	Reference SNP ID
SPSS	Software package for social science
SNP	Single nucleotide polymorphism
SD	Standard deviation
STEMI	ST-segment elevation myocardial infarction
TC	Total Cholesterol
TG	Triglyceride
TBE	Tris borate EDTA
Taq polymerase	Thermus aquaticus polymerase
UV	Ultra violet
VLDL-C	Very Low Density Lipoprotein Cholesterol
V	Voltage
WHO	World Health Organization

Chapter One Introduction and Literature Review

1. Introduction

1.1 Myocardial Infarction

1.1.1 Definition

Myocardial infarction (MI) is the most common vascular disease and the main cause of death among all cardiovascular diseases (CVD) in the world [1].

Myocardial infarction is one of the most frequently encountered reasons for hospital admission and is commonly seen in all populations world [2], MI means interruption of blood supply to a part of the heart, and it is almost due to the formation of occlusive thrombus at the site of the rupture or erosion of an atheromatous plaque in coronary artery causing heart cell to die and without treatment the infarct related artery remains permanently occluded in 30% of patients [3]. The Figure 1-1 shows the Myocardial infarction.

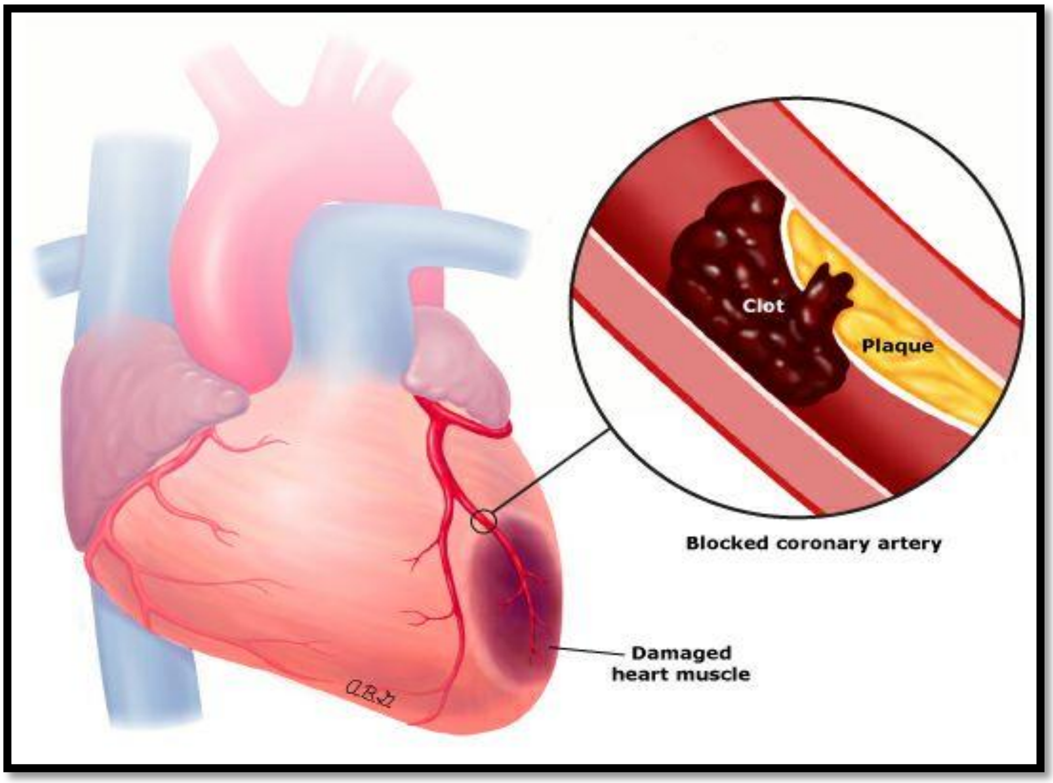


Figure 1-1 Myocardial infarction [4].

Myocardial infarction also defines a necrosis of myocardial secondary to interruption of coronary blood supply caused by ischemia. As a consequence of ischemia, necrosis occurs first in the sub-endocardial Myocardium, that begin early in 15-20 minutes after coronary artery occlusion [5].

Deprivation of oxygen and nutrients in cardiac myocytes lead to quick depletion of ATP in cells, resulting in mitochondrial dysfunction, decreased intracellular pH, and energetic imbalance as the cells shift from aerobic to anaerobic respiration. Compromised myocytes rapidly become necrotic and die, mitochondrial and cytoplasmic proteins are released [6]. the complications Myocardial infarction are heart failure (HF), myocardial rupture or arrhythmias [7].

In adolescence, MI uncommon, especially when there is no family history of early coronary disease, familial hyperlipidemia, diabetes mellitus, or cocaine use [8].

Approximately 90% of all cases of MI are the result of acute thrombus, causing obstruction of an artery vessel in places of atherosclerosis plaque rupture [9].

Acute MI has require a quick intervention since time is an essential factor in successfully preserving the cardiac muscle. Management in the initial (24 hours) include admit to the hospital, oxygen administration, continuous multiplied ST-segment monitoring for arrhythmias and ischemia, observation of vital signs, bed rest and pain relief [10].

1.1.2 Classification of Myocardial Infarction

- **ST segment elevation myocardial infarction (STEMI)**

associated with atherosclerosis involving a major coronary artery**.** Transmural Infarcts extend through the whole thickness of the heart muscle and are usually a result of complete occlusion of coronary artery, where ST - segment elevation and Q waves are seen on electrocardiograph (ECG) [11].

- **Non ST-segment elevation myocardial infarction (NSTEMI)**

involving a small area in the subendocardial wall of the left ventricle, ventricular septum, or papillary muscles. The subendocardial area is particularly susceptible to ischemia, where ST segment depression is seen on ECG [12].

1.1.3 Epidemiology

Myocardial infarction is the most common cause of death in worldwide. MI is a common presentation of coronary artery disease. The World Health Organization estimated in 2008, that 12.2% of worldwide deaths were from ischemic heart disease (IHD) [13]. IHD is becoming a more common cause of death in the world. In Iraq WHO 2016 reports 33% death because of cardiovascular diseases [14].

According to the Third Report by the World Health Organization, 12 million people die annually of CVD worldwide, and it is estimated that by 2025, cardiovascular mortality on worldwide scale will likely surpass that of every major disease group, including infection, cancer and trauma [15].

1.1.4 Pathophysiology of Myocardial Infarction

The most common triggering event is the disruption of an atherosclerotic plaque in coronary artery, which leads to a clotting cascade, sometimes resulting in total occlusion of the artery [16]. Atherosclerosis is the gradual buildup of cholesterol and fibrous tissue in plaques in the wall of arteries (in this case, the coronary arteries), typically over decades [17]. blood stream column irregularities visible on angiography reflect artery lumen narrowing as a result of decades of advancing atherosclerosis [18]. Plaques can become unstable, rupture and additionally promote the formation of a blood clot that occludes the artery;

this can occur in minutes. When a severe enough plaque rupture occurs in the coronary vasculature, it leads to MI (necrosis of downstream myocardium) [19]. If impaired blood flow to the heart lasts long enough, it triggers a process called the ischemic cascade; the heart cells in the territory of the occluded coronary artery die (chiefly through necrosis) and do not grow back. A collagen scar forms in their place. A study indicates that another form of cell death, apoptosis , also plays a role in the process of tissue damage subsequent to MI . As a result, the patient's heart will be permanently damaged [20]. The Figure 1-2 shows Pathophysiology of myocardial infarction.

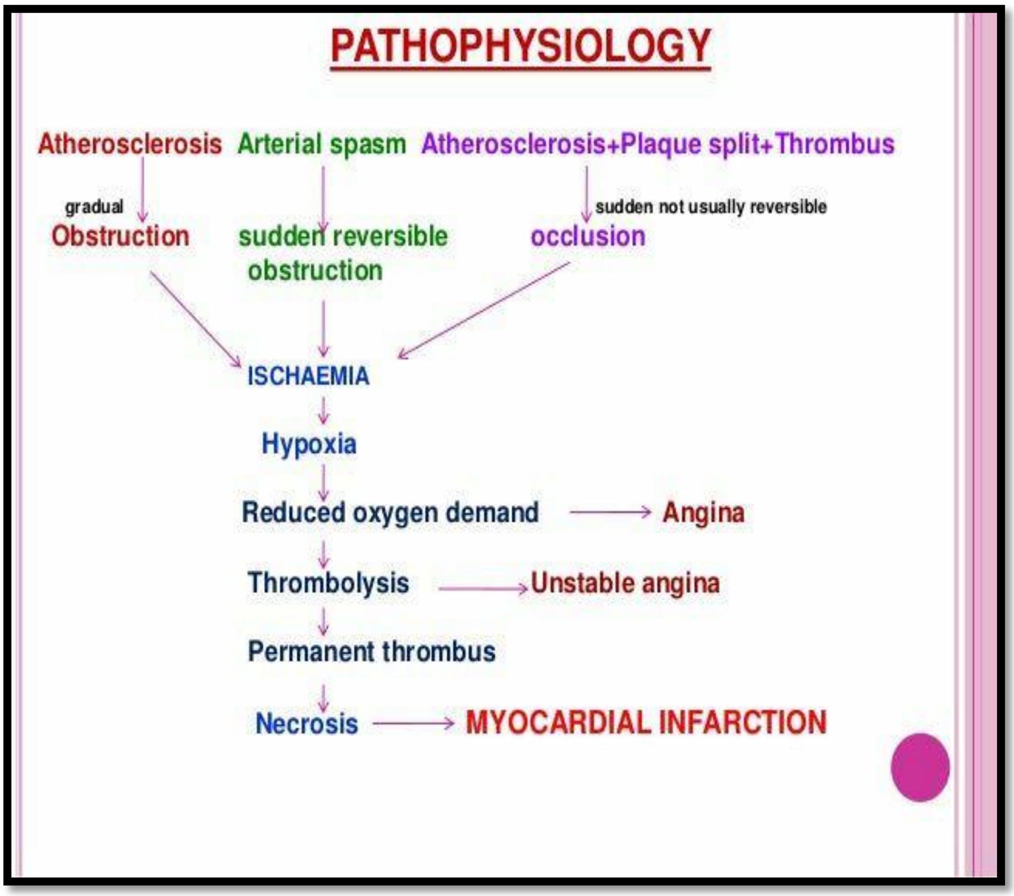


Figure 1-2 Pathophysiology of Myocardial infarction [21].

1.1.5 Diagnosis of Myocardial Infarction

The diagnosis of MI depends on three factors, clinical manifestation, ECG and measurement of cardiac biomarkers.

1.1.5.1 Clinical Manifestation of Myocardial Infarction

The onset of symptoms in MI is usually gradual, over several minutes, and rarely instantaneous [22]. Chest pain is the most common symptom of acute MI and is often described as a sensation of tightness, pressure, or squeezing, (dyspnea) occurs when the damage to the heart limits the output of the left ventricle, causing left ventricular failure and consequent pulmonary edema. Other symptoms include diaphoresis (an excessive form of sweating), weakness, light-headedness, nausea, vomiting, palpitations and Loss of consciousness (due to inadequate blood flow to the brain and cardiogenic shock) and sudden death (frequently due to the development of ventricular fibrillation) can occur in MI [23].

1.1.5.2 Electrocardiogram Changes

The Electrocardiogram (ECG) serves as the first and most immediate available diagnostic tests in determining the likelihood of Myocardial ischemia. The ECG is easily available, non-invasive, not costly. In the early stage of MI the ECG is necessary in the diagnosis of MI [24].

1.1.5.3 Biomarker Evaluation

Myocardial cell death can be recognized by the appearance in the blood of different proteins released into the circulation from the damaged myocytes myoglobin, cardiac troponin T and I, creatine kinase (CK), lactate dehydrogenase (LDH). Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers such as cardiac troponin or CK-MB are increased in the clinical setting of acute myocardial ischemia. elevations in these biomarkers reflect myocardial necrosis [25].

1.1.6 Risk Factors of Myocardial Infarction

1.1.6.1 Age

The most potent independent factor for the development of MI is the age. Older persons have a higher the incidences of MI than do younger ones. Age consider as a risk factors for ischemic heart disease (IHD), nearly 4 to 5 case of deaths recorded due to heart disease occurs among old person within (65) years, old age may affect the hearts function, hearts may not work as well as young. Physiological changes such as the walls of the heart may be thickened and the arteries may be stiffened and become hard, which make the heart lose the ability to pump blood all over the body [26].

1.1.6.2 Gender

The incidence of atherosclerosis and myocardial infarction in men is higher than women in all age groups but this difference narrows with advanced age. This difference is due to lack or a very low amount of estrogen and particularly 17β-estradiol (E2) in males in comparing with females in premenopausal period, this hormone in addition to its role in sexual development and reproduction implicated in a large number of physiological processes, particularly in the cardiovascular system [27], it has vasculoprotective action. 17β-estradiol (E2) has been shown to strongly prevent fatty streak deposition in vascular endothelium [28], increase in basal nitric oxide (NO) production, has a role in acceleration of re-endothelialization with all of the above reasons male gender is more liable for atherosclerotic changes [29].

1.1.6.3 Family History

Family history of CHD increases the risk of atherosclerosis and MI in individuals and the etiology of familial coronary events is multifactorial and included other elements such as genetic component and acquired general health practices as smoking and high fat diet. Several types of inherited hyperlipidemia, inherited vascular pathies, inherited aneurismal disorders and inherited coagulopathies associated with family history of premature CAD [30].

1.1.6.4 Diabetes Mellitus

Diabetes is a metabolic disorder which is characterized by hyperglycemia due to insulin deficiency or insulin resistance. Diabetes mellitus increases the risk of MI and stroke by 2 to 3 times. Obesity, insulin resistance associated with diabetes, high cholesterol and high blood pressure form the most important risk factors for cardiovascular disease. The vascular manifestations associated with diabetes mellitus result from the dysfunction of several vascular physiological components, mainly involving the endothelium, vascular smooth muscle and platelets [31].

Diabetes is associated with an increase in LDL particles, high TAG and low HDL levels. Diabetes mellitus accelerates coronary and peripheral atherosclerosis and is frequently associated with dyslipidemia and increases in risk of angina and sudden coronary death, this is a potent risk factor of atherosclerosis, where Endothelial function is abnormal in patients with diabetes [32].

hyperglycemia accelerate generation of free radical that mediate LDL oxidation that lead atherosclerosis. Oxidized LDL is cytotoxic to endothelium, It impairs endothelium dependent vasodilatation by inactivating nitric oxide (NO) production and causes endothelial disruption [33].

1.1.6.5 Hypertension

Hypertension is the most common risk factor for MI, affecting more than 60 million individuals in the US alone and 1 billion globally. According to the

World Health Organization (WHO), hypertension is now the leading cause of preventable death worldwide Importantly, the prevalence and complications of hypertension increase significantly with age. Hypertension can cause MI or stroke through many mechanisms, A high intraluminal pressure will lead to extensive alteration in endothelium and smooth muscle function in intracerebral and coronary arteries. The increased stress on the endothelium can increase permeability of arteries, This may lead to local thrombi formation and ischemic lesions [34].

High blood pressure increases the pressure in the blood vessels, when the heart act against this resistance the myocardium needs to work harder. with time, the heart muscle become thick, the muscle needs more power to pump blood, elevated blood pressure can cause IHD because heart muscle needs an increased supply of oxygen, high blood pressure can contribute to change of the blood vessel walls, This may increase cholesterol deposits in the blood vessels [35]. High blood pressure increases the occurrence of CVD, also can contribute as a cause of IHD, as elevated blood pressure can be harmful to the heart and lead with time to heart damage and IHD [36].

1.1.6.6 Smoking

Cigarette smoking is the most important modifiable risk factor for CAD. Risk from smoking is dose dependent; depends on the age at which the person started smoking and the number of cigarettes smoked per day. Smoking enhances oxidation of LDL, reduces HDL, increases aggregation and adhesion of platelets. Nicotine of cigarette will cause lipolysis thereby increasing the acetyl - CoA and cholesterol synthesis. Nicotine also causes transient constriction of coronary and carotid arteries [37].

1.1.6.7 Obesity

The body mass index (BMI) of >30 kg/m2 (obesity) and overweight in the general population, are associated with increased risk of developing CVD. As the authors mention, elevated BMI may be associated with a more favorable neuro-hormonal profile, such as reduced levels of B-type natriuretic peptide, which is a strong predictor of mortality in both MI and heart failure [38].

Therefore, this group of patients has higher morbidity and mortality associated with diseases of the cardiovascular system. because weight reduction is associated with an improved cardiovascular risk profile [39].

The effects of obesity on the cardiovascular system are varied and include increased insulin resistance, elevated blood pressure, systemic inflammation and pro coagulant state, dyslipidemia, heart failure, endothelial dysfunction, this lead CAD. More studies found a correlation between BMI and total mortality and sudden cardiac death in patients with MI [40].

The main mechanisms of increased mortality in obese individuals (BMI>40 kg/m2) are increased pressure, and increased activation of sympathetic system. In addition, the pro inflammatory and thrombotic state make up an important mechanism of increased mortality in extremely obese patients. Additionally, the increased cardiac mass predisposes to arrhythmias and sudden death, increasing mortality in these patients [41].

