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Study of some physiological Biomarkers and genetic polymorphism in Ischemic Heart disease Patients in Karbala Governorate

A Thesis Submitted to the Council of College of Education For Pure Sciences University of Karbala in Partial Fulfillment of the Requirements for the Degree of Doctor in Biology/ Zoology

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Summary

In all countries of the world, myocardial infarction remains a cause of morbidity and mortality. This may occur when complete myocardial ischemia, which is called reduced blood flow to the heart muscle, bypasses the healing mechanisms intended to maintain the body's homeostasis and regular functions. This has led to improved ability to identify individuals at high risk, in order to accurately predict and diagnose. In individuals with myocardial infarction, it is necessary to examine various biochemical markers. Some biochemical markers, such as fibroblast growth factor-21 (FGF-21), are associated with myocardial infarction, gelatinase-associated lipocalin (NGAL), and subtilisin/kexin adapter protein. 9 (PCSK9) in heart patients.

The aim of the current study was to estimate some biochemical markers associated with ischemic heart disease to predict and consider it a prognostic marker for this disease, and the study evaluated VF-21, fibroblast growth factor-21 (FGF-21), myocardial infarction, and gelatinase-associated lipocalin (NGAL). And the converting protein subtilisin/kexin 9 (PCSK9) in sixty patients (60) suffering from ischemic heart disease. Samples were collected from the Karbala Cardiology Center in Imam Hussein Medical City in the holy city of Karbala from the period of November 1, 2022 to July 30, 2023 from infected males. With ischemia disease and the control group (apparently healthy group) were thirty (30), and this group was matched in age from (30) - (69) as a patient group.

The criteria for the current study included a lipid profile and four biochemical markers. Patients were divided into four subgroups according to age, disease type, smoking, and body mass index. The results of the current results showed a significant increase in the level of cholesterol, triglycerides, and LDL level, and a significant decrease in the HDL level in patients with ischemic heart disease compared with the control group. Biochemical markers were also elevated (differential growth factor 15, tryptase and interleukin 1 beta) in patients compared to controls. According to age, the oldest ages (60-69) years had a high significant increase in all scores compared to (50-59), (40-49) and (30-39) years. In types of ischemic heart disease patients, the results of the current study showed a significant increase in all vital signs in myocardial infarction compared to patients with unstable angina and stable angina, and there were significant differences between

myocardial infarction, unstable angina, and stable angina patients. All biomarkers increased significantly in smoking patients compared with non-smoking patients.

In obese patients, all biochemical markers were significantly elevated compared with overweight and normal weight patients. The current correlation study indicated that there is a positive significant relationship between (Protein Von Factor - (PCSKA) - Lipocalin - Fibroblast Growth Factor 21) with the level of cholesterol, LDL-C and TG, while there is a negative significant relationship between all biomarkers and the HDL level. The present study concluded that all biomarkers (PCSKA-lipocalin-fibroblast growth factor 21) are risk prognostic and predictive of atherosclerosis associated with ischemic heart disease .

Results from a study of ACE polymorphisms in patients with CVD were consistent with some groups reporting a positive association between the DD genotype and/or D allele and stroke, whereas others reported the opposite. Al's duet. A significant association between ACE gene polymorphisms and incidence and mortality of ischemic cardiovascular disease has been reported in patients aged 30 to 60 years or younger in unaffected patients. reported a positive association between ACE gene polymorphisms and cerebrovascular diseases. Early studies showed a strong relationship between the D allele and levels of circulating intracellular and tissue activity of the enzyme ACE. Because both alleles have common dominant effects on ACE levels, the homozygous DD genotype leads to the highest levels of the enzyme, while the homozygous II genotype leads to the lowest level, and the heterozygous DI genotype leads to an intermediate level. In the current study, we found that variant genotypes of ACE I/D polymorphisms were associated with higher cardiovascular disease in patients. We have demonstrated that patients with a large amount of PCSK9 protein, partial PCSK9 deficiency causes obesity, and an association between PCSK9, hypercholesterolemia, and coronary heart disease

Increase in cardiovascular disease, and its effect on adipogenesis in the coating on a high-fat diet. The importance of the PCSK9 gene in MT1-MMP activation in cardiometabolic syndrome is further supported by data. This indicates for the first time that monocytes in CVD patients were upregulated toward the pro-migratory/pro-inflammatory state that characterizes CVD PCSK9, and its levels

were not significantly changed, supporting a disturbance of LDL and inhibitor homeostasis in CVD and obese patients. This PCSK9 may be a contributor to regulation. Interestingly, regulation of hepatic LDL receptors by resistin involves the related PCSK family member PCSK9, which was recently identified as a novel target in patients with severe dyslipidemia and CVD .

The results of the current results showed a significant increase in cholesterol, triglycerides, and LDL levels, and a significant decrease in HDL levels in patients with ischemic heart disease compared with the control group. Biochemical markers (growth differentiation factor 15, tryptase, and interleukin 1 beta) were also raised in patients compared to the control group. By age, the elderly (60-69) years had a highly significant increase in all scores compared to (50-59), (4940) and (30-39) years. In the types of ischemic heart patients, the results of the current study showed a significant increase in all biomarkers in myocardial infarction compared to unstable angina and stable angina patients with the exception of L-1betal. There were no statistically significant differences between myocardial infarction, unstable angina, and stable angina. Sedentary patients. All biomarkers increased significantly in smoking patients compared to non-smoking patients. In patients with increased BMI, both biochemical markers were significantly increased compared to patients with overweight and normal weight . The current correlation study indicated that there is a positive significant relationship between (PCSK9 - lipocalin - fibroblast growth factor 21) with the level of cholesterol, LDL-C and TG, while there is a negative significant relationship between all biomarkers and the level of HDL-C.

The present study concluded that all biomarkers of PCSK9-lipocalin-fibroblast growth factor 21 are risk factors for diagnosing and prognosticating atherosclerosis associated with ischemic heart disease, and levels of FGF-21, NGAL, and PCSK9 can be used to identify and diagnose myocardial infarction the heart

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Abbreviations	Meaning
HFGF21	Human Fibroblast Growth Factor 21
PCSK9	Convertase Subtilisin Proprotein /Kexin Type 9
NGAL	Human Lipocalin 1
HVWF	Human Von Willebrand Factor
(CVH)	Cardiovascular health
CAD	Coronary artery disease
PAD	Peripheral Artery Disease
(CHF)	Congestive heart failure
(IHD)	ischemic heart disease
(ACS)	acute coronary syndrome
(CAD)	Coronary artery disease
MI	Myocardial Infraction
(ESC/	European Society of Cardiology
ACCF	American College of Cardiology Foundation
/AHA	American Heart Association
WHF	World Heart Foundation
DLV	Density Lipoprotein Very
(LDL)	low density lipoprotein
Ucps	Uncoupling proteins
ECG	Electrocardiograms
TC	Total cholesterol
(IDL),	Low-Density Lipoprotein
(VEGFs)	vascular endothelial growth factors
(PPARa)	Peroxisome proliferator-activated receptor a
(SFAs)	saturated fatty acids
NGAL	Neutrophil gelatinase associated lipocalin
(ApoB)	apolipoproteins B
factor (vWF)	Von Willebrand
vWD	von Willebrand Disease
(AMI)	acute myocardial infarction
(ACE),	Angiotensin-converting enzyme

1. Introduction

Coronary artery disease is a major cause of mortality and ill-health. It presents in different ways. The underlying cause is a process of atherosclerosis, which leads to narrowing of the coronary arteries, restricting the blood flow to the heart muscle. Coronary heart disease prevalence is increasing all over the world including our community that is not only a need for better recognition of the warning signs of a heart attack, but also a tremendous need for more efforts targeting prevention(Ziv-Baran *et al.*, 2023) .

Myocardial infarction is one of the main types of high incidence of cardiovascular disease and is one of the most important causes of mortality in humans (Virani et al., 2020). According to Yang et al. (2022) Myocardial infarction can be defined as heart tissue death, and heart muscle cells caused by progressive ischemia in acute as well as chronic and also may occur as a result of hypoxia in the coronary arteries. More than four million people die from cardiovascular disorders each year in Europe alone, accounting for over half of all mortality globally (Tieuwnsend *et al*., 2015). Acute myocardial infarction is one of most important diseases that lead to deaths that result from coronary artery disease, and it may also be among the heart attacks (Mendis et al., 2011). The high incidence of such diseases, including AMI, is Significantly in our days, and it is so noticeable that it was noticed with the annual increase in human growth and it may reach 3.5%. The reasons can be caused by changes in human behavior such as eating and exercise, as well as some daily activities and in general in the lifestyle, and this is evident in countries emerging (Benjamin *et al.*, 2017).

Recent cardiovascular research has focused on fibroblast growth factor 21 (FGF21), another anti-inflammatory cytokine released by the liver. Previous research has connected FGF21 to anti-inflammatory, anti-oxidative stress, and glycolipid Prievious reseairch has shown inflammation, oxidatiave striess, and chainges in

glycolipid metabolism as key contributions to the pathogenesis of AMI (Neri et al., 2017; Tao et al., 2015; Zhang et al., 2018). As a result, we believe FGF21 and AMI Fibroblast growth factors (FGFs), together with their receptors, are prognosis are found in a variety of cardiac cell types and control the development and operation of the heart in both mice and humans in an autocrine/paracrine way , FGF-21 is a possible biomarker for cardiovascular disorders since, among them, a high level of circulating FGF-21 has been shown in human heart diseases such as acute myocardial infarction, coronary heart disease, heart failure, and diabetic cardiomyopathy(Gillum *et al.*, 2020).

