Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbala

College of Medicine

Department of Biochemistry

Serum Levels and Role of Von Willebrand Factor Gene Polymorphism in Pathogenesis of ST-Elevation Myocardial Infarction in Iraqi Patients and its Relations with ADAMTS13 Levels

A Thesis

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

BY

Haneen Abd Ali Ouda

(B SC in Science Chemistry - University of Karbala, 2016)

(Supervisor)

Dr. Shatha Mohammed Jawad Al-Khateeb

Mustansiriya University

Department of Biochemistry

College of Medicin

(Supervisor)

Dr.Ahmed Qasim Al-Haideri

Physician – International Cardiologist

Kerbala Heart Center

2021 AD **1443 AH**

Certification of Supervisors

I certify that the preparation of this thesis entitled:

Serum Levels and Role of Von Willebrand Factor Gene Polymorphism in Pathogenesis of ST-Elevation Myocardial Infarction in Iraqi Patients and its Relations with ADAMTS13 Levels

was presented by "**Haneen Abd Ali Ouda Al-Salammi**" and made under my supervision at the Department of Clinical Biochemistry, College of Medicine, University of Kerbala, as a partial fulfillment of the requirements for the degree of Master in Clinical Chemistry

Supervisors

Dr. Shatha Mohammed Jaw
Department of Biochemistry
College of Medicin

Dr.Ahmed Qasim Al- HaderiAl-Khateeb
Physician –International Cardiologist
Kerbala Heart Center

Dr. Rana M. Hameed

Head of Department of Biochemistry

College of Medicine – University of Kerbala

Dedication

To the ideal in my life, to whom I raised my head high in pride for my father, may God protect him.

For those under her feet are heaven, the joy of my life, my mother, may God extend her life

To my support, my strength, my help and my refuge after God _ Almighty _my dear brothers

To everyone who helped and encouraged me to complete this research, I dedicate my humble work

Haneen Abd Ali Oudha

Acknowledgments

In the name of God, the most merciful and most gracious.

First of all I thank my God, Allah, for inspiring me with strength, patience and willingness to perform this research.

I would like to thank all the patients who have cooperated with me and also the patients' families. I wish them healing, health and wellness.

I would like to express my sincere gratitude to my supervisor Assist. Prof. **Dr. Shatha Muhammad Jawad / Department of Biochemistry / College of Medicine, Al-Mustansiriya University**, who was more generous in giving her time, support and advice

Then I would like to express my sincere gratitude to my supervisor, **Dr. Ahmed Qassem Al-Haidari / Karbala Heart Center / Physician – International cardiologist**

for providing support, advice and instructions and for facilitating the task of work in the hospital

I would also like to thank the staff of the Karbala Center for Cardiac Diseases and Surgery at the Imam Hussain Medical Educational Center Hospital in Holy Karbala, with thanks to all the participants.

Haneen Abd Ali Oudha

List of Contents

List of Figures

List of Tables

List of abbreviations

Abbreviations	Description
ACS	acute coronary syndrome
ADAMTS13	A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13
AH	arterial hypertension
ARF	acute rheumatic fever
CABG	coronary artery bypass grafting
CHD	coronary heart disease
CK	creatine kinase
CVD	Cardiovascular disease
ECG	electrocardiogram
EF	ejection fraction
HDL	high-density lipoprotein cholesterol
HF	Heart failure
HGP	Human Genome Project

HHD	hypertensive heart disease
LDL	serum low-density lipoprotein cholesterol
LV	Left ventricular
MI	myocardial infarction
PAD	peripheral artery disease
PCI	percutaneous coronary intervention
RHD	Rheumatic heart disease
TC	total cholesterol
TG	triglyceride
TIA	transient ischemic attack
TTP	Thrombotic thrombocytopenic purpura
VTE	venous thromboembolism
VWF	von Willebrand factor

Abstract

Background: ST-elevation myocardial infarction (STEMI) is a clinical syndrome defined by characteristic symptoms of myocardial ischemia in association with persistent electrocardiographic ST elevation (STE) and subsequent release of biomarkers of myocardial necrosis

.

Aim of the Study:

To see the serial variations in the levels and gene polymorphism of thrombo-inflammatory biomarker von Willebrand factor (VWF) and A Disintegrin and Metalloprotease with Thrombo-Spondin motif (ADAMTS13) over the course of ST- elevation myocardial infarction and to determine their relationship with the another cardiovascular risk markers in Iraqi patients

Materials and Methods: The study design is a case-control study.
A total of 42 samples of ST-elevation myocardial infarction patients are collected from Kerbala Heart Center with age ranged between(25-75)years, and other 48 apparently healthy adults also as a control group and determination some biochemical marker creatine kinase and lipid profile was measure by using dirui device. Troponin is measure by cobas e411.
Enzyme-Linked immunosorbent assay (ELISA) was performed using Sandawich method to measure the concentrations of serum VwF and Adamts13

Result: The current study, found that the proportion of men with st elevation MI is greater in men than women. there was a significant difference between blood glucose and lipid profile in st-elevation MI patient and control group. There was statistical significant increase in serum cholesterol, triglycerides and LDL while for VLDL not statistically significant and there was statistical significant increase in serum VWF and reduced level ADAM where in the genetic study The AA, AT and TTgenotypes were significantly raised the risk of ST_Elevation MI

Conclusion: The AA, AT and TTgenotypes were significantly raised the risk of ST_Elevation MI, The results of the current study of genotype distribution of the (rs216311) SNP exhibited a significant associations were noticed between AT, AA,

and TT genotype and incidence of ST Elevation patients when compared with those of the control group.

found that the proportion of men with st elevation mi is greater than women. the total number of patients who have blood group A is the largest percentage for O and B in patient with st elevation myocardial infraction. Patients with diabetes mellitus have higher levels of TG and LDL cholesterol. while HDL level was normal, In the current study there was statistical significant increase in serum VWF and reduced level ADAM in St-Elevation MI patient.

CHAPTER ONE

Introduction and literature Review

Introduction and literature Review

1.1.Heart disease

The term "heart disease" encompasses a variety of conditions affecting the heart. Coronary artery disease, which can result in a heart attack, is the most common type. Other types of cardiac illness may affect the heart's valves, or the heart may not pump effectively, resulting in heart failure. Heart disease is a condition that some people are born with. **(Control, 2009).** In coronary artery disease, one or more of the coronary arteries becomes narrow or blocked.**(Heart et al., n.d.)**

1.1.2. signs and symptoms

The symptoms of heart disease differ based on the kind. For many people, chest pain or a heart attack is the first indicator of a heart attack. A person suffering from a heart attack may have a variety of symptoms, including:
Pain or discomfort in the chest that persists after a few minutes
Pain or discomfort in the jaw, neck, or back**.**
Weakness, light-headedness, nausea (feeling sickto in stomach), or a chilly sweat are among symptoms to watch out for.
Discomfort or pain in the arms or shoulder. **(Control, 2009)**

1.1.3. Blood Vessels

The cardiovascular system consists of the pump and vessels that distribute blood to all areas of the body. This system allows for the delivery of needed substances to the cells of the body as well as for the removal of wastes. Organs**(Smith, 2013)**
The primary structures that comprise the cardiovascular system:
blood vessels, arteries, capillaries, veins.

Blood is pumped around the body through a network of blood vessels:
• Arteries transport oxygen-rich blood from the heart to all parts of the body. The arteries get smaller as they get further away from the heart.
• Capillaries are the smallest of blood vessels.They connect the smallest arteries to the smallest veins. This is where oxygen, carbon dioxide, nutrients and waste products are exchanged.

• Veins carry blood, lackingin oxygen, back towards the heart. The veins get bigger as they get nearer the heart.**(Series, n.d.)**

Blood vessels not only act as a transport conduit system but also play important roles in organ development, tissue morphogenesis, inflammation, barrier formation, and wound healing. In addition, active involvement of blood vessels in the pathogenesis of a number of diseases suggests a fundamental need to understand these versatile transport networks in the body. blood vessels form an integral part of the skeletal system playing multiple roles in the maintenance of bone homeostasis. **(Ramasamy, 2017)**

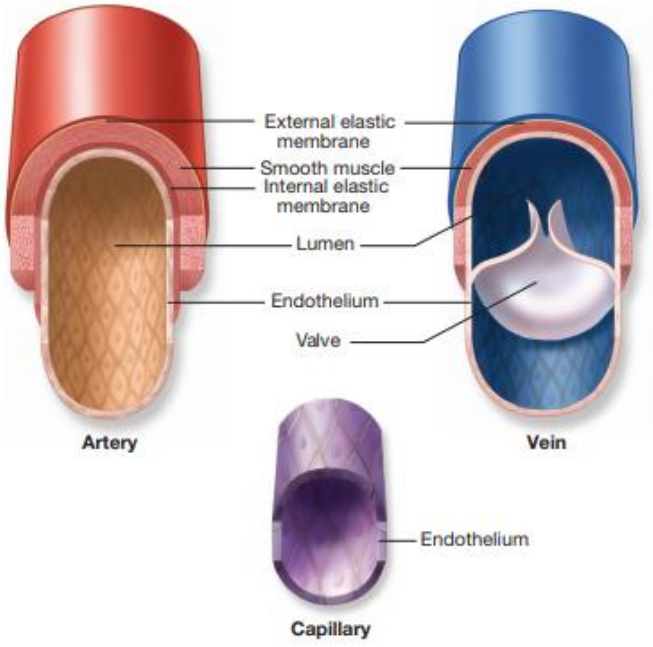


Figure1.1: Comparative structure of arteries, capillaries, and veins(Smith, 2013)

1.1.4. Biomechanics of Cardiac Function

To keep all organs functioning normally, the heart pumps blood and nourishment to them. The continual repeated pumping activity of the myocardium creates pressure to push blood flow in arteries to distant organs, and the synchronized contraction of the myocardium generates pressure to drive blood flow in arteries to all organs. Biomechanical concepts are crucial in regulating heart function. Many illnesses can damage the heart's complicated pumping system, and they can be caused by a number of causes such as genetic abnormalities, aging, and exposure to environmental stimuli. Two primary types of heart disease are left ventricular (LV) myocardial infarction (MI) caused by coronary heart disease and diastolic dysfunction caused by hypertension and LV hypertrophy. Biomechanics is important in controlling cardiac function in both healthy and sick hearts. Understanding the origins and effects of cardiac disorders requires an understanding of heart biomechanics. **(Voorhees et al., 2016).**

The LV's main job is to pump blood to all of the body's distal organs and extremities. The myocardium's contraction force, loading circumstances, ventricular size and shape, and valve function all influence the pump's performance. When the LV can't keep up with the body's needs, it's called heart failure. Biomechanics is an important factor in heart function. As a result, being able to define and quantify heart function is critical..**(Voorhees & Han, 2015)**

The mechanical forces produced by the beating heart are continually applied to the circulatory network. Because the beating heart begins early in embryonic development, cardiovascular development is dynamic: endothelial cells rearrange and migrate to build an efficient vascular network, while blood flow rises in tandem with the beating heart's growing efficacy. This implies that relationships between hemodynamic forces, heart function, and cardiovascular morphogenesis are changing, according to developmental biology. As the effects of mechanical forces become more understood, these interactions are becoming increasingly significant. **(Boselli et al., 2015)**

1.2: Cardiovascular Disease

Cardiovascular disease (CVD) is an umbrella term for a number of linked pathologies, commonly defined as coronary heart disease (CHD), cerebrovascular

disease, peripheral arterial disease, rheumatic and congenital heart diseases and venous thromboembolism. Globally CVD accounts for 31% of mortality, the majority of this in the form of CHD and cerebrovascular accident**(Stewart et al., 2017)**.

Cardiovascular diseases affect many people in middle age, very often severely limiting the income and savings of affected individuals and their families. Lost earnings and out of pocket health care payments undermine the socioeconomic development of communities and nations (**WHO, 2017).**

CVD is the largest cause of death in the world, with around 16.7 million fatalities each year, mainly from heart attacks and strokes.this number is predicted to increase to approximately 25 million deaths by 2020 **(Dahlöf, 2010).** Cardiovascular diseases are the leading cause of disease burden and deaths globally. the UN, alarmed by the increasing burden of non-communicable diseases (NCDs) and high disease severity and case-fatality in low-income and middle-income countries compared with high-income countries, acknowledged in 2012 that the rising burden of NCDs was one of the major threats **(Prabhakaran et al., 2018)**

1.2.1. Risk factors for cardiovascular disease

More information on the key CVD risk factors. The section starts with lipid and inflammation-related variables, then moves on to behavioral risk factors like smoking, dietary issues, alcohol, and physical activity**.** The key biological risk factors that modulate the impact of these behaviors in CVD, such as obesity, blood pressure, blood lipids, and diabetes, are next discussed. **(Amani & Sharifi, 2012).** Treatment of high CVD risk was defined as self-reported use of antihypertension, lipid lowering and antiplatelet medicines **(Maharani et al., 2019)**

1.2.2. High Blood Pressure

Blood pressure is the amount of force on the artery wall when the heart pumps and relaxes with each heart beat , Normal Blood Pressure is 120/80, Narrowed blood vessels increase the pressure causing the heart to work harder, Hypertension was defined as the presence of elevated systolic (≥140 mmHg) and/or diastolic (≥90 mmHg) blood pressure or the current use of antihypertensive drugs. **(Žaliaduonytė-Pekšienė et al., 2017)**

1.2.3 Diabetes Mellitus

Diabetes occurs when the pancreas does not produce enough insulin or the body cannot use insulin properly. With diabetes there is an abnormal amount of lipoprotein which speeds up atherosclerosis and raises the risk of heart attack

1.2.٤ Obesity

Obesity increases blood cholesterol, triglyceride levels, blood pressure and the risk for diabetes. It also decreases HDL cholesterol levels, Extra weight makes the heart work harder to supply the body with the needed oxygen. **(Pressure, n.d.)**

1.2.٥ -Abnormal blood lipids

High total cholesterol, LDL-cholesterol and triglyceride levels, and low levels of HDL cholesterol increase risk of coronary heart disease and ischaemic stroke**(*Risk Factors*, n.d.)**

Obesity and its associated comorbidities, such as cardiovascular and metabolic disorders, are promoted early in childhood and adolescence by changes in lifestyle and physical activity levels. Knowing the CVD risk factors is important for developing preventive and treatment methods. Excess weight, which is common in children and teenagers, is one of them. Dyslipidemia is linked to excess weight. The primary predictors of CVD include hypercholesterolemia, namely elevated LDL and reduced HDL values. Another risk factor for CVD is a sedentary lifestyle, which is prevalent in childhood and adolescence and is justified by changing behaviors. Obesity development and maintenance are closely linked to an unhealthy lifestyle that includes less physical exercise and more sedentary behavior. **(Faria et al., 2015)**

1.3. Cardiovascular Disease and Diabetes

Type 2 diabetes mellitus acts as an independent risk factor for several forms of CVD (micro- and macro vascular diseases), and people with T2DM are more likely to develop CVD due to a variety of risk factors. Preclinical manifestations of macro vascular diseases are developed much earlier in newly diagnosed, never-treated T2DM patients , and such macro vascular changes are also observed even in

normoglycemic and normotensive offspring of parents with T2DM **(Rahman et al., 2017)** .

Cardiovascular diseases (CVD) are the most prevalent cause of mortality and morbidity among people with T2Dand T1D. Adult people with diabetes present rates of mortality due to heart disease and stroke from two to four times higher than those without diabetes. It has been stated that patients with T2D without a previous history of myocardial infarction have the same risk of coronary artery disease (CADs) as nondiabetic subjects with a history of myocardial infarction, **(Matheus et al., 2013),** Individuals with type 2 diabetes (T2D) have a twofold increased risk for cardiovascular disease (CVD) (myocardial infarction, stroke, peripheral vascular disease), and CVD is the principal cause of death in T2D patients **(Abdul-Ghani et al., 2017)**.