1.1.6.8 Hyperlipidemia

Hyperlipidemia means that high lipid levels in the blood. It acts as a risk factor of obesity associated with a high incidence of coronary heart disease. Elevated cholesterol in the blood over time builds up a fat-like substance in the walls of arteries causing arteries to become narrow and the blood flow to the heart is slowed or blocked [42]. Many factors increase the level of cholesterol in the blood such as, diet, weight, age, gender, sedentary behaviors and heredity. High cholesterol level can be controlled and managed by lifestyle changes, improving physical activities, healthy diet, loss of weight because increasing the level of cholesterol plays a great risk factor to increase the developing of heart disease. hyperlipidemia can be classified either primary or secondary, depending on its cause [43].

1-primary hyperlipidemia is due to genetic defect that decreasing activity of lipid metabolism, like (type I hyperlipoproteinemia, type III hyperlipoproteinemia and type IIa hyperlipoproteinemia.

2-Secondary hyperlipidemia is due to diet (food rich with cholesterol), obesity and diseases (diabetes mellitus, renal diseases, liver diseases, hypothyroidism. Elevated LDL-C is a strong risk factor for Myocardial Infarction, decreased HDL-C levels and elevated triglyceride levels are also associated with the risk of coronary heart disease, results from atherosclerosis. The MI patients seems to be related to endothelial damage, atherosclerosis, insulin resistance [44].

1.1.6.9 Physical Inactivity

Increased physical activities and exercise decrease the risk of cardiac disease. Several studies find out that playing video games, using computer and even watching television for long hours associated with gaining weight this sedentary behavior increase the risk to expose to cardiac disease. Physical activity increases serum HDL-C and decreases serum (TG) [45].

Lack of exercise has been linked to 7-12% of MI. physical activity (exercise) is a primary prevention against 35 chronic conditions (obesity, insulin resistance, type2 diabetes, coronary heart disease, hypertension, endothelial dysfunction, arterial dyslipidemia, and deep vein thrombosis) [46].

1.1.6.10 Excessive Alcohol Intake

Heavy alcohol consumption of more than 14 drinks per week is a risk Factor for development of atherosclerosis and coronary calcification [47]. While mild to moderate amount of alcohol may have a protective effect on cardiovascular risk by increase the production of HDL and NO. alcohol consider one of the risk factors which increase the risk of cardiac diseases. calories which are release from alcohol may produce fat to the body which may elevate the occurrence of cardiac diseases [48].

1.2 Plasma Lipids

Lipids are heterogeneous group of compounds which are water insoluble but soluble in organic solvents such as alcohol. Lipids are presented in the plasma as fatty acids, triglycerides, cholesterol and phospholipids, they can be transported as lipoprotein particles, i.e. associated with proteins. lipids play role as coenzymes, storage form of energy, emulsifying agents in the digestive tract, hormones and intracellular messengers [49].

1.2.1 Cholesterol

Cholesterol is an essential component of mammalian cells and is either obtained from foods of animal origin (e.g., milk, cheese, meat, eggs, etc.) or synthesized in the endoplasmic reticulum. It is required for building and maintaining cell membranes, regulates their fluidity and may act as an antioxidant [50]. It is highly fat soluble but only slightly soluble in water and it is specifically capable of forming esters with fatty acids. Moreover, about 70 per cent of the cholesterol in the lipoproteins of the plasma is in the form of cholesterol esters, It is often found as cholesteryl ester where the hydroxyl group on position 3 is esterified with along chain fatty acid. Figure (1-3) showed the structure of cholesterol [51].

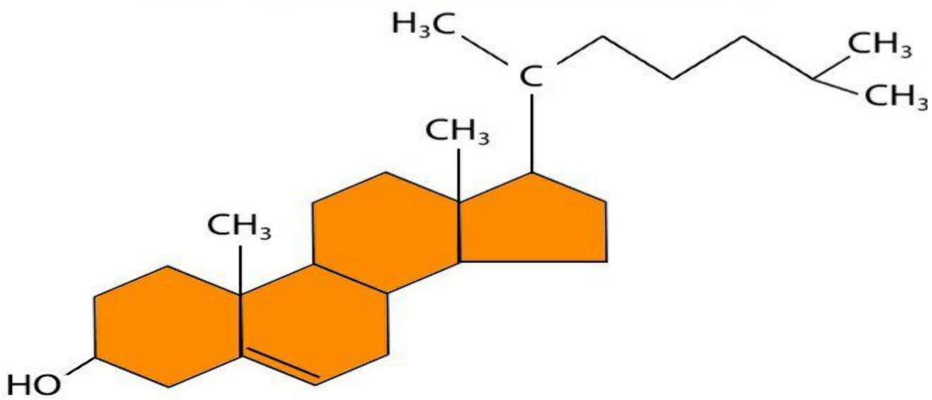


Figure 1-3 Structure of cholesterol [51].

Cholesterol served as a precursor of bile acids, steroid hormones and vitamin D, it is synthesized by all tissues in human including, liver, intestine, adrenal cortex, and reproductive tissues (ovaries, testis, and placenta). It is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. Cholesterol can also serve as a component of plasma lipoproteins sent to peripheral tissues. this deposition leads to plaque formation, causing the narrowing of blood vessels and increased risk of CAD [52].

1.2.2 Triglyceride (TG)

Triglyceride are fatty acid esters of glycerol, each containing three fatty acids. They are derived from three primary sources: the diet, storage depots in adipocytes and biosynthesis particularly in the liver High levels of triglycerides is linked to heart disease. Triglyceride is the most common type of fat in the body. Many people who have heart disease have high triglyceride levels. A high triglyceride level combined with low HDL cholesterol or high LDL cholesterol seems to speed atherosclerosis, which is the buildup of fatty deposits in artery walls that increase the risk for heart attack and stroke [53].

1.3 Lipoproteins

Lipoproteins are spherical macromolecular complexes of lipids and proteins that carry various lipids in plasma. The lipids are mainly free and esterified cholesterol, triglycerides and phospholipids. Lipoproteins are composed of a neutral lipid core (containing TAG and cholesteryl esters) surrounded by a shell of amphipathic apolipoproteins, phospholipid and non-esterified (free) cholesterol [54], These amphipathic compounds are oriented such that their polar portions are exposed on the surface of the lipoprotein, thereby rendering the particle soluble in aqueous solution. Lipoproteins function both to keep their component lipids soluble as they transport them in the plasma and to provide an efficient mechanism for transporting their lipid contents to (and from) the tissues [55].

The apolipoproteins associated with lipoprotein particles have a number of diverse functions, such as providing recognition sites for cell-surface receptors and serving as activators or coenzymes for enzymes involved in lipoprotein metabolism such as Apo-C-II activates lipoprotein lipase. The apoproteins also increase the solubility of lipids. Some of the apolipoproteins are required as essential structural components of the particles, whereas others are transferred freely between lipoproteins such as Apo-E. Apo B100 is required for secretion of hepatic-derived VLDL, IDL and LDL. Apo B48 is a truncated form of Apo B100 that is required for secretion of chylomicrons from the small intestine [56].

1.3.1 Classification of Lipoproteins

Lipoproteins have been classified according to their density into five classes. Chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) , low density lipoprotein (LDL) and high density lipoprotein (HDL).

1.3.1.1 Chylomicron

Dietary lipids are carried from the intestinal mucosa cells to other tissues by lipoproteins called chylomicron. Chylomicron are large and have the lowest protein to lipid ratio and hence have the lowest density of all the lipoproteins. The principle Apo proteins of nascent chylomicron are Apo B - 48. In circulation, the nascent chylomicrons acquire Apo - CII and Apo - E from plasma HDL. The acquisition of Apo - CII from HDL is essential to activate lipoprotein lipase (LPL) [57]. Chylomicron bind to membrane bound (LPL) located on adipose and muscle tissues where the triacylglycerols are hydrolysed into fatty acids and glycerol. The fatty acids are transported into the adipose cell where they are once again resynthesized into triacylglycerols and stored or used for energy in muscle. The glycerol is taken up by the liver, converted to dihydroxyacetone phosphate (an intermediate of glycolysis), and used in lipid synthesis or gluconeogenesis. As the tissues absorb the fatty acids, the chylomicron progressively shrink until they are reduced down to cholesterol enriched remnant. The remnants are rapidly absorbed by the liver [58].

1.3.1.2 High Density Lipoprotein Cholesterol (HDL-C)

High- density lipoproteins are spherical micelles [59]. Plasma HDL is a small particle and dense, protein-rich, almost 50% total mass of HDL is a lipid component [60]. HDL is synthesized in the liver and it serves as a source of Apo lipoproteins for other lipoproteins , as the site of action for the conversion of cholesterol to cholesterol ester in the plasma by the enzyme lecithincholesterol acyl transferase (LCAT) and delivers cholesterol esters derived from peripheral membranes to the liver. It is commonly called the good cholesterol [61]. HDL play a wide spectrum of biological activities, of which cellular cholesterol efflux activity, anti-inflammatory and anti-oxidative actions. The functionality of HDL is potentially highly vasculoprotective, they maintain endothelial vasoreactivity, attenuate oxidative stress, inhibit endothelial cell apoptosis, contribute to the repair of damaged endothelium , inhibit monocyte activation , and reduce the expression of adhesion molecules and cytokines. All of these actions may potentially attenuate component steps of atherosclerotic plaque formation [62].

High density lipoprotein remove excess cholesterol from tissues and vessel walls and carry it to the liver where it is removed from blood and discarded. So HDL help keep cholesterol from building up in the walls of the arteries. The amount of serum HDL is inversely correlated with the incidence of CVD, the higher level the lower risk of atherosclerosis and vice versa [63].

High-density lipoproteins particles are considered protective against CVD mainly due to a role in reverse cholesterol transport. Reduced levels of HDL are correlated with a high risk of CAD and MI. More than 40% of patients with MI have low HDL [64].

1.3.1.3 Low- Density Lipoprotein Cholesterol (LDL-C)

Low density lipoprotein is synthesized from the degradation of VLDL- by the action of lipoprotein lipase. LDL are ingested by a receptor-mediated endocytosis by both peripheral tissues and the liver, It is commonly called the bad cholesterol. High serum levels of LDL are strongly associated with atherosclerosis [65]. LDL carries cholesterol in the blood and deposits it in the body tissues and in the walls of blood vessels, a condition known as atherosclerosis. LDL carries most of the cholesterol in the blood, and the cholesterol from LDL is the main source of damaging buildup and blockage in the arteries, Thus the more LDL we have in our blood, the greater our risk of heart disease [66].

Low density lipoprotein plays a vital role in endothelial dysfunction, induces the endothelial injury, increases monocyte adhesion, and platelet aggregation and inhibits eNOS expression/activity, all of which contribute to the atherosclerotic process [67].

1.3.1.4 Very Low Density Lipoprotein Cholesterol (VLDL-C)

Very low density lipoprotein are synthesized in the liver. They are composed predominantly of endogenous TAG (~60%), and their function is to carry this lipid from the liver (site of synthesis) to the peripheral tissues. There, the TAG is degraded by LPL [68]. when VLDL reach the peripheral tissues, apo C-II activates LpL which liberates fatty acids that are taken up by adipose tissue and muscle. The VLDL remnant is now designated as IDL (intermediate density lipoprotein) and contains less of TAG and more of cholesterol. The major fraction of IDL further loses triglyceride, so as to be converted to LDL .This conversion of VLDL to IDL and then to LDL is referred to as lipoprotein cascade pathway. A fraction of IDL is taken up by the hepatic receptors. VLDL, act to promote atherosclerosis, similar to LDL [69].

1.3.1.5 Intermediate Density Lipoprotein (IDL)

intermediate density lipoprotein is an intermediate metabolite of VLDL that is normally further degraded to low density lipoprotein (LDL) with the cleavage of triglycerides by lipases. IDL can also be taken up by liver cells through receptor-mediated endocytosis that uses apo E as the ligand [70]. patients with defect in apo E are deficient in the clearance of IDL and chylomicron remnants. These individuals have familial type III hyperlipoproteinemia. IDL and LDL have high affinity for macrophages, which theoretically promote their entry into the vascular wall to participate in the formation of foam cells and atherosclerotic plaques. The plasma levels of Apo B , which is the major Apo lipoprotein of LDL and IDL, are strongly correlated with levels of these lipoproteins [71].

1.4 The Genetic Basis of Myocardial Infarction

The evidence for heritability of MI is striking, with a positive family history being one of the most important risk factors for this complex trait [72].

Tanaka, (2016) has pointed out that more than 80% of CHD may be accountable by lifestyle issues, such as weight, diet, exercise, and control of risk factors such as blood pressure and smoking. Yet, the influence of genetics is also quite important, and recent work in this field has led to determining key genes associated with increased risk of susceptibility for acute MI. coronary artery disease (CAD) and MI are complex diseases that result from a life-long interplay between genetic and environmental factors [73].

Through genome-wide associated studies have identified more than 50 genetic loci to coronary artery diseases, these genetic variants account for only 10% of cases. To date, genetic causes and underlying molecular mechanisms for CAD remain largely unclear. It has been hypothesized that low frequency and rare variants with large effects may account for some of the missing heritability for CAD [74].

1.5 Gene Polymorphisms

The human genome consists of more than 3 billion base pairs which reside in every nucleated cell of the body [75]. The genome, which has remained well conserved through evolution, is at least 99.5% identical between humans. Recent genomic material have revealed that they are more complex, diverse and dynamic than previously concept, although limitation of variation (between 0.1% and 0.4%) of genome [76]. Sequencing changes, even in unencrypted regions of DNA proteins, began to change our conception of the human genome [77], while some studies have linked some variables to their predictability of disease susceptibility and drug response, the most of diseases have a complex genetic signature. The variation in the DNA sequence that occurs in a population with a frequency of 1% or higher is termed a polymorphism [78]. Polymorphism can be also taken with one or more nucleotide changes just like mutations. Single nucleotide polymorphism (SNP) represents the most common form of polymorphism, and is believed to originate every 1000 pairs of bases in the human genome [79]. usually found in the vicinity of protein encoding genes, regions now recognized as critical regulation of gene/protein expression [80].

1.5.1 Detection of Single Nucleotide Polymorphism (SNP)

The polymerase chain reaction (PCR) is an amplification-based technique for DNA detection. The standard protocol involves rising the temperature of the reaction to 95 C to separate the DNA strands, lowering to the annealing temperature which is specific for the oligonucleotide primers to hybridize, and then raising to the optimal DNA polymerase temperature 72C for primer extension. This process is repeated cyclically, creating billions copies of the target sequence [81].

1.6 Arginase-I (ARGI)

Arginase-I (EC 3.5.3.1) or arginine transamidinase is a manganese-containing enzyme. ARGI was discovered in mammalian liver tissue by Kossel and Dakin in 1904, It acts on the final step that leads to the production of urea [82], (As shown in Figure 1-4). Its importance in this cycle has long been recognized. ARGI is thought to have appeared first in bacteria, but it has persisted through evolution and is found in yeasts, plants, invertebrates and vertebrates [83]. The transfer of ARGI from bacteria to eukaryotic cells has been suggested to have occurred via the mitochondria. The majority of animals that metabolize excess nitrogen as urea also express ARGI, and it is localized in the cytosol. In some vertebrates, ARGI is expressed in the liver, red blood cells, cardiomyocyte and specific immune cells [84].

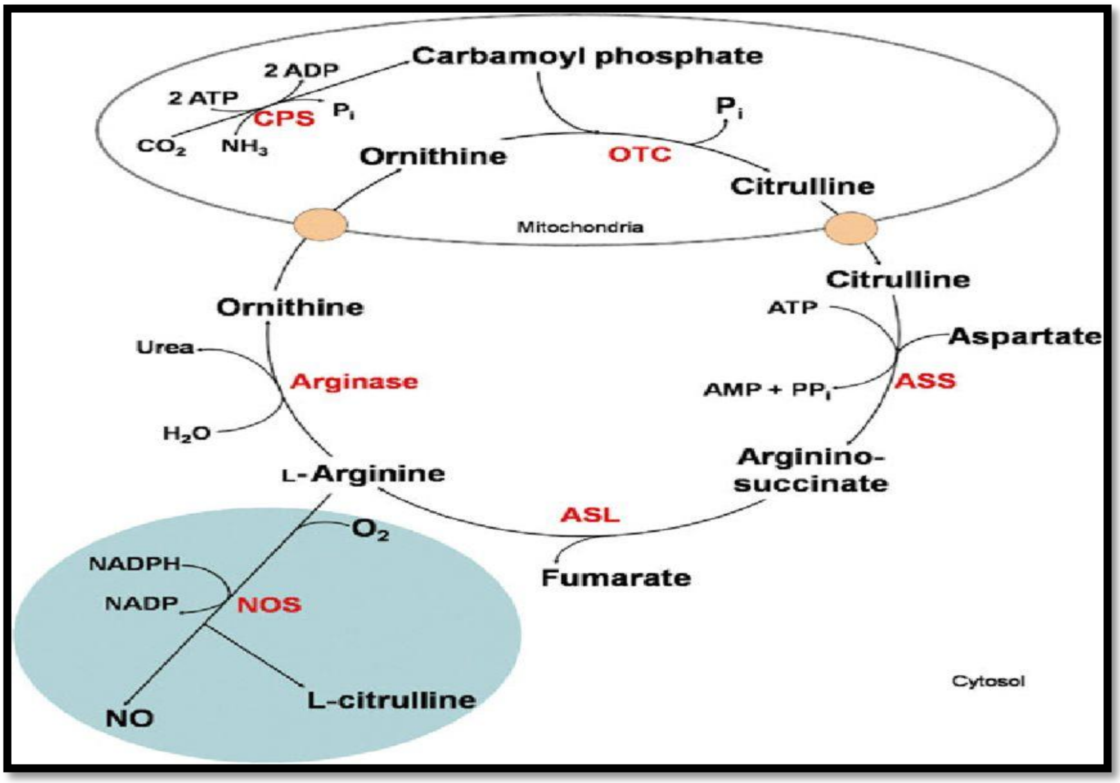


Figure 1-4 urea cycle [82].