Neiutrophophil gielatinase-associated lipocalin (NGAL), a 25 kDA glycoprotein of the liapocalin supeirfamily, is synthisized by granulociyte progenitors one the bone marrow during a brief period of development, it is retained in mature neutrophil granules in conjunction with gelatinase. Because it rises in plasma and urine levels before creatinine levels, NGAL has recently been identified as a key predictor of acute kidney injury (AKI), increased systemic and myocardial expression of NGAL after acute MI has also been associated to cell death, inflammation, and matrix degradation (Helanova *et al*., 2014)

The ninth member of the proprotein convertase family, proprotein convertase subtilisin/kexin9 (PCSK9), is specifically developed to target and destroy the LDL receptor (LDLR) (Artenstein & Opal, 2011). The majority of the PCSK9 detected in peripheral blood was generated by the liver (Zaid *et al*., 2008). LDLR is prevented from recycling to the cell membrane by the PCSK9-LDLR complex, which takes it to the lysosome for destruction (Xiao et al., 2019).PCSK9 has also been found in a number of biological organs, including the heart, brain, and lung (Ding et al., 2018) (Fagerberg et al., 2014). PCSK9 inhibitors have been linked to early plaque development, late plaque rupture, thrombosis, and angiogenesis in addition to their ability to lower LDL (Norata *et al*., 2016). According to Sun et al. (2012), PCSK9 interacts with apo-lipoprotein B and inhibits it from degrading within cells regardless of the presence of the low-density lipoprotein receptor. A

clinical investigation discovered that PCSK9 levels, regardless of LDL plasma levels, are linked to an increased risk of future cardiovascular events. (Leander et al., 2016). PCSK9 levels in people with stable coronary artery disease were linked to white blood cell count, and PCSK9 affected sepsis and rheumatoid arthritis (Schreckenberg *et al*., 2022) .

PC2 (PCSK2)-defective mice also seem normal at birth but present severely impaired pancreatic processing of proglucagon, prosomatostatin, and proinsulin; altered pancreatic islet morphology as well as a small decrease in growth rate , they thus activate or inactivate receptors, ligands, enzymes, growth factors or viral glycoproteins (Canuel *et al*., 2013

Von Willebrand Factor serves as an important part to the coagulation cascade to promote hemostasis. In the event of an injury, vWF binds to the subendothelial collagen through its A3 domain, followed by binding to the inactive platelets via its A1 domain to the platelet glycoprotein (GP) receptor; thereby recruiting additional platelets to the site of injury(Kovacevic *et al*., 2019).

Genetic factors help in explaining the molecular basis of the disorder and in designing prevention and treatment of the disease. Several polymorphisms in different genes in the renin-angiotensin-aldosterone system (RAAS) have been linked to heart disease. The RAAS system is essential for vascular homeostasis and plays an important role in the development of cardiovascular diseases (Fuster, 1994). Angiotensin I-converting enzyme (ACE) is a key component of the RAAS. Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase that cleaves the C-terminal dipeptide (His-Leu) from angiotensin 1 and generates a vasoconstrictor (angiotensin II) The ACE gene maps to chromosome 17q 23, and consists of 26 exons and 25 introns (Schreckenberg *et al*., 2022) .

Some studies on the association between ACE genotypes and the risk of CAD have provided controversial results, especially when combined with bad food choices that contains high fat and salt methods. To the best of our knowledge, the

study is the first to report on possible association of ACE polymorphisms as predisposing factors in the development of the CAD in the West Bank, Palestine(Ozawa *et al*., 2023).

1.1Aim of study:

This study aimed to provide insight to know the physiological changes that help in prediction and diagnosis of coronary artery diseases (CAD). To assess the role of biochemical tests evaluation of patients with moderate risk for coronary artery Such information is no doubt necessary as background for any programs disease devised in the future for studying CAD and treating this disease also. This study will aid in gaining a better understanding of pathophysiology of CAD

1 - Estimate biomarkers ((Proprotein convertase subtilisin Kexin 9 (PCSKA) , Fibroblast growth factor 21 ,Lipocalin -2 ,Von Willebrand protein factor (levels in serum)

2 - Measuring Blood pressure for all patients and control , Compare between biomarkers in patients and control group .

3- Estimation of lipid profile **A**- Cholesterol , **B**-Triglyceride , **C**-HDL-C . **E** - LDL-C

4 - Compare between each biomarker in patients according to ages and BMI and types of disease , level of smoker and blood pressure Compare between stable angina and unstable and myocardial infarction .

5- correlation between biomarker and lipid profile .

The genetic side:

The study includes the study of allelic forms and genotypes of genes

1- ACE gene 2- PCSK9 gene

And find the relationship that connects these polymorphisms with the level of the protein encoded for these genes.

2.Literature review

2.1. Cardiovascular health definition

Cardiovascular health (CVH) is defined by the absence of clinical manifestation of CVD together with the presence of optimal levels of all life's Simple 7 (LS7). These include four health behaviors (not smoking, healthy diet pattern, sufficient physical activity, normal body weight), and three health factors (normal level of total cholesterol, of blood pressure, and fasting blood glucose) in the absence of drugs treatment (Ahmed & Abdalla, 2017).

2.1.1. **Cardiovascular disease definitions**

CVDs, refers to various chronic pathology or events that have in common a pathophysiology related to atherosclerosis and including

• Coronary artery disease (CAD): stable angina, unstable angina, myocardial infraction, sudden death

• Cerebral vascular accident: stroke: hemorrhagic, ischemic or transit ischemic attack

• Peripheral Artery Disease (PAD): Lower Extremity Artery Disease (LEAD), aortic aneurysm

• Congestive heart failure (CHF) The burden of coronary artery disease, cerebral vascular accident and lower extremity artery disease will be displayed in this thesis.(Cheng *et al.*, 2018) .

2.1.2. Coronary artery disease

Also, known as ischemic heart disease (IHD) refers to conditions that involve impairment of coronary artery blood flow that can result in silent ischemia, angina pectoris, acute coronary syndrome (ACS) or sudden cardiac death. Coronary artery disease (CAD) is a common public health problem associated with high mortality and increased health cost (Years, 2022) .

2.1.3. Myocardial infraction

Myocardial infriaction is defined as thief necrosis when the heart's coat is nourished by the coronary arteries, the myocardium suffers from severe

myocardial necrosis ،It is sometimes referred to be a heart attack since the blood flow in the corona arteries may be bannock. In extreme cases, the heart may cease beating (Blekhman *et al*., 2015) .

Depending on clinical opinion, there are two types of MI: ST-Elevation Myocardial Infraction (STEMI) and Non ST-Elevation Myocardial Infraction (NSTEMI). Additionally, MI is categorized into 5 groups based on its pathogenesis, clinical features, and prognostics (Domouzoglou et al., 2015)

Type I: Unexpected MI Disruption of the decreased myocardial blood flow, atherosclerotic plaque causing thrombus to form, or distant platelet emboli causing myocyte necrosis. Other diseases including coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachy- and brady-arrhythmias, heart failure, anemia, respiratory failure, hypotension, hypertension, and renal failure can cause(Al-Hadraawy et al., 2019) .

type II MI. which causes an ischemic imbalance between the supply and demand of oxygen to the myocardium.

Type III: Death due to myocardial infarction when biomarker results are not available. Myocardial infraction brought on by

Type IVa describes percutaneous coronary intervention .

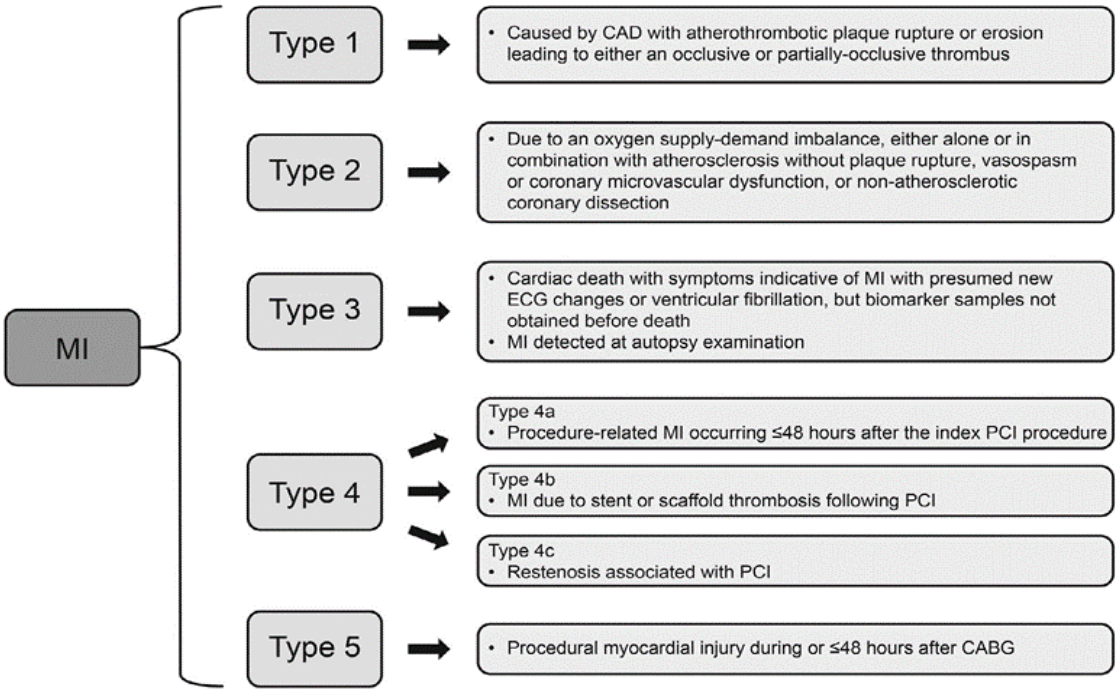
Type IVb: Stent thrombosis-related myocardial infarction

Type V: Coronary artery bypass grafting (CABG)-related myocardial infarction (Eilenberg *et al*., 2016)

2.1.4 . MI subtypes

Over the past 20 years, advances in cardiovascular (CV) imaging, updated ECG criteria, and the accessibility of high-sensitivity Cardiac troponin tests have increased the accuracy of MI diagnosis and made it possible to conduct more thorough analyses of underlying pathophysiology. With their most recent criteria for each MI type published in 2018 Figure (2.1), the European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Foundation (ESC/ACCF/AHA/WHF) Task Force for

the Redefinition of MI divided MI into five main categories according to etiology. These new MI categories, which were then further classified into subgroups according to etiology, were developed as a result of the better MI diagnosis (Thygesen *et al.*, 2018).