In the United States, roughly one in every three persons has high blood pressure, and middle-aged and elderly people have a 90 percent lifetime chance of developing hypertension. Hypertension can develop to hypertensive heart disease (HHD), which is a condition that represents an essential underlying mechanism for the incidence of CVD morbidity and mortality associated with high blood pressure **(Nwabuo & Vasan, 2020).** hypertensive individuals with hypertensive heart disease are more prone to myocardial infarction, congestive heart failure, stroke, and sudden death than persons with hypertension alone. **(Díez, 2013)**

1.4 Obesity and Cardiovascular Disease

Obesity can increase CVD morbidity and mortality directly and indirectly. Direct effects are mediated by obesity-induced structural and functional adaptations of the cardiovascular system to accommodate excess body weight **(Koliaki et al., 2019).** Obesity pathogenesis is not only about how excess body fat is acquired, but also about how this excess is biologically assimilated. Several metabolic parameters (glucose, insulin, fatty acids, adipocytes, gut microbiome) are involved in the obesity pathogenesis, as well as all the systems (gastric, nervous) that regulate appetite control or food intake. Genetic factors and age are also parameters that can modulate the phenotypic expression of obesity (Cercato & Fonseca, 2019). Body mass index (BMI) has been reported to be the most common index of obesity**(Lin et al., 2020).**Central obesity more strongly associated with hypertension than peripheral

obesity in both men and women
(Ortega et al., 2016)

1.5. Smoking

Inhaling tobacco smoke causes several immediate responses within the heart and its blood vessels. Within one minute of starting to smoke, the heart rate begins to rise. This is partially attributable to nicotine, the addictive substance in cigarettes, it stimulates the body to produce adrenaline, making the heart beat faster, Nicotine also increases blood pressure. The increase in heart rate and blood pressure means that smokers' hearts often have to work harder than nonsmokers hearts, resulting in an increased risk of heart disease or stroke**.(*Smoking , the Heart and Circulation*, 2018)**.

The risk of cardiovascular disease increases with smoking duration and the quantity and kind of smoked tobacco products ingested. Even at modest levels of exposure, the risk of CHD is significantly raised — individuals who smoke only one cigarette per day have half the risk of CHD as those who smoke at least 20 cigarettes per day. Smoking tobacco, in addition to being a significant independent risk factor for CHD, has a synergistic effect with other major CHD risk factors such as high blood cholesterol, untreated hypertension, and diabetes mellitus **(Smoke, 2020).**

1.6. Types of cardiovascular disease

1.6.1 Atherosclerosis

Disease of the arterial vasculature that is characterised by the dysrupted balance and abnormal accumulation of lipids, inflammatory cells, matrix deposits and smooth muscle cell proliferation in the wall of medium- and large-calibre arteries. This accumulation is most commonly detected during the second decade of life and develops further with age. The implication of ageing and early development of atherosclerotic lesions makes a significant difference between chronic and acute plaque characteristics **(Mota et al., 2017)**.
For example:

1.6.2. Angina

Angina pectoris is defined as a substernal chest pain, pressure, or discomfort that is typically exacerbated by exertion and/or anxiety or other emotional or mental stress, lasts greater than 30 to 60 seconds, and is relieved by rest and/ or nitroglycerin

1.6.3. Coronary artery disease (CAD)

The most common form of heart disease, It is the result of atheromatous changes in the vessels supplying the heart. CAD is used to describe a range of clinical disorders from asymptomatic atherosclerosis and stable angina to acute coronary syndrome (unstable angina, NSTEMI, STEMI) **(Sherazi & Block, 2009).** A common symptom of coronary artery disease (CAD) is angina, Angina is chest pain or discomfort that occurs if an area of the heart muscle doesn't get enough oxygen-rich blood.**(National Institute Of Health, 2016)**

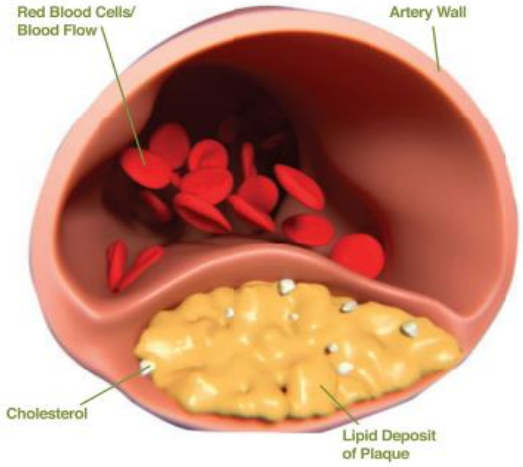


Figure1.2: Coronary artery disease (MAYRA, 2013)

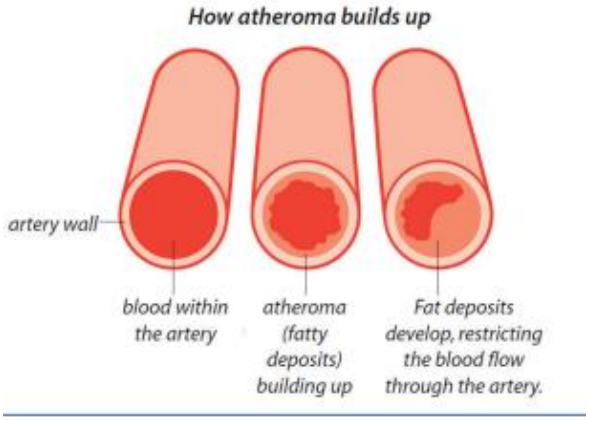


Figure 1.3: Coronary heart disease (CHD) occurs when fatty deposits line the coronary arteries(Barrett & Davenport, 2014)

1.6.4. The Pathophysiology of Chronic CAD

Previously thought to be a cholesterol storage illness, atherogenesis is now understood to be a complex interplay of risk factors, including arterial wall cells, blood, and the molecular messages that they communicate. Inflammation has a key role in all stages of atherogenesis, according to a valuable organizing theme that arose initially in laboratory investigations and has recently acquired credence in the clinic. Inflammation also plays a role in atherosclerosis' local, myocardial, and systemic consequences. When the arterial endothelium is exposed to bacterial products or risk factors such as dyslipidemia, vasoconstrictor hormones implicated in hypertension, glycoxidation products linked to hyperglycemia, or proinflammatory cytokines derived from excess adipose tissue, these cells increase the expression of adhesion molecules that promote blood leukocyte adhesion to the endothelium. The production of chemoattractant cytokines, which is controlled by signals linked to conventional and new atherosclerosis risk factors, is crucial for adherent leukocyte transmigration **(Libby & Theroux, 2005) .**

Another source of intraluminal coronary thrombus development is plaque erosion. A thrombus develops on a defect in the endothelium layer that covers a plaque in this situation. These plaques might be inflammatory or not, and the cap is generally

thick. Plaque erosions are prevalent among smokers and women under the age of 50, and the prothrombotic milieu is thought to play an essential role in the process. It is unknown how frequently this procedure occurs asymptomatically at this time. The majority of evidence on plaque erosion comes from patients who have had a symptomatic acute coronary episode **(Ambrose & Singh, 2015)**. CHD mainly occurs due to atherosclerosis and its progression is associated with environmental and genetic factors). Atherosclerosis is a chronic process, characterized by progressive accumulation of lipids, fibrous elements, and inflammatory molecules in the walls of the large arteries. Atherosclerosis starts with the efflux of low density lipoprotein (LDL) cholesterol to the sub-endothelial space, which can be changed and oxidized by various agents.**(Themistocleous et al., 2017**)

1.6.5. Heart failure (HF)

Heart failure is a clinical syndrome caused by structural and functional defects in myocardium resulting in impairment of ventricular filling or the ejection of blood. The most common cause for HF is reduced left ventricular myocardial function; however, dysfunction of the pericardium, myocardium, endocardium, heart valves or great vessels alone or in combination is also associated with HF**(Inamdar & Inamdar, 2016).**

The clinical diagnosis of heart failure in the later stages of the syndrome is both highly specific and highly sensitive. For the experienced physician, the presence of the symptoms of breathlessness and fatigue, together with the clinical signs of raised jugular venous pressure, pulmonary congestion, tachycardia with gallop rhythm, hepatomegaly and angle oedema are of sufficient diagnostic validity to require little further evaluation**(Taylor, 1996) .**

Diagnosis Measure ejection fraction (EF) to determine if the etiology of the heart failure is systolic dysfunction (rather than diastolic dysfunction or valvular heart disease and Check serum B-type natriuretic peptide (BNP) to help determine if dyspnea is due to heart failure **(Serdahl, 2008) .**

heart failure may be considered as a progressive disorder that starts from a trigger or "index event" that damages the heart muscle and consequently impairs cardiac myocytes, therefore reducing the myocardium's ability to fill with or eject blood, and finally precluding the heart from contracting normally. This inciting event may have a sudden onset (e.g. myocardial infarction) or a gradual or insidious onset (e.g.

haemodynamic pressure or volume overloading), or it may be hereditary **(Lainscak et al., 2017).**
The HF prevalence in the general population in the developed countries is estimated to be in the range of 0.4% to 2%. **(Maciejak et al., 2015)**

1.6.6. Stroke

Stroke is a neurological disorder characterized by blockage of blood vessels. Clots form in the brain and interrupt blood flow, clogging arteries and causing blood vessels to break, leading to bleeding. Rupture of the arteries leading to the brain during stroke results in the sudden death of brain cells owing to a lack of oxygen. Stroke can also lead to depression and dementia **(Kuriakose & Xiao, 2020) .** Stroke prevention can be divided into primary and secondary prevention. Primary prevention de-scribes the prevention of a first ischemic stroke, whereas secondary prevention covers the prevention of stroke among survivors of ischemic stroke or TIA. Primary and secondary stroke preventions both include cardiovascular risk factor reduction and treatments of specific conditions causing stroke such as atrial fibrillation and carotid artery stenosis.

1.6.7. Hypertensive Heart Disease

Interactions between genetic and hemodynamic factors cause hypertensive heart disease in patients with arterial hypertension. hypertensive individuals with hypertensive heart disease are more prone to myocardial infarction, congestive heart failure, stroke, and sudden death then persons with hypertension alone**(Diamond & Phillips, 2005).** Hypertension can lead to hypertensive heart disease (HHD), which is a condition that represents an important underlying mechanism for the occurrence of blood pressure-related CVD morbidity and mortality. HHD is a diagnostic and prognostic marker that can appear as a subclinical condition that foreshadows negative outcomes later in life or as an overt trait in people. **(Nwabuo & Vasan, 2020)**

1.6.8. Peripheral artery disease

Peripheral artery disease is one of the most prevalent conditions, and it frequently coexists with vascular disease in other parts of the body. Early diagnosis is important for improving the patient's quality of life and for reducing the risk of serious secondary vascular events such as acute myocardial infraction (AMI) or strok**e(Serrano Hernando & Conejero, 2007)**. The term peripheral artery disease (PAD) broadly encompass the vascular diseases caused primarily by atherosclerosis and thromboembolic pathophysiologic processes that alter the normal structure and function of the aorta, its visceral arterial branches, and the arteries of the lower extremity. PAD is the preferred clinical term and should be used to denote stenotic, occlusive and aneurysmal diseases of the aorta and its branch arteries, exclusive of the coronary arteries.**(Beckman et al., 2011).**

The basic method for diagnosis and assessment of patients with symptomatic peripheral arterial disease includes assessing medical history with walking distance palpation, and a simple physical examination with a stethoscope sphygmo-manometer cuff and Doppler probe to compare blood pressure in the arms and legs. Such examinations, which may be performed at any health centre or hospital, can identify most patients with peripheral arterial disease. **(Engström-laurent, 2007)**

1.6.9. Arrhythmias

are abnormalities of the heart rate and rhythm (sometimes felt as palpitations). They can be divided into two broad categories: fast and slow heart rates. Some cause few or minimal symptoms. Others produce more serious symptoms of lightheadedness, dizziness and fainting.**(Heartbeat, n.d.).** Normal sinus rhythm is the natural rhythm of the heart. Synchronous contraction of the atria, followed by that of the ventricles optimises blood flow. If the heart rate is too fast or too slow, insufficient blood may be pumped to meet the demands of the organs of the body**. (Humphreys et al., 2013).**

The normal rhythm of the heart originates in the sinus node, a collection of cells at the junction of the right atrium and the superior vena cava with the unique property of automaticity, shared with few other cardiac tissues. Automatic cells in the sinus node discharge at rates affected by autonomic influences through the parasympathetic nervous system, which regulates heart rates during most normal

activities and at rest, and by sympathetic stimulation, which raises the heart rate during exercise**(Kibos et al., 2013)**

1.7.1. myocardial infarction

An unstable ischemia syndrome causes an acute myocardial infarction, which is characterized by myocardial necrosis. Clinical examination, electrocardiogram (ECG), biochemical tests, invasive and noninvasive imaging, and pathological evaluation are used to diagnose and assess it. Acute myocardial infarction is classified into six types based on the presence or absence of ST-segment elevation on the ECG: infarction due to coronary atherothrombosis (type 1), infarction due to a supply–demand mismatch that is not the result of acute atherothrombosis (type 2), infarction causing sudden death without the opportunity for biomarker or ECG confirmation (type 3), infarction causing sudden death without the (type 5) (**Anderson and Morrow, 2017)**.

The biggest risk of death occurs in the first few hours after the beginning of AMI. As a result, early detection of myocardial ischemia is crucial for efficient care of AMI patients. Incorrect diagnosis of patients with chest pain frequently results in improper hospitalization of individuals without AMI, and vice versa. Physical examination, correct ECG results, and measurement of cardiac biomarkers, in addition to clinical history, play an essential role in the early identification of acute ischemia. **(Elsayed Azab, 2017**). Coronary atherosclerosis, dynamic coronary artery changes and intimal injury are regarded as the principal events leading to acute thrombotic occlusion, which causes myocardial infarction **(Pepine, n.d.)**

1.7.2. Pathophysiology

Myocardial ischemia can be caused by an increase in the myocardial's demand for oxygen, a reduction in oxygen delivery to the myocardium, or both. Myocardial oxygen demand is raised during exercise, tachycardia, or emotions, and if there is a coronary occlusion, this will result in a temporary imbalance. The most common cause of chronic stable angina is demand ischemia, which is also known as demand ischemia. This imbalance can also develop as a result of an abrupt drop in oxygen delivery owing to elevated coronary vascular tone (i.e., coronary vasospasm) or an apparent reduction or blockage of a coronary artery due to platelet aggregation or

thrombi. This is referred to as supply Ischemia can cause a heart attack or unstable angina (UA). Ischemia is caused by both an increase in oxygen demand and a decrease in oxygen delivery in many cases **(Picchio et al., 2020).**

Atherosclerosis is a chronic condition that can manifest itself in the coronary arteries as eccentric or concentric lesions. Through various pathophysiological processes, they might cause myocardial ischemia or infarction. Compensatory outward expansion of atherosclerotic arteries to "accommodate" plaque while avoiding lumen constriction as a result, extensive plaques can develop in the walls of afflicted arteries without generating symptoms or prompting arteriograms to show up **(eccentric lesion).**

Acute coronary syndrome (ACS), also known as Type 1 myocardial infarction (MI), and persistent myocardial oxygen supply-demand imbalance in the context of stable CAD (Type 2 MI) are the two different identities accountable for the development of myocardial ischemia. **(Smit et al., 2019)**

1.7.3. Genetics and myocardial infarction

Genetic information is increasingly being used as part of personalized clinical treatment, and genomic medicine is having an influence in a variety of medical areas, including cancer, pharmacology, and cardiology, to name a few. The Human Genome Project (HGP) has made it possible to use genetic information to enhance health, but translating new discoveries into patient treatment can take years. Since the completion of the HGP, researchers have concentrated on determining how variations in an individual's DNA affect disease and health, elucidating disease etiologies and prognoses, identifying disease susceptibility variants, and improving the efficacy and safety of pharmacological treatments. **(Melo, 2018)** . All of these investigations assume that proteins involved in the pathophysiology of atherosclerosis have mutations that make them a possible contributing cause of myocardial infarction. Although there have been over 5000 papers in this subject to far, only a handful of them have found a consistent link between genes involved in lipid metabolism and an increased risk of myocardial infarction. **(Erdmann et al., 2010)**. with the heritability of CAD and MI estimated at approximately 50% to 60%. Understanding the genetic architecture of CAD and MI has proven to be difficult and costly due to the heterogeneity of clinical CAD and the underlying multi-decade

complex pathophysiological processes that involve both genetic and environmental interactions.**(Dai, 2016) .**