The ARGI gene located on chromosome 6q23 consists of eight exons, has a genomic length of 18.1 kb and encodes a protein of 322 amino acids which an estimated molecular mass of a 105 kDa [85]. High-resolution crystallographic analysis has shown that ARG1 consist of three identical subunits, and the active site is located at the bottom of a 15 Å cleft. Binding of manganese ions at the bottom of the cleft is essential for enzyme activity [84], [86], as shown in Figure 1-5.

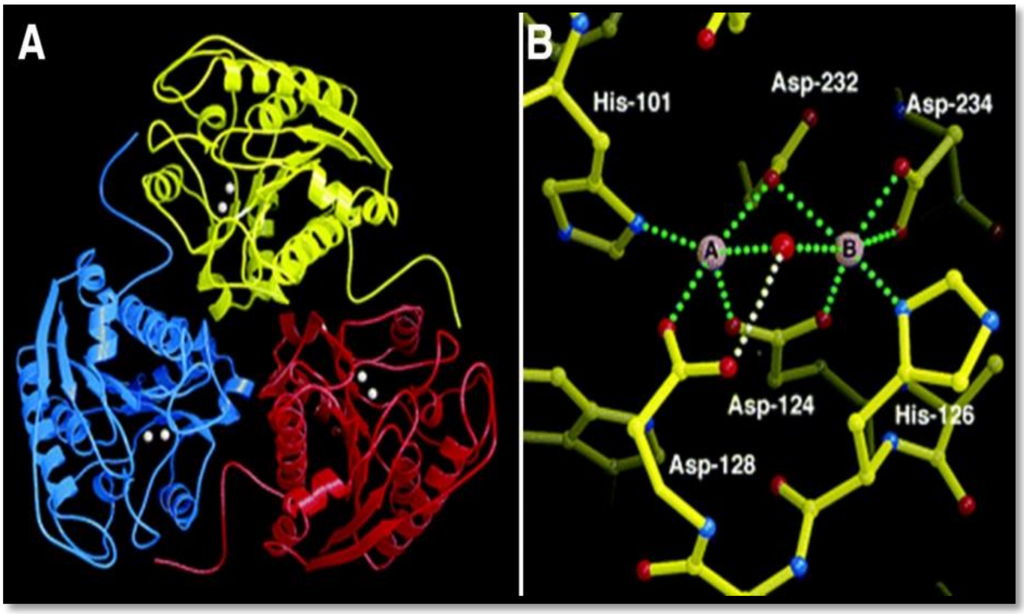


Figure 1-5 shown the three subunit of ARGI [84].

arginase-I is also expressed in tissues originating outside the liver and are involved in regulation of arginine and nitric oxide metabolism. Recently, the enzyme was detected in various blood cell types, including macrophages, cardiomyocyte, endothelial cells, and smooth muscle cells [87].

1.6.1 Arginase-I Function

arginase-I activity has two major homeostatic aims: first, to detoxify of ammonia through urea synthesis, and second, to produce ornithine, the precursor for prolines and polyamines [88]. L-Arginine hydrolysis to form L-Ornithine and urea by the action of ARGI. L-Ornithine will convert to L-proline by ornithine aminotransferase (OAT) and polyamines by the ornithine decarboxylase (ODC) pathway, as shown in Figure 1-6.

In mammals, the arginine-derived ornithine is utilized for the synthesis of glutamine in mitochondria and proline, polyamines in the cytoplasm. They have an essential role in cell proliferation and growth, and they are also needed for inflammation, wound healing, tissue repair, and neuronal development [84] .

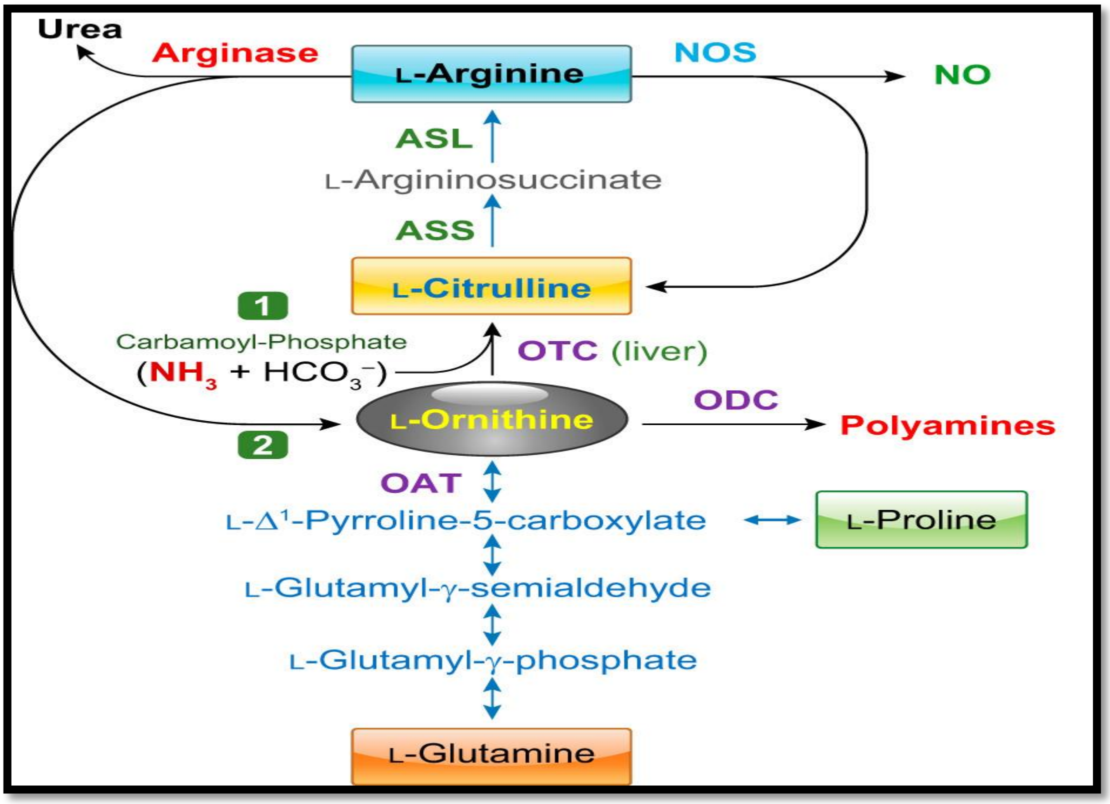


Figure 1-6 shown the production of polyamines and proline from ornithine [84] .

1.6.2 Clinical Significance of Arginase-I

Increased ARGI activity in mammals has been associated with cardiovascular dysfunction. Two relevant aspects of this excessed activity may be engaged in these disease states. First, increased ARGI activity decreases the availability of L-arginine needed by nitric oxide (NO) synthase to produce NO. second ,increased production of ornithine leads to vascular problems [88].

endothelial Nitric Oxide Synthase (eNOS) and ARGI compete for arginine, Therefore relative changes in their enzymatic activities serve as major determinants of nitric oxide and polyamine production in many cell types, including smooth muscle cells, endothelial cells, this leads endothelial dysfunction [89].

arginase-I was originally detected from hepatocytes and was also observed to be expressed in various vascular cell types, including vascular smooth muscle cells and endothelial cells. Reported experimental data showed that ARGI could be a candidate marker that might increase individual susceptibility to cardiac abnormalities [90]. Consequently, ARGI has gained attention as a novel therapeutic target in cardiac patients because it seems to play a key role in progression of atherosclerosis through endothelial cell dysfunction [89].

1.6.3 Role of ARGI in Myocardial Infarction

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in much of the world today. CAD is a complex condition, resulting from genetic and environmental contributions. Many genes are suspected to be involved in MI, However remain to be clarified. Some very recent studies support a role for ARGI in the initiation, development and complications of CAD [91], [92]. Recently, data from the National Center for Biotechnology Information/single-nucleotide polymorphism (SNP), a total of 10 SNPs spaced throughout the ARGI gene were chosen on the basis of their location and/or putative functionality and were studied. One of them rs2781666G/T located within the 5 promoter sequence has been reported to be associated with Myocardial infarction in French population [93]. Ethnic background is known to influence polymorphism frequencies and their effects on the disease, therefore in the present study aims to investigate the possible association between the ARGI rs2781666G/T polymorphism and MI in the Babylon population.

Because genetic contribution to CHD has been estimated to range from 20% to 60% [94]. It is essential to establish the genetic bases of CHD to understand the pathophysiology of this disease. Recent data support a role for ARGI in the initiation, development, and complications of CHD. in addition to hepatocytes, the gene encoding ARGI has been shown to be expressed in various vascular cell types, including endothelial cells, vascular smooth muscle cells. Numerous studies have suggested that ARGI overexpression may be deleterious by contributing to the endothelial dysfunction observed in vascular disorders including atherosclerosis [92]. This effect has been related, at least in part, to the competition between ARGI and the endothelial nitric oxide synthase NOS for their shared substrate L-arginine, where is the NO cause coronary arteries to dilate and prevents platelet aggregation [95].

1.6.4 Arginase-I in endothelial dysfunction

main consequences of over activation of the vascular ARGI pathway is impaired NO production, resulting in endothelial dysfunction. This dysfunction is attributable to endothelial eNOS uncoupling, a situation in which, owing to L-arginine depletion, eNOS generates predominantly superoxide anions rather than NO. A clue for the involvement of ARGI in NO-dependent vascular dysfunction in humans is provided by a dynamic study with radioisotopes in pulmonary arterial hypertension (PAH) [96].

The mechanisms involved in the up-regulation of vascular ARGI include inflammatory factors such as interleukin-4 (IL-4), IL-6, IL-8, IL-10 and tumor necrosis factor-α (TNFα), endothelin-1, oxidative species, oxidized lipoproteins, glucose or hypoxia. These factors activate various intracellular signaling pathways, such as the protein kinase pathways. In addition, genetic mechanisms through histone deacetylation or DNA methylation, as well as post-transcriptional regulation by miRNA, have been recently described in vascular cells [97], [98].

1.6.5 Significance of ARGI in Myocardial Ischemia

Ischemic injury of the myocardium, damage caused by coronary artery occlusion, is a major cause of morbidity and death, especially in patients with diabetes and atherosclerosis. Current strategies to revert myocardial infarction are focused on achieving rapid and effective reperfusion to minimize tissue damage [99]. However, reperfusion may itself induce additional myocardial damage accompanied by induction of proinflammatory factors, and death of cardiomyocytes [100]. Impaired endothelial-dependent vasodilation due to a reduction in bioavailability of NO is a primary mechanism of ischemia- injury. Pervious study have demonstrated the critical role of increased ARGI expression and activity in myocardial injury [101].

Studies have shown that treatment with the ARG1inhibitor before ischemia, during late ischemia, and in early reperfusion restores NO bioavailability and markedly decreases the size of cardiac infarcts. However, it is important to note that the normal function of ARGI in “healing” is important for tissue repair [84]. MI sets off an inflammatory response involving endothelial cell activation and neutrophil adhesion, which can further result in myocardial cell death [102].

Aim of Study

The aim of the present study was to investigate the potential association between the arginase-I activity and rs2781666 G/T polymorphism of arginase-I gene and myocardial infarction in the Babylon Province/ Iraq.

Chapter Two
Materials and Methods

2. Materials and Method

2.1 Chemicals

All chemicals and standard kits used in this study were shown in Table 2-1.

Table 2-1 Chemical substances and kits used in this study

No.	Chemical substance	Origin
1	Agarose	Himedia (india)
2	PCR Master Mix kit	Promega (USA)
3	Primers	Macrogen (Korea)
4	DNA ladder 100bp	Promega (USA)
5	DNA extraction kit (blood)	Favorgen (Korea)
6	Ethidium Bromide	Biotech (USA)
7	Loading dye (blue)	Promega (USA)
8	HDL-cholesterol kit	Biolabo SA (France)
9	TBE buffer (Tris-Borate-EDTA)(10X)	Promega SA (USA)
10	Total cholesterol kit	Biolabo SA (France)
11	Triglyceride kit	Biolabo SA (France)
12	Human Arginase-I ELISA Kit	Bioassay (china)
13	Absolute Ethanol	J.T.Baker (Netherlands)

2.2 Instruments and equipment

The instruments and equipment used in this study were shown in Table 2-2.

Table 2-2 Instruments and equipment used in this study

No.	Instruments and equipment	Origin
1	Spectrophotometer SP-3000	Optima (Japan)
2	Centrifuge	Hitachi (Germany)
3	Deep Freeze	GFL (Germany)
4	EDTA tube	AFCO (Jordan)
5	Eppendorf tube	China
6	Micropipettes	Slamed (Germany)
7	Gel tube	AFCO (Jordan)
8	PCR Thermocycler	Analytic Jena (Germany)
9	Vortex	Digisystem (Taiwan)
10	Water bath	GFL (Germany)
11	Horizontal Gel electrophoresis	Bioneer (USA)
12	UV transiluminator	Analytic Jena (Germany)
13	ELISA reader and washer	Paramedical (Italy)
14	Plane tube	AFCOVAC (Jordan)
15	Microwave	LG (Korea)
16	Distiller	GFL (Germany)
17	Incubator	Memmert (Germany)
18	Disposable syringe (5 ml)	Jordan
19	Scan drob	Analytic Jena (Germany)
20	Sensitive balance	Sartorius (Germany)

2.3 Subjects

In this case-control study, there are two groups: the first includes patients with MI, and the second includes those who appear to be healthy. The sample size was determined according to the Daniel formula for sample size [103].This formula is:

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

where n= sample size

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

P= Prevalence of MI which is 2.1 % in the Iraq.

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

This research was carried out at the laboratory of the College of Medicine at the University of Babylon. The collecting of samples carried out from November 2021 to June 2022. Questionnaires were created to collect data from the control and patients group.

2.3.1 Ethical Issues

This study was approved by the College of Medicine and the Department of Biochemistry.

The verbal consent and permission were taken from all subjects of (patients and control) groups after they were told about the aim and advantages of this study.

2.3.2 Patients Group

The MI group who subjected to this study were (45) individuals in the age group ranging from (42-73) years. They were obtained at Shaheed Al-Mehrab center, in Hilla City of Iraq. All patients were pre-diagnosed by the doctors by ECG and biochemical markers such as troponin. All patients underwent a questionnaire including the following: name, age, gender, weight, length, medications and residence. The verbal consent and permission were taken from all subjects of (patients and control) groups after they were told about the aim and advantages of this study.

2.3.3 Control Group

Forty five individuals were taken as a control group. They were selected from the population of Babylon Province. The age and sex of this group were matched to age and sex of patients groups, a permission was taken from all subjects of patients and control group after they were told around aim of the study. Each person who contributed to the control group underwent full history and physical examination including: age, gender, smoking, past history of diseases and medications. The subjects included in the control group apparently had not suffered from any diseases, pregnancy, cancer/malignancies or chemotherapy drug**.**

2.3.4 Exclusion Criteria

- Subjects with renal disease
- Diabetic ketoacidosis (DKA)
- Cardiogenic shock
- Liver disease
- Systemic immune disease
- Patients with malignancies or chemotherapy drug
- Drug dependency such as glucocorticoid

2.3.5 Body Mass Index (BMI)

Body Mass Index (BMI) is a ratio of person weight to a height; it commonly used to classify weight as health or unhealthy. BMI calculated by the equation [104]. as follow

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

Table 2-3 the range of BMI in adult is classified according to the following

Weight standard	BMI
Under weight	Below 18.5
Healthy weight	18.5 – 24.9
Overweight	25.0 – 29.9
Obese	30.0 and higher

2.4 Methods

Methods used in the present study were summarized in the following figure 2-1.

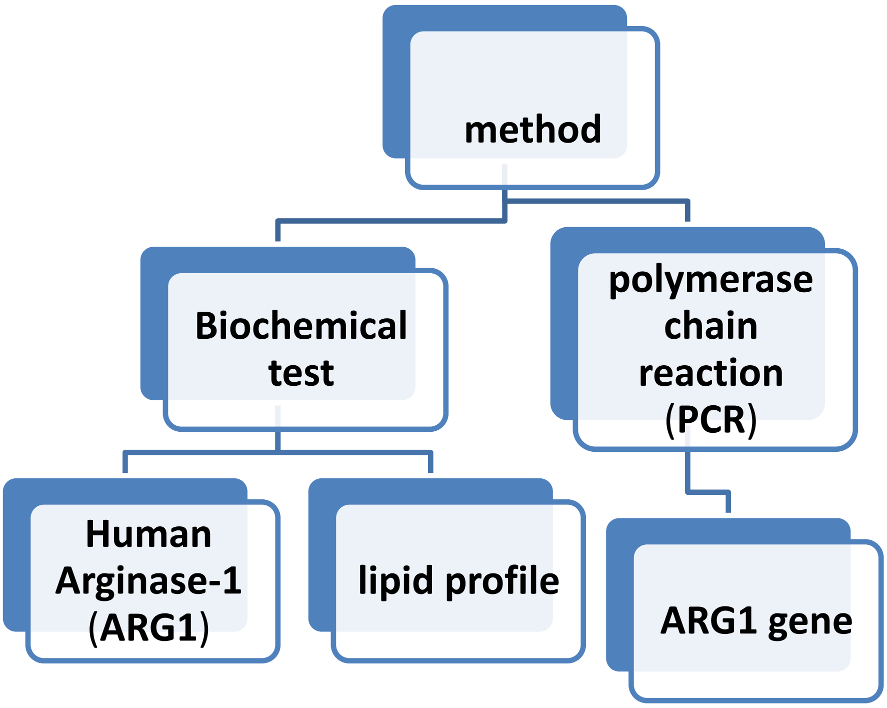


Figure 2-1 Summary of Practical Part of the Study.