A blood clot (thrombosis) that develops inside a coronary artery or one of its branches and prevents blood flow to a certain area of the heart is the most frequent reason for a MI. However, a clot may occur if there is atheroma (the medical word for fatty patches or "plaques") within the lining of the artery Blood clots typically do not form in normal arteries (Lee *et al*., 2023) .

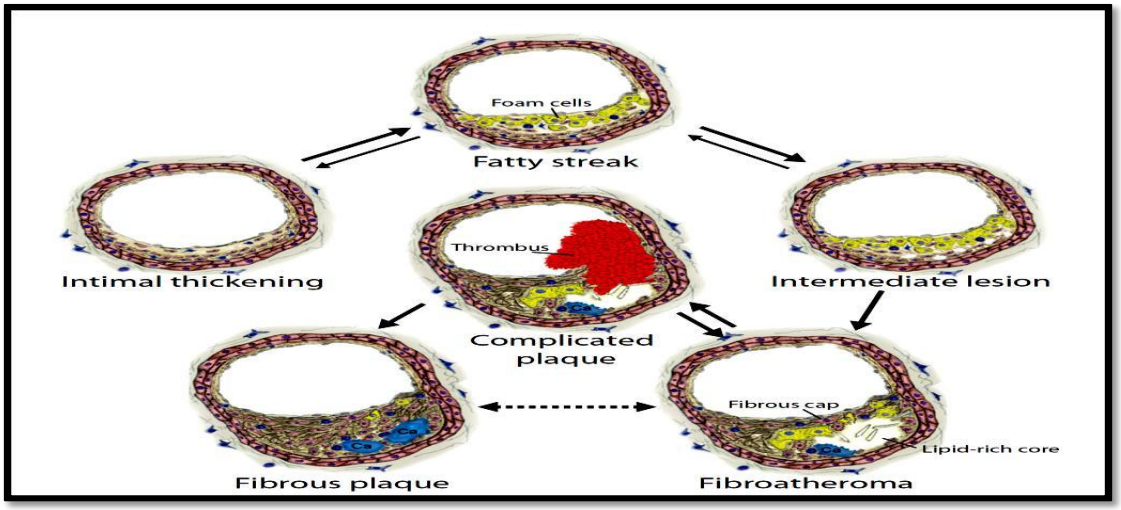
Coronary atherosclerosis is a chronic illness with stable and unstable periods, and during unstable times with active inflammation in the vascular wall, patients may suffer a myocardial infarction Myocardial infarction is a major cause of mortality and disability globally (Kafkas *et al*., 2012) .

A heart attack (myocardial infarction), which typically happens when there is a sudden total blockage of a coronary artery and is caused by a blood clot that forms over a patch of atheroma and blocks the blood supply to a segment of heart muscle, is more likely to occur in angina patients than it would normally(Blekhman et al., 2015; Bruns & Stalder, 2019; Spindel et al., 2012)

2.1.5. Atherosclerosis

Atherosclerosis a slow development of fatty deposits in the walls of the coronary arteries, is what causes ischemic heart disease (IHD) (BHF, 2010). These fatty deposits, also known as plaques, are generated first as a result of injury to the endothelium, which lines the inside of the artery. They cause the artery to constrict and restrict blood flow to the heart , in addition, if a piece of the plaque separates from the endothelium, it can lead to the formation of a clot, which blocks the artery and deprives the heart of blood and oxygen, which is known as a myocardial infarction (MI)(Landmesser *et al*., 2022) .

Hypercholesterolemia, hypertension, diabetes, and smoking are a few coronary risk factors that have an impact on this process Endothelial dysfunction, which is brought on by these risk factors, affects the blood vessel's endothelium and plays a key role in the beginning of the atherosclerotic process. Dysfunctional endothelium is characterized by decreased nitric oxide bioavailability and by excessive endothelin production, which compromises vascular hemostasis; increased expression of adhesion molecules (such as selectins, vascular cell adhesion molecules, and inter(Jakovljevic *et al*., 2011) .



Atherosclerosis stage is shown in Figure (2-2). (Feder *et al*., 2021)

2.1.6 . Myocardial infarction pathogenesis

Cell death is classified pathologically as coagulation and/or contraction band necrosis, which typically evolves through oncosis but can result to a lesser

extent from apoptosis Careful analysis of histological sections by an experienced observer is essential to distinguish these entities ,it takes a certain amount of time (as little as 20 minutes or less in some cases) for cell death to manifest after the commencement of myocardial ischemia. Depending on the presence of collateral circulation to the ischemic zone, persistent or intermittent coronary arterial occlusion, the sensitivity of the myocytes to ischemia, pre-conditioning, and/or, finally, individual demand for myocardial oxyglycation, complete necrosis of all myocardial cells at risk requires at least 2-4 h or longer *(Spiel et al*., 2008).

Table(2-1): Classification and Severity of Angina

Classification and Severity of Angina according to the Canadian Cardiovascular Society

Class I	(no limitation of ordinary activity)	Angina reproduced with strenuous exertion
Class II	(slight limitation of ordinary activity)	Angina reproduced on walking rapidly
Class III	(marked limitation of ordinary activity)	Angina reproduced on walking 100-200m
Class IV	(inability of activity)	Angina reproduced for any activity

Typical angina(Definite) all three of the following characteristics Substernal chest discomfort of characteristic quality and duration Provoked by exertion or emotional stress relived by rest and /or nitrates within Minutes.

Atypical angina (probable) two of these characteristics

Non-anginal chest pain Lacks or meets only one or none of the characteristics(Krychtiuk *et al*., 2021).

2.2. Cardiovascular risk factors in the world

2.2.1. Hypertension

Approximately one billion of people worldwide have HTN which corresponds to more than40% , and projected to increase by 30% in 2025 (Kearney *et al.*, 2005). In all world regions the prevalence is similar in males and females and rises with increasing age and BMI (Gerrits *et al*., 2020) .

2.2.2. Hypertension and cardiovascular disease

Several studies have reported the association between either SBP or DBP and the increase CVD risk. There is no threshold at which the risk becomes apparent. Stroke, CAD, left ventricular hypertrophy, LEAD and chronic kidney disease are the main complications of HTN. An increase of 20 mmHg in SBP or a 10 mmHg increase in DBP was associated with a 2-fold increased risk of death from stroke, heart disease or other vascular disease , individuals with high-normal BP (130-139/85-89mm Hg) have a 3-fold greater risk of progression to HTN and 2-fold increase risk of CVD (Association, 2022) .

The risk of CVD associated with hypertension is observed from 30 years to 80 years of age , in the Framingham Heart Study during 36-years of follow up HTN was associated with a 2 to 4 fold increase of cardiovascular events in men and women equally , HTN is closely associated with the risk of stroke, and it is the commonest factor for end stage renal disease. Controlling BP alone decreases the risk of stroke by 30% and MI by 20%-25%(Kaba *et al*., 2004) .

2.2.3. Obesity

In 2016, 39% of adults aged 18 years (39% of men and 40% of women) were overweight and 13% of the world's adult population (11% of men and 15% of women) were obese (WHO, 2017a), the prevalence of obesity in 2015-2016 among American adults was 39.6% and 4 in10 adults were obese , the Middle East region is affected by alarming increase in the prevalence of obesity at all ages, mainly in the Arab countries , where the prevalence is close to that found in western countries. The areas with the higher rate were Jordan (49.7%),

Palestine (41.5%), Qatar (40.8%),Tunis (34%) and Oman (30.8%) (Crudele *et al*., 2023) .

2. 2.4. Obesity and cardiovascular disease

Overweight or abdominal obesity causes or exacerbates other cardiovascular metabolic risk factors including hypertension, diabetes, dyslipidemia , these risk factors in turn, increase the likelihood of morbidity and mortality from CVD and contribute to increased health care costs , Adiposity is the result of the balance between energy intake and energy expenditure , the rapid rise in the rate of obesity is driven by increased total energy intake, sedentary life or both , Higher body mass index (BMI) was associated with premature mortality, non-smokers who were obese at age 40 years died 6-7 years earlier than no obese , another important factor associated with obesity is the socio-economic status (SES)(Yang *et al*., 2016).