1.7.4. ST-Elevation Myocardial Infarction

Myocardial ischaemia results from the reduction of coronary flow to such an extent that supply of oxygen to the myocardium does not meet the oxygen demand of myocardial tissue. When this ischaemia is prolonged and irreversible then myocardial cell death and necrosis occurs which is defined as myocardial infarction (MI)**(Nigam, 2007).** ST-elevation myocardial infarction (STEMI) is a clinical condition characterized by myocardial ischemia symptoms in the presence of prolonged electrocardiographic ST elevation (STE) and the subsequent release of biomarkers of myocardial necrosis. The single best surrogate measure for identifying acute total coronary artery blockage without collateral circulation, indicating a large area of damaged myocardium at imminent danger of permanent infarction and needing urgent reperfusion treatment is STE. **(Hwang & Levis, 2014)**

ST segment elevation myocardial infarction reflects acute myocardial infarction resulting from the rupture or erosion of an atherosclerotic plaque with thrombotic occlusion of an epicardial coronary artery and transmural ischaemia. The size of the resulting infarction depends on (i) the size of the ischaemic area at risk, (ii) the duration and intermittency of coronary occlusion, and (iii) the magnitude of residual collateral blood flow and the extent of coronary microvascular dysfunction **(Heusch & Gersh, 2017).**

Clinical symptoms, electrocardiographic (ECG) alterations, and a particular pattern of changes in blood enzymes including creatine kinase (CK), creatine kinase isoenzyme MB (CKMB), lactate dehydrogenase isoenzyme 1 (LD1), as well as cardiac specific proteins like troponins, are used to diagnose MI. ECG is the most frequently used method of diagnosing myocardial infarction since clinical signs are not always trustworthy. However, the relevance of serum biochemical indicators of myocardial damage to establish the diagnosis of myocardial injury emerges when the ECG reveals an ambiguous pattern.**(Nigam, 2007)**

1.7.5. Non-ST elevated myocardial infarction

a myocardial infarction that does not produce elevations in the ST segments of the electrocardiogram. ST segment elevation of the ECG is often used in determining the treatment protocol **(Mannsverk, 2019).**

1.7.6. Association Between ABO Blood Groups and Myocardial Infarction

The ABO blood group has a blood cell surface protein and an endothelial cell-associated Willebrand factor, and there is evidence that an individual's blood type might predict their thrombosis risk. Because of the importance and great incidence of coronary artery disease, a link between myocardial infarction and ABO blood types has yet to be established, and there are even some doubts **(Hassan et al., 2017)** . CHD frequency was usually greater in blood types A than in the other ABO blood groups, according to research. whereas al found that people with blood group phenotypic AB have a substantially greater rate of ischemic heart disease than those with blood groups O, A, or B. & et al. discovered that the O and B blood groups were more common in MI patients, suggesting that these blood types may play a role in the disease's development. Patients with CHD had a higher prevalence of blood group A than the general population.**(Garg et al., 2012).** Among all of those studies, the mechanism of relationship between ABO blood group and venous thrombosis is elucidated and its major determinants are von Willebrand factor (vWF) and coagulation factor VIII7 which result in thrombosis. This interesting finding makes a theoretical hypothesis that ABO blood group may also be related to risk of coronary artery disease (CAD) and myocardial infarction (MI).**(Chen et al., 2016)**

1.7.7. The electrocardiogram (ECG)

ECG is an effective diagnostic tool for a variety of cardiovascular diseases (CVDs), including myocardial infarction (MI). The ECG records the electrical activity of the heart, and these signals can be used to detect aberrant heart function. The ECG signals, however, are difficult to visually interpret due to their limited amplitude and length.**(Acharya et al., 2017).** As a result, an early identification of MI will enable patients to receive timely treatment, lowering the risk of death. Because cardiac

muscle damage is irreversible, it's critical to get detected early. An electrocardiogram can be used to make an early diagnosis of MI (ECG). The ECG is a noninvasive, cost-effective primary technique for diagnosing heart problems **(Acharya et al., 2017)**

1.9. BIOMARKERS IN MYOCARDIAL INFRACTION

1.9.1. Creatine Kinase MB (CK-MB)

Creatine kinase with a molecular weight of 87.0 kDa, is an enzyme found in heart muscle. Creatine Kinase is a dimeric molecule made up of two subunits labeled "M" and "B" that combine to generate three isoenzymes: CK-MM, CK-BB, and CK-MB. CKMB is the Creatine Kinase isoenzyme that is particularly active in cardiac muscle tissue metabolism. Within 3-8 hours of the onset of symptoms, the release of CK-MB into the blood can be identified. It peaks in 9 to 30 hours and then drops back to baseline in 48 to 72 hours. CK- MB is one of the most essential cardiac indicators and is widely known as the conventional MI marker. The Atlas One Step CK-MB Test Device (Whole Blood/Serum/Plasma) is a simple test that detects CK-MB in whole blood, serum, or plasma using a combination of anti-CK-MB antibody coated particles and capture reagent. The detection threshold is set at 5 ng/ml **(*MATERIALS*, n.d.)** . CK-MB levels rise in response to myocardial cell injury and can be detected four to eight hours after the beginning of chest discomfort, peaking at 18-24 hours and reverting to baseline in 24 to 48 hours. Increased CK-MB levels show a high specificity for myocardial infarction, and early clearance can assist detect re-infarction. The CK-MB (creatine kinase myocardial band) is widely available and used in resource-poor places around the world. Increased CKMB levels have been linked to a greater mortality rate in AMI patients. **(Carvalho & Rassi, 2016).** Subsequent prospective clinical trials have confirmed that serial CK-MB results can be provided during ED chest pain patient evaluation.**(Hedges, 1995)**

1.9.2 Cardiac troponins

the cardiac troponin (cTn) assays. Whilst troponin is found in all forms of striated muscle, troponin in the heart is distinguished by regions of different amino acid sequences. Identifying the subtle dissimilarities between cardiac and skeletal

troponin enabled the raising of antibodies against specific epitopes. These antibodies were exploited to develop myocardial-specific assays. cTn assays have been regarded for the past decade as the gold-standard biomarker for detecting acute myocardial necrosis, the pathological hallmark of acute myocardial infarction (AMI). **(Park et al., 2017)**

Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue. They are not present in smooth muscle tissue. With the proteins actin and tropomyosin, they are part of the thin filaments within the myofibrils and are essential for the calcium-mediated regulation of muscle contraction. The troponin complex consists of 3 interacting and functionally distinct proteins (troponin I, T, and C) **(Wells & Sleeper, 2008)**

There are three parts to the troponin complex:

Troponin C (TnC):is a calcium-binding protein that regulates thin filament activation during contraction by eliminating troponin I inhibition. It has an 18 kDa molecular weight.

Troponin I (TnI):is an inhibitory subunit of actinomyosin that suppresses its ATPase activity. It has a molecular weight of 22 kDa and is encoded on the 19q13.3chromosome.

Troponin T (TnT): is a structural component that connects the troponin complex to tropomyosin and serves a structural role. Actinomyosin ATPase activity is also activated by TnT. Its gene is found on chromosome 1q32 and has a molecular weight of 37 kDa **(Sodi, 2006)**

1.9.3 Lipid Profile

The pathogenesis of acute myocardial infarction (AMI) is multifactorial; however, several studies have implicated impaired lipid metabolism as one of the crucial factors in the development of this disease. observed signicantly higher total cholesterol (TC) and triglyceride (TG) levels and lower high-density lipoprotein cholesterol (HDL) levels in AMI patients. In a series of 50 male AMI patients, serum low-density lipoprotein cholesterol (LDL) levels and the ratio of LDL to HDL**(Khan et al., 2013**). However, more and more clinical trials have revealed that after controlling for deterministic risk factors such as LDL-C, the risk for coronary heart disease (CHD) remained, while the increase in triglycerides (TG) was

significantly correlated with the increase in mortality, the incidence of myocardial infarction (MI) and the recurrence rate of coronary artery disease**(Jiao et al., 2018)** Lipids, which include phospholipids, cholesterol, triglycerides (TG), and fatty acids, are considered essential to the human body because they form the basic structure of cell membranes (phospholipids), act as a precursor to steroid hormones, bile acids, and vitamin D, and are a constituent of cell membranes, influencing their fluidity and activation (cholesterol). TG, on the other hand, is made up of three fatty acids coupled to a glycerol molecule and is deposited in adipose and muscle tissue as one of the most important forms of energy storage in the body. **(Freitas et al., 2013).** According to epidemiological studies, atherosclerosis caused by dyslipidemia is directly linked to an increased risk of IHD. Hypercholesterolemia, specifically high cholesterol levels in low-density lipoproteins, has been associated to coronary artery disease (CAD) (LDL-C). Patients with low plasma levels of high-density lipoprotein (HDL-C) cholesterol have a higher risk of AMI **(N. Kumar et al., 2019)**

1.9.4 Glucose levels

Hyperglycemia's significance in the development of cardiovascular problems in myocardial infarction (MI) patients is still controversial. In the medical literature, there are two major perspectives on the role of hyperglycemia. Hyperglycemia is thought to be caused by the activation of adrenergic receptors in patients with acute coronary syndrome (ACS). Another hypothesis is that hyperglycemia in MI patients is a sign of pre-existing carbohydrate metabolism problems that have not to be recognized **(Karetnikova et al., 2016)** .Hyperglycemia on admission in patients with acute coronary syndromes (ACS) is common, and it is a powerful predictor of survival and increased risk of inhospital complications in patients both with and without diabetes mellitus. Despite the findings from prior studies, many gaps in knowledge currently exist in our understanding of the association between elevated glucose levels and adverse outcomes in patients with ACS(artery coronary syndrome **) (Deedwania et al., 2008)**

1.9.5 Family History of Myocardial Infarction

family history of myocardial infarction (FHMI) is an established risk factor for MI, and atherosclerotic risk factors (eg, smoking, hypertension, hypercholesterolemia,

obesity, and diabetes mellitus) are known to modify the association between FHMI and MI slightly. Recently, FHMI has also been shown to be associated with increased risk of venous thromboembolism (VTE) **(Lind et al., 2014)**

1.9.6. Genetic study

1.9.7. Genetics and myocardial infarction

Genomic medicine is having an impact in several medical fields, including oncology, pharmacology, and cardiology, among others. Genetic information is increasingly being used as part of individual clinical care, and genomic medicine is having an impact in several medical fields, especially in rare and undiagnosed diseases. The Human Genome Project (HGP) has made it possible to use genomic information to enhance health, but translating new discoveries into patient treatment can take years. Since the completion of the HGP, researchers have concentrated on determining how variations in an individual's DNA affect disease and health, elucidating disease etiologies and prognoses, identifying disease susceptibility variants, and improving the efficacy and safety of pharmacological treatments **(Melo , 2018).**

A positive family history for myocardial infarction is known to be a major cardiovascular risk factor. As a result, current European guidelines propose that siblings and children of people who have had a MI receive increased primary prevention. Although the genes underpinning the heritable component of MI were previously unknown, the introduction of modern molecular genetic approaches, particularly genome-wide association (GWA) research, has led to the discovery of multiple genetic variations linked to an increased risk of MI.**(Erdmann et al., 2010).** Years of experience in conducting wide-genomic association studies (GWAS) have demonstrated that polygenic inheritance of common genetic variants with small effect is a significant part of the risk of developing multifactorial diseases. Based on GWAS data, a polygenic scale has been developed that allows the risk of developing, early MI. It was shown that in patients with early MI, a high polygenic score (inheritance of many genetic variants with small **(Goncharova et al., 2020)**

1.9.8. Von Willebrand factor (VWF)

Is a multimeric protein that aids in hemostasis by promoting platelet adhesion and thrombus formation in the aftermath of vascular injury. Von Willebrand disease, a bleeding illness defined by quantitative or qualitative defects in VWF, demonstrates this function of VWF. VWF can be found in the blood, platelets, and endothelial cells. It is stored in Weibel-Palade bodies in endothelial cells as ultra large VWF (UL-VWF). **(Rutten et al., 2015).** Von Willebrand factor is synthesized in vascular endothelial cells and then released into the plasma as unusually large VWF multimer (UL-VWFM), 4which has most potent Biological activities interacted with platelet, and is rapidly degraded into smaller VWF multimers by ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13),4,9a metalloproteinase that specifically cleaves multimeric VWF between Tyr1605 and Met1606 within the VWF A2 domain. 9Loss-of-function mutation of ADAMTS13 leads to Upshaw–Schulman syndrome**,(Horii et al., 2008).** vWF exists in the bloodstream in one of two conformations—globular and unfolded. vWF conformation depends on the shear rate of blood flow in vessels. The shear rate is the rate of change of velocity ,at which one of layer of fluid passes over another and it measured in inverse or reciprocal , seconds (s^{-1}) **(Okhota et al., 2020).**

vWF also binds to blood coagulation factor VIII, another blood clotting protein, and acts as its carrier in the circulation . In addition to homeostasis vWF has recently beenrecognized as a critical regulator in angiogenesis, inflammation and cell proliferation. The vWF gene is located on chromosome 12p and comprises 52 exons spanning ~178 kb of genomic sequence. Mutations in the vWF gene are responsible for von Willebrand disease a bleeding disorder that prolongs the blood clotting process**(Li et al., 2015)**

1.9.9 VWF in Vascular Inflammation

The VWF/ADAMTS13 axis exerts a pivotal role in vascular inflammation and thrombosis. Thrombosis, with the recruitment of platelets to the site of vessel's injury, and immune response, with the recruitment of leukocytes in inflamed tissues, have traditionally been considered two distinct pathways. **(Gragnano et al., 2017)**. An further issue is that elevated VWF levels are not specific for an inflammatory

acute phase response, but may also be regarded as an indicator of vascular dysfunction**(Kawecki et al., 2017)**

1.9.9.1. ADAMTS 13

ADAMTS disintegrin-like metalloproteinases with thrombospondin type 1 motifs are a family of proteinase enzymes found in the ECM and plasma. The ADAMTS family consists of 19 enzymes with various functions. vWF encompassing a wide range of physiological activities. ADAMTS13, also known as von Willebrand factor cleaving protease, is one of the most well-known members. It cleaves abnormally large von Willebrand factor (UL-VWFM) multimers (UL-VWFM) into small VWF fragments, which lowers VWF coagulation activity. Endothelial cells (ECs) and hepatic cells are the main locations for the synthesis and secretion of ADAMTS13 **(Akyol et al., 2016)** . Clinical studies revealed that low ADAMTS13 (and high VWF) levels are associated with an increased risk of MI. ADAMTS13 polymorphisms that decrease ADAMTS13 activity are also associated with an increased risk of death in patients with coronary artery disease**(De Meyer et al., 2012).**

ADAMTS13 not only inhibits thrombogenesis by inactivating VWF during its release, but it also inhibits thrombogenesis by cleaving VWF after vascular injury. The distal carboxyl-terminal domains of ADAMTS13 are necessary for the regulation of thrombus development, according to previous study. Increased levels of inactive VWF in the blood have been linked to a variety of cardiovascular disorders, and researchers are working to create inhibitors of VWF's interaction with platelets**(Rutten et al., 2015).**

A lack of ADAMTS-13 leads to thrombotic thrombocytopenic purpura, in which large multimers of vWF accumulate and induce platelet aggregation and vascular occlusions.

Furthermore, cleavage of vWF by ADAMTS-13 can attenuate thrombus formation in atherosclerotic plaques**(Whan et al., 2012)**

Figure (1-4): Schematic representation the domain structure of ADAMTS13. Signal peptide (S), propeptide (P), metalloprotease (M), disintegrin-like (D), TSP1 (T), cysteine-rich (Cys), CUB (C) domains (Sargent et al., 2017)

Regulation of ADAMTS-13 function The modulation of coagulation enzymes by cofactors is well known, and it enhances the rate of an enzymatic process by several orders of magnitude. ADAMTS-13 is released as a constitutively active protease, unlike other clotting factors, which are produced as inactive zymogens. To date, no inhibitor has been discovered. Many other matrix metalloproteases, such as ADAMTS-4, -5, -7, and -12, are inhibited by plasma a2- macroglobulin, however it does not appear to bind and impair ADAMTS-13 action toward VW. As a result, the function of ADAMTS-13 must be controlled at the substrate level. **(Zheng, 2013).** Severe deficiency of ADAMTS13 (such as autoantibodies, thrombotic thrombocytopenic purpura) leads to circulating ULVWF and, thus, aberrant platelet aggregation, thrombus formation and microvascular occlusion**(Kling et al., 2008)**

Aim of the Study:

To see the serial variations in the levels and gene polymorphism of thrombo-inflammatory biomarker von Willebrand factor (VWF) and A Disintegrin and Metalloprotease with Thrombo-Spondin motif (ADAMTS13) over the course of ST-elevation myocardial infarction and to determine their relationship with the another cardiovascular risk markers in Iraqi patients.