2.4.1 Blood Collection

The blood sample was collected from participants vein by needle puncture about 5ml. 2ml of blood was placed in EDTA tubes and the other 3ml in gel tubes. Blood-containing EDTA tubes were stored at temperature (- 40˚C) in (deep freeze) in order to be used later in genetic part of the study, ARGI gene polymorphisms. While blood in the gel containing tubes was allowed to clot at room temperature for 10-15 minutes and then centrifuged at 3500×g for approximately 10-15 minutes, then the serum were obtained and stored at around (-20°C) until analysis begun and to be used for estimation of biochemistry test.

2.4.2 Determination of Human Arginase-I

The Enzyme Linked Immunosorbent Assay (ELISA) technology was used to measurement of ARGI with kit provided by Bioassay-China.

Principle

The sandwich-ELISA technique is used in this ELISA kit. This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human ARGI antibody. ARGI present in the samples were added and binds to antibodies coated on the wells. Then biotinylated Human ARGI antibody was added and binds to ARGI in the samples, then StreptavidinHRP was added and binds to the Biotinylated ARGI antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human ARGI. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm [105].

Reagents Preparation

1- All reagents were placed at room temperature before use.

2- standard: The 120µl of standard (240ng/ml) was reconstituted with 120µl of standard diluent to generate a 120ng/ml standard stock solution. The standard was allowed to site for 15 minutes with gentle agitation prior to making dilutions. The standard points was prepared by serially diluting the standard stock solution (120ng/ml) 1:2 with standard diluent to produce 60ng/ml, 30ng/ml, 15ng/ml and 7.5ng/ml solutions. Standard diluent serves as the zero standard (0ng/ml). Any remaining solution was frozen at (-20°C) and used within one month. Dilution of standard solutions using as described in Table 2-4.

Table 2-4 Standard preparation steps for ARGI

Original Standard	240ng/ml	
Standard No.5	120ng/ml	120µl Original Standard+120µl Standard diluent
Standard No.4	60ng/ml	120µl Standard No.5+120µl Standard diluent
Standard No.3	30ng/ml	120µl Standard No.4+120µl Standard diluent
Standard No.2	15ng/ml	120µl Standard No.3+120µlStandard diluent
Standard No.1	7.5ng/ml	120µl Standard No.2+120µl Standard diluent

3- Wash Buffer: A volume of 20ml of wash buffer concentrate 25X was diluted using deionized water to yield 500ml of 1X wash buffer, and mixed gently until the crystals have completely dissolved.

Reagents

Table 2- 5 shows the kit components for ARGI

Components	Quantity
Standard solution (240ng/mL)	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human ARGI antibody	1ml x1
User instruction	1
Plate sealer	2 pics

Assay procedure

1- All reagents, standard solution and samples were prepared according to the instructions. All reagents was bring at room temperature before use. All assay was done at room temperature.

2- The number of strips required for the assay was determined. The strips was inserted in the frames for use. The unused strips was stored at 2-8°C.

3- A volume of 50μl of each standards were added to standard wells, antibody was not added to a standard well because the standard solution contains Biotinylated antibody.

4- A volume 40μl of sample was added to the well and then added 10μl anti-ARGI antibody to sample well, then added 50μl streptavidin-HRP to sample wells and standard wells. The wells were mixed. The plate was covered with a sealer and incubated for 60 minutes at 37°C.

5- The sealer was removed and washed the plate 5 times with washing buffer. wells were washed after discarded their components with 300μl washing buffer for 30 seconds to 1 minute for each wash.

6- A volume of 50μl of substrate solution A was added to each well and then 50μl of substrate solution B were added to each well. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.

7- A volume of 50μl of stop solution was added to each well, the blue color would immediately change into yellow.

8- The optical density (OD) of each well was determined instantly using a micro plate reader set to 450 nm within 10 minutes after the addition of stop solution.

Calculation of Results

The standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the ARGI level on the horizontal (X) axis and was draw a best fit curve through the points on the graph. This standard curve is depicted in Figure 2-2.

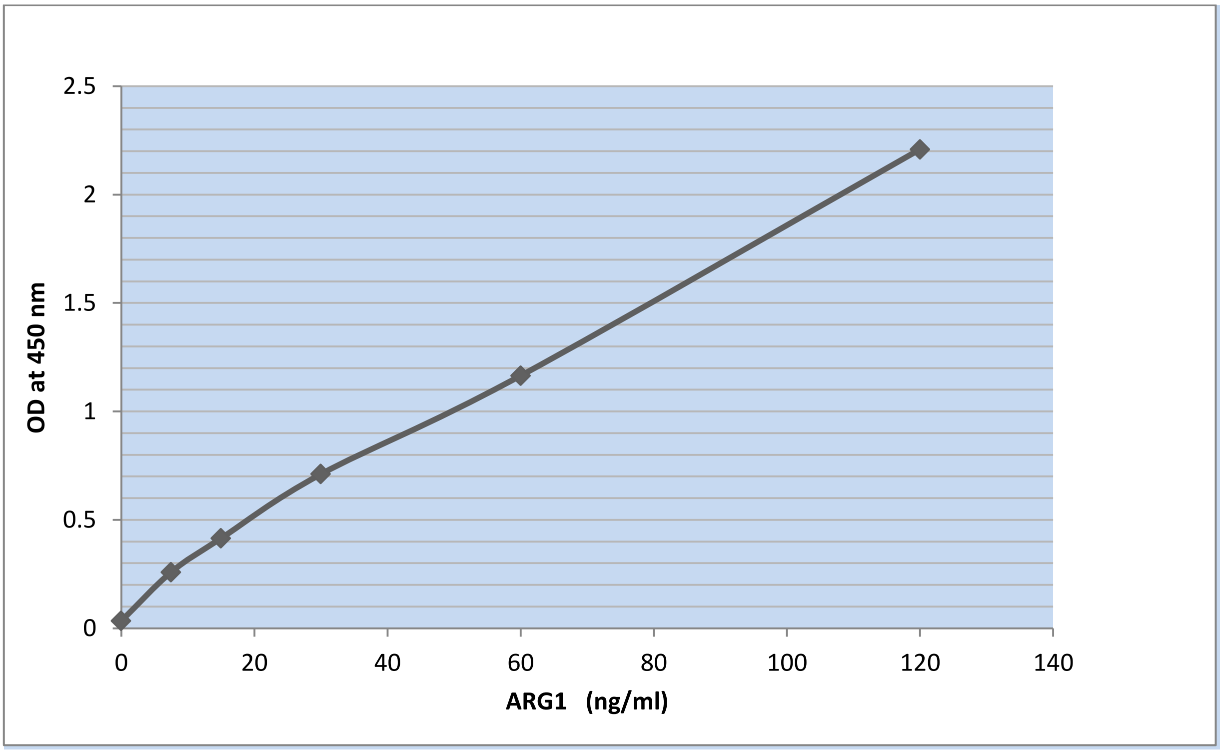


Figure 2-2 standard curve for ARGI

2.4.3 Determination of Total Cholesterol (TC)

Total cholesterol was measured by spectrophotometric method

principle

Cholesterol concentration was determined enzymatically according to the method described by Allain C. *et al*. [106]. as shown in the following reactions

$$\textbf{Cholesterol esters} \xRightarrow{\textbf{cholesterol esterase}} \textbf{Cholesterol} + \textbf{free fatty acids}$$

$$\textbf{Cholesterol} + \mathbf{O_2} \xRightarrow{\textbf{cholesterol oxidase}} \textbf{Cholesten4} - \textbf{3 one} + \mathbf{H_2O_2}$$

$$\mathbf{2\ H_2O_2} + \textbf{Phenol} + \textbf{PAP} \xRightarrow{\textbf{peroxidase}} \textbf{Quinoneimine (pink)} + \mathbf{4\ H_2O}$$

Components of kit

Table 2-6 shows the contents of kit used to determine total cholesterol

Reagents	Composition	Concentration
Reagent 1 (Buffer)	Phosphate buffer Chloro-4-phenol Sodium chloride Triton x 100 Preservative	100 mmol/L 5.0 mmol/L 2.3 mmol/L 1.5 mmol/L
Reagent 2 (Enzymes)	Cholesterol oxidase Cholesterol esterase peroxidase Phenol aminophenazone Polyethylene glycol 6000	100 IU/L 170 IU/L 1200IU/L 0.25mmol/L 167 µmol/L
Reagent 3 (standard)	Cholesterol 200 mg/dL or	5.17 mmol/L

Procedure

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (buffer), gently mixed until complete dissolution (approximately 2 minutes) to prepare working reagent. The procedure was carried out as follows.

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

the tubes were mixed and then let for 5 minutes at 37˚C . Absorbance was Record at 500 nm against blank. The color is stable for 1 hours.

Calculation of Results

$$\text{Cholesterol (mg/dL)} = \frac{\text{Sample absorbance (at 500 nm)}}{\text{Standard absorbance}} \times 200$$

2.4.4 Determination of serum HDL-C

Principle

Chylomicron, LDL and VLDL from were precipitated by phosphotungstic acid and magnesium chloride. HDL-cholesterol obtained in the supernatant after centrifugation is then measured with TC reagent [107].

Table 2-7 shows the contents of kit used in this procedure

Reagents	Composition	Concentration
Reagent 1 (precipitant)	phosphotungstic acid Magnesium chloride pH 6.2	13.9 mmol/L 490 mmol/L
Reagent 2 (Standard)	Cholesterol	100mg/dL (2.58mmol/L)

Procedure

1- A volume of 0.5ml of samples (serum) was added into clean plane tubes, then 50µL of precipitant was also added. The tubes were mixed vigorously, then let stand for 10 minutes at room temperature. tubes were Centrifuged for 15 minutes at 2000×g.Then the next procedure was applied, which include the measurement of cholesterol in the supernatant.

Reagents	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Distilled water	25µl	-	-
Standard	-	25µl	
Supernatant	-	-	25µl

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

Calculation of Results

$$\mathrm{HDL-C\ (mg/dL)} = \frac{\mathrm{Abs\ (Assay)}}{\mathrm{Abs\ (Standard)}} \times \mathrm{Standard\ concentration}$$

(Standard concentration = 100 mg/dl)

2.4.5 Determination of Serum Triglyceride (TG)

Principle

Triglyceride concentration was determined by an enzymatic procedure corresponding to the method expressed by Fossati P. and prencipe method associated with Trinder reaction, as shown in the following reactions.

$$\textbf{Triglycerides} \xRightarrow{\textbf{lipase}} \textbf{Glycerol} + \textbf{free fatty acids}$$

$$\mathbf{Glycerol + ATP \xLeftrightarrow{Glycerol\ kinase} Glycerol\ 3\ phosphate + ADP}$$

$$\mathbf{Glycerol\ 3\ phosphate + O_2}$$

$$\mathbf{\xLeftrightarrow{Glycerol\ 3\ phosphate\ oxidase} Dihydroxyacetonphosphate + H_2O_2}$$

$$\mathbf{H_2O_2 + 4 - Chlorophenol + PAP \xRightarrow{Peroxidase} Quinonimine\ (pink) +}$$

$$\mathbf{4H_2O_2}$$

The absorbance of the colored complex (quinonimine), at 500nm proportional to the amount of triglycerides in the specimen.

Contents of kit

Table 2-8 shows the contents of kit used to determine triglycerides

Reagents	Composition	Concentration
Reagent 1 (Buffer)	PIPES Magnesium chloride Chloro-4-phenol Preservative	100 mmol/L 9.8 mmol/L 3.5 mmol/L
Reagent 2 (Enzymes)	Lipase Peroxidase Glycerol-3-p-oxidase Glycerol kinase Phenol aminophenazone adenosine triphosphate	1000 IU/L 1700 IU/L 3000 IU/L 660 IU/L 0.5 mmol/L 1.3 mmol/L
Reagent 3 (Standard)	Glycerol equivalent to triglycerides	200 mg/Dl

Procedure

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

Reagents	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Deionized water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

The tubes were mixed, then let to stand for 5 minutes at 37˚C.The tubes were mixed, then let to stand for 5 minutes at 37˚C. Absorbance was recorded at 500 nm against the blank. The color is stable for 1 hour.

Calculation of Results

$$Triglyceride\ (mg/dl) = \frac{Abs\ (Assay)}{Abs\ (Standard)} \times Standard\ concentration$$

(Standard concentration = 200 mg/dl)

2.4.6 Determination of Serum VLDL-C

Concentration of VLDL-C was determined by dividing the triglyceride value by 5

$$\mathbf{VLDL - cholesterol\ \ (mg/dl) = \frac{TG}{5}}$$

2.4.7 Determination of Serum LDL-C

Concentration of LDL was calculated by using Friedewald equation [108].

$$\mathbf{LDL-C\ (mg/dl) = Total\ cholesterol\ -\ HDL\ -\ \frac{TG}{5}}$$

2.4.8 DNA Extraction

Principle

The DNA Extraction kit can rapidly and conveniently be extracted of total DNA from a many source (whole blood, frozen blood, Buffy coat). DNA can be extracted from peripheral blood monocytes. Chaotropic salt is acting in lysis of cells and degrade proteins, and permit the binding of the DNA pieces with a glass fiber matrix of the spin column. Wash Buffer who containing ethanol used to remove the Contaminants from DNA, then eluted the purified DNA by a Elution Buffer.

Table 2-9 Components of DNA extraction kit.

Item	Quantity
FATG Buffer	30 ml
FABG Buffer	40 ml
W1 Buffer	45 ml
Wash Buffer* (concentrated)	25 ml
Elution Buffer	30 ml
FABG Column	100 pcs
2 ml Collection Tube	200 pcs

Preparation

1- Proteinase K dissolved in 1.1 ml of deionized water.

2- Before the first used, a volume of 100 ml of absolute ethanol was put in washing buffer.

3- Elution buffer (EL) was preheated in a 70ºC water bath, before starting the extraction process,

Procedure

Step 1-Sample Preparation

1- A volume of 200 µl of blood sample was put into the 1.5 ml microcentrifuge tube.

2- A volume of 30µl of Proteinase K (10 mg/ml) was putted into the tube containing whole blood, and mixed briefly, then incubated for 15 minutes at 60ºC.

Step 2 – Cell Lysis

3- A volume of 200µl of (FABG Buffer) was put in the sample and mixed immediately by vortex mixer.

4- The mixture was incubated in a water bath at 70ºC for 15 minutes to lysate this sample, during incubation, the sample inverted every 3 minutes.

Step 3- Binding

5- A volume of 200µl of ethanol (98%) was putted into the sample and mixed for 10 seconds.

6- A FABG column was placed into 2ml collection tube. The sample mixture was transferred (including any precipitate) carefully to FABG column. Then Centrifuged for 5 minutes at full speed (18.000 ×g) and discarded the 2ml collection tube. The FABG column was Placed in a new 2ml collection tube.

Step 4 – Washing

7- FABG column was washed with 400µl W1 Buffer, then centrifuged for 30 second at full speed (18.000×g) and discarded the flow-through.

8- the FABG column was back placed in the 2ml collection tube. centrifuged for an additional 3 min at full speed (18.000×g) to dry the column.

Step 5 – Elution

9- The dry FABG column was placed in a new 1.5ml micro centrifuge tube.

10- A volume of 100µl of preheated Elution Buffer was added to the membrane center of FABG column. FAGB column was let to stand for 3~5 min or until the buffer is absorbed by the membrane.

11- Centrifuged for 1 minute at full speed (18.000×g)to elute the DNA.

Final Step - Pure DNA

12- The DNA fragment was stored at -20ºC.

2.4.9 Determination of DNA Concentration and Purity

The concentration and purity of extracted DNA were determined by a Nano drop spectrophotometer. DNA concentration measurements were recorded as (ng/µl) at absorption 260 nm. while the purity of DNA solution is indicated by ratio of absorption 260/280nm which is in the range of 1.8 ± 0.2 for pure DNA.

2.4.10 Agarose Gel Electrophoresis

agarose gel electrophoresis is the most effective way and standard method to separate, identify, purify DNA fragments (DNA fragments of varying sizes ranging from 100 bp to 25 kb) and it is simple, rapid to perform. The location of DNA within the gel can be determined directly using staining. Bands containing as little as 1-10 mg of DNA can be detected by direct examination of the gel in ultraviolet light. Electrophoresis is the movement of a charged molecules, chiefly proteins and nucleic acids under the influence of an electric field, Loading dyes used in gel electrophoresis serve three major purposes:

1- They add density to the sample , allowing it to sink into the gel.

2- The dyes provide color and simplify the loading process.

3- The dyes move at standard rates through the gel, allowing for the estimation of the distance that DNA fragments have migrated.

The rate of migration of a DNA molecule through a gel is determined by the following: 1- size of DNA molecule; 2- agarose concentration; 3- DNA conformation; 4- voltage applied; 5- presence or use of staining, 6- type of agarose and 7- electrophoresis buffer [109].