2. 2.5. Smoking

The most common form of tobacco use are cigarette smoking, electronic cigarette (e-cigarette) involving the inhalation of a vaporized liquid that includes nicotine, solvents, and flavoring cigarillos, water pipe and hookahs , This addictive practice is a well-known cause of cancers, cardiovascular, and respiratory diseases (Office of the Surgeon General (US) & Office on Smoking and Health (US), 2004). Cigarette smoking increases inflammation and thrombosis leading to oxidative stress manifestation, prothrombotic activity, platelet aggregation, leukocyte activation, lipids peroxidative and smooth muscle proliferation(Ahmed & Abdalla, 2017)

2. 2.6. Risk factors

The relative risk associated with the most important risk factors for stroke are displayed (Al-Fatlawi *et al*., 2020)

2. 2.7.1. Age

By 2030 20% of the population will be aged > 65 years. In this age group CVD will result in40% of all deaths and will be the leading cause , the cardiovascular system is strongly affected by the ageing process leading to progressive

deterioration in structure and function of the heart and vasculature that contribute to the development of CVD . Epidemiologic studies revealed that at any age the risk of cardiovascular events varies widely (4-5-fold) depending on the associated risk factors(Costa *et al*., 2016).

2. 2.8. Serum lipids

Dyslipidemia is defined by the elevation or attenuation of serum lipids Cholesterol and triglycerides are the major lipoproteins. To date, there is no evidence that fasting is superior to non-fasting in evaluating a lipid profile for cardiovascular risk prediction, Many countries are currently in the process of modifying their guidelines for measuring a lipid profile in the nonfasting state, which facilitates blood collection for patients, laboratory technicians and clinician , All lipoproteins have a common basic structure but they differs in their size, density composition and chemical proprieties , The different lipoproteins are including chylomicrones, Intermediate Density Lipoprotein (IDL), Very Low-Density Lipoprotein (VLDL), LDL, HDL, and apolipoproteins such as (Apo A, apo B, apo C and apo E) (Julius *et al*., 2019) .

2. 2.9. Stress

Everyone feels stress in different ways and reacts to it in different waysHow much stress you experience and how you react to it can lead to a wide variety of health problems , The concept of psychological stress produces a physiological change by the activation of the hypothalamic-pituitary-adrenocortical and sympathetic nervous system, which triggers pathophysiological mechanisms that include inflammation, hemostasis, and dysfunction of metabolic and cardiac autonomic control (Kivimäki *et al*., 2017).

2.3 . Biological indicators

2.3.1 .FGF-21, or fibroblast growth factor

2.3.1.1 Definitions and roles

The endocrine subfamily of FGFs, which also includes hormone-like FGFs, includes FGF21 . FGF21 is mostly released by the liver but is also expressed in the pancreas, adipose tissue, and skeletal muscle . In a physiological sense, fatty acid oxidation and ketogenesis are mediated by endogenous activation of FGF21 during fasting or starving circumstances has systemic advantageous metabolic effects in target organs including adipose tissues, pancreas, and liver (Kotsiou, Kotsios, *et al*., 2018)

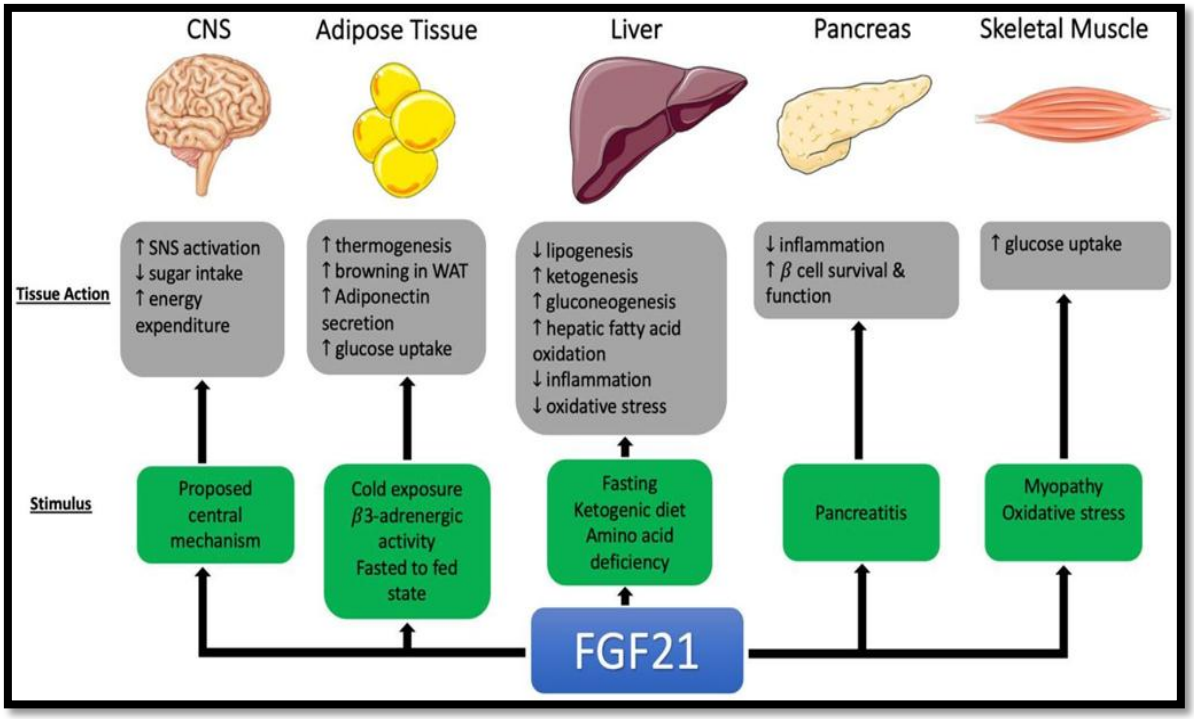


Figure (2-3) shown are Tissue-specific activities of FGF21 in animal studies (Villarroya *et al*., 2018) .

Fibroblast growth factors (FGFs), together with their receptors, are found in a variety of cardiac cell types and control the development and operation of the heart in both mice and humans in an autocrine/paracrine way , FGF-21 is a possible biomarker for cardiovascular disorders since, among them, a high level of circulating FGF-21 has been shown in human heart diseases such as acute

myocardial infarction, coronary heart disease, heart failure, and diabetic cardiomyopathy(Gillum *et al.*, 2020) .

2.3.1.2 Organization

A large family of polypeptides known as FGFs has significant biological effects. Humans have 22 members of the FGF family, each of which is found in a distinct tissue and has a particular purpose. Seven subfamilies of FGFs that are functionally categorized according to their intracrine/intracellular, paracrine, and endocrine effects have been found by phylogenetic study of the human FGF gene family (Kotsiou, Kotsios, *et al.*, 2018) .

The paracrine and endocrine factors work by attaching to certain FGF receptors (FGFRs), which are secreted or released from the cell. Because of their extremely low heparin-binding affinity, endocrine FGFs can be released into the bloodstream. , Numerous FGFs have been investigated as potential therapeutics for cardiovascular illness, along with other cytokines such vascular endothelial growth factors (VEGFs) (Kiluk *et al.*, 2019).

The chromosome 19 region contains the FGF21 gene. Peroxisome proliferator-activated receptor a (PPARa) in the liver and PPARg in adipose tissue are the main regulators of its expression , Additional transcription factors also have a role in controlling the expression of FGF21, including Jumonji-D3, ATF4, Kruppel-like factor 15, retinoic acid receptor-related orphan receptor a, Hirai et al. 2019, retinoic acid receptor-related orphan receptor 4, and carbohydrate-responsive element binding protein (Aleem *et al*., 2021) .

Only homologous FGF receptors (FGFRs) in the target tissues can be activated by FGF21 when co-receptor b-klotho is present. The targeting signal of FGF21 is mediated by the cell surface protein b-Klotho, whereas the intracellular signal is mediated by FGFR , the N- and C-termini of FGF21 bind to FGFR and b-klotho, respectively. Although not widely distributed, klotho protein is highly expressed in the pancreas, liver, gall bladder, colon, and adipose tissues (Seaman *et al*., 2015).

2.3.1.3. FGF21's mode of action in cardiovascular diseases

The association between FGF21 and CVDs, including CHD (, and HF , has been found in a number of investigations. FGF21 has cardioprotective properties in part because of modulating oxidative stress, lipid metabolism, autophagy, and apoptosis (X. Zhang *et al*., 2008) .