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1. Subjects

The current study is case-control study comprised from 42 subjects of the patients have st-elevation myocardial infraction as cases and 48 apparently healthy as control groups. The sample collected from karbala heart center in the AL-Imam AL_Hussein medical city in the period from20-1-2021 to 1-3-2021 ,the parameters is done worked in the laboratory of AL-Imam AL_Hussein medical city. The research was done in the molecular laboratory of Biochemistry Department.

-Inclusion Criteria:

ST-elevation myocardial infarction patients

-Exclusion Criteria:

Suffering of subjects from kidney disease, Liver disease, cancer, pregnancy and lactating mothers, cerebrovascular accidents, alcoholics, rheumatoid arthritis, autoimmune disease, patients with type 1 diabetes mellitus were excluded.

2.1.1. Patient group

The patients of current study have st- elevation MI to detected Von Willebrand Factor Gene Polymorphism in patient in age ranged between (25–70) years.

2.1.2. Control group

The control group was healthy from myocardial infraction, their ages ranged between (25–70) years. They were collected from the blood collection unit at Imam Hussain Medical Hospital, after making sure that they are not infected with any diseases

2.1.3. Blood sampling

Five milliliters of blood ware drawn by vein puncture from all individuals participated in this study after taking the patient's consent. The collected blood was divided into three parts:

1. One ml of blood that used for gene analysis, collected in EDTA containing tube and used for DNA extraction, after that, they were directly tested to obtain high-purity DNA.

2. One ml placed in EDTA containing tube for analyzing blood sugar test and blood group.

3. Three ml of blood placed in gel tube. It was left 15 minutes at room temperature for coagulation. Blood was centrifuged for 15 minutes at 3000 xg. Serum was collected then frozen till analyses for measuring the test (Troponin I, Ck-mb, Lipid profile) and measure serum level vwf and Metalloprotease with Thrombo-Spondin motif (ADAMTS13) using ELISA technique

The samples were put it in package containing ice for frozen samples after collected and transfer it to the laboratory

2.2. Materials

2.2.1. Chemicals

Chemical and kits used in current study are presented in table (2.1)

Table 2.1: The kits and chemicals

NO	**Chemicals**	**Source (Country)**
1	Genomic DNA extraction kit	**Geneaid (UK)**
2	Master Mix Kit	**Sentol (Russia)**
3	Nuclease free water	**Promega (U.S.A**

4	Primers	**AlphaDNA(Canada)**
5	100 - 1000 bp DNA leader	**Sentol (Russia)**
6	Ethidium bromide	**promega (U.S.A)**
7	Agarose analytical grade	**promega (U.S.A)**
8	Tris borate EDTA (TBE) Buffer x10	**Promega (U.S.A)**
9	ADMATS13 kit	**China**
10	Vwf kit	**American**
11	Lipid profile kit	**China**
12	HS Troponin T kit	**China**
13	Ck-mb kit	**China**

2.2.2. Apparatus and Equipment's

Apparatus and Equipment's used in current study are presented in table (2.2).

Table 2.2: Instruments and apparatus

NO	Apparatus	Source
1	Autoclave	Hirayama (Germany)
2	Bench Centrifuge	Hettichi (Germany)
3	Gel electrophoresis system	Biometra (Germany)
4	Hood	C.B.S scientific (USA)
5	Magnetic stirrer	Japan
6	Minispin centrifuge	Eppendorf (Germany)
7	Oven	Binder (Germany)
8	PCR-thermocycler	Biometra (Germany)
9	Photo documentation	UVP (UK)
10	Rotater (Rotisserie mixer)	GreinerLaborgerate (England)
11	Sensitive balance	Sartorius (Germany)
12	UV Transilluminator	USA
13	Vortex mixer	Cyan (Belgium)
14	Water bath	Memmert (Germany)
15	CST180 Auto Chemistry Analyzer	China
16	cobas e 411 analyzer	China

2.3. Methods

2.3.1. Determination of Serum human von willebrand factor cleaving protase (ADAMTS_13)

This test to determined the quantitative detection of adamts-13 in serum

Assay Principle

this kit is an enzyme linked immunosorbent assay (ELISA). The plate was already coated with human ADAMTS13/vWF-cp present in the sample is added and binds to antibodies coated on the wells and after that biotinylated human ADAMTS-13/vWF-cp antibody is added and binds to ADAMTS-13 in the sample, then streptavidin-HRP is added and binds to the biotinylated ADAMTS13/vWF-cp antibody. after incubation unbound streptavidin-HRP is washed away during a washing step. Substrate soulation is then added and color develops in proportion to the amount of human ADAMTS13/vWF-cp. The reaction is terminated by addition of acidic stop solution and absorance is measured at 450 nm.

Reagent Preparation

- Before using, all reagents should be brought to room temperature.
- Standard to make a 40ng/ml standard stock solution, reconstitute 120 μg/L of the standard (80ng/ml) with 120 μg/L of standard diluent. Before producing dilutions, allow the standard to sit for 15 minutes with gentle agitation.
- Prepare duplicate standard points by serially diluting the standard stock solution (40ng/ml) 1:2 with standard diluent to produce 20ng/ml, 10ng/ml, 5ng/ml and 2.5ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any the remainder of the solution should be frozen at -20°C and utilized within one month of freezing.
- Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

Procedure

1. All reagents were prepared, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2.The number of strips required for the assay is determined. Strips were inserted into tires for use. Unused strips were stored at a temperature of 2-8 ° C
3. A standard 50 µl was added to the standard well.
4. A 40 µl sample was added to sample wells, then 10 µl of ADAMTS-13 / vWF-cp antibody is added to. Well samples, then add 50 µl of Streptavidin-HRP to sample wells and standard wells (not empty Control well). Mixing well and cover the board with sealant. Incubate 60 minutes at 37 ° C.
5. The seal was removed and the panel is washed 5 times with a washing solution. Soak the wells with a ratio of at least 0.35ML wash buffer for 30 seconds to 1 minute per wash. For machine wash, pull out all Wash and wash 5 times with wash buffer, and fill wells with wash buffer. Smudge painting On paper towels or other absorbent materials
6. Add 50 µl of substrate A solution to each well, then add 50 µl of substrate B solution to each.
7. Add 50 µl of stop solution to each well, and the blue color turns yellow immediately.
8. The optical density (OD value) of each well is determined immediately using a microplate reader Set to 450 nm within 10 minutes after adding stop solution.

2.3.2. Determination Human von willebrand factor (vwf)

Reagent preparation:

20 x wash solution: Dilute with distilled or deionized water 1:20
١. All reagents were prepared before starting assay procedure
2. add standard: set standard wells testing sample wells, add standard 50 µl to standard wells
3. sample was add: add testing sample 10 µl , and then add sample diluent 40 µl to testing sample well ,blank well doesn’t add anything

4. 100 µl HRP conjugate reagent is added to each well, covered with adhesive and incubated for 60 min at 37 °C

5. Withdrawing each well and washing it, the process is repeated four times for a total of five washes. After the last wash, any remaining washing solution is removed by suction or decanting, Invert the plate and wipe with clean paper towels

6. Chromogen A 50 µl solution and Chromogen B 50 µl solution were added to each well, mixing gently and incubated for 15 minutes at 37 ° C, keeping away from light.

7. 50 µl stop solutions was added to each well, in which the color of the well was changed from blue to yellow

8. read the optical density at 450 nm using a microtiter plate reader within 15 minutes**(Lorenz, 2012)**

2.3.3. Determination Lipid Profile

Cholestrol reagent was applied to the invitro quantitive determination of total cholesterol concentration in human serum **(*Tc.Pdf*, n.d.)**

Triglycerides is applied to the invitro quantitive determination of triglycerides concentration of serum and plasma.triglycerides belong to lipid substance which can be absorbed feom food or generated from the internal metabolic of carbohydrate **(*Tg.Pdf*, n.d.)**

the significance of triglycerides testing was for the dia Using a device CS-T180 Auto- Chemistry Analyzer the level of lipid profile was measured, by taking 50 microliters and placing them in the device and entering the samples information, the measurement was done and the results were read, was measured TG ,TC ,HDL,LDL,VLDL

2.3.4. Determination Of Creatine Kinase

Creatine kinasewas applied to the invitro quantitive measurement (Ck) activity of human serum or plasma.ck is the dipolymerase comprised by monomer subunits of M(muscle) and B(brain) mode **(*Ck.Pdf*, n.d.)** The test method of CK-MB It was done using the CS-T180 Auto- Chemistry Analyzer device using 500 µl and the information of the samples was entered and the results were read

2.3.5. Determination Of High Senstive Troponin T

In vitro quantitative measurement of cardiac HS troponinT in human serum and plasma using an immunoassay. This assay is intended to aid in the diagnosis and treatment of myocardial infarction and cardiac muscle damage. Cardiac troponin I determinations aid in the risk stratification of patients with unstable angina pectoris or non-ST-segment elevation acute coronary syndrome with respect to relative risk of mortality, myocardial infarction, or increased probability of ischemic eventsrequiring urgent revascularization procedures **(Cobas, 2019).**

The test method of troponin it was done using the Cobas e411 device using 500 µl and the information of the samples was entered and the results were read

2.4. DNA extraction

In EDTA tubes, blood samples are obtained from patients and healthy controls.then DNA was extracted from whole blood by using (Geneaid kit) according to the kit instruction**.**

Quick Protocol Diagram:

1.Sample preparation and cell lysis
2.DNA binding to membrane while contaminants remain suspended
3.Wash for removal of contaminants while DNA remains bound to membrane
4.Elution of pure genomic DNA which is ready for subsequent reactions.

Procedure:

1. Sample Preparation:

The frozen blood samples are left to completely thaw. completely mixed the blood samples for at least 10 minutes in a rotisserie shaker at room temperature. Transfer up to 200 µl of whole blood to a 1.5 ml microcentrifuge tube. Adjust the vol ume to

200 µl with distilled water. and then is added 20 µl of Proteinase K. the sample tubes are Incubated at 60ºC for 5 minutes.

2. Cell Lysis:

A 200 µl of GSB Buffer is added to the tube then mixed by vortex. Then the sample tubes are Incubated at 60ºC for 5 minutes, inverting the tube every 2 minutes. During incubation, is transferred the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).

3. DNA Binding:

A 200 µl of absolute ethanol is added to the sample lysate and mix immediately by shaking vigorously for 10 seconds.A GS Column was Placed in a 2 ml Collection Tube. Then is transferred all of the mixture to the GS Column. and put them in Centrifuge at 14-16,000 x g for 1 minute. The collection tubes containing flow-through were removed, and the liquid discarded. then was transferred the GS Column to a new 2 ml Collection tube.

4. Wash:

Wash Buffer 400 µl was added to the GS Column. then is put in Centrifuge for 30 seconds then discarded the flow-through. the GS Column is placed back in the 2 ml Collection Tube. A 600 µl of Wash Buffer is added to the GS Column. and put in Centrifuge for 30 seconds then discard the flow-through. the GS Column is placed back in the 2 ml Collection Tube. then is put in Centrifuge for 3 minutes to dry the column matrix.

5. Elution:

The dried GS Column is transferred to a clean 1.5 ml microcentrifuge tube. 100 µl of pre-heated Elution Buffer1 is added into the center of the column matrix. then was leaved for at least 3 minutes to allow Elution Buffer to be completely absorbed. then was putted in Centrifuge for 30 seconds to elute the purified DNA.

Figure (2.1): DNA Extract

2.4.1 Amplification refractory mutation system – ARMS-PCR

The allele-specific PCR also called as an ARMS- PCR (amplification refractory mutation system) or PASA (PCR amplification of specific alleles) or AS-PCR used to detect the SNPs **(Darawi, Ai-Vyrn et al. 2013),** ARMs – PCR protocol was used for SNPs detection of Vwf gene

2.4.1.1. Principles of ARMS-PCR

Amplification Refractory Mutation System PCR (ARMS-PCR) provides an exciting methodology to simultaneously detect and type haplotype/single nucleotide polymorphism based on differential size of PCR products for a particular allele when resolved on agarose gel electrophoresis**(Chander et al., 2016)**. Template DNA, primers, nucleotides, and DNA polymerase are all required for each PCR assay. The DNA polymerase is the enzyme responsible for joining individual nucleotides to generate the PCR product. The nucleotides include the four bases – adenine, thymine, cytosine, and guanine (A, T, C, G) – that are found in DNA. These serve

as the building blocks for the DNA polymerase to utilise in producing the PCR product. the primers in the reaction specify the exact DNA product to be amplified. The primers are short DNA fragments having a specific sequence that complements the target DNA to be identified and amplified. These serve as a base for the DNA polymerase to construct upon **(Carriço et al., 2013)**

The basic ARMS-PCR steps are :- Initiation step, Denaturation step, Annealing step, Extension / Elongation step, Final Extension **(Lorenz, 2012)**

2.4.2. Primers for PCR

Polymorphisms	SNIPs	Primers	Base Pair	GC%	Tm	Product size
vWF gene (rs216311)	**T allele (wild) Forward**	**CCAACGGATGTCCCGGAACTTT**	**22 bp**	**55%**	**61c**	**458 bp**
	A allele Forward	**CCAACGGATGTCCCGGAACTTA**	**22 bp**	**55%**	**61c**	
	Common Reverse	**AGTACTGCAGCACCGTGACGT**	**24 bp**	**42%**	**57c**	

Table 2.3: primer sequence for three SNPs of VWF gene, number of base pair (bp) of primers, number of product size of three genes and the percentage of number of guanine and cytosine (GC)

2.4.3. Primers reconstitution and dilution

The primers are lyophilized before use. A mass in Picomoles is the unit of measurement for a lyophilized primer. The subsequent steps are done for the reconstitution and dilution of the primers:

• The tube is centrifuged at 10000 xg for 5-10 min before decapping.

•The chosen volume from nuclease free water are added according to the manufacturer to obtain a 100 pmoles /µL (master stock).

• The Primers are re- mixed by suitable vortexing.

• To obtain a 10 pmoles/L concentration, ten microliters of the master stock are transferred to a 0.5 mL eppendorf tube containing 90 mL of nuclease-free water (working stock). Both the master and working stocks are held at -20 degrees Celsius. The working stock is warmed up and kept on ice for use in PCR and then stored at -20 ^{o}C after each use.

2.4.4. ARMS-PCR master mix

PCR Master Mix includes Nuclease-Free Water and PCR Master Mix, 2X. PCR Master Mix is a premixed, ready-to-use solution that contains appropriate quantities of Taq DNA polymerase, dNTPs, MgCl2, and reaction buffers for effective PCR amplification of DNA templates **(Promega Corporation, 2012)**

2.4.5. Optimization of ARMS-PCR conditions

The following methods are used to optimize PCR conditions:

_ Different volumes of primer (1 µL, 1.5 µl)

_ Different volumes of template DNA (3 µl, 5 µl)

_Different annealing temperatures. (58°C, 59°C, 60°C, 65°C).

The preferred condition which provided the best result was addition of:

1. Thirteen µL master mix

2. 1.5 µL outer forward primer

3. 1.5 µL inner reverse primer

4. 4 µL DNA sample

5.5 µL nuclease free water. A total reaction volume is a 25 µL that added to the 500µl

PCR tube at 25° C then centrifuged for 30 seconds at 2000 xg in a micro centrifuge for mixing the sample tubes and then placed in thermocycler.

2.4.6. Thermocycler program for DNA amplification

To obtain the the best results of amplification of alleles of VWF gene, The two alleles put in one program of the PCR thermocycler is shown in table (2.4) and the melting temperature for primer 50 – 65 C.