2.4.10.1 Gel Electrophoresis for Analyze DNA Quality

The molecular weight and the integrity of extracted DNA were determined by Agarose gel electrophoresis, the electrophoresis was carried out as describe below in brief [110].

1- The gel (1%) was prepared by dissolving 1gram of agarose in 100 ml of 1X TBE buffer and heated by microwave oven for 2minutes.

2- The homogenized agarose then cooled to 55 C by water bath.

3- A 5µl of Ethidium Bromide stock (10mg/ml) solution was added to the gel and mixed by swirling.

4- The gel then poured to the gel try and let to polymerize for 30minutes .

5- The polymerized gel then transferred to the electrophoresis devise and submerged with 1X TBE running buffer.

6- Five microliters of extracted DNA was mixed with 1µl of bromophenol blue and loaded carefully by mechanical pipet to the gel wells.

7- Five microliters of DNA Ladder was added to wells as a size marker.

8- The electrophoresis was carried out by setting the device on 75 volts for 30 minutes.

Agarose gel photodocumentation

Agarose gel was visualized in a UV transilluminator provided with gel documentation unit , agarose gel was placed above the UV transilluminator device . The gel was exposed to UV light at 260 nm and the photos was captured using a Canon digital camera , as indicated in Figure 2-3.

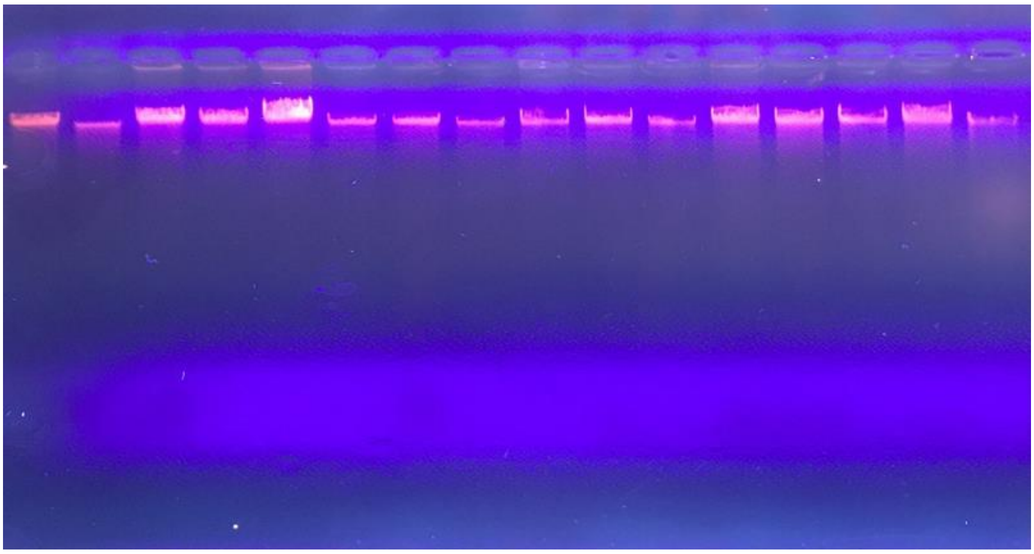


Figure 2-3 Electrophoresis for Genomic DNA

2.4.11 Primers Preparation

Specific primers were used for the PCR protocol. Primer pairs were provided by Macrogen company as a lyophilized product of various concentrations of pico moles. According to Macrogen company, at first primer wall centrifuged at 10000 ×g for 1 min because the material distributed on the walls of tube. Lyophilized primers were dissolved in deionized distilled water to give final concentration of 100pmol/µl as stock solution.

This solution was well mixed by vortex and centrifuged for five seconds at 4000 ×g. Then 10µl of each primer (stock solution) diluted with 90µl of nuclease free DW to prepare working solution with concentration of 10pmol/µl and kept at (– 20 °C) until used. It was recommended that the primers should be distributed into single-use aliquots to limit the freeze-thawing of primers and therefore will extend their life.

2.4.12 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a biochemical technique used to amplify a DNA piece for generating thousands to millions copies of a particular DNA sequence. The components are required for PCR: (DNA, Master mix, nuclease free water (NFW) and forward primer and reverse primer). The components consist of target DNA (DNA template) that includes the region of DNA to be amplified, primers (forward and reverse primer) which are complementary to the DNA template, Thermus aquaticus polymerase (Taq polymerase), deoxynucleoside triphosphates (dNTPs) and buffer solution which provide an appropriate chemical environment for most favorable stability and activity of the DNA polymerase [111].

2.4.12.1 The Polymerase Chain Reaction (PCR) steps

1- Initialization step

The reaction was heated to about 94-96 ° C (this study used 95.0 ° C). It required for DNA polymerases activation.

2- Denaturation step

This step includes heating the reaction to about 94-98 ° C (this study used 95.0 ° C) for melting the DNA template by distraction of hydrogen bonds. The single stranded DNA molecules are yielded in this step.

3- Annealing step

The temperature of reaction is lowered to 50-65 °C (this study used 60.0 ° C). allowing for the primers to annealing with single stranded of DNA template. The Taq polymerase attaches to the primer - template hybrid and begins the formation of DNA.

4- Extension / Elongation step

The optimum activity of Taq polymerase occurs at 72-80 ° C and (this study used 72.0 ° C) is used with this enzyme. DNA polymerase synthesizes a new DNA strand that complementary to the DNA template strand by adding dNTPs in 5 to 3 direction . The extension time depends on both the length of the DNA target and the type of DNA polymerase.

2.4.13 PCR Components of Amplification

The PCR G0 Taq® G2 Green Master - Mix Kit was used for amplification of ARG1gene. The PCR G0 Taq® G2 Green Master - Mix Kit is a premixed ready - to - use solution containing G0 Taq® G2 DNA Polymerase, MgCl2, dNTPs, and reaction buffers at optimal concentrations for efficient amplification of a wide range of DNA templates by PCR. G0 Taq® G2 Green Master Mix contains two dye (blue and yellow) that allow monitoring of progress during electrophoresis. The reactions assembled with G0 Taq® G2 Green Master Mix have sufficient density for direct loading onto agarose gels. G0 Taq® G2 DNA Polymerase exhibits 5 _' 3 ' exonuclease activity. The reaction components for PCR amplification of ARGI gene that give the best results are described in Table 2-10.

Protocol

1- Template DNA and primers (Forward and Reverse primers) were added into PCR G0 Taq® G2 Green Master - Mix in eppendroff tubes.

2- Nuclease free water was added into the tubes to a total volume of 25µL.

Table 2-10 Components of PCR for Amplification of ARGI gene by PCR Master - Mix Kit.

Component	Volume
Genomic template DNA	5 µl
G0 Taq® G2 Green Master mix	12.5 µl
Forward primer* (10pmol)	2µl
Reverse primer* (10pmol)	2µl
Nucleases free water	3.5 µl
Final reaction volume	25µl

2.4.14 Thermocycler Program of Amplification

The PCR Thermocycler program that gave the results of amplification of ARGI gene are shown in Table 2-11.

Table 2-11 Thermocycler program for PCR Amplification of ARGI gene

Name of cycle	Temp °C	Time	No of cycle
Initial denaturation	95	5 min	1 Cycle
Denaturation	95	1min	35 cycle
Annealing	60	1 min	35 Cycle
Extension	72	1 min	35 cycle
Final extension	72	5 min	1 Cycle

The Primer sequence (forward and reverse) as shown in Table 2-12

Table 2-12 shows the Sequences of Primers for PCR.(Tm: Melting Temperature, R: Reverse Primer, F: Forward Primer) [112].

Primer	Primer sequences (5'-3')	Length	Tm °C
F	**5’-CGGAAGGATCTTTAAGGTGCC-3’)**	21	61.3
R	**(5’-CCATGTGTCCGATGCAGTTCTG-3’)**	22	64

2.4.15 Agarose Gel Electrophoresis for PCR products

After execution PCR, 5 µL of each sample were loaded on 1.5 % agarose gel and stained with Ethidiume Bromide. The electrophoresis was performed at (55V for 45 minutes) to evaluate the PCR product [113]. The amplified PCR products were visualized under UV light as shown in figure 2-4.

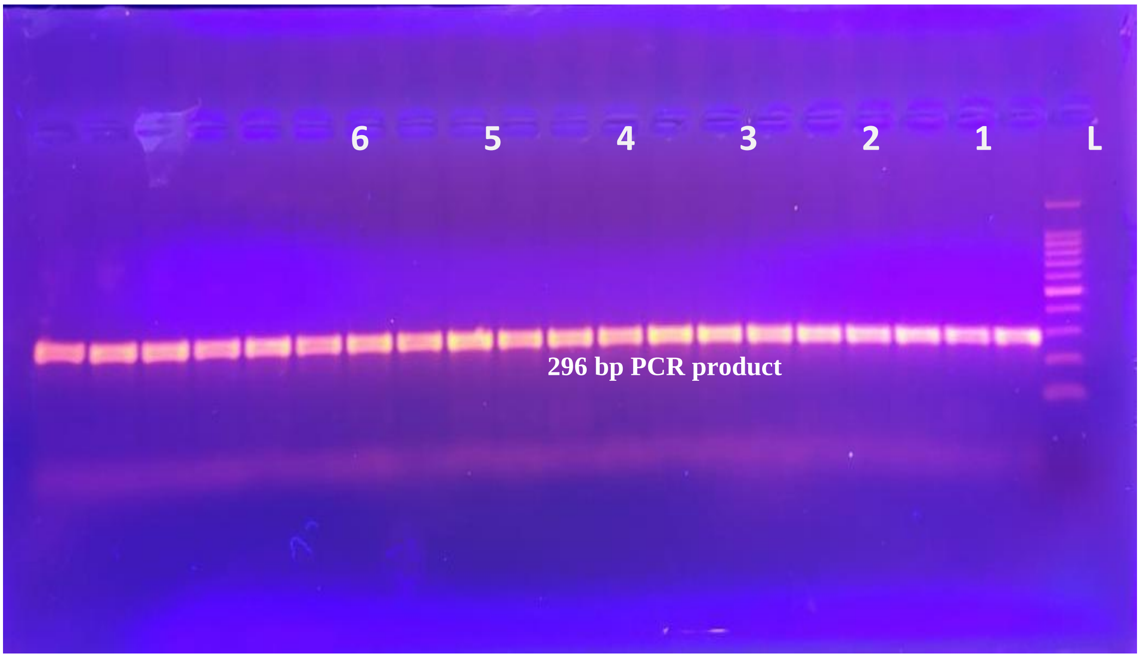


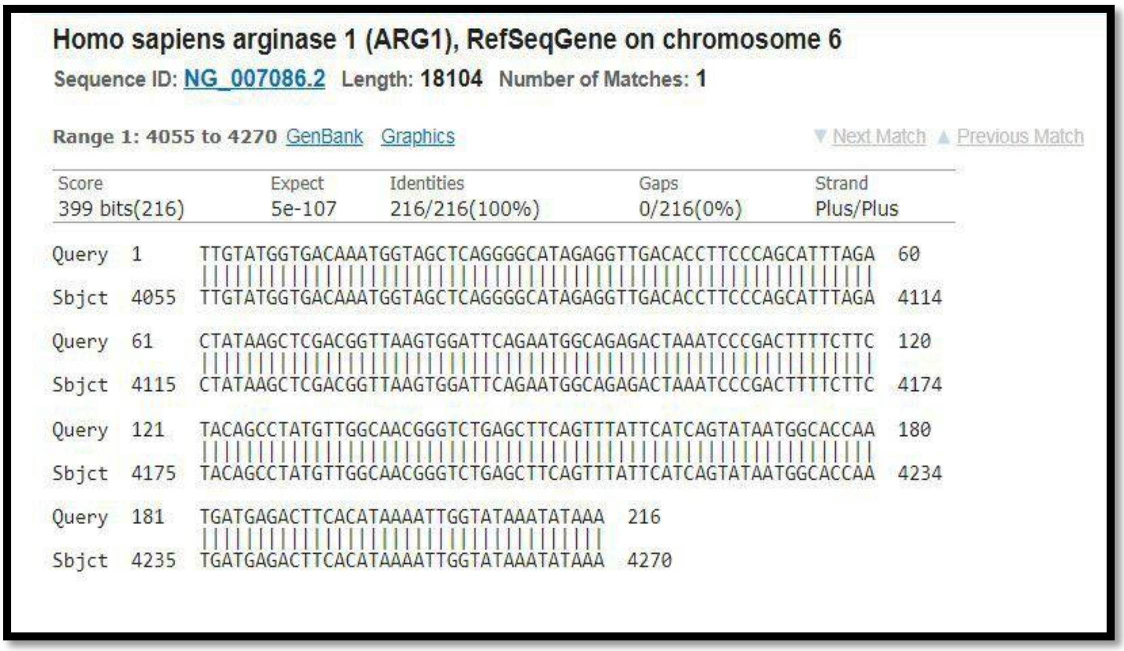
Figure 2-4 Electrophoresis for PCR products

The presence of bands at 296 bps indicate the presence ARGI genotype, whereas the absence of these bands indicates null genotypes for these gene.

2.4.16 DNA Sequencing of ARGI gene Polymorphism

The molecular detection was carried out by using the technology of DNA sequencing, where the sequence of nitrogen bases was determined in ARGI polymorphism region, that amplified by PCR technique. The results were obtained after they were done by Macrogen company / Korea (Macrogen Inc. Geumchen, Seoul, South Korea) and it was reading by using the Snap Gene Viewer 3.3.1 software. The results were compared with data obtained from the

National Center for Biotechnology Information (NCBI). As shown in Figure 2-5.


Homo sapiens arginase 1 (ARG1), RefSeqGene on chromosome 6
Sequence ID: NG_007086.2 Length: 18104 Number of Matches: 1

Range 1: 4055 to 4270 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
399 bits(216)	5e-107	216/216(100%)	0/216(0%)	Plus/Plus

```
Query  1     TTGTATGGTGACAAATGGTAGCTCAGGGGCATAGAGGTTGACACCTTCCCAGCATTTAGA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4055  TTGTATGGTGACAAATGGTAGCTCAGGGGCATAGAGGTTGACACCTTCCCAGCATTTAGA  4114

Query  61    CTATAAGCTCGACGGTTAAGTGGATTCAGAATGGCAGAGACTAAATCCCGACTTTTCTTC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4115  CTATAAGCTCGACGGTTAAGTGGATTCAGAATGGCAGAGACTAAATCCCGACTTTTCTTC  4174

Query  121   TACAGCCTATGTTGGCAACGGGTCTGAGCTTCAGTTTATTCATCAGTATAATGGCACCAA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4175  TACAGCCTATGTTGGCAACGGGTCTGAGCTTCAGTTTATTCATCAGTATAATGGCACCAA  4234

Query  181   TGATGAGACTTCACATAAAATTGGTATAAATATAAA  216
             ||||||||||||||||||||||||||||||||||||
Sbjct  4235  TGATGAGACTTCACATAAAATTGGTATAAATATAAA  4270
```

Figure 2-5 DNA sequencing of ARGI polymorphism gene region.

2.5 Statistical Analysis

Statistical analysis was carried out using SPSS version 26. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. ANOVA test was used to compare means between three groups or more. chi-square test were used to find the association between categorical variables. A p-value of ≤ 0.05 was considered as significant.

Chapter Three

Results and Discussion

3. Results and Discussion

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

3.1 Demographic Characteristics of Patients and Control subjects

3.1.1 Age

The comparison of mean age between patients and control group according to age as shown in Table 3-1, The mean ±SD age of patients group was (60.13 ± 9.42) years and that of control group was (57.82 ± 9.45) years and there was statistically no significant difference in mean age between patients and control group (p = 0.249), this age matching helps to eliminate differences in parameters' results that may originate due to the significant variation in age.

Table 3-1 age distribution in patients and control group.

Variable	Study Group	N	Mean ± SD	P-value
Age (years)	Patients	45	60.13 ± 9.42	0.249
	Control	45	57.82 ± 9.45	

A study done in Turkey, about arginase-I and its association with left ventricular function after myocardial infarction also found no significant difference in mean age between patients and control groups [90].

The results of present study were disagreement with a previous study done by Zhang *et al.*,2020 about MI which found significant difference in mean age between patients and control groups [114].

Regarding patients group, there were 13 (28.8 %) less than 55 years of age, 32 (71.2 %) more than 55 years of age as shown in Figure 3-1.

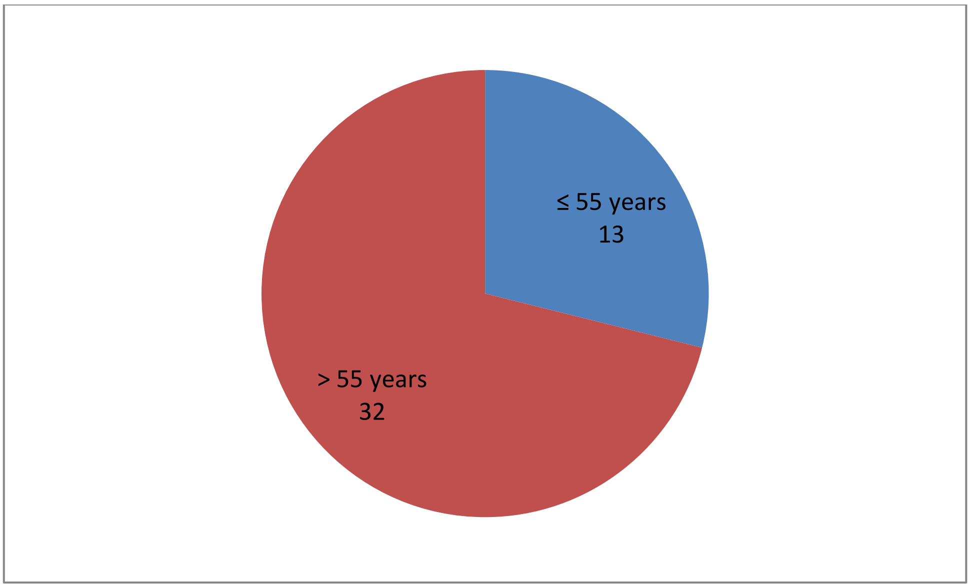


Figure 3-1 frequency of age distribution in patients group.