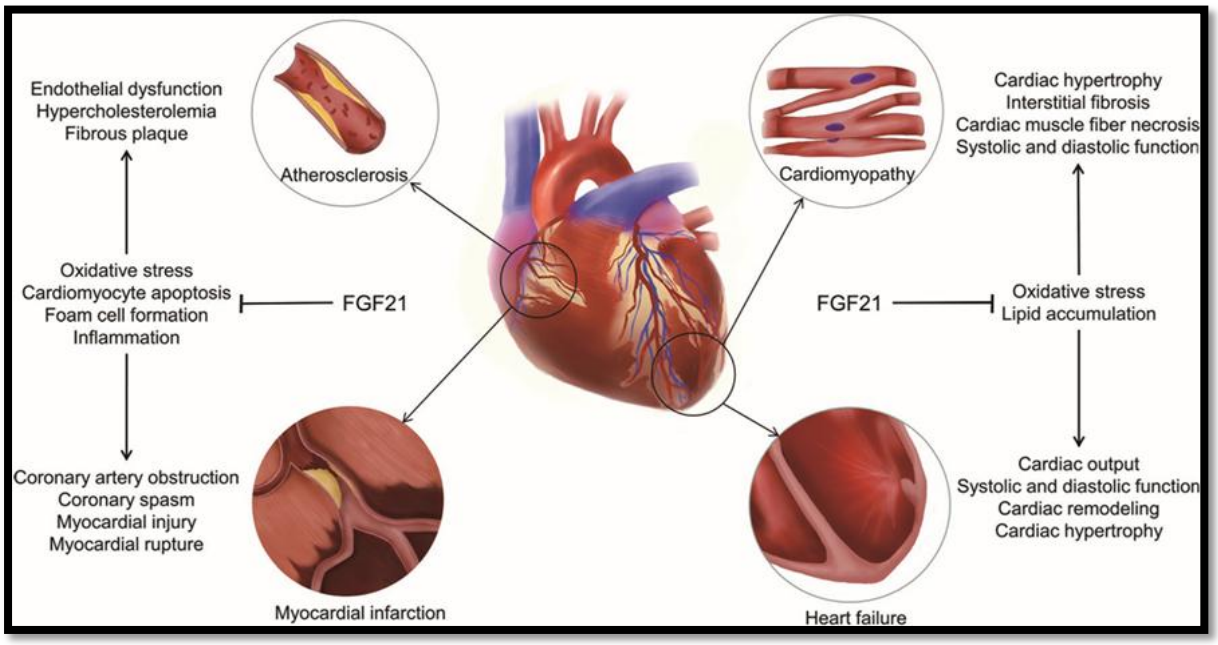


Figure (2- 4) Pathophysiological alterations in cardiovascular disorders and the part FGF21 plays in these processes are shown (Jin *et al*., 2018 ; Kovacevic *et al*., 2019)

The link between FGF21 and heart disease Atherosclerotic plaque development in the coronary arteries is the main pathological alteration seen in CHD Through the activation of cellular antioxidant defense, the antioxidant response element (ARE) and transcription factor E2-related factor 2 (Nrf2) signaling pathways have an anti-atherosclerotic impact ,When FGF21 was upregulated in atherosclerotic rats, it enhanced the expression of markers associated to the antioxidant system, the Nrf2/ARE pathway, and endothelial dysfunction while decreasing endothelial dysfunction when FGF21 was downregulated (Madrigano, 2008).

Through ATF4 and CCAAT enhancer-binding protein homologous protein (CHOP), ERS stimulated the production and secretion of FGF21 , The

expression of ERS-specific proteins, including as glucose-regulated protein-94, caspase-12, and CHOP, was reduced by FGF21. By preventing ERS-induced apoptosis, FGF21 further reduced atherosclerosis in apolipoprotein E (ApoE) animals (Chong *et al*., 2019).

The primary pathogenic characteristics of atherosclerosis are vascular endothelial cell damage and apoptosis. FGF21 accelerated vascular remodeling brought on by ischemia and an eNOS-dependent mechanism Hyperglycemia-induced damage to aortic endothelial cells is lessened by FGF21 (Paciullo *et al*., 2019).

2.3.1.4. The mechanism of action of FGF21 in MI

According to certain research, cardiac ischemia causes the sympathetic nervous system to become activated, which leads to the lipolysis of adipose tissue and a rise in catecholamines and saturated fatty acids (SFAs) , Then, in vitro studies demonstrated that catecholamines and SFAs caused AMPK to become activated, increasing the amount of FGF21 that was produced and released by cardiomyocytes and creating a cardiac AMPK-FGF21 feed-forward loop. According to this protective signaling mechanism is efficient and durable against ischemia stress(Schlein *et al*., 2016).

In addition, according FGF21 prevented cardiomyocyte death via lowering caspase-3 activity and altering galectin-3 in cardiomyocytes. Additionally, mice who received intramuscular injections of exogenous FGF21 had higher plasma levels of the cardioprotective adipokine adiponectin. Additionally, it was shown that the marginal infarction zone's capillary development improved while cardiomyocyte apoptosis was simultaneously inhibited. Figure 2.6 provides an illustration of the molecular pathways behind the cardiac effects of FGF21 in relation to the development of HF(Le *et al*., 2023).

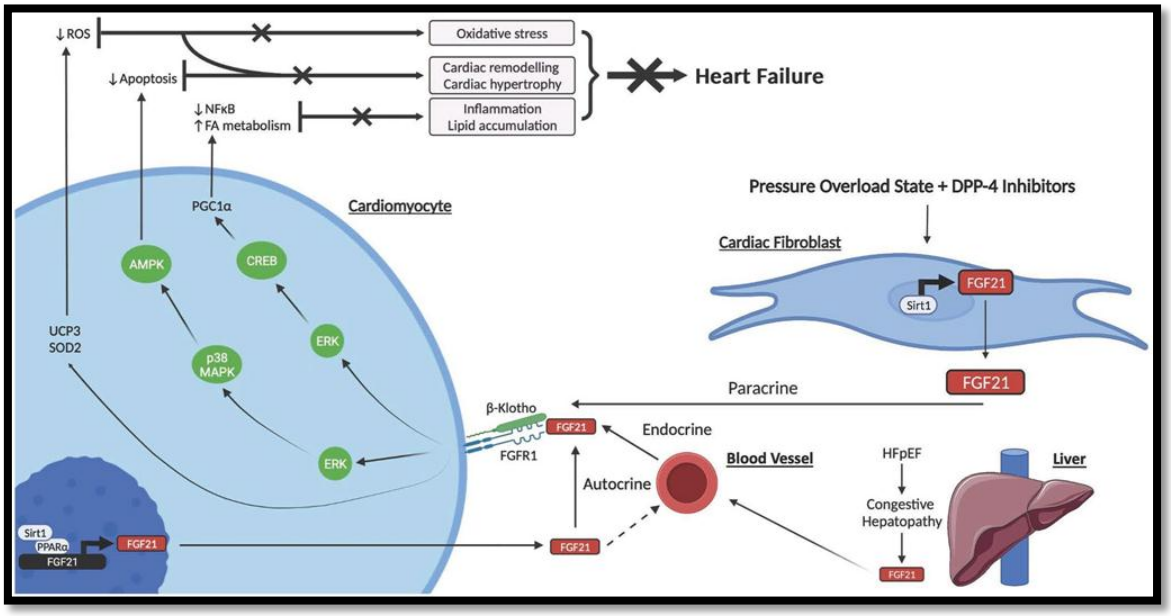


Figure (2-5) shown are Mechanisms of FGF21 cardioprotection against the development of HF (Schlein *et al.*, 2016)

2.3.1.5 . Physiological Role of FGF21

FGF21 in the Population**:** The first observation of FGF21 in humans was described in patients with type 2 diabetes mellitus (T2DM) , Paradoxical to its glucose and lipid regulatory effects, plasma FGF21 was increased in patients with diabetes compared to non-diabetic controls. Following that report, elevated serum FGF21 was observed in overweight and obese patients compared to lean controls and positively correlated with risk of metabolic syndrome, adiposity, fasting insulin, and triglycerides , Investigators also found FGF21 expression in subcutaneous adipose tissue correlated with serum FGF21 in a subset of this population , giving rise to FGF21 as an adipokine. Introducing more complexity, reported that circulating FGF21varied nearly 250-fold males and females and failed to find relationships between FGF21 and BMI, lipids, or plasma glucose(Panahi *et al*., 2016) .

2.3.2 . Neutrophil gelatinase associated lipocalin or NGAL

Glycoproteins play a key role in the body's defense against multiple diseases. From being structural components of the cell membrane to antigenic determinants on immune cells, glycoproteins serve an important functional role in the body. Circulating glycoproteins are also commonly used as blood-based biomarkers to detect and follow the progression of both benign and malignant diseases. Most of these glycoproteins are large molecules , there is a family of small, secreted glycoproteins that are important in the maintenance of health and in combating diseases effectively. This family of proteins is called "lipocalins". A prototype of this family called Neutrophil gelatinase associated lipocalin or NGAL (also called lipocalin 2 or 24p3) has emerged in recent years as a biomarker in several benign and malignant diseases. Further, studies in cultured cells and inmurine models have revealed a pivotal role for this molecule both in health and disease(Abdelrazek *et al*., 2020).

2.3.2.1. The Lipocalin family

Lipocalins are a diverse family of small secreted proteins that act as carriers, transporting predominantly small lipophilic molecules, in recent years, several additional functions have been discovered for these proteins, including regulation of cell division (e.g. α1- microglobulin), differentiation, cell to cell adhesion and survival (e.g. Purpurin), Unlike most other protein families, whose members are identified on the basis of similarities in their amino acid sequence, the members of the lipocalin family share much less sequence identity, in some cases as low as 20%. However, they all share a common secondary and tertiary(Helanova *et al*., 2014)

2.3.2.2. Inflammatory diseases—NGAL expression is upregulated in several acute and chronic inflammatory diseases . NGAL was expressed at a higher level in the skin of patients with psoriasis compared to patients with atopic dermatitis or eczema. A significant negative co-relation was observed between the expressions of NGAL and the degree of differentiation of keratinocytes . Staining of skin tissues underneath areas of parakeratosis (i.e abnormal differentiation) revealed a strong positivity for NGAL while that for filaggrin, a marker of terminal epidermal differentiation was absent, suggesting that NGAL is expressed at a higher level by undifferentiated epidermal cells(Valluru *et al*., 2011 ; Buonafine *et al*., 2018) .