Table 2.4: Thermocycler program for DNA amplification

Type of Cycle	Temperature °C	Time	No. of Cycles
Initial denaturation	95	4 min	1 cycle
Denaturation	95	25 sec	35 cycle
Annealing	65	35 sec	
Extension	72	55 sec	
Final extension	72	5 min	1 cycle
Hold	12	5 sec	

Total time: 1 hours and 45 minutes

2.4.7. Agarose gel electrophoresis

Agarose gel electrophoresis is a widely used procedure in various areas of biotechnology. This simple, but precise, analytical procedure is used in research, biomedical and forensic laboratories. Of the various types of electrophoresis, It is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it is a convenient analytical method for

determining the size of DNA molecules in the range of 500 to 30,000 base pairs**(Taha, 2016)**

Procedure: A 1.5 g agarose in 100 ml of a solution was prepared by the following steps

A. Preparation of solution

10X TBE buffer (tris borate EDTA) was dilute with deionized water to make 1X TBE buffer (tris borate EDTA). (one volume of 10X TBE buffer with 9 volume of deionized water: 1:10 dilution).

B. Preparation of agarose gel

1. Firstly 1. 5 g of agarose were weighted and placed into a conical flask, and then added 90 ml of distilled water and 1o ml of 1X TBE bufferThis gel was utilized to identify the PCR product's band.

2. While waiting for the gel solution to appear clear and pure, the solution was agitated over a hot plate, then allowed to warm before being poured into the tray.

3. At 60-65°C, five liters of stock ethidium bromide (10 mg/mL) were added to the gel, resulting in a fluid content of 0.5 Lml.

4. Adhesive tape was used to seal the ends of the gel chambers

5. About 1 inch from one end of the tray, a comb was placed into the gel chamber

6. A gel solution was put into the chamber and allowed to solidify at room temperature for around 30 minutes.

7.The comb was removed, and the chamber was placed in a horizontal electrophoresis tank filled with TBE buffer, which was utilized for the gel agarose preparation

8. Approximately 4 ml of sample was placed into each well with extreme caution to avoid well damage and cross contamination of nearby wells.

9. The negative pole was connected to the unit's negative side, while the positive pole was connected to another.

10. According to the size of the DNA fragment, electrophoresis was performed at (70 volts for 1.5 hours) and (80 volts for 60 minutes) or while waiting for dye indicators to travel to the necessary distance.

Loading of samples: The comb was removed after the gel had become clear and solid, and the glass plates were placed in a submarine horizontal gel electrophoresis chamber. 1 X TBE buffer was added to the chamber until it reached above the level of the wells, and the sample was loaded into the gel wells using a 4l micropipette.

Electrophoresis conditions:

An electric field (70 volts for 1.5 hours) and (80 V for 1 hour) were applied to the system after the sample was applied, allowing the negatively charged nucleic acids to migrate across the gel to the positive electrode (anode)

2.4.8. DNA ladder

In current study, 4 μL of DNA ladder was used [Sentol (Russia)] and band size ladder was 100- 1000 bp. Without the need for sample loading dye, the ladder type employed in this investigation can be given directly.

2.4.9. Gel- band visualization

The agarose gel was placed in a UV transilluminator device and exposed to UV light to view the DNA bands, and images were taken with a digitcaera connected to a PC

Figure (2.2): Prepare agarose gel

2.5. Statistical analyses

Analysis of data was carried out using the available statistical package of SPSS-24 (Statistical Packages for Social Sciences- version 24). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means Pearson's correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate strength, >0.7 strong correlation. In addition to correlation the r2 was calculated (The coefficient of determination), i.e. when value of r=0.58, then r2=0.34, this means that 34% of the variation in the values of y may be accounted for by knowing values of x or vice versa Receiver Operating Characteristic "ROC" curve technique was used in order to determine the use of any parameter as diagnostic or screening tool for disease and the ability to determine the "cut-off value" for optimal sensitivity and specificity of a diagnostic test **.** Sensitivity refers

to the proportion of those who have the condition ,that received a positive result on this test.

Specificity refers to the proportion of those who do not have the condition ,that received a negative result on this test.

cut-off values are the dividing points on measuring scales where the test results are divided into different categories

CHAPTER Three

Results and Discussion

3. Results and Discussion

Biochemical Study

3.1 Demographic and clinical characteristics of the study groups

The clinical demographic characteristics and laboratory parameters of both patient groups and the healthy control group were summarized in table (3.1). Gender distribution among the studied groups were: 30 male, 12 female for patient group, while 32 male and 15 female for control group. and the blood group divide to three group (A group 51 % , O group 17% and B group 34%) and three group in patient(A group 61.9% , O group 21.5% and B16.5%)

Table 3.1: The relationship between age, gander, body mass and blood group

	Controls (n= **48**)	**Patients** n=(42)
Age Range (year)	25-75	25-75
BMI (medain)	24	26
Blood group n (%)	A group 24 (51%) O group 8 (17%) B group 16 (34 %)	A= 26 (61.9%) O= 9 (21.5%) B= 7 (16.5%)
Gander Male, Female n (%)	32 (68%), 15 (32%)	30 (71.5 %), 12 (28.5%)

3.2 Number of ST- Elevation patients based on age groups

The age groups that appeared in figure (3.1) divided into two group
A_ forty two patients their age range25-75
B_ forty eight healthy subjects as control group age range 25-75

The current study, found that the proportion of men with st elevation MI is greater in men than women.
While other studie was found age were higher in females than in male with a higher incidence of heart failure in females than in males In addition, the mortality rate was higher in female than in male patients **(Alharbi et al., 2020).**
Clinical profiles and presentations differ between women and men with AMI. Women have less typical symptoms of AMI than men **(Kosuge et al., 2006).**

3.3 Blood Group relationship between patients and controls group

The results of present study showed the distribution of sample according to the blood group. It was found that the total number of patients who have blood group A is the largest percentage than O and B in patients with St- Elevation MI.
While other studies found patients with blood group A had a greater risk of heart rupture HR after AMI than those with other blood groups **(Fu et al., 2020)** .
Patients with blood groups A and O were at a higher risk, and there was a substantial difference in risk between blood groups A and O and B and AB. **(Hassan et al., 2017)**

3.4 Correlation the difference between biomarkers among study groups

In table (3.2) there was a significant difference between blood glucose and lipid profile in st-elevation MI patient and control group (P value=o.oo1).
The current study, found he statistical significant increase in serum blood glucose level in patients, statistically significant with (P value 0.001)
In the current study, the blood glucose level was measured for diabetic and non-diabetic patients after making sure of the patient's medical history, where it was found that the rise in sugar is the largest percentage.

Table (3.2): Correlation the difference between biomarkers among study groups

			P Value
	Patients n=(42)	Controls (n= 48)	
Sugar mg/ dl	203	97	< 0.05
Cholesterol mg/ dl	186	176	< 0.05
Triglyceride mg/ dl	184.46	162.94	< 0.05
HDL mg/ dl	29.8	36.2	< 0.05
LDL mg/ dl	113.75	95	< 0.05
VLDL mg/ dl	37	32.5	> 0.05

There was statistical significant increase in serum cholesterol, triglycerides and LDL with (P value < 0.05), while for VLDL(P value > 0.05) not statistically significant. Observed Patients with diabetes mellitus have higher levels of TG and LDL cholesterol,in a previous study, it say in STEMI patients a lower HDL-C was paradoxically associated with a lower risk of death during hospitalization **(Sia et al., 2020)**

3 .5 Correlation of ADAMTS13 Level with other biomarkers among St-elevation MI patients group

Table 3.3: Correlation of ADAMTS13 Level with other biomarkers among St-elevation MI patients group

Biomarkers	ADAMTS13 Level	
	r_s	P value
VWF Level	0.3	0.05
HS Troponin T	0.4	0.02
CK-MB	0.4	0.02
TC	0.5	0.0005
LDL	0.5	0.003
HDL	0.6	0.0024
VLDL	-0.3	0.03
TG	-0.3	0.07
B.sugar	-0.3	0.1

The nonparametric (Spearman rank test) (Coefficient rs) was used for the analysis of the difference in quantitative data between markers.

The current study observed weak positive relationship between ADAMT with VWF, HS Troponin T and CK-MB with P value ≤0.05 .

Strong positive relationship between TC, LDL and HDL with P value >0.001 and ADAMT, and weak negative relationship between VLDL, TG and B.sugar with ADAMT

The current study shown a significant increase CK-MB in serum. Other studies was found CK-MB is the second best marker in the absence of troponins assays**(Al-Hadi & Fox, 2009),** CK-MB represents an alternative when it comes to prognostic predictors of combined outcomes including myocardial re-infarction, re-intervention or death **(Carvalho & Rassi, 2016).**

other studies found the creatinine kinase (CK)-MB assay can be used for the early diagnosis of acute coronary syndrome**(Ota et al., 2020) .**

There was statistical significant increase in serum HS Troponin (p vale0.001), While other studies was opposite the serum level of HS Troponin along with the level of the CK-MB fraction is assessed for the diagnosis of myocardial infarction**(MYTHILI & MALATHI, 2015)** , It was found that a high level CK_MB and troponin as a sign of heart disease , the current study found that the proportion of patients with diabetes had higher levels of heart disease than patients who did not have diabetes.

Other studies found Most patients had elevated troponin levels, while Seven out of the total patients showed normal troponin levels and 35patient had markedly elevated troponin levels. High admission troponin in STEMI permits early identification of patients at increased risk of major cardiac complications and death**(Naser Zangana et al., 2017).** Mortality increases with elevated admission troponin levels, regardless of baseline clinical risk **(Wanamaker et al., 2019).** Troponin has both diagnostic and prognostic significance in the setting of acute coronary syndrome (ACS). Increased troponin levels in the absence of ACS should prompt an evaluation for an alternative, non-thrombotic mechanism of troponin elevation and direct management at the underlying cause **(Daubert & Jeremias, 2010)**

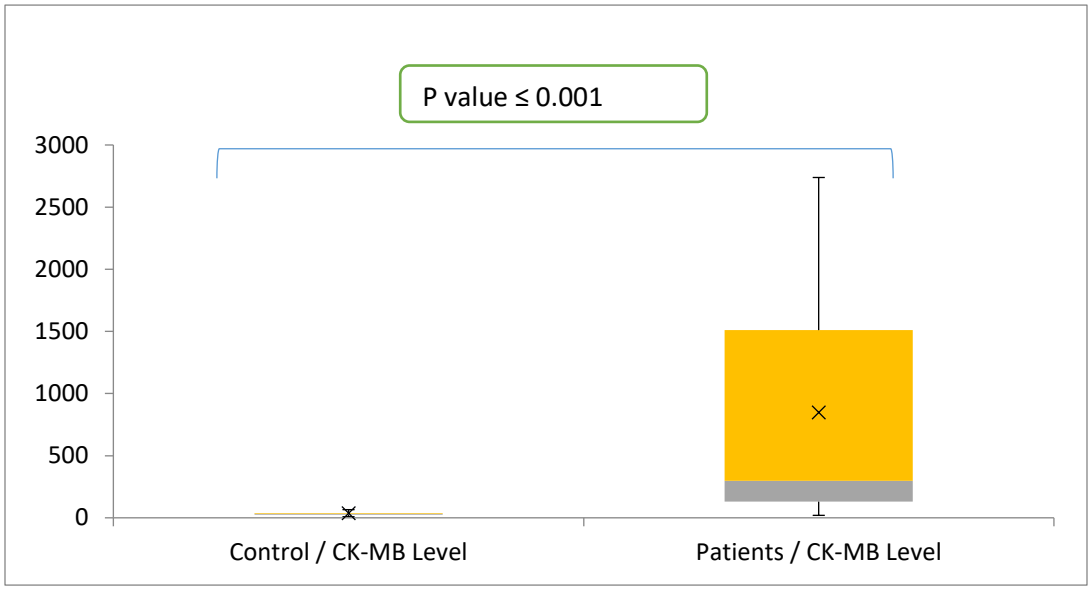


Figure (3.1): Distribution of serum CK-MB level in St-elevation MI patients compared to control group

the median levels of the CK-MB were higher in ST-Elevation MI Patient compared to control group with P value ≤ 0.001

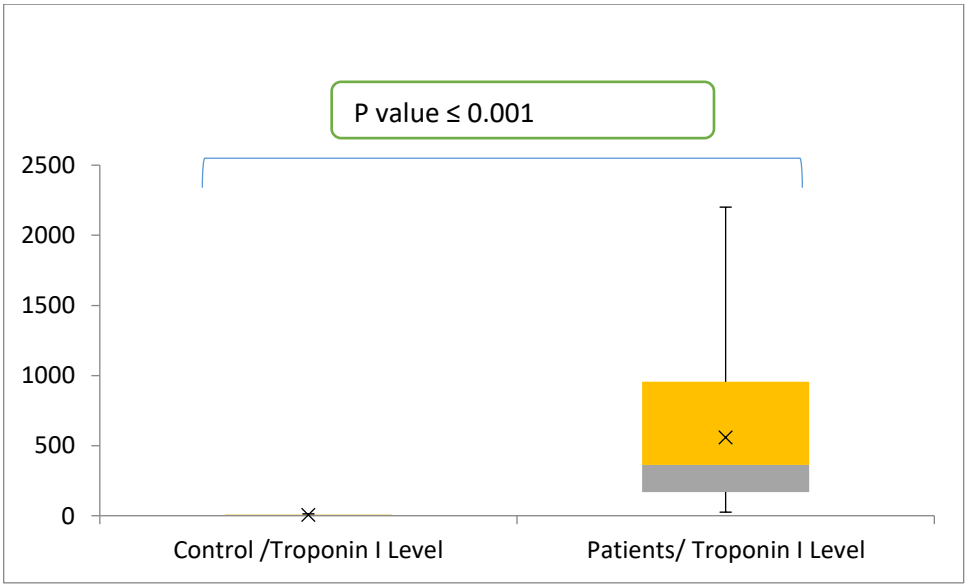


Figure (3.2):Distribution of serum Troponin T level in St-elevation MI patients compared to control group.

the median levels of the HS Troponin Twere higher in ST-Elevation MI Patient compared to control group with P value ≤ 0.001

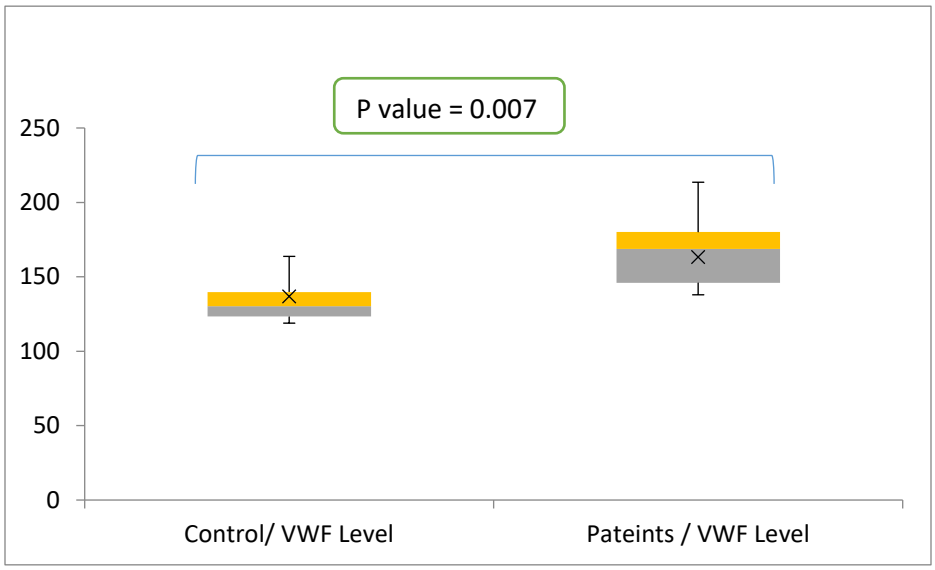
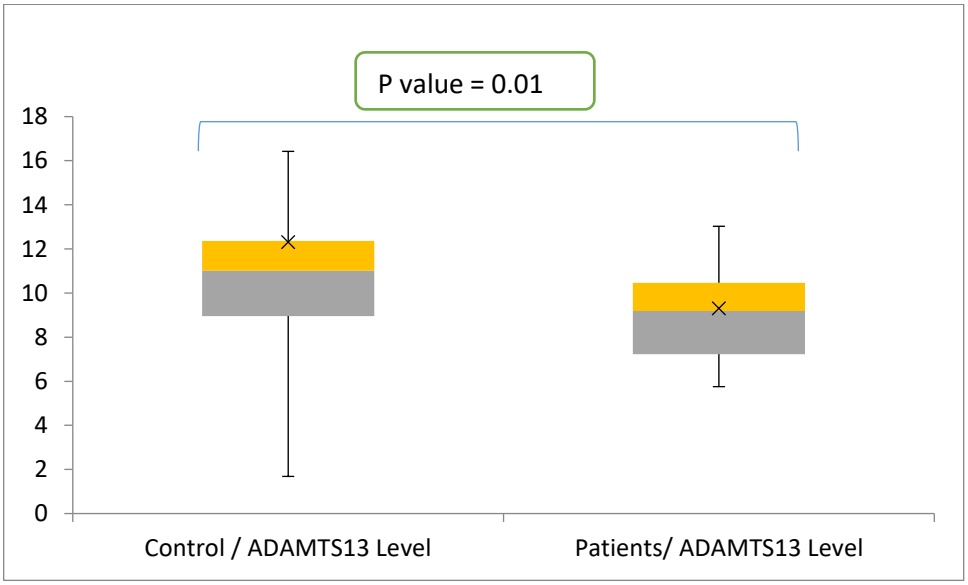


figure (3.3):Distribution of serum VWF level in St-elevation MI patients compared to control group.