In the this study, it was found that most patients with MI fall within the age group more than 55 years. cardiovascular disease (CVD) is a prevalent disease in the older population. The incidence of atherosclerotic coronary artery disease (CAD) increases with age and is present in over 50% of people over 60 years old As such, older patients have a higher incidence of MI, which is accompanied by increased morbidity and mortality. old age may affect the heart function, heart may not work as well as young. Physiological changes such as the walls of the heart may be thickened and the arteries may be stiffened and become hard, which make the heart lose the ability to pump blood all over the body [115].

3.1.2 Gender

The number of males in the patients group was 34(75.5%) and females were 11(24.5%), while the number of males in the control group was 33 (73.4%) and the females were 12 (26.6%), as shown in Figure 3-2.

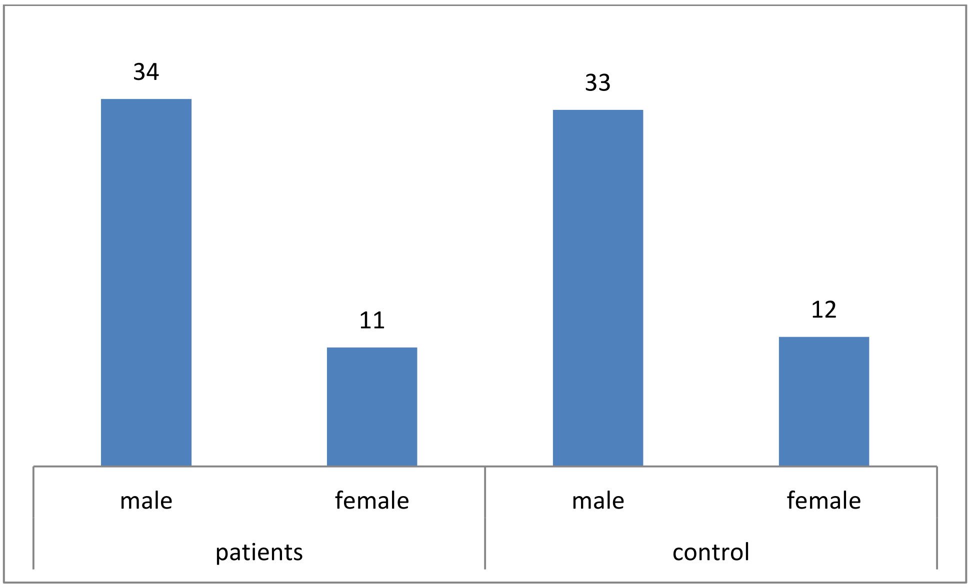


Figure 3-2 frequency of gender distribution in patients and control group.

In present study there was increase in the rate of males compared to females and consistent with some studies that consider males have higher rates of cardiovascular disorders than females. In the general population, men have been found to experience more ischemic MI more than females. The incidence of MI in men is higher than women in all age groups but this difference narrows with advanced age. women suffer less from heart disease due to vasculo-protective action of estrogen which helps in preventing atherosclerosis [116].

Many studies debated about the interaction between gender, risk factors and incidence of myocardial infarction this study result is goes with study in Department of Cardiology, Zhongda Hospital, in China that shown statistically a significant relation with (p-value < 0.05) between male and myocardial infraction [114].

Ischemia heart disease affects men more than women. Natural protection from cardiac disease is provided by the estrogen hormone in women during their reproductive years. normally, following menopause, the protection declines and the risk gradually increases [117].

gender differences in the production and release of NO in female higher than males where Estrogen maintains the bioavailability of NO by increasing the expression of eNOS mRNA and protein, thus increasing the production of NO in endothelial cells and vascular smooth muscle cells [118]. NO plays a direct role in tissue oxygen balance, organ perfusion, prevent platelet aggregation vascular remodeling, and metabolic requirements by regulating vascular tension and diameter [119].

3.1.3 Residency

The frequency of patients and control subjects according to residency is shown in Figure 3-3. Patients group included 28 (62.2 %) subjects from urban areas and 17 (37.8 %) subjects from rural areas whereas, control group included 27 (60 %) subjects from urban areas and 18 (40 %) subjects from rural areas. There was no significant difference in the frequency distribution of patients and control subjects according to residency (p = 0.831).

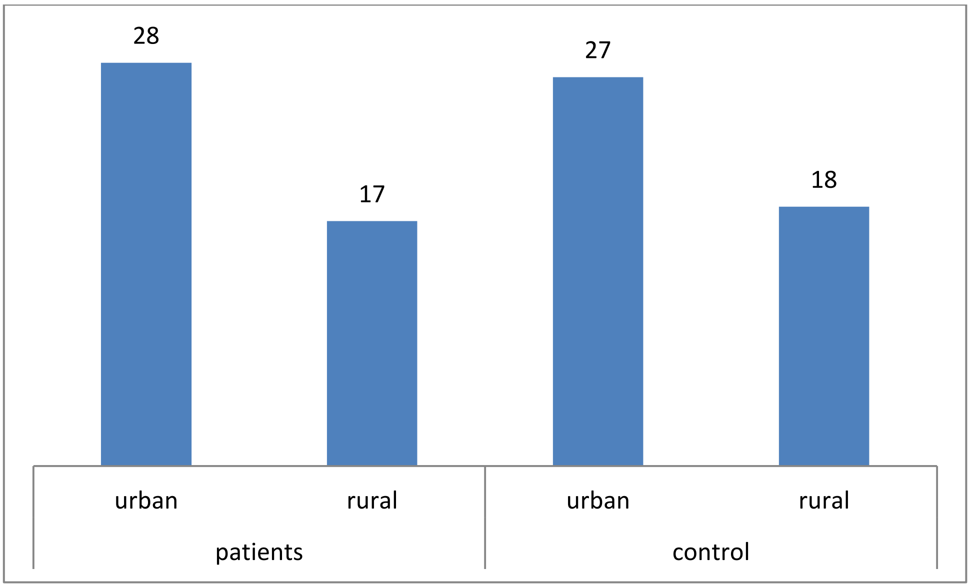


Figure 3-3 the frequency of patients and control group according to residency

In this study was found that most patients with MI are from urban areas, The explanation of this finding may be due in part to sedentary life style of the urban communities relative to rural ones, this agreed with Warren TY *et al.*, 2010, who reported increased MI risk attributed to increased urbanization [120].

This results agreed with other previous study done by Saleem *et al*., 2011, stated that the majority of the study subjects are living in urban residential area and the remaining participant are living in the rural ones. Those persons in urban residential area related to complex life, than in those from rural. Also those persons in rural residential area often experience a physical exercise every day as compared with those in urban that make them less risky to get ischemic heart diseases. Furthermore, the individuals in rural residential areas are less prone to get ischemic heart diseases [121].

3.1.4 Body Mass Index (BMI)

The mean ± SD of BMI for patients‘ group was (30.1 ± 3.74) kg/m^2 and that of control group was 29.4 ± 3.56 kg/m^2 and there was no significant difference between patients and control groups ($p = 0.382$), as shown in Table 3-2.

Table 3-2 the frequency distribution of patients and control group according to BMI.

Subjects	N	Mean ± SD	P value
Patients	45	30.1 ± 3.74	0.382
Control	45	29.4 ± 3.56	

Regarding patients group, there were 6 (13.3%) as normal weight, 22(49%) as overweight and 17 (37.7%) as obese, as shown in Figure 3-4.

In this study was found that most patients with MI are overweight/obese. Yusuf *et al*., 2005 compared normal and overweight in their study and found that the risk of MI in overweight/obese patients increased compared to normal weight patients and there was a strong link between obesity and MI [122].

This results agreed with Foussas S. *et al*., 2016 who reported, obesity is associated with increased mortality, Obese individuals have a higher incidence of cardiovascular risk factors, such as hypertension, dyslipidemia and diabetes mellitus [41].

The effects of obesity on the cardiovascular system are varied and include increased insulin resistance, elevated blood pressure, systemic inflammation and pro coagulant state, dyslipidemia, endothelial dysfunction, this lead CAD. More studies found a correlation between BMI and total mortality and sudden cardiac death in patients with MI [40].

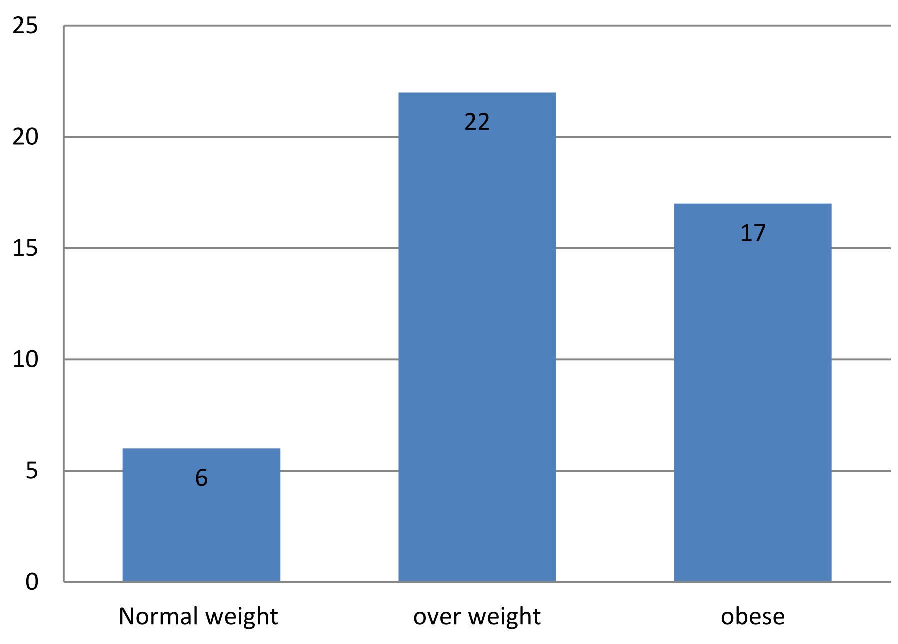


Figure 3- 4 Frequency of BMI distribution in patients group.

3.2 Serum Human Arginase-I in Patients and Control Group

The results of the present study revealed that there was a significant difference P-value (0.001) in human ARGI among MI patient and control group. human ARGI found to be higher in the MI group than the control group, as shown in the Table 3-3.

Table 3-3 Comparison of ARGI in patients and control group.

Parameter	Study group	N	Mean ± SD	P value
ARGI (ng/ml)	Patients	45	33.5 ± 11.08	0.001
	Control	45	24.9 ± 6.56	

The present study results were agreement with a previous study done by Shah *et al*., 2019 which reported that, serum arginase-I showed an increased activity in myocardial infarction when compared with healthy control. Significant association was observed between serum arginase-I and myocardial infarction [112].

Additional study by Tengbom *et al*., 2021 also shown a statistically significant relation and conclude that arginase-I is increased in patients with MI and suggests that arginase-I may be involved in the development of MI [123].

Other research by Wernlya *et al.*, 2020 conclude that increased arginase-I activity is associated with atherosclerosis. ARGI however, is not just an innocent bystander, but it directly contributes to endothelial dysfunction [124].

There is increasing evidence that endothelial nitric oxide synthase (eNOS) is responsible for regulating generation of NO from arginine [125]. However, recent developments revealed that arginase-I has a potential role in limiting arginine for NO production in the cardiovascular system. In fact, early literature showed that arginine metabolism, by activating macrophages, consumed arginine for production of urea rather than NO [125], [126]. In present study observed increased of ARGI in patients as compared control group. In Previous research observed a reduced concentration of NO and increased ARGI in CAD patients, suggesting that constitutive expression of ARGI counteracts NO-mediated dilation of vascular endothelium. Moreover, several mechanisms regulating arginase-I activity appear to be involved in atherosclerosis [127]. Ming *et al.,* 2004 showed that high arginase-I activity in endothelial cells was regulated by Rho/ROCK activation in human vascular endothelium. Immunohistochemical studies showed that oxidized LDL (OxLDL) amplified the expression of arginase-I in the vascular wall without affecting eNOS [127].The attempt to inhibit arginase-I has markedly improved endothelium-dependent vasodilation in arteries of CAD patients, suggesting that high arginase-I is a key factor in development of atherosclerosis [128]. The present study showed that high arginase-I activities were strongly associated with development of CAD among patients. Similarly, ARGI concentrations also significantly increased the risk of CAD among patients. The notion that high arginase-I is the key factor regulating the risk of cardiovascular disease has been supported in MI patients from a Turkish population [90]. Similarly, high ARGI activities were found in hypertensive patients [129]. The effect of ARGI on cardiac function is also supported by the association of ARGI with a high risk of heart failure in MI patients [89]. Therefore, in the present study, it is reasonable to accept that high arginase-I activity has a role in development of CAD.

Arginase-I expression and activity are increase in atherosclerosis and during myocardial ischemia-reperfusion [125]. A recent study in patients with MI demonstrated a shift in plasma amino acid levels, indicative of a switch in arginine metabolism from eNOS to increased arginase-I [130]. Experimental studies have shown that arginase-I is up-regulated in endothelial cells, smooth muscles cells and cardiomyocytes following ischemia-reperfusion [131].

Previous research revealed that ARGI could compete with nitric oxide synthase (eNOS) for the common substrate-L-arginine and inhibited of biosynthesis of nitric oxide (NO), thus induce endothelial dysfunction and promote progress of atherosclerosis [132]. increased arginase-I activity will, via substrate competition, limits the availability of arginine for eNOS, resulting in decreased NO production [125]. The decreased bioavailability of NO results in endothelial dysfunction, plaque instability and increased susceptibility to myocardial ischemia. Nitric oxide (NO) is an important protective factor against atherosclerosis and a regulator of cardiovascular homeostasis by its vasodilatory, anti-inflammatory and anti-thrombogenic effects [133].

ornithine, the primary metabolite of the ARGI pathway, may itself play an important part in atherogenesis. In addition, ornithine is involved in the formation of polyamines that play an important role in the mitogenic response of vascular smooth muscle cells Thus, various metabolites of ornithine play an important role cellular composition of the atherosclerotic plaque [134].

3.3 Lipid Profile

3.3.1 Total Cholesterol (TC)

The results of the present study revealed that there was a statistically significant difference with P-value (0.03) in serum concentration of TC among MI patients and control group, were TC concentration is higher in the MI group than the control group as shown in the Table 3-4.

Table 3-4 Comparison of total cholesterol level in patients and control group

Parameter	Study group	N	Mean ± SD	P value
TC (mg/dl)	Patients	45	205.1 ± 53.64	0.03
	Control	45	181.5 ± 52.46	

The present study results were agreement with a previous study done in Turkey, by Yaoshan *et al.*, 2019 that shown a statistically significant relation of high TC levels and myocardial infraction incidence with p-value (<0.05). They reported that increase TC in patients with MI than control group are linked with development of MI [135].

Additional study by Mohd. *et al., 2019* in Bangladesh concludeds that hypercholesterolemia are the most prevalent dyslipidemia or lipid disorders in patients with myocardial infarction in Banglades [136].

High cholesterol concentration participates to build-up of plaque in the arteries, blood vessels that carry oxygen-rich blood to body organs and various portions of the body. Additionally, a plaque solidifies and narrows the arteries, subsequently forming of atherosclerosis. Atherosclerosis restrictions the movement of oxygen-rich blood and can influence in any body arteries, including arteries in the heart [137].

3.3.2 Triglyceride (TG)

Results of the present study revealed that there was a statistically significant difference with P-value (0.002) in serum concentration of TG among myocardial infarction patient and control group were TG concentration is higher in the MI group than the control group as shown in the Table 3-5.

Table 3-5 Comparison of Triglyceride level in patients and control group.

Parameter	Study group	N	Mean ± SD	P value
TG (mg/dl)	Patients	45	226.1 ± 68.03	0.002
	Control	45	184.6 ± 49.26	

Study in Turkey, done by Yaoshan, *et at.*, 2019 shown a statistically significant relation of high TG levels and myocardial infraction incidence with p-value (<0.05) [135].

Other study carry out by Michael V. Holmes *et al.*, 2018 in China shown a statistically significant relation of high TG levels and myocardial infraction incidence with p-value (<0.05) [54].

Also study by Aaron *et al.*, 2020 concludes that TG strongly associates with future MI events, whereas LDL-C strongly associates with MI alone [138]. Triglycerides are the most common forms of fats in the body and the metabolic disorder related to the abnormal concentration of TGs in the blood is hypertriglyceridemia (HTG) [139].