2.3.2.3. Ischemic diseases—A second group of diseases associated with significant elevation in NGAL levels are ischemic disorders, i.e. diseases characterized by a decrease in blood supply to a particular organ with resultant hypoxia and either temporary (e.g. fatty change) or permanent (e.g. apoptosis and necrosis) tissue damage. The major ischemic diseases associated with an elevation in NGAL include cerebrovascular accidents and myocardial infarction(Ahmed & Abdalla, 2017 ; Zhang *et al*., 2008).

2.3.2.4. NGAL receptors

While it is known that exogenous NGAL (or Lcn2 in mouse) can produce effects similar to that produced by overexpression of the protein, it is not yet clear if there is a definite receptor for NGAL. Two candidate proteins have been however come to the forefront(Chong *et al.*, 2019).

2.3.2.5. Cardiovascular disorders Acute decompensated heart failure (ADHF) which is defined as “either a gradual or a rapid alteration in the signs and symptoms of heart failure that necessitate emergent institution of appropriate therapy” constitutes a major public health problem in the United

States . A single center study involving 91 patients with ADHF observed that patients who developed WRF had significantly higher serum NGAL levels at the time of admission. Further, higher NGAL levels at admission were an associated with a greater risk of developing WRF(Chapin, 2018)

2.3.2.6. NGAL - physiological functions

Neutrophil gelatinase-associated lipocalin (NGAL), also called lipocalin-2, is a representative of a large group of lipocalins, which are small extracellular proteins with a variety of functions. This acute phase protein is a 25 kDa large glycosylated monomer of simple protein chains. It was originally identified as a protein isolated from specific neutrophil granules, and subsequently it was proved to be covalently bound with neutrophil gelatinase (an enzyme of the matrix metalloproteinase group – collagenase IV of 92 kDa, contained in neutrophils(Valluru *et al*., 2011) .

2.3.2.7 .NGAL and cardiovascular diseases

In cardiovascular diseases, there may be more mechanisms causing elevated NGAL levels, and they may be active in several ways , increased levels of NGAL were found in patients with hypertension compared to those with normal blood pressure, which were related to renal function (expressed by glomerular filtration and the level of creatinine or cystatin C), age and the duration of hypertension (Helanova *et al*., 2014).

2.3.2.8. Organization

A glycoprotein of 198 amino acids, lipocalin-2 (LCN-2) is a new adipokine. LCN-2 is also known by the names siderocalin, Uterocalin and neutrophil gelatinase-associated lipocalin (NGAL). The lipocalin superfamily, which LCN-2 belongs to, is a group of circulatory proteins that transports tiny, hydrophobic compounds such hormones, retinoids, prostaglandins, fatty acids, and steroids LCN-2's atomic structure The human LCN-2 protein, as well as its equivalents

in mouse and rat, have a signaling 20-amino acid peptide at their N-termini that is released before being detached from the molecule(Kotsiou, Kotsios, *et al*., 2018).

The lipocalin domain, which is where lipocalin binds to their ligands, borders this region. The eight-stranded beta-barrel that forms the closing calyx in the antiparallel direction of the extremely conserved LCN-2 structure serves as the internal ligand-binding site that enables the lipocalin moiety to attach to its ligands. Compared to other lipocalin proteins, LCN-2 has a substantially bigger and more polar binding cavity. By binding to larger and less hydrophobic ligands like mammalian proteins, LCN-2 can bind to receptors on the surface of plasma membranes to form large complexes of molecules, which in turn allows the cell to carry out important functions in cellular division and regulation(Chong *et al*., 2019) .

2.3.2.9. – Mechanism of action

Megalin /glycoprotein GP330, which binds to human LCN-2 , and SLC22A17 or 24p3R, which binds to the LCN-2 mouse protein, have both been proposed as cell surface receptors for this protein , there is a gene on chromosome 9 (locus 9q34.11) that encodes LCN-2. Numerous functional transcripts from the LCN-2 gene eventually code for a secreted protein of 198 amino acids. LCN-2 was first isolated from mice kidney cells and neutrophil granules produced at sites of infection and inflammation in humans(Fan *et al*., 2020)

Infection, damage, and inflammation are protected against by this , as well as other different kinds of cellular stress , additionally, it has the ability to interact with and stabilize matrix metalloproteinase 9 (MMP-9) in human neutrophils. The LCN-2 and MMP-9 combination increases in vitro MMP-9 activity while inhibiting MMP-9's auto-degradation , The extracellular matrix and basement

membranes can both be destroyed by MMP-9, according to the LCN-2/MMP-9 complex is thought to promote the growth and spread of tumors. Prior research using animal models suggests that LCN-2 plays significant roles in a variety of physiological and pathological processes , including cell differentiation, apoptosis, organogenesis, inflammation, kidney damage, and liver injury, in addition to its bacteriostatic capabilities. Additionally, LCN-2 may have a part in the development and spread of cancer(Pérez-Gómez *et al*., 2010)

2.3.3. Proprotein convertase subtilisin/kexin type 9 (PCSK9)

Structure 2.3.3.1.

The protein structure of all PCs begins with a signal peptide followed by a prosegment and a catalytic domain, which contains the typical catalytic triad residues Asp (D), His (H) and Ser (S) as well as the Asn (N) comprising the oxyanion hole (which is replaced by Asp for PC2), the first seven PCs also harbor a β-barrel-containing P domain downstream of the catalytic domain, which is thought to stabilize the catalytic pocket and regulate the calcium and pH dependence of the PCs , the C-terminal domain of each PC harbors a specific sequence dictating their cellular localization and trafficking. For example, PC5 and PACE4 contain a C-terminal Cys-rich domain (CRD) allowing them to bind to heparin sulphate proteoglycans both at the cell surface and in the extracellular matrix(Tavori *et al*., 2013).

2.3.3.2. Function

PC2 (*PCSK2*)-defective mice also seem normal at birth but present severely impaired pancreatic processing of proglucagon, prosomatostatin, and proinsulin; altered pancreatic islet morphology as well as a small decrease in growth rate , PC4, furin, PC5/6, PACE4 and PC7 cleave and activate/inactivate secretory precursor proteins in the TGN, cell surface or endosomes. PC4 is a membrane-bound PC which has a reproductive function. Indeed, its transcripts have been

localized exclusively in round spermatids in mice In contrast, furin, PC5/6, PACE4 and PC7 are widely or even ubiquitously expressed and are implicated in processing events occurring in the constitutive secretory pathway, They thus activate or inactivate receptors, ligands, enzymes, growth factors or viral glycoproteins(Canuel *et al*., 2013) .

In cellular studies and in human genome-wide association studies, it was demonstrated that PC7 regulates iron metabolism by shedding transferrin receptor-1 . PC7 loss-of-function (LOF) was also associated with high HDL levels, low TG, low atherogenic small dense LDL and insulin resistance reduction, indicating a decreased CVD risk (Qiu *et al*., 2017).

2.3.3.3. Mechanism of PCSK9 Inhibitors

In the human body, LDL-C levels are regulated primarily through LDL receptors found on hepatocytes , as mentioned above, PCSK9 is a proteolytic enzyme that destroys LDL receptors and hence indirectly modulates serum LDL-C. Blocking or binding circulating PCSK9 using alirocumab or evolocumab leads to more LDL receptors and a decrease in serum LDL-C, extrahepatic organs such as the kidney, gut, and central nervous system contribute to PCSK9 production and, presumably, local modulation of LDL-R expression, despite its hepatic origin, if PCSK9 attaches to the LDL-R before the LDL particle, the entire complex enters the hepatocyte and gets destroyed by the lysosome, this process is demonstrated in Figure *2* below, this implies that lowering the amount of free PCSK9 available to attach to the LDL-R will reduce the destruction of the receptor, therefore, resulting in less LDL-C in the plasma(Ferri, 2012) .

Cardiovascular disease is a major cause of mortality worldwide, the annual death rates from coronary heart disease (CHD) are the highest among cardiovascular patients . There are many well characterized CHD risk factors

that can lead to atherosclerosis, including high blood cholesterol and triglycerides levels .Under normal physiological conditions, dietary fat form a complex with apolipoproteins B (ApoB) into chylomicrons, which are lipolyzed to hydrolyze triglycerides, and stored in adipose tissue for future energy expenditure(Rojas *et al*., 2019).