Figure(3.4) Distribution of serum ADAMTS13 level in St-elevation MI patients compared to control group.

the median levels of theVWF level were higher in ST-Elevation MI Patient compared to control group with P value ≤ 0.001 and reduced level ADAM with P value =0.01

Other studies found no correlation was found between VWF/ADAMTS13 and infarct size in patients. However, patients suffering from IMH had significantly higher VWF activity and lower ADAMTS13 activity **(Eerenberg et al., 2016).**
The lowestADAMTS13levelswerefoundinacuteischemic stroke patients **(Denorme et al., 2017)**
In the current study there was statistical significant increase in serum VWF and reduced level ADAM, this result agree with studies found increased levels of VWF and reduced levels of ADAMTS13 activity may contribute to the pathogenesis of acute myocardial infarction and might prove to be important mediators of AMI progression **(Al-masri et al., 2020**). findings confirm the presence of VWF abnormalities in patients with STEMI and may be used to develop new therapeutic approaches **(Rutten et al., 2015)** , increased reduced levels of ADAMTS13 activity may contribute to the pathogenesis of acute myocardial infarction **(Al-Masri et al., 2020)**
The causal effect of lower ADAMTS13 activity on the increased odds of having cardiovascular diseases was coronary heart disease and myocardial infarction**(Ye & Zheng, 2021)**
A high concentration of von Willebrand factor was a novel index of increased risk for reinfarction and mortality in survivors of myocardial infarction**(Blann et al., 1992)**

3.6 Receiver Operating Characteristic "ROC" ADAMTS_13 And VWF

ROC curve was used to choose the most appropriate cut-off for the proposed marker. The best cut-off has the highest true positive rate together with the lowest false positive rate. Area under the ROC curve was measured for the usefulness of a test in general. The areas under ROC curves were used to compare the usefulness of tests.

The ROC test for the ADAMTS13 and VWF diagnostic performance in **St-elevation MI cases** was shown in Figures (3.4) & (3. 5).

Table (3.4) demonstrated the ADAMTS13 performance with (0.7) area under the curve (AUC) (95% CI: 0.566-0.787), 85% sensitivity and 62% specificity at > 8.2 cutoff value, whereas VWF showed a performance with (0.85) AUC (95% CI: 0.755- 0.938), 95% sensitivity and 31% specificity at 136.1 cutoff value as shown in table (3.4)

Table (3.4): AUC, optimal threshold, Sensitivity and specificity of ADAMTS13 **obtained by the ROC curves for prediction of St-elevation MI cases**

Test Variable	AUP	Sensitivity %	Specificity %	Cut-off points	CI (95%)
ADAMTS13	0.7	0.85	0.62	8.2	0.566 -0.787

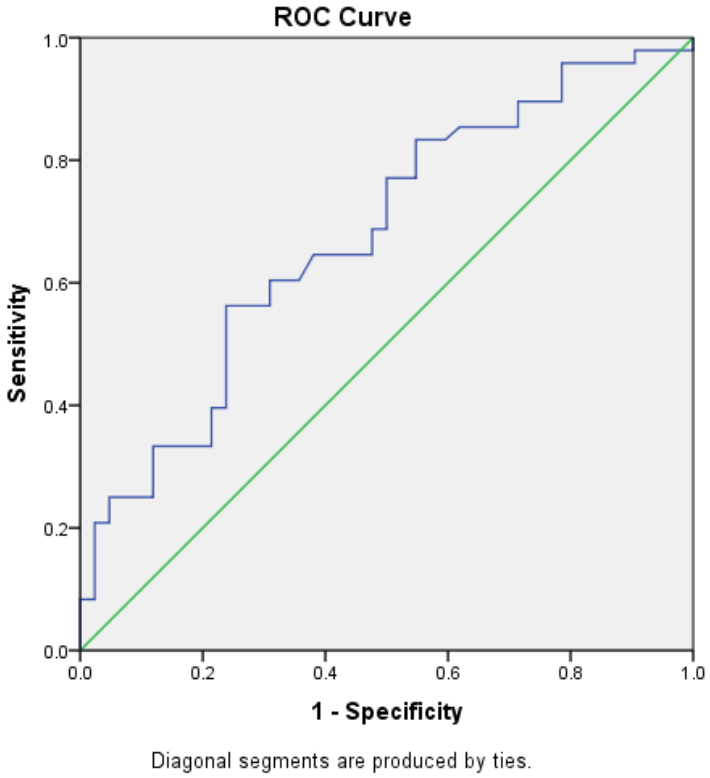


Figure (3.5) Receiver operating characteristics (ROC) curve analysis of ADAMTS13 in St-elevation MI cases

	AUP	Sensitivity %	Specificity %	Cut-off points	CI (95%)
ADAMTS13	0.7	0.85	0.62	8.2	0.566 -0.787

Table (3.5): AUC, optimal threshold, Sensitivity and specificity of ADAMTS13 obtained by the ROC curves for prediction of St-elevation MI cases

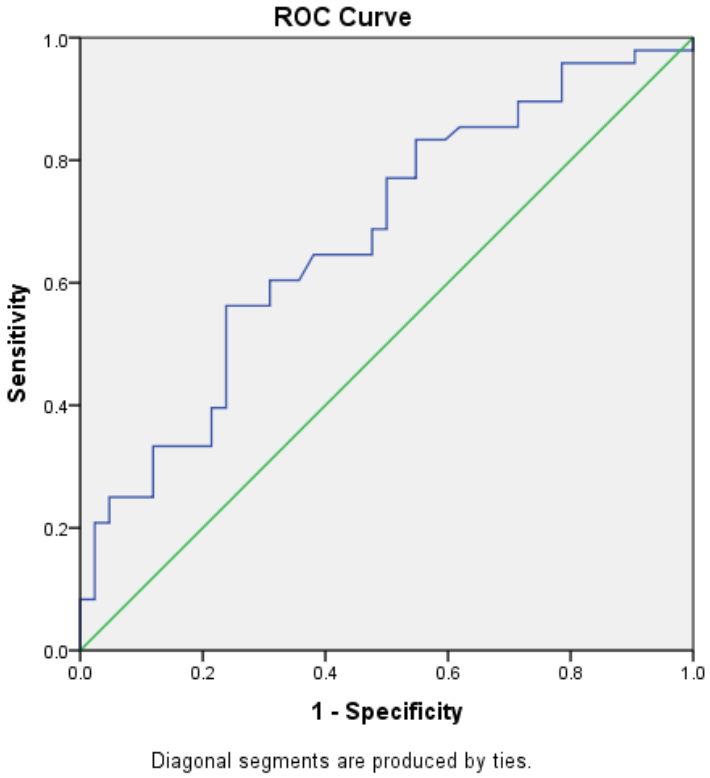


Figure (3.6) Receiver operating characteristics (ROC) curve analysis of ADAMTS13 in St-elevation MI cases

3.7 VWF genotypes correlated with St_Elevation MI patients

The subjects enrolled in present study were classified into three genotypes
The subjects enrolled in present study were classified into three genotypes, one homozygous for the A allele (AA) wild type, heterozygous (AT) and the last was homozygous for the allele T (TT).
The results of the current study for genotype distribution of the (rs216311) SNP exhibited a significant associations were noticed between
AT, AA, and TT genotype and incidence of ST Elevation patients when compared with those of the control group.

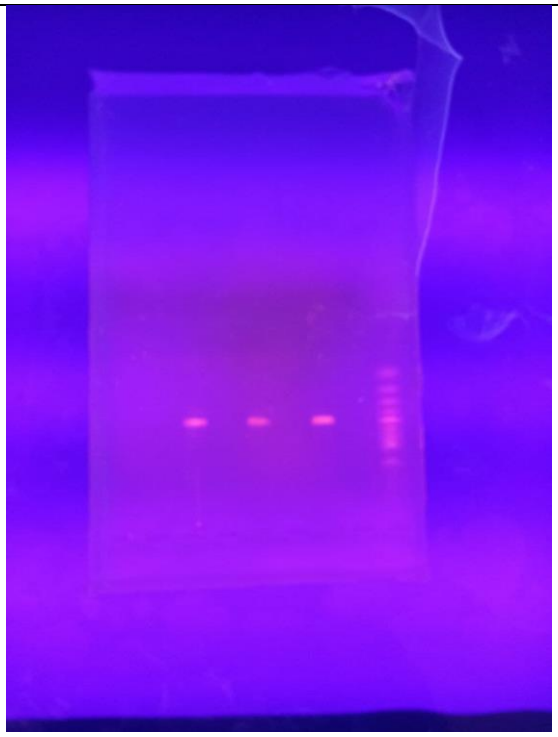


Figure (3.7):AT, AA, and TT genotype

Table 3.6: Comparison of (TT_0) allele between ST elevation MI patient and healthy group

	Patients vs. controls TT 0		P Value
	Controls (n=48)	Patients (n= 42)	
ADAM (Median) Level	11.33	9.5	0.04
VWF (Median) Level	128.84	170.52	0.07

The median of ADAM Level was significant for group patients who do not have TT allele with P value 0.04 while the median of VWF was non significant in patients compared to healthy group

Table 3.7: Explains the comparison between patients, control, and the absence of the allele(TT1)

	Patients vs. controls TT 1		P Value
	Controls (n= 48)	Patients (n=42)	
ADAM (Median) Level	11.01	8.67	0.13
VWF (Median) Level	131.10	150.6	0.025

The median of ADAM Level was non significant who have TT allele for patient group with P value 0.13 while the median of VWF was ignificant compared with healthy group

Table 3.8: Explains the comparison between patients, control (AT 0)

	Patients vs. controls AT 0		P Value
	Controls (n=48)	Patients (n=42)	
ADAM (Median) Level	10.145	9.504	0.15
VWF (Median) Level	131.102	157.382	0.004

The median of ADAM Level was non significant who do not have AT allele for patient with P value 0.15 while the median of VWF was significant compared with healthy group

Table 3.9: Explains the comparison between patients, control, and the absence of the allele(AT1)

	Patients vs. controls AT 1		P Value
	Controls (n=48)	Patients (n=42)	
ADAM (Median) Level	11.88	8.86	0.001
VWF (Median) Level	129.29	169.62	0.73

The median of ADAM Level significant who have AT allele for patient with P value 0.001 while the median of VWF was non significant compared with healthy group

High plasma VWF levels are associated with an increased risk of arterial thrombosis, including myocardial infarction and ischemic stroke **(Van Schie et al., 2011).** Increased levels of VWF and reduced levels of ADAMTS13 activity may contribute to the pathogenesis of acute myocardial infarction and might prove to be important mediators of AMI progression **(Al-masri et al., 2020)**

The causal effect of lower ADAMTS13 activity on the increased odds of having cardiovascular diseases was coronary heart disease and myocardial infarction**(Ye & Zheng, 2021)**

CHAPTER FOUR

Conclusion and Recomendatio

4. Conclusion and Recommendation

4.1. Conclusion

1. In the current study there was statistical significant increase in serum VWF and reduced level ADAM in St-Elevation MI patient

2. the (rs216311) SNP exhibited a significant association were noticed between AT, AA, and TT genotype and incidence of ST Elevation patients when compared with those of the control group

3. the proportion of men with st elevation MI is greater than women. the total number of patients who have blood type A is the largest percentage for O and B in patient with st elevation myocardial infraction.

4. Patients with diabetes mellitus have higher levels of TG and LDL cholesterol. while HDL level was normal, in a previous study, it say in STEMI patients a lower HDL-C was paradoxically associated with a lower risk of death during hospitalization.

4.2. Recommendation

1. Study other SNPs to determine its correlation with VwF gene due to very effective on ST-Elevation MI and their treatment.

2. Use more advance molecular techniques for detection of other VwF polymorphisms (Real time PCR and sequencing).

3. Increase sample size and collect samples from other different hospitals and Provinces

Refrences

REFRENCES

1. Abdul-Ghani, M., DeFronzo, R. A., Del Prato, S., Chilton, R., Singh, R., & Ryder, R. E. J. (2017). Cardiovascular disease and type 2 diabetes: Has the dawn of a new era arrived? *Diabetes Care*, *40*(7), 813–820. https://doi.org/10.2337/dc16-2736
2. Acharya, U. R., Fujita, H., Oh, S. L., Hagiwara, Y., Tan, J. H., & Adam, M. (2017). Application of deep convolutional neural network for automated detection of myocardial infarction using ECG signals. *Information Sciences*, *415–416*, 190–198. https://doi.org/10.1016/j.ins.2017.06.027
3. Akyol, O., Akyol, S., & Chen, C. H. (2016). Update on ADAMTS13 and VWF in cardiovascular and hematological disorders. In *Clinica Chimica Acta* (Vol. 463, pp. 109–118). Elsevier B.V. https://doi.org/10.1016/j.cca.2016.10.017
4. Al-Hadi, H. A., & Fox, K. A. (2009). Cardiac markers in the early diagnosis and management of patients with acute coronary syndrome. *Sultan Qaboos University Medical Journal*, *9*(3), 231–246.
5. Al-Masri, A. A., Habib, S. S., Hersi, A., & Al Zamil, H. (2020). Effect of Acute Myocardial Infarction on a Disintegrin and Metalloprotease with Thrombospondin Motif 13 and von Willebrand Factor and Their Relationship with Markers of Inflammation. *International Journal of Vascular Medicine*, *2020*. https://doi.org/10.1155/2020/4981092
6. Al-masri, A. A., Habib, S. S., Hersi, A., & Zamil, H. Al. (2020). *Effect of Acute Myocardial Infarction on a Disintegrin and Metalloprotease with Thrombospondin Motif 13 and Von Willebrand Factor and Their Relationship with Markers of Inflammation*. *2020*.
7. Alharbi, M. S., Alanazi, B. K., Alquhays, I. A., Alhamied, N. A., &

Al Shimemeri, A. (2020). Effect of Gender on the Outcomes of ST-Elevation Myocardial Infarction at a Tertiary Care Hospital in Riyadh, Saudi Arabia. *Cureus*, *12*(8). https://doi.org/10.7759/cureus.10118

8. Amani, R., & Sharifi, N. (2012). *Cardiovascular Disease Risk Factors*. *April*. https://doi.org/10.5772/34374
9. Ambrose, J. A., & Singh, M. (2015). Pathophysiology of coronary artery disease leading to acute coronary syndromes. *F1000Prime Reports*, *7*(January), 1–5. https://doi.org/10.12703/P7-08
10. Anderson and Morrow. (2017). *ACUTE MIOCARDIO The New Englad Journal*. *21*, 2053–2064. https://doi.org/10.1056/NEJMra1606915
11. Barrett, J. E., & Davenport, J. (2014). Managing coronary heart disease in older people: age is no barrier. *Nursing and Residential Care*, *16*(5), 258–264. https://doi.org/10.12968/nrec.2014.16.5.258
12. Beckman, J. A., Findeiss, L. K., Golzarian, J., Gornik, H. L., Halperin, J. L., Hirsch, A. T., Jaff, M. R., Misra, S., Moneta, G. L., Olin, J. W., Rooke, T. W., Sidawy, A. N.-, Stanley, J. C., White, C. J., White, J. V., & Zierler, R. E. (2011). Management of Patients With Peripheral Artery Disease - Lower extremity, Renal, Mesenteric and Abdominal Aorta. *American College of Cardiology Foundation*, *November*, 1–66.
13. Blann, A., Porter, M., & McCOLLUM, C. N. (1992). von Willebrand factor in plasma: A novel risk factor for recurrent myocardial infarction and death. *Heart*, *68*(12), 635. https://doi.org/10.1136/hrt.68.12.635
14. Boselli, F., Freund, J. B., Vermot, J., Atherosclerosis, V. Á., & Biomechanics, Á. K. Á. (2015). Blood flow mechanics in cardiovascular development. *Cellular and Molecular Life Sciences*,