Numerous biological mechanisms supply a possible description for the link between postprandial TG levels and cardiovascular disease, Following food consumption, TG are transported from the small intestines by chylomicrons within the bloodstream. Lipoprotein lipase (LPL) catalyze of Lipolysis of the TG within chylomicrons in tissues, defect in LPL or in Apo CII lead increase

TG in blood. The patients in the highest TG level is a notably serious risk of cardiac mortality than those in the lowest TG level [140].

3.3.3 High-Density Lipoprotein Cholesterol (HDL-C)

The results of the present study revealed that there was a statistically significant difference with P-value (0.001) in serum concentration of HDL-C among myocardial infarction patients and control group were HDL-C concentration is lower in the MI group than the control group as shown in the Table 3-6.

Table 3-6 Comparison of HDL-C level in patients and control group

Parameter	Study group	N	Mean ± SD	P value
HDL-C (mg/dl)	Patients	45	28.4 ± 9.82	0.001
	Control	45	52.3 ± 12.48	

This results goes with study in Department of Cardiology, Zhongda Hospital in China that shown a statistically significant relation of low HDL-C levels and myocardial infraction incidence with p-value (<0.05) [114].

Also study in Turkey, Istanbul Faculty of Medicine by Yesim Unlucerci *et al.*,2011 shown a statistically significant relation of low HDL levels and myocardial infraction incidence with p-value (<0.05) [90].

Results of present study also agreed with a study by Jin Sup Park *et al.*, 2019 which shown a statistically significant relation of HDL-C levels and myocardial infraction with p-value (<0.05) and conclude that low HDL-C level increases the risk of cardiovascular death and recurrent myocardial infarction, whereas high HDL-C level likely reduces the risk of cardiovascular events [141].

Other study by Byung-Hun *et al*.,2020 concludes that low mean of HDL-C is associated with an increased risk of MI and mortality [142].

This results agreed with other studies Suggest the decrease of HDL-C level in the blood stimulates cardiac disease and that increasing HDL-C plasma level may be beneficial, Several arguments have suggested a protective role of HDL-C against LDL-C oxidative modification [143]. HDL-C characterizes an important cardioprotective factor assumed the role in reverse cholesterol transport, its effects on endothelial cells, and its antioxidant activity [139]. The low HDL-C may be a fairly public baseline in lipid abnormality among the general population that it increases MI. HDL-C also shows potent anti inflammatory and antioxidant properties that inhibit the atherogenic process. Alteration in lipid metabolism including a decrease in HDLC can result in endothelial damage and trigger an increase in blood pressure which may partially account for its strong predictive power for CHD [144], The protectivety of HDL-C against cardiovascular disease predominantly the function in reverse cholesterol transport, more than 40% of MI patients have Low level of HDL-C in blood [145].

3.3.4 Low-Density Lipoprotein Cholesterol (LDL-C)

Results of the present study revealed that there was statistically significant difference with P-value (< 0.01) in serum concentration of LDL-C among myocardial infarction patients and control group were LDL-C concentration is higher in the MI group than the control group, as shown in the Table 3-7.

Table 3-7 Comparison of LDL-C level in patients and control group

Parameter	Study group	N	Mean ± SD	P value
LDL-C (mg/dl)	Patients	45	131.9 ± 30.22	0.01
	Control	45	92.3 ± 31.23	

This goes with a study done by Winter MP *et al*.,2018 shown a statistically significant relation of LDL-C levels and myocardial infraction with p-value (<0.05) and conclude that LDL-C is strongly associated with premature MI [146].

The present study results were also in agreement with a previous study done at Mayo Clinic in Rochester, Minnesota, in 2018 in Turkey, by Yaoshan Dun *et al*.,2019 shown a statistically significant relation of LDL levels and myocardial infraction incidence with p-value (<0.001) [135].

Other study by Michael V. Holmes, Iona Y. Millwood *et al.*,2018 in China shown a statistically significant relation of LDL-C levels and myocardial infraction incidence with p-value (<0.05) [54].

The elevation of plasma LDL cholesterol may does not reflect impaired LDL catabolism but rather implies increased production of LDL [147]. The LDL-C act as a transporter of lipids, cholesterol in the body. The oxidation of LDL-C to become oxidized (ox-LDL) leading to becoming atherogenic in the arterial wall. Ox-LDL, identified as a small dense LDL (sd-LDL), activates the cascade of local inflammation [148]. ox-LDL, alternatively, has been linked with formation of plaque and increased risk of stroke and heart attack when build up in the endothelium [149].

3.3.5 Very Low-Density Lipoprotein Cholesterol (VLDL-C)

Results of the present study revealed that there was a statically significant difference with P-value (0.002) in serum concentration of VLDL among Myocardial Infarction patient and control group were VLDL concentration is higher in the MI group than the control group as shown in the Table 3-8.

Table 3-8 Comparison of VLDL-C level in patients and control group.

Parameter	Study group	N	Mean ± SD	P value
VLDL-C (mg/dl)	Patients	45	45.2± 13.62	0.002
	Control	45	36.9 ± 9.85	

This results agreed with a study done by George Davey Smith *et al.*,2021 which concludes that hazard ratio for myocardial infarction was 3.5-fold for VLDL-C and 1.3-fold for IDL and LDL-C combined. Biological implications include that VLDL-C particles are more atherogenic than LDL particles [150].

Other study by Michael V. Holmes, Iona Y. Millwood *et al.*,2018 in China shown a statistically significant relation of high VLDL-C levels and myocardial infraction incidence with p-value (<0.05) [54].

VLDL-C is the precursor of LDL-C, that have cholesterol and triglycerides, and these are also a risk factor for MI [151]. Increase production of VLDL or reduce VLDL clearance, or more commonly the dual effect of both processes, lead to hyperlipidemia. The endogenous TG-rich VLDL particles produced in the liver and secreted to the circulation, and then transported to peripheral tissues where they are metabolized by lipoprotein lipase and used for energy by muscle tissue or stored in adipose tissue [152].

3.4 Molecular Genetics

3.4.1 Arginase-I genotyping

The conventional PCR was used to amplify a target DNA using specific primer pairs, The result of amplification, which was conducted to determine the ARGI polymorphic site by using specific primer resulted in one band when visualized on agarose gel electrophoresis, these band was 296 base pair (bp) representing the presence of ARGI genotyping, as shown in Figure 3-5.

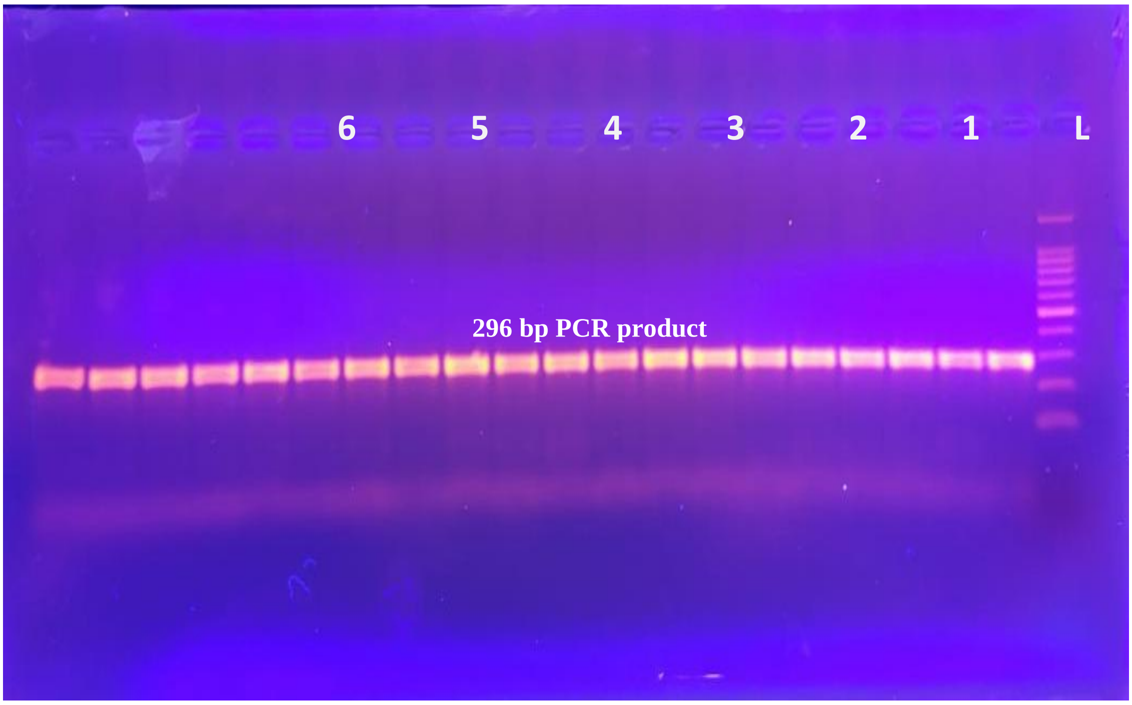


Figure 3-5 PCR products the band size 296 bp, the product was electrophoresis on 1.5% agarose: DNA ladder (100).

3.4.2 Genotype and Allele Frequency

The genotype and allele frequencies of the ARGI (G→T) variant in the study subjects are shown in the Table 3-9.

Table 3-9 genotype and allele frequencies of the patients and control group

Mode	Arginase-1 genotype or allele	Patients	Control	p. value	OR	95% CI
Codominant	GG	18(40%)	32 (71.1%)		REFERENCE	
	GT	22 (48.9%)	11 (24.4%)		3.55	1.4 -8.97
	TT	5 (11.1%)	12 (4.5%)	0.037	2.3	1.73–3.07
Dominant	GG	18(40%)	32 (71.1%)		REFERENCE	
	GT + TT	27 (60%)	13 (28.9%)	0.0036	0.27	0.11 - 0.65
Recessive	GG +GT	40 (88.9%)	43 (95.5%)	0.25	REFERENCE	
	TT	5 (11.1%)	2 (4.5%)		2.68	0.49 - 14.6
Allele frequency	G	58(64.5%)	75 (83.3%)		0.58	0.24 -1.05
	T	32 (35.5%)	15 (16.6%)	0.006	2.3	1.73–3.07

According to the results of genotyping, ARGI polymorphism was identified as homologous genotype (GG) in 18 (40%) sample, of patients group and in 32 (71.1%) sample, of the control group.

The heterozygous genotype (GT) identified in 22 (48.9%) samples of patients group and in 11 (24.4%) sample of the control group, while homologous genotype (TT) of the rare allele is identified in 5 (11.1%) samples, of patients group and in 2 (4.5%) samples, of the control group as observed in Figure 3-6.

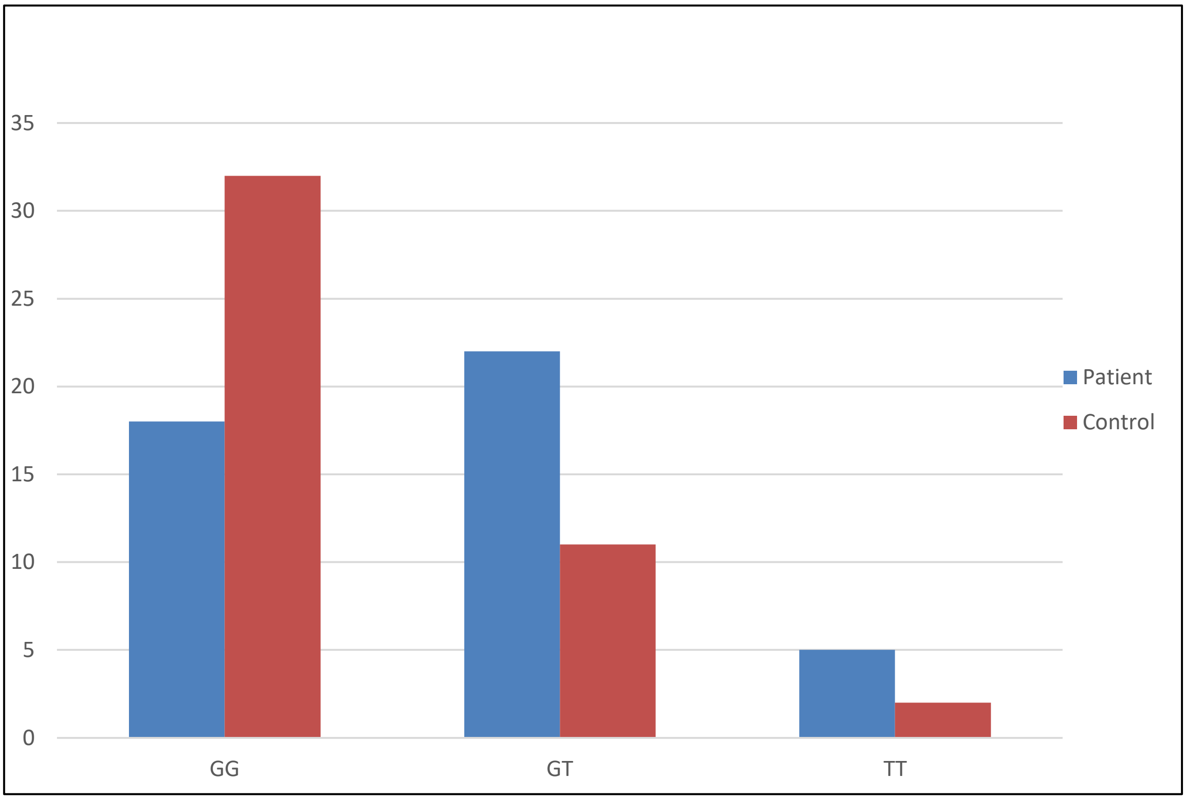


Figure 3-6 genotype and allele frequencies of the control and patients group

From Table 3-9, the patients with MI had significantly higher frequency of TT genotype compared to control group, that 5 (11.1%) patients samples had TT genotype compared to 2 (4.5%) control samples had TT genotype with p-value =0.037.

The MI patients showed higher frequency of T allele compared to the control group that 32 (35.5 %) patients samples had T genotype compared to 15(16.6%) control samples had T genotype with p-value =0.006.

The result of this study is goes with a study by Julie Dumont, Mahmoud Zureik *et al.,* 2006 in France that conclude that The ARGI rs2781666G/T polymorphism was associated with MI, and other study in Hospital la Rabta, Cardiology, Tunis, by Yousra *et al*., 2009 reported that significant association between rs2781666 G/T polymorphism of ARGI gene and MI in the Tunisian population [93], [153].

Ethnicity is well known to impact the frequencies of SNPs and their effects on the disease, therefore significant developments have been achieved in understanding the association of these tagged SNPs with cardiovascular genotypes through genome-wide association studies [154]. However, before the current study, genetic variations in ARGI and their association with CAD and family history have not been studied extensively. Interestingly, data from the current investigation revealed a significant association between the said SNPs and CAD. The novel finding is that the T allele at rs2781666 increased the risk of CAD in patients. Patients carrying the T allele at rs2781666 had a 2.3-fold increased risk of developing the disease. Contradictions have been reported in the literature regarding the rs2781666G/T polymorphism and its association with cardiovascular disease genotypes. Sediri *et al.*, 2010 showed a significant link between the variant genotype and allele at rs2781666 with myocardial infarction in patients from a Tunisian male population [153]. Similarly, Dumont *et al.,* 2007 showed an increased risk for developing myocardial infarction with a variant genotype of the rs2781666G/T polymorphism in patients [155], while others have reported a non-significant link with the disease in the Algerian population [154]. The control subjects in present study showed an rs2781666G allele frequency of 83.3%, which is comparable with those in case–control studies from France at 81.7% and Tunisia at 78.3% [155]. The G allele frequency in healthy individuals of a Iraqi population was in the range of those in other populations. However, this contrariety between the findings has always been attributed to the difference in ethnic origin, disease phenotype, and gene–environment interaction [153].

3.5 Quantitative analysis of serum Arginase-I in different genotypes of ARGI.

To demonstrate the effect of ARGI polymorphism on arginase-I activity in study group, an ANOVA test was conducted, as shown in Table 3-10.

Table 3-10 Mean value of arginase-I by ARGI genotype in study group

ARGI	Patients			Control		
	N	Mean ± SD	P.value	N	Mean ± SD	P.value
GG	18	27.5 ± 5.33	< 0.001	32	24.4 ± 5.3	0.729
GT	22	30.7 ± 5.74		11	25.2 ± 7.13	
TT	5	49.4 ± 7.14		2	21.6 ± 0.59	

In the present study, there was highly significant association with p-value of (< 0.001) between ARGI polymorphism and arginase-I activity in patients group and no significant association with p-value of (0.729) between ARGI polymorphism and arginase-I activity in control group.

myocardial infarction subjects showed a varying activity of arginase-I in rs2781666G/T as shown in (Table 3-10). Subjects with the ARGI variant genotype at rs2781666 showed higher activity of arginase-I than the cases with GG genotype. Carriers of the TT genotype showed maximal (mean ±SD 49.4 ± 7.14 ng/ml), carriers of GT (mean ± SD 30.7 ± 5.74ng/ml) and GG (mean ±SD 27.5 ± 5.33ng/ml) genotype, respectively. There was significant difference in arginase-I activity among the carriers of different genotype of rs2781666G/T ($P< 0.001$ for GG vs GT; GG vs TT, and GT vs TT).

This results agreed with study in Shifa College of Medicine, Shifa International Hospital, Islamabad, Pakistan, by Syed Fawad Ali Shah *et al*., 2018 about ARGI gene Polymorphisms and their association in individuals with myocardial infarction [129].