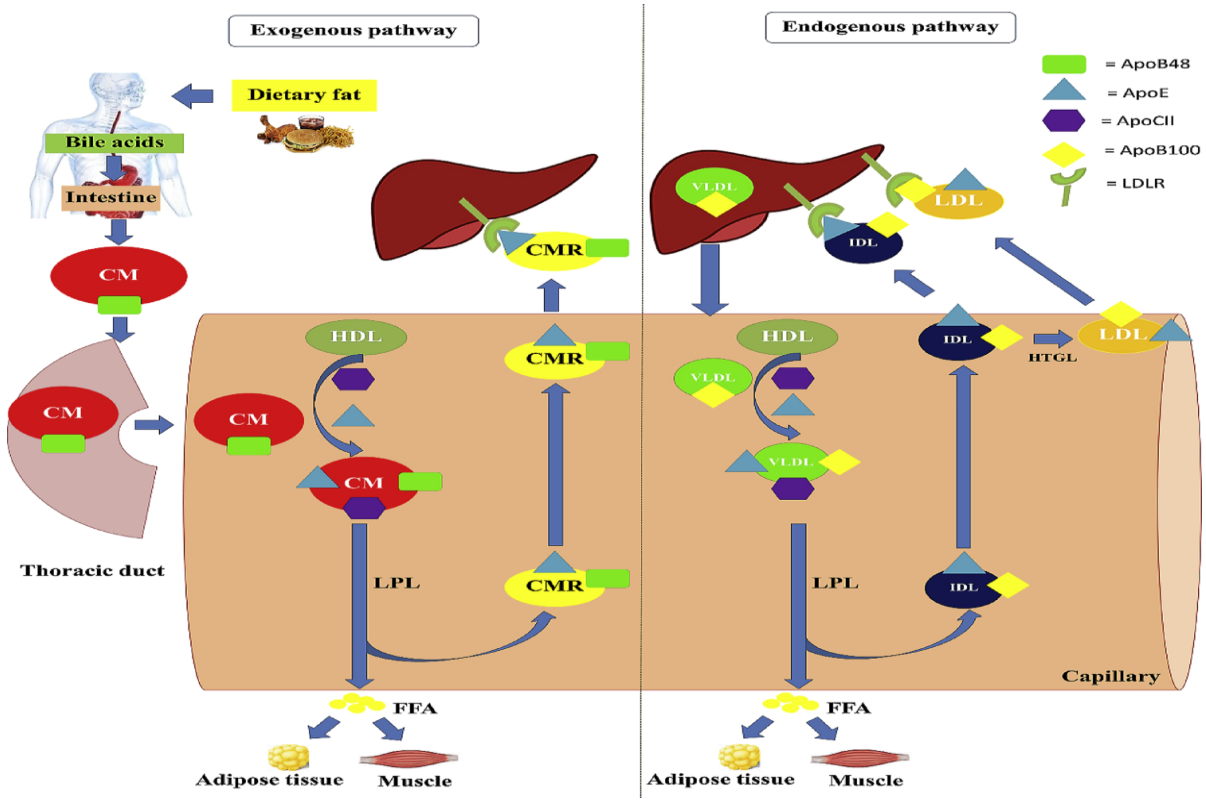


Fig. (2-6) Lipid metabolism is mainly regulated via two pathways: the exogenous pathway and the endogenous pathway. (Tavori *et al*., 2013).

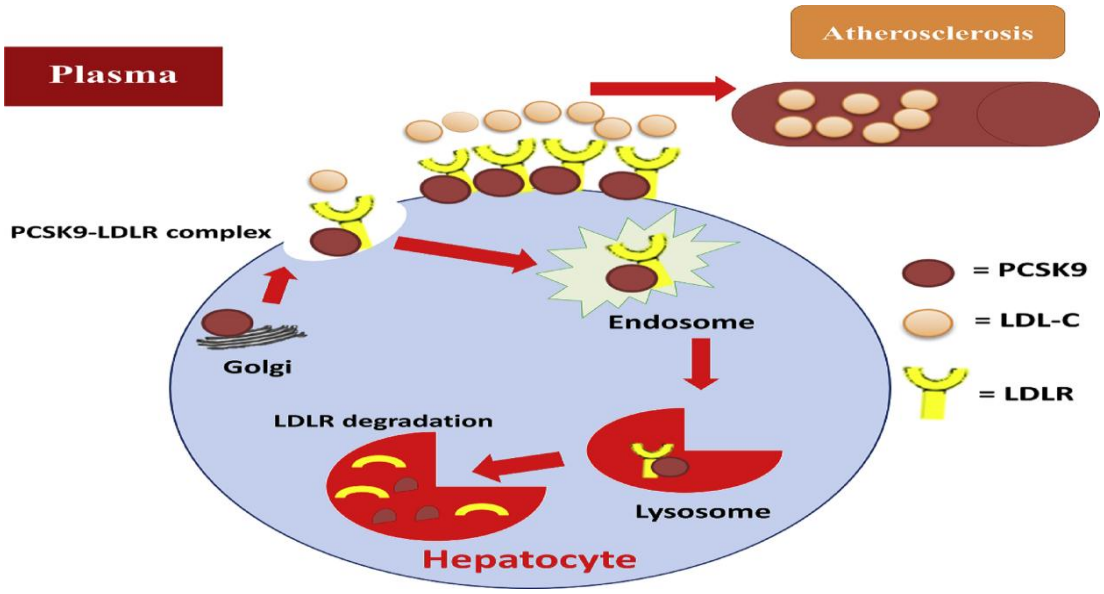


Fig. (2-7) PCSK9 mediated degradation of the LDLR. PCSK9 is secreted by the Golgi apparatus and binds to the LDLR on the hepatocyte surface, before this LDLR-PCSK9 complex undergoes endocytosis and is degraded by lysosomes. (Kovacevic et al., 2019) .

2.3.3.4. The effects of PCSK9 inhibitors on PCSK9 expression and cholesterol regulation: reports from *in vitro* studies

The effects of PCSK9 inhibitors on PCSK9 expression and cholesterol regulation in in vitro studies are summarized . Gain-of-function mutations of PCSK9 genes (S127R, D374Y) or PCSK9 overexpression accelerates the degradation of the LDLR, resulting in low LDL level in the cell(Kovacevic *et al*., 2019) .

In contrast, loss-of-function mutations to PCSK9 genes lead to increased LDLR expression, resulting in increased LDL level in the cell. Therefore, PCSK9 inhibition can increase LDLR density, thus improving lipid metabolism . Consistently, PCSK9 inhibition by siRNA transfection can reduce PCSK9 expression and increase LDLR expression in Huh-7 cells . Moreover, anti-PCSK9 antibodies, which block the binding capacity of PCSK9 to the LDLR, can effectively inhibit the function of PCSK9 . This leads to a decline in LDLR degradation and an increase in LDL-C uptake in the PCSK9 overexpression cellular system (Amput *et al*., 2019).

2.3.3.5. The effects of PCSK9 inhibitors on PCSK9 levels and cholesterol regulation: reports from *in vivo* studies

The effects of PCSK9 inhibitors on PCSK9 levels and cholesterol regulation in *in vivo* studies are summarized Previous studies have shown that high plasma PCSK9 levels result in hepatic LDLR degradation and increased TC respectively In addition, annexin A2 knockout mice had high plasma PCSK9 levels, and had reduced LDLR expression and increased LDL-C levels , additionally, PCSK9 inhibitors can increase LDLR expression via the inhibition of PCSK9 function, resulting in decreased plasma cholesterol levels and an accelerated clearance of circulating cholesterol in cynomolgus macaques fed with high fat diet These *in vivo* reports indicated the potential therapeutic roles of PCSK9 inhibition in hyperlipidemic conditions which could exert beneficial effects and may potentially be used in the prevention of cardiovascular disease in hyperlipidemic patients (Page & Watts, 2016) .

2.3.3.6. The effects of PCSK9 inhibitors on PCSK9 levels and cholesterol regulation: reports from clinical studies

The effects of PCSK9 inhibitors on PCSK9 levels and cholesterol regulation in clinical studies are summarized . Novel PCSK9 inhibitors including alirocumab, evolocumab, bococizumab and RG7652 have recently become a forefront of cardioprotective medicine for regulating cholesterol levels inhypercholesterolemia patients with a significant cardiovascular disease risk. Alirocumab, evolocumab, bococizumab and RG7652 are human monoclonal antibodies targeted to PCSK9, with function to inhibit its binding to the LDLR. Anti PCSK9 antibodies effectively regulate cholesterol incardiovascular disease patients exhibiting hypercholesterolemia levels by demonstrating efficacy in the reduction of TC, LDL-C, triglycerides, non-HDL-C and increased HDL-C Moreover, anti PCSK9 antibodies show higher efficacy in the reduction of LDL-C, triglycerides and elevation of HDL-C, as compared to current standard lipid

lowering drugs(Amput *et al.*, 2019)

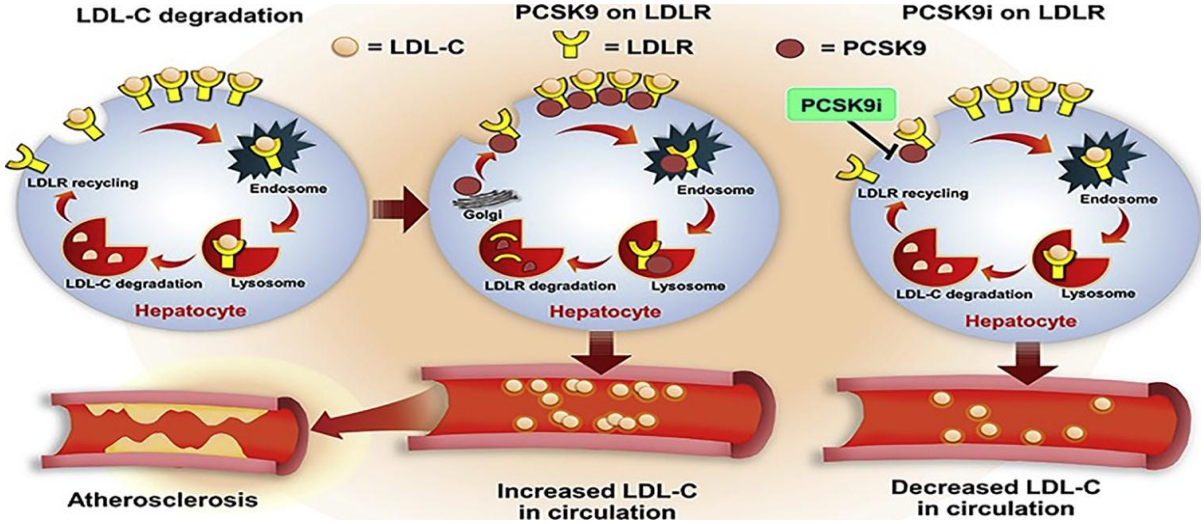


Fig.(2-8). A diagram summarizes the potential benefits of PCSK9 inhibitor. PCSK9 inhibitor decreases the LDL-R degradation, resulting in improved lipid metabolism, reducing plasma LDL-C, leading to reduced risk of atherosclerosis PCSK9: (Amput et al., 2019).