72(13), 2545–2559. https://doi.org/10.1007/s00018-015-1885-3

15. Carriço, J. A., Sabat, A. J., Friedrich, A. W., & Ramirez, M. (2013). Bioinformatics in bacterial molecular epidemiology and public health: Databases, tools and the next-generation sequencing revolution. *Eurosurveillance*, *18*(4), 1–8. https://doi.org/10.2807/ese.18.04.20382-en
16. Carvalho, G., & Rassi, S. (2016). The Prognostic Value of CK-MB in Acute Myocardial Infarction in Developing Countries: A Descriptive Study. *Angiology: Open Access*, *4*(3). https://doi.org/10.4172/2329-9495.1000183
17. Cercato, C., & Fonseca, F. A. (2019). Cardiovascular risk and obesity. *Diabetology and Metabolic Syndrome*, *11*(1), 1–15. https://doi.org/10.1186/s13098-019-0468-0
18. Chander, V., Chakravarti, S., Gupta, V., Nandi, S., Singh, M., Badasara, S. K., Sharma, C., Mittal, M., Dandapat, S., & Gupta, V. K. (2016). Multiplex Amplification Refractory Mutation System PCR (ARMS-PCR) provides sequencing independent typing of canine parvovirus. *Infection, Genetics and Evolution*, *46*, 59–64. https://doi.org/10.1016/j.meegid.2016.10.024
19. Chen, Z., Yang, S. H., Xu, H., & Li, J. J. (2016). ABO blood group system and the coronary artery disease: An updated systematic review and meta-analysis. *Scientific Reports*, *6*(December 2015), 1–11. https://doi.org/10.1038/srep23250 *ck.pdf*. (n.d.). Cobas. (2019). *Elecsys Troponin I STAT*. *7*, 1–5.
20. Control, C. for D. (2009). Centers for Disease Control: Know the Facts About Heart Disease. *Final Data for 2009. Www.Cdc.Gov/Nchs/ Data/Nvsr/Nvsr60/Nvsr60_03.Pdf Having High Cholesterol, High Blood Pressure, or Diabetes Also Can Increase Your Risk for Heart Disease. Ask Your Doctor about Preventing or Treating These Medical Conditions. What Are* , 1–2.

21. Dahlöf, B. (2010). Cardiovascular Disease Risk Factors : Epidemiology and Risk Assessment. *AJC*, *105*(1), 3A-9A. https://doi.org/10.1016/j.amjcard.2009.10.007
22. Dai, X. (2016). Genetics of coronary artery disease and myocardial infarction. *World Journal of Cardiology*, *8*(1), 1. https://doi.org/10.4330/wjc.v8.i1.1
23. Daubert, M. A., & Jeremias, A. (2010). The utility of troponin measurement to detect myocardial infarction: Review of the current findings. *Vascular Health and Risk Management*, *6*(1), 691–699. https://doi.org/10.2147/vhrm.s5306
24. De Meyer, S. F., Savchenko, A. S., Haas, M. S., Schatzberg, D., Carroll, M. C., Schiviz, A., Dietrich, B., Rottensteiner, H., Scheiflinger, F., & Wagner, D. D. (2012). Protective anti-inflammatory effect of ADAMTS13 on myocardial ischemia/reperfusion injury in mice. *Blood*, *120*(26), 5217–5223. https://doi.org/10.1182/blood-2012-06-439935
25. Deedwania, P., Kosiborod, M., Barrett, E., Ceriello, A., Isley, W., Mazzone, T., & Raskin, P. (2008). Hyperglycemia and acute coronary syndrome: A scientific statement from the american heart association diabetes committee of the council on nutrition, physical activity, and metabolism. *Circulation*, *117*(12), 1610–1619. https://doi.org/10.1161/CIRCULATIONAHA.107.188629
26. Denorme, F., Kraft, P., Pareyn, I., Drechsler, C., Deckmyn, H., Vanhoorelbeke, K., Kleinschnitz, C., & De Meyer, S. F. (2017). Reduced ADAMTS13 levels in patients with acute and chronic cerebrovascular disease. *PLoS ONE*, *12*(6), 1–10. https://doi.org/10.1371/journal.pone.0179258
27. Diamond, J. A., & Phillips, R. A. (2005). Hypertensive heart disease. *Hypertension Research*, *28*(3), 191–202. https://doi.org/10.1291/hypres.28.191

28. Díez, J. (2013). Hypertensive heart disease. *Hypertension*, *28*(3), 153–166. https://doi.org/10.2217/EBO.12.497
29. Eerenberg, E. S., Teunissen, P. F. A., Born, B. Van Den, Meijers, J. C. M., Hollander, M. R., Jansen, M., Tijssen, R., Belie, J. A. M., Ven, P. M. Van De, Aly, M. F., Kamp, O., Niessen, H. W., Kamphuisen, P. W., Levi, M., & Royen, N. Van. (2016). *The role of ADAMTS13 in acute myocardial infarction : cause or consequence ?* 194–203. https://doi.org/10.1093/cvr/cvw097
30. Elsayed Azab, A. (2017). Acute Myocardial Infarction Risk Factors and Correlation of its Markers with Serum Lipids. *Journal of Applied Biotechnology & Bioengineering*, *3*(4). https://doi.org/10.15406/jabb.2017.03.00075
31. Engström-laurent, A. (2007). *Peripheral Arterial Disease – Diagnosis and Treatment The Swedish Council on Technology Assessment in Health Care SBU Board of Directors and Scientific Advisory Committee Secreteriat Board of Directors*.
32. Erdmann, J., Linsel-Nitschke, P., & Schunkert, H. (2010). Übersichtsarbeit: Genetische ursachen des herzinfarktes. *Deutsches Arzteblatt*, *107*(40), 694–699. https://doi.org/10.3238/arztebl.2010.0694
33. Faria, F. R. De, Faria, E. R. De, Franceschini, C., & Priore, S. E. (2015). *Cardiovascular risk and associated factors in adolescents*. *32*(2), 897–904. https://doi.org/10.3305/nh.2015.32.2.8824
34. Freitas, R. W. J. F. de, Araújo, M. F. M. de, Lima, A. C. S., Pereira, D. C. R., Alencar, A. M. P. G., & Damasceno, M. M. C. (2013). Study of lipid profile in a population of university students. *Revista Latino-Americana de Enfermagem*, *21*(5), 1151–1158. https://doi.org/10.1590/S0104-11692013000500019
35. Fu, Y., Chen, M., Sun, H., Guo, Z., Gao, Y., Yang, X., Li, K.,

& Wang, L. (2020). Blood group A: a risk factor for heart rupture after acute myocardial infarction. *BMC Cardiovascular Disorders*, *20*(1), 1–9. https://doi.org/10.1186/s12872-020-01756-y

36. Garg, P., Kumar, J., Choudhary, R., & Chawla, V. (2012). Association Between ABO Blood Groups And Myocardial Infarction In Jodhpur City of India. *Journal of Bangladesh Society of Physiologist*, *7*(1), 13–17. https://doi.org/10.3329/jbsp.v7i1.11154
37. Goncharova, I. A., Nazarenko, M. S., Babushkina, N. P., Markov, A. V., Pecherina, T. B., Kashtalap, V. V., Tarasenko, N. V., Ponasenko, A. V., Barbarash, O. L., & Puzyrev, V. P. (2020). Genetic Predisposition to Early Myocardial Infarction. *Molecular Biology*, *54*(2), 196–203. https://doi.org/10.1134/S0026893320020041
38. Gragnano, F., Sperlongano, S., Golia, E., Natale, F., Bianchi, R., Crisci, M., Fimiani, F., Pariggiano, I., Diana, V., Carbone, A., Cesaro, A., Concilio, C., Limongelli, G., Russo, M., & Calabrò, P. (2017). *Review Article The Role of von Willebrand Factor in Vascular Inflammation : From Pathogenesis to Targeted Therapy*. *2017*.
39. Hassan, I., Bream, A. S., El-Sayed, A., & Yousef, A. M. (2017). International Journal of Advanced Research in Biological Sciences Assessment of disinfection by-products levels in Aga surface water plant and its distribution system, Dakhlia, Egypt. *Int. J. Adv. Res. Biol. Sci*, *4*(4), 37–43. https://doi.org/10.22192/ijarbs
40. Heart, Y., Causes, T. H. E., & Disease, O. F. H. (n.d.). *Your heart and the causes of heart disease*.
41. Heartbeat, N. (n.d.). *Arrhythmias*.
42. Hedges, J. R. (1995). The role of CK-MB in chest pain decision-making. *Journal of Accident and Emergency Medicine*,

12(2), 101–106. https://doi.org/10.1136/emj.12.2.101

43. Heusch, G., & Gersh, B. J. (2017). *The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion : a continual challenge*. 774–784. https://doi.org/10.1093/eurheartj/ehw224

44. Horii, M., Uemura, S., Uemura, M., Matsumoto, M., Ishizashi, H., Imagawa, K., Iwama, H., Takeda, Y., Kawata, H., Nakajima, T., Fujimura, Y., & Saito, Y. (2008). Acute myocardial infarction as a systemic prothrombotic condition evidenced by increased von Willebrand factor protein over ADAMTS13 activity in coronary and systemic circulation. *Heart and Vessels*, *23*(5), 301–307. https://doi.org/10.1007/s00380-008-1053-x

45. Humphreys, M., Warlow, C., & McGowan, J. (2013). Arrhythmias and their Management. *Nursing the Cardiac Patient*, *January*, 132–155. https://doi.org/10.1002/9781118785331.ch10

46. Hwang, C., & Levis, J. T. (2014). ECG diagnosis: ST-elevation myocardial infarction. *The Permanente Journal*, *18*(2), e133. https://doi.org/10.7812/TPP/13-127

47. Inamdar, A., & Inamdar, A. (2016). Heart Failure: Diagnosis, Management and Utilization. *Journal of Clinical Medicine*, *5*(7), 62. https://doi.org/10.3390/jcm5070062

48. Jeemon, P., Chacko, M., Sarma, P. S., Harikrishnan, S., & Zachariah, G. (2019). Family history of cardiovascular disease and risk of premature coronary heart disease: A matched case-control study. *F1000Research*, *8*, 1–14. https://doi.org/10.12688/wellcomeopenres.15829.1

49. Jiao, Z. Y., Li, X. T., Li, Y. B., Zheng, M. L., Cai, J., Chen, S. H., Wu, S. L., & Yang, X. C. (2018). Correlation of triglycerides with myocardial infarction and analysis of risk factors for myocardial infarction in patients with elevated triglyceride. *Journal*

of Thoracic Disease, *10*(5), 2551–2557. https://doi.org/10.21037/jtd.2018.04.132

50. Karetnikova, V., Gruzdeva, O., Uchasova, E., Osokina, A., & Barbarash, O. (2016). Glucose levels as a prognostic marker in patients with ST-segment elevation myocardial infarction: A case-control study. *BMC Endocrine Disorders*, *16*(1), 1–6. https://doi.org/10.1186/s12902-016-0108-8
51. Kawecki, C., Lenting, P. J., & Denis, C. V. (2017). von Willebrand factor and inflammation. *Journal of Thrombosis and Haemostasis*, *15*(7), 1285–1294. https://doi.org/10.1111/jth.13696
52. Khan, H. A., Alhomida, A. S., & Sobki, S. H. (2013). Lipid profile of patients with acute myocardial infarction and its correlation with systemic inflammation. *Biomarker Insights*, *8*(February 2014). https://doi.org/10.4137/BMI.S11015
53. Kibos, A. S., Knight, B. P., Essebag, V., Fishberger, S. B., Slevin, M., & Tintoiu, I. C. (2013). Cardiac arrhythmias: From basic mechanism to state-of-the-art management. *Cardiac Arrhythmias: From Basic Mechanism to State-of-the-Art Management*, 1–678. https://doi.org/10.1007/978-1-4471-5316-0
54. Kling, S. J., Judd, C. A., Warner, K. B., & Rodgers, G. M. (2008). Regulation of ADAMTS13 expression in proliferating human umbilical vein endothelial cells. *Pathophysiology of Haemostasis and Thrombosis*, *36*(5), 233–240. https://doi.org/10.1159/000252818
55. Koliaki, C., Liatis, S., & Kokkinos, A. (2019). Obesity and cardiovascular disease: revisiting an old relationship. *Metabolism: Clinical and Experimental*, *92*, 98–107. https://doi.org/10.1016/j.metabol.2018.10.011
56. Kosuge, M., Kimura, K., Ishikawa, T., Ebina, T., Hibi, K., Tsukahara, K., Kanna, M., Iwahashi, N., Okuda, J., Nozawa, N.,

Ozaki, H., Yano, H., Nakati, T., Kusama, I., & Umemura, S. (2006). Differences between men and women in terms of clinical features of ST-segment elevation acute myocardial infarction. *Circulation Journal*, *70*(3), 222–226. https://doi.org/10.1253/circj.70.222

57. Kumar, N., Kumar, S., Kumar, A., Shakoor, T., & Rizwan, A. (2019). *Lipid Profile of Patients with Acute Myocardial Infarction (AMI)*. *11*(3), 1–8. https://doi.org/10.7759/cureus.4265

58. Kumar, R. K., Antunes, M. J., Beaton, A., Mirabel, M., Nkomo, V. T., Okello, E., Regmi, P. R., Reményi, B., Sliwa-Hähnle, K., Zühlke, L. J., & Sable, C. (2020). Contemporary Diagnosis and Management of Rheumatic Heart Disease: Implications for Closing the Gap: A Kuriakose, D., & Xiao, Z. (2020). Pathophysiology and treatment of stroke: Present status and future perspectives. *International Journal of Molecular Sciences*, *21*(20), 1–24. https://doi.org/10.3390/ijms21207609

59. Lainscak, M., Spoletini, I., & Coats, A. (2017). Definition and Classification of Heart Failure. *International Cardiovascular Forum Journal*, *10*, 3–7. https://doi.org/10.17987/icfj.v10i0.419

60. Li, Y. A. N., Li, L., Dong, F., Guo, L., Hou, Y., Hu, H., Yan, S., Zhou, X., Liao, L. I. N., Allen, T. D., & Liu, J. U. (2015). *Plasma von Willebrand factor level is transiently elevated in a rat model of acute myocardial infarction*. 1743–1749. https://doi.org/10.3892/etm.2015.2721

61. Libby, P., & Theroux, P. (2005). Pathophysiology of coronary artery disease. *Circulation*, *111*(25), 3481–3488. https://doi.org/10.1161/CIRCULATIONAHA.105.537878

62. Lin, C. J., Chang, Y. C., Cheng, T. Y., Lo, K., Liu, S. J., & Yeh, T. L. (2020). The association between metabolically healthy obesity and risk of cancer: A systematic review and meta-analysis of prospective cohort studies. *Obesity Reviews*, *21*(10), 1–11.