Sediri and colleagues showed a significant link of variant genotype and allele T at rs2781666 G/T with myocardial infarction in patients from a Tunisian population [156]. Similarly, reports showed a significant association of ARGI rs2781666G/T polymorphism in cardiovascular disease patients, [93], while some have reported a non-significant link with the cardiovascular disease from the Algerian population [154].

Pervious research showed that ARGI variant genotypes, GT+TT at rs2781666, are significantly associated with reduced NO metabolites, and higher activity of arginase-I in CAD cases compared with the carriers of wild genotype GG [157]. Little is known regarding the functional activities of different genotypes in ARGI and their relationship with the disease. Data from the present study showed that high arginase-I activity and variants of ARGI may be associated with an increased risk of atherosclerosis by promoting an inflammatory response CAD [156].

3.6 Significance of ARGI in Diagnosis of MI

To further investigate the efficiency of ARGI as potential biomarker of MI, Receiver operating characteristic (ROC) curve between MI patients and control was performed to analyze the sensitivity and specificity of ARGI in diagnosing of MI. The result showed that areas under the curve (AUC) of ARGI was 0.776, while AUC of TnI was 0.998. The optimal cut-off value of the relative level of ARGI was 25.5(ng/ml) with a sensitivity of 77.1 % and a specificity of 68.8 %. P-value 0.001, 95% CI (0.674-0.870), as shown in Figure 3-7.

Accuracy of test is measured by the area under the ROC curve. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test.

▶ 0.90 - 1 = excellent

▶ 0.80 - 0.90 = good

▶ 0.70 - 0.80 = fair

▶ 0.60 - 0.70 = poor

▶ 0.50 - 0.60 = fail

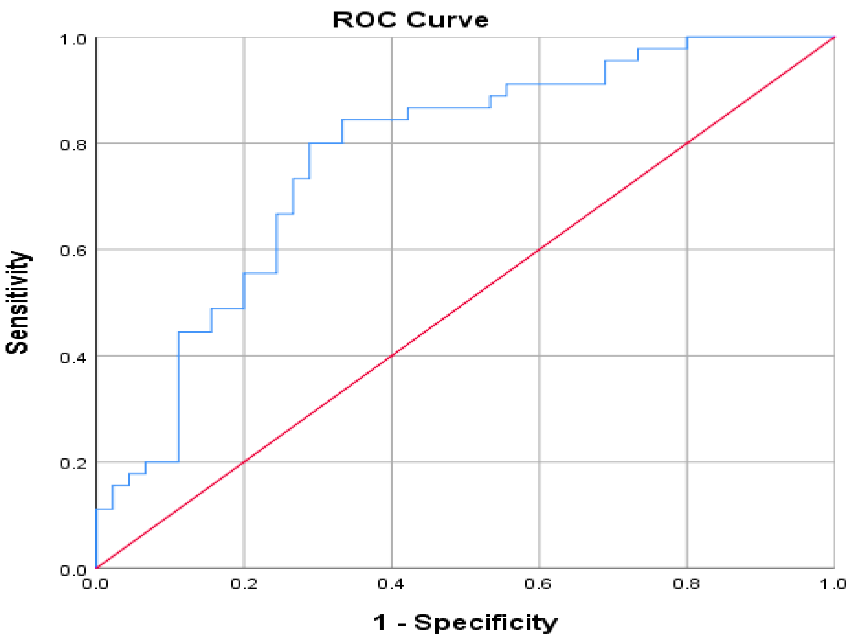


Figure 3-7 ROC Curve for arginase-I

The ROC curve used to find out the diagnostic value of a particular test therefore, it is possible to use any test in diagnosis the disease by depended on a specificity and sensitivity for this test. May be use ARGI in the diagnosis of MI but not depend on it only because of low specificity and sensitivity.

Chapter Four

Conclusions and Recommendations

Conclusions

1- A significant association between rs2781666G/T polymorphism of ARGI gene and MI may be is important risk factor in the development of coronary artery disease.

2- The high of ARGI activity in the patients compared to control may give an impression it may play a role in the pathogenesis of MI This supported by the a significant association between rs2781666G/T polymorphism of ARGI gene and MI in case group.

3- According to ROC curve of arginase-I activity cannot be used in to diagnose MI at the levels of >25.5ng/ml.

Recommendations

1-Study other candidate genes for myocardial infarction (such as lipoprotein lipase).

2- Further study using genetic polymorphisms of other SNP of ARGI gene to clarify the relationship genetic and MI.

3- Study the same SNP of ARGI in other regions of Iraq to give a complete picture about genotype distribution in the Iraqi population, and with a larger sample size.

4- Study of other biochemical parameters in addition to ARGI activity such as nitric oxide (NO).

5- Other study about treatment with the ARGI inhibitor that restores NO bioavailability and markedly decreases the size of cardiac infarcts.

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Appendix

Questionnaires

Name [ ]

Age [ ] years **Gender:-** male [ ] female [ ]

Residence:- Urban [ ] Rural [ ]

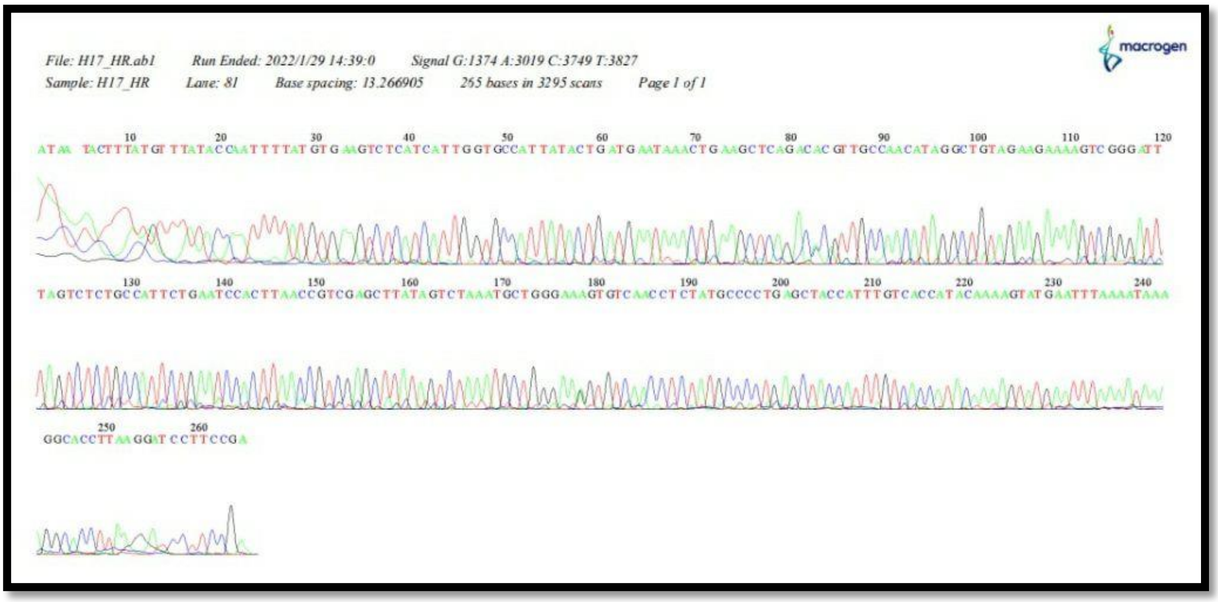
Phone [ ]

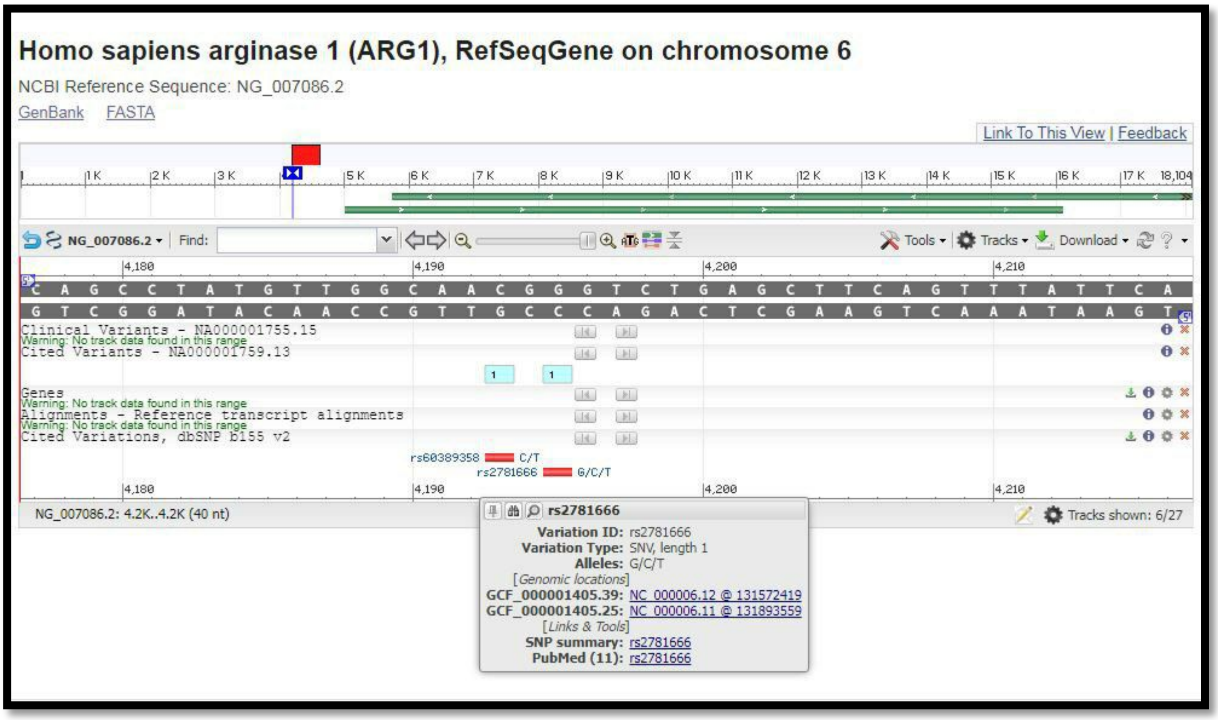
File [ ] ID [ ]

BMI [ ]

Exclusion Criteria : -

- Subjects with renal disease
- Diabetic ketoacidosis (DKA)
- Cardiogenic shock
- Liver disease
- Systemic immune disease
- Patients with malignancies or chemotherapy drug
- Drug dependency such as glucocorticoid


File: H17_HR.ab1 Run Ended: 2022/1/29 14:39:0 Signal G:1374 A:3019 C:3749 T:3827
Sample: H17_HR Lane: 81 Base spacing: 13.266905 265 bases in 3295 scans Page 1 of 1
macrogen


Homo sapiens arginase 1 (ARG1), RefSeqGene on chromosome 6
NCBI Reference Sequence: NG_007086.2
GenBank FASTA
Link To This View | Feedback
NG_007086.2 Find:
Tools Tracks Download
Clinical Variants - NA000001755.15
Warning: No track data found in this range
Cited Variants - NA000001759.13
Genes
Warning: No track data found in this range
Alignments - Reference transcript alignments
Warning: No track data found in this range
Cited Variations, dbSNP b155 v2
rs60389358 C/T
rs2781666 G/C/T
NG_007086.2: 4.2K..4.2K (40 nt)
Tracks shown: 6/27
rs2781666
Variation ID: rs2781666
Variation Type: SNV, length 1
Alleles: G/C/T
[Genomic locations]
GCF_000001405.39: NC_000006.12 @ 131572419
GCF_000001405.25: NC_000006.11 @ 131893559
[Links & Tools]
SNP summary: rs2781666
PubMed (11): rs2781666

الخلاصة

احتشاء عضلة القلب هو تلف في خلايا عضلة القلب نتيجة لانقطاع إمداد الدم التاجي الناجم عن نقص التروية ، يحدث تلف عضلة القلب عندما يكون إمداد القلب بالأكسجين غير كافياً لتلبية احتياجات التمثيل الغذائي. السبب الأساسي الأكثر شيوعًا لنقص تروية عضلة القلب هو انسداد الشرايين التاجية بسبب تصلب الشرايين.

تؤدي زيادة نشاط الارجنيز-1 إلى انخفاض إنتاج أكسيد النتريك (NO) وزيادة تكوين أنواع الأوكسجين التفاعلية بسبب عدم اقتران إنزيم تخليق اكسيد النتريك البطاني (eNOS) الذي ينتج أكسيد النيتروجين هذا يساهم في الخلل البطاني ، عدم استقرار اللويحة وزيادة التعرض لاحتشاء عضلة القلب.

كان الهدف من هذه الدراسة هو التحقق من العلاقة المحتملة بين نشاط الارجنيز-1 وتعدد الأشكال rs2781666 G / Tلجين arginase-I واحتشاء عضلة القلب في محافظة بابل / العراق.

تم تصميم هذه الدراسة كدراسة مرضى واصحاء وتم تصميمها لدراسة دور الارجنيز- 1 في المرضى الذين يعانون من احتشاء عضلة القلب. ولتحقيق هذا الهدف ، بلغ عدد المصابين باحتشاء عضلة القلب 45 مريضاً تتراوح أعمارهم بين (42-73 سنة) ، 34 منهم ذكور و 11 إناث ، و 45 مريضاً يبدو أنهم أصحاء كمجموعة ضابطة ، 33 منهم ذكور و 12 إناث.

جمعت عينات المرضى من مستشفى المرجان التعليمي / مركز الشهيد المحراب في مدينة الحلة بمحافظة بابل من تشرين الثاني 2021 الى كانون الثاني 2022 ، فيما جمعت عينات الاصحاء من خارج المستشفيات وأجريت هذه الدراسة في مختبر قسم الكيمياء الحياتية في كلية الطب - جامعة بابل.

تم استخدام الأمصال التي تم الحصول عليها من دم جميع المجموعات لقياس مستوى الأرجنيز -1 وتركيز الدهون ، بينما تم استخدام عينات الدم الكاملة من الأشخاص الخاضعين للدراسة لاستخراج الحمض النووي لدراسة تعدد الأشكال في جين الارجنيز-1.

تم قياس مستوى الارجنيز-1 في المصل بواسطة تقنية الاليزة (ELISA) وتم قياس تركيز الدهون باستخدام طريقة الطيف الضوئي. بينما تم استخدام PCR- Sequencing للكشف عن النمط الجيني الحالي لـ arginase-I والنمط الجيني الخالي في وقت واحد.

أظهرت النتائج أن هناك زيادة معنوية في arginase-I بين مجموعة المرضى و الاصحاء (P = 0.001) كان مستوى الأرجنيز-1 أعلى في مجموعة المرضى (Mean ± SD 33.5 ± 11.08) مقارنة بالاصحاء (Mean ±SD 24.9 ± 6.56).

كانت هناك زيادة كبيرة (P <0.05) في الكوليسترول الكلي (TC) ، والدهون الثلاثية (TG) ، والبروتين الدهني منخفض الكثافة (LDL) والبروتين الدهني منخفض الكثافة جدا (VLDL) في مجموعة المرضى بمعدل mean±SD

(53.64 ± 205.1 ، 68.03 ± 226.1 ، 30.22 ± 131.9 و 13.62± 45.2) على التوالي مقارنة بمجموعة التحكم بمعدل

(46 .52 ± 181.5، 49.26 ± 184.6 ، 31.23 ± 92.3, 9.85± 36.9) على التوالي.

بينما وجد أن تركيز البروتين الدهني عالي الكثافة (HDL) ينخفض معنوي (p=0.001) في مجموعة المرضى (9.82 ± 28.4) مقارنة بمجموعة التحكم (12.48 ± 52.3).

فيما يتعلق بالتحليل الجيني ، كان هناك فرق معنوي في تواتر النمط الجيني بين المرضى ومجموعات المراقبة في الأنماط ، السيادة (P <0.05) ولا يوجد فرق كبير في الوضع المتنحي (ع = 0.25). بالإضافة إلى ذلك كان هناك فرق كبير في تكرار الأليلات بين المرضى ومجموعة المراقبة (p=0.006).

أظهرت مجموعة المرضى نشاطًا متنوعًا من الارجنيز-1 في rs2781666G/T. أظهرت الموضوعات ذات التركيب الوراثي المتغير في rs2781666 نشاطًا أعلى من arginase-1 من الحالات ذات النمط الجيني GG. أظهرت ناقلات النمط الوراثي TT الحد الأقصى (7.14 ± 49.4) , GT (5.74 ± 30.7) وGG (5.33 ± 27.5). كان هناك اختلاف كبير في نشاط arginase-1 بين ناقلات النمط الجيني المختلف (P <0.001) rs2781666G / T لـ GG مقابل GT ، GG مقابل TT و GT مقابلTT.

في الاستنتاجات، قد تعطي المستويات العالية من arginase-I في المرضى مقارنة مع مجموعة التحكم انطباعًا أنه قد يلعب دورًا في التسبب في احتشاء عضلة القلب وهذا مدعوم بالارتباط الكبير بين rs2781666G / T تعدد الأشكال لجين arginase-I واحتشاء عضلة القلب.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية الطب

فرع الكيمياء والكيمياء الحياتية

تعدد أشكال الأرجنيز-1 ونشاطه لدى مرضى احتشاء عضلة القلب في محافظة بابل

رسالة

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

من قبل

علي عبدالكاظم عبدالله عباس

بكالوريوس علوم كيمياء / جامعة بابل/ 2011

بأشراف

الأستاذ الدكتور

عبدالسميع حسن الطائي

الأستاذ المساعد الدكتور

شكري فائز السعد

2022م

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