https://doi.org/10.1111/obr.13049
63. Lorenz, T. C. (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments*, *63*, 1–15. https://doi.org/10.3791/3998
64. Maciejak, A., Kiliszek, M., Michalak, M., Tulacz, D., Opolski, G., Matlak, K., Dobrzycki, S., Segiet, A., Gora, M., & Burzynska, B. (2015). Gene expression profiling reveals potential prognostic biomarkers associated with the progression of heart failure. *Genome Medicine*, *7*(1). https://doi.org/10.1186/s13073-015-0149-z
65. Maharani, A., Sujarwoto, Praveen, D., Oceandy, D., Tampubolon, G., & Patel, A. (2019). Cardiovascular disease risk factor prevalence and estimated 10-year cardiovascular risk scores in Indonesia: The SMARThealth Extend study. *PLoS ONE*, *14*(4), 1–13. https://doi.org/10.1371/journal.pone.0215219
66. Mannsverk, J. (2019). *The epidemiology of myocardial infarction Trends in incidence , risk factors , severity , treatment and outcomes of myocardial infarction in a general population* . 1–105.
67. *MATERIALS*. (n.d.). 1–3.
68. Matheus, A. S. D. M., Tannus, L. R. M., Cobas, R. A., Palma, C. C. S., Negrato, C. A., & Gomes, M. D. B. (2013). Impact of diabetes on cardiovascular disease: An update. *International Journal of Hypertension*, *2013*(April 2014). https://doi.org/10.1155/2013/653789
69. MAYRA, G. (2013). **済無**No Title No Title. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699.
70. Melo, J. B. (2018). *Cardiologia*. *37*(9), 737–738.
71. Mota, R., Homeister, J. W., Willis, M. S., & Bahnson, E. M. (2017). Atherosclerosis: Pathogenesis, Genetics and Experimental Models. *ELS*, *November*, 1–10. https://doi.org/10.1002/9780470015902.a0005998.pub3

72. MYTHILI, S., & MALATHI, N. (2015). Diagnostic markers of acute myocardial infarction. *Biomedical Reports*, *3*(6), 743–748. https://doi.org/10.3892/br.2015.500
73. Naser Zangana, S., A AL-Othman, A. kareem, & G AL-Tawil, N. (2017). Role of Cardiac Troponin I Level in Predicting in Hospital Outcomes in Patients with ST-segment Elevation Myocardial Infarction in Erbil-Iraq. *Diyala Journal of Medicine*, *13*(1), 1–7. https://doi.org/10.26505/djm.13013150123
74. National Institute Of Health. (2016). Coronary artery disease management. *Clinical Care Planning and Resource Guide CORONARY ARTERY DISEASE MANAGEMENT*, *May*, 1–8.
75. Nigam, P. K. (2007). Biochemical markers of myocardial injury. *Indian Journal of Clinical Biochemistry*, *22*(1), 10–17. https://doi.org/10.1007/BF02912874
76. Nwabuo, C. C., & Vasan, R. S. (2020). Pathophysiology of Hypertensive Heart Disease: Beyond Left Ventricular Hypertrophy. *Current Hypertension Reports*, *22*(2). https://doi.org/10.1007/s11906-020-1017-9
77. Okhota, S., Melnikov, I., Avtaeva, Y., Kozlov, S., & Gabbasov, Z. (2020). *Shear Stress-Induced Activation of von Willebrand Factor and Cardiovascular Pathology*.
78. Ortega, F. B., Lavie, C. J., & Blair, S. N. (2016). Obesity and cardiovascular disease. *Circulation Research*, *118*(11), 1752–1770. https://doi.org/10.1161/CIRCRESAHA.115.306883
79. Ota, T., Hasegawa, Y., Murata, E., Tanaka, N., & Fukuoka, M. (2020). False-Positive Elevation of CK-MB Levels with Chest Pain in Lung Adenocarcinoma. *Case Reports in Oncology*, *13*(1), 100–104. https://doi.org/10.1159/000505724
80. Park, K. C., Gaze, D. C., Collinson, P. O., & Marber, M. S. (2017). Cardiac troponins: From myocardial infarction to chronic

disease. *Cardiovascular Research*, *113*(14), 1708–1718. https://doi.org/10.1093/cvr/cvx183

81. Pepine, C. J. (n.d.). *Acute Myocardial Infarction*. *64*.

82. Picchio, V., Cammisotto, V., Pagano, F., Carnevale, R., & Chimenti, I. (2020). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intechopen*, *Cell Interaction-Regulation of Immune Responses, Disease Development and Management Strategies*, 1–15. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

83. Prabhakaran, D., Jeemon, P., Sharma, M., Roth, G. A., Johnson, C., Harikrishnan, S., Gupta, R., Pandian, J. D., Naik, N., Roy, A., Dhaliwal, R. S., Xavier, D., Kumar, R. K., Tandon, N., Mathur, P., Shukla, D. K., Mehrotra, R., Venugopal, K., Kumar, G. A., … Dandona, L. (2018). The changing patterns of cardiovascular diseases and their risk factors in the states of India: the Global Burden of Disease Study 1990–2016. *The Lancet Global Health*, *6*(12), e1339–e1351. https://doi.org/10.1016/S2214-109X(18)30407-8

84. Pressure, H. B. (n.d.). *Cardiovascular Disease Risk Factors Risk Factors How it affects the heart*. 5200.

85. Promega Corporation. (2012). *GoTaq® Green Master Mix Certificate of Analysis*. *6*, 2–3. www.promega.com

86. Rahman, S., Majumder, M. A. A., Kabir, R., Haque, M., Gupta, S., Arafat, S. M. Y., Ojeh, N., & Dalvi, P. (2017). Cardiovascular Disease and Diabetes: Two Sides of the Same Coin! *Recent Trends in Cardiovascular Risks*. https://doi.org/10.5772/intechopen.69038

87. Ramasamy, S. K. (2017). Structure and Functions of Blood Vessels and Vascular Niches in Bone. *Stem Cells International*,

2017(Figure 1). https://doi.org/10.1155/2017/5046953

88. *Risk factors*. (n.d.). 1616.

89. Rutten, B., Maseri, A., Cianflone, D., Laricchia, A., Cristell, N. A., Durante, A., Spartera, M., Ancona, F., Limite, L., Hu, D., Li, H., Uren, N. G., de Groot, P. G., Mannucci, P. M., & Roest, M. (2015). Plasma levels of active Von Willebrand factor are increased in patients with first ST-segment elevation myocardial infarction: a multicenter and multiethnic study. *European Heart Journal. Acute Cardiovascular Care*, *4*(1), 64–74. https://doi.org/10.1177/2048872614534388

90. Sargent, C. R., Pieper, I. L., Heart, S. R., & Kanamarlapudi, V. (2017). Encyclopedia of Signaling Molecules. *Encyclopedia of Signaling Molecules*, *December*. https://doi.org/10.1007/978-1-4614-6438-9

91. Serdahl, S. A. (2008). Heart failure: managing systolic dysfunction. *Rn*, *71*(6).

92. Series, H. (n.d.). *UNDERSTANDING*.

93. Serrano Hernando, F. J., & Conejero, A. M. (2007). Peripheral artery disease: Pathophysiology, diagnosis and treatment. *Revista Espanola de Cardiologia*, *60*(9), 969–982. https://doi.org/10.1157/13109651

94. Sherazi, S., & Block, R. (2009). Coronary Artery Disease Prevention. *Manual of Heart Failure Management*, *November*, 17–32. https://doi.org/10.1007/978-1-84882-185-9_2

95. Sia, C. H., Zheng, H., Ho, A. F. W., Bulluck, H., Chong, J., Foo, D., Foo, L. L., Lim, P. Z. Y., Liew, B. W., Tan, H. C., Yeo, T. C., Chua, T. S. J., Chan, M. Y. Y., & Hausenloy, D. J. (2020). The Lipid Paradox is present in ST-elevation but not in non-ST-elevation myocardial infarction patients: Insights from the Singapore Myocardial Infarction Registry. *Scientific Reports*, *10*(1), 1–13.

https://doi.org/10.1038/s41598-020-63825-8

96. Smit, M., Chb, M. B., Anes, M., Sa, F. C. A., Coetzee, A. R., Chb, M. B., Anes, M., Sa, F. F. A., & Lochner, A. (2019). The Pathophysiology of Myocardial Ischemia and Perioperative Myocardial Infarction. *Journal of Cardiothoracic and Vascular Anesthesia*, *000*. https://doi.org/10.1053/j.jvca.2019.10.005

97. Smith, K. R. (2013). Cardiovascular System. *Small Animal Toxicology Essentials*, 89–93. https://doi.org/10.1002/9781118785591.ch12

98. Smoke, S. (2020). *CORONARY HEART DISEASE*. *September*.

99. *Smoking , the heart and circulation*. (2018). *August*.

100. Sodi, R. (2006). Cardiac Troponins: Clinical and Analytical Aspects. *Advances in Clinical Chemistry*, *41*(August), 49–122. https://doi.org/10.1016/s0065-2423(05)41003-3

101. Stewart, J., Manmathan, G., & Wilkinson, P. (2017). Primary prevention of cardiovascular disease: A review of contemporary guidance and literature. *JRSM Cardiovascular Disease*, *6*, 204800401668721. https://doi.org/10.1177/2048004016687211

102. Taha, K. M. (2016). *Agarose Gel Electrophoresis Agriculture College-Animal Resources Preparation of Agarose Gel*. *January*, 1–2. https://doi.org/10.13140/RG.2.2.27626.72644

103. Taylor, S. H. (1996). Congestive heart failure. Towards a comprehensive treatment. *European Heart Journal*, *17*(SUPPL. B), 43–56. https://doi.org/10.1093/eurheartj/17.suppl_b.43 *tc.pdf*. (n.d.).

104. *tg.pdf*. (n.d.).

105. Van Schie, M. C., Van Loon, J. E., De Maat, M. P. M., & Leebeek, F. W. G. (2011). Genetic determinants of von Willebrand factor levels and activity in relation to the risk of cardiovascular

disease: A review. *Journal of Thrombosis and Haemostasis*, *9*(5), 899–908. https://doi.org/10.1111/j.1538-7836.2011.04243.x

106. Voorhees, A. P., & Han, H. C. (2015). Biomechanics of cardiac function. *Comprehensive Physiology*, *5*(4), 1623–1644. https://doi.org/10.1002/cphy.c140070

107. Voorhees, A. P., Han, H., & Program, E. (2016). *HHS Public Access*. *5*(4), 1623–1644. https://doi.org/10.1002/cphy.c140070.Biomechanics

108. Wanamaker, B. L., Seth, M. M., Sukul, D., Dixon, S. R., Bhatt, D. L., Madder, R. D., Rumsfeld, J. S., & Gurm, H. S. (2019). Relationship Between Troponin on Presentation and In-Hospital Mortality in Patients With ST-Segment–Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention. *Journal of the American Heart Association*, *8*(19), 1–9. https://doi.org/10.1161/JAHA.119.013551

https://doi.org/10.1016/j.jacc.2018.06.063

109. Wells, S. M., & Sleeper, M. (2008). Cardiac troponins. *Journal of Veterinary Emergency and Critical Care*, *18*(3), 235–245. https://doi.org/10.1111/j.1476-4431.2008.00307.x

110. Whan, C., Ilseon, L., Park, H. C., Lee, H., Kang, D. P. S., Kim, S. L. Y., & Park, S. P. S. (2012). *Expression of ADAMTS-2 , -3 , -13 , and -14 in culprit coronary lesions in patients with acute myocardial infarction or stable angina*. 362–370. https://doi.org/10.1007/s11239-011-0673-7

111. Ye, Z., & Zheng, J. (2021). Verification of the Role of ADAMTS13 in the Cardiovascular Disease Using Two-Sample Mendelian Randomization. *Frontiers in Genetics*, *12*(July), 1–9. https://doi.org/10.3389/fgene.2021.660989

112. You, I., & Problems, H. (n.d.). *Human von willebrand factor cleaving protease (ADAMTS-13 / vWF-cp) ELISA Kit*. 1–25.

113. Žaliaduonytė-Pekšienė, D., Lesauskaitė, V., Liutkevičienė, R., Tamakauskas, V., Kviesulaitis, V., Šinkūnaitė-Maršalkienė, G., Šimonytė, S., Mačiulskytė, S., Tamulevičiūtė-Prascienė, E., Gustienė, O., Tamošiūnas, A., & Žaliūnas, R. (2017). Association of the genetic and traditional risk factors of ischaemic heart disease with STEMI and NSTEMI development. *JRAAS - Journal of the Renin-Angiotensin-Aldosterone System*, *18*(4), 1–9. https://doi.org/10.1177/1470320317739987

114. Zheng, X. L. (2013). Structure-function and regulation of ADAMTS-13 protease. *Journal of Thrombosis and Haemostasis*, *11*(SUPPL.1), 11–23. https://doi.org/10.1111/jth.12221

بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

☼☼

صدق الله العظيم

☼☼

سورة المجادلة (الآية ١١)

الخلاصة

الخلفية:هو متلازمة إكلينيكية (STE) ST الخلفية: احتشاء عضلة القلب الناجم عن ارتفاع STE محددة من خلال الأعراض المميزة لنقص تروية عضلة القلب بالترافق مع ارتفاع والإفراج اللاحق عن المؤشرات الحيوية لنخر (STE) المستمر في تخطيط القلب الكهربائي عضلة القلب

:الهدف من الدراسة

لمعرفة الاختلافات التسلسلية في المستويات وتعدد الأشكال الجينية لعامل فون ويلبراند احتشاء عضلة القلب المرتفع -ST على مدار (ADAMTS13) و A Disintegrin و (VWF) وتحديد علاقتهم مع علامات أخرى لخطر الإصابة بأمراض القلب والأوعية الدموية لدى المرضى العراقيين

المواد والطرق: تصميم الدراسة هو دراسة حالة وضبط
تم جمع ٤٢ عينة لمرضى احتشاء عضلة القلب المشخصين بارتفاع مقطع ال ST الذين تراوحت اعمارهم بين(٢٥-٧٥) سنة و ٤٨اخرين من البالغين الاصحاء كمجموعة ضابطة وتحديد بعض العلامات البيوكيميائية للكرياتين كيناز وملف الدهون بأستخدام جهاز dirui وقياس التروبونين بأستخدام جهاز
ADAMT, Vwf.411 Cobes e , وبأستخدام طريقه الاليزا تم قياس تركيزات

النتيجة: وجدت الدراسة الحالية أن نسبة الرجال المصابين بارتفاع احتشاء عضلي أعلى عند الرجال منها عند النساء. كان هناك فرق كبير بين مستوى السكر في الدم وملف الدهون في ومجموعة المراقبة. كانت هناك زيادة ذات دلالة إحصائية في MI مجموعة مرضى ذو دلالة إحصائية وكانت VLDL بينما لم يكن لـ LDL الكوليسترول في الدم والدهون الثلاثية و هناك زيادة ذات دلالة إحصائية في مصل

ADAM وانخفاض مستوى VWF

بشكل ملحوظ من خطر TT و AT و AA حيث في الدراسة الجينية ، زادت الأنماط الجينية الإصابة بـ

ST_Elevation MI

بشكل كبير من خطر الإصابة بـ TT و AT و AA الخلاصة: زادت الأنماط الجينية (rs216311) ، أظهرت نتائج الدراسة الحالية لتوزيع النمط الجيني لـ ST_Elevation MI مرضى .ST النمط الجيني وحدوث TT و AA و AT ارتباطات مهمة لوحظت بين SNP .الارتفاع عند مقارنتهم بمجموعة التحكم

وجدت أن نسبة الرجال الذين لديهم ارتفاع ميل أكبر من النساء. العدد الإجمالي للمرضى A الذين لديهم فصيلة الدم

B و O هو أكبر نسبة ل

في المرضى الذين يعانون من ارتفاع في عضلة القلب. مرضى السكري لديهم

طبيعيًا ، في الدراسة الحالية كانت HDL بينما كان مستوى LDL و TG مستويات أعلى من ADAM وانخفاض مستوىVWF هناك زيادة ذات دلالة إحصائية في مصل

St-Elevation MI في مريض

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب
فرع الكيمياء الحياتية

مستويات المصل ودور تعدد الأشكال الجينية لعامل فون ويلبراند في التسبب في احتشاء عضلة القلب ST-Elevation وعلاقته بمستويات ADAMTS13 في المرضى العراقيين

رسالة

مقدمة الى مجلس كلية الطب جامعة كربلاء

كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية من قبل

حنين عبد علي عوده

بكالوريوس علوم كيمياء_جامعة كربلاء٢٠١٦

اشراف
الأستاذ المساعد الدكتورة
شذى محمد جواد الخطيب
كلية الطب_الجامعة المستنصرية

اشراف
الدكتور احمد قاسم الحيدري
طبيب اختصاص امراض القلب
مدينة الامام الحسين الطبية

١٤٤٣هـ

٢٠٢١م