2.3.3.7.PCSK9 Structure and Function in Regulating LDL Levels

PCSK9 is one of nine mammalian serine proteases aptly named due to their relation to the bacterial subtilisin and yeast kexin family, The first eight proteases, PCSK1-8, catalyze the proteolytic maturation of inactive secretory precursors to mature proteins such as hormones, enzymes and peptides(reviewed elsewhere), although PCSK9 is synthesized and secreted primarily by the liver, it is expressed to a lesser extent in the small intestine, kidney, and central nervous system. In the hepatocyte, PCSK9 is synthesized as a 75-kDa zymogen consisting of a signal peptide, a prosegment, a catalytic domain, and a cysteine- and histidine-rich C-terminal domain that is required for trafficking of the PCSK9/LDL-R complex to the lysosome(Ferri, 2012). (Amput *et al.*, 2019).

2.3.3.8. PCSK9 related to cholesterol metabolism

In 2003, PCSK9 mRNA was shown to be downregulated by dietary cholesterol like a cholesterogenic target of SREBP2 in vivo , However, HepG2 cells (hepatocarcinoma human cell line) stably overexpressing PCSK9 or its

natural S127R mutant presented decreased LDLR levels , indicating a direct link between PCSK9 activity and LDLR regulation ,This reduction was abrogated upon incubation of cells with 5 mM ammonium chloride, suggesting that PCSK9 overexpression might increase the turnover rate of the LDLR by enhancing its intracellular degradation in acidic compartments , importantly, several in vivo studies demonstrated that adenoviral-mediated expression of murine or human PCSK9 induced a post-transcriptional degradation of the LDLR, accordingly, mouse models lacking PCSK9 presented decreased cholesterol levels as well as increased hepatic LDLR protein levels(Ferri, 2012 ; (Kovacevic *et al.*, 2019).

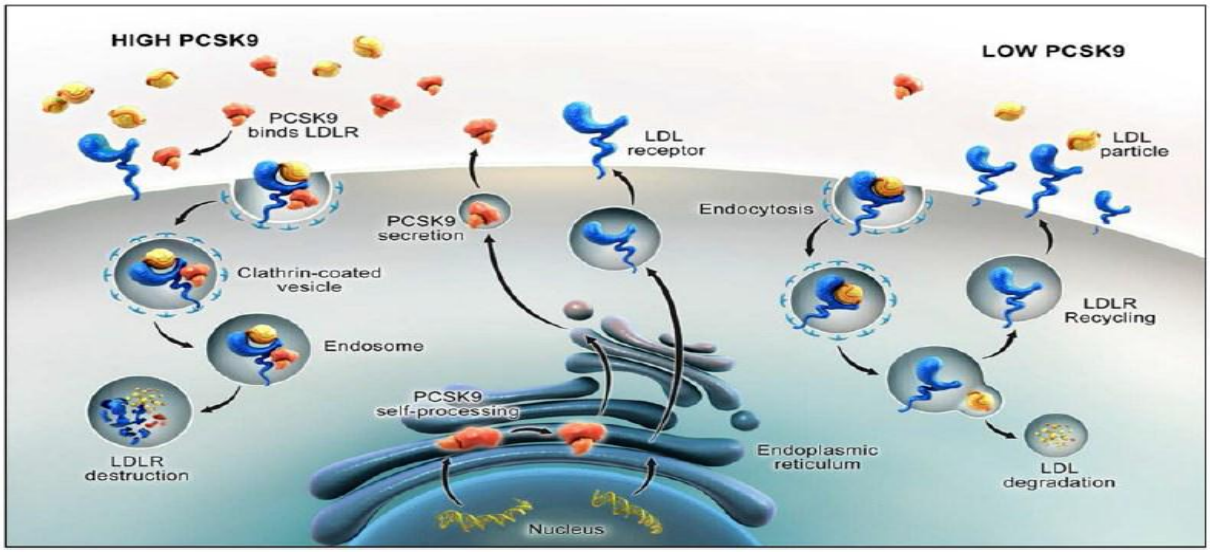


Figure (2-10). Schematic of the extracellular pathway of PCSK9-induced degradation of the LDLR(Canuel *et al*., 2013).

2.3.3.9. Role of PCSK9 in disease

Many studies demonstrated that loss of PCSK9 reduces the incidence of several physiological disorders , Compared to nondiabetic patients, those with T2D are exposed to >5-fold higher CVD-related mortality , This risk can be treated by lowering excess LDLc characterized by this T2D-associated dyslipidemia, also known as diabetic or atherogenic dyslipidemia, using statins , Nonetheless, statin treatment has also been associated with a higher incidence of new-onset of T2D . Many researchers thus tried to study the link between PCSK9 and T2D in several

vitro and *in vivo* experiments and clinical trials, resulting in conflicting (Canuel *et al*., 2013 ; Bergeron *et al*., 2015)

2.3.3.10. PCSK9 gene

The PCSK9 gene on chromosome 1 in humans encodes the enzyme known as proprotein convertase subtilisin/kexin type 9 (PCSK9), according to it is the ninth member of the family of proteins called proprotein convertases that make other proteins active (Zhang *et al*., 2016). Numerous species have orthologous genes, which are similar genes. When PCSK9 is initially created, it is inactive due to a segment of peptide chains (Schreckenberg *et al*., 2022).

Inhibits its activity; proprotein convertases remove this region to make PCSK9 active , One of the 27 loci linked to a higher risk of coronary artery disease is also found in the PCSK9 gene (Kovacevic *et al.*, 2019).

PCSK9 was first identified in humans as a brain-expressed protein . However, the kidney has also been mentioned as having it. the small intestine, liver, and pancreas , according to recent research, PCSK9 has a local effect that can control vascular homeostasis and atherosclerosis in arterial walls, including endothelium, smooth muscle cells, and macrophages , as a result, it is now abundantly obvious that PCSK9 modulates lipoprotein synthesis and has pro-atherosclerotic effects (Kivimäki *et al*., 2017)

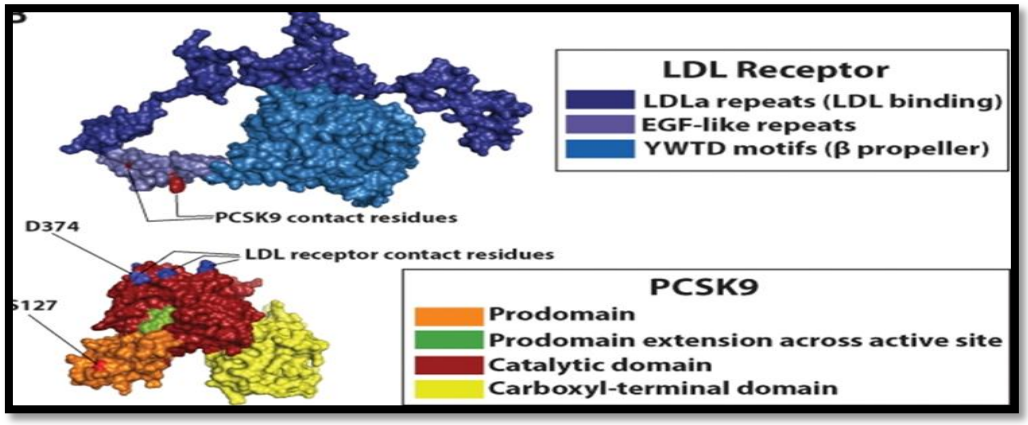
In the endoplasmic reticulum, PCSK9 is processed by autocatalytic intramolecular processing after it is produced as a soluble zymogen , PCSK9 co-localizes with sortilin as it passes through the Golgi and trans-Golgi complex after being processed in the ER , according to research , PCSK9 cellular secretion is thought to depend on a PCSK9-sortilin interaction. Sterol-response element has the ability to control PCSK9 gene expression. the small intestine, the pancreas, and the liver , Recent research suggests that PCSK9 is abundantly expressed in the endothelium, smooth muscle cells, and macrophages of arterial

walls, with a local impact that can control atherosclerosis and vascular homeostasis). It is now abundantly obvious that PCSK9 affects the production of lipoproteins and has pro-atherosclerotic effects (Bergeron *et al*., 2015).

• a fully developed monomeric protein that circulates only in an LDL-bound state.

• Self-associated, multimeric form with likely higher activity

• Inactive, furin-cleaved fragment a method of action



.Figure 2.10 The structure of the LDL receptor and PCSK9 (Fan *et al*., 2020)

PCSK9 is a key player in cholesterol homeostasis as a negative post-translational regulator of the low-density lipoprotein receptor (LDLR), The LDLR-LDL complex is internalized when low-density lipoprotein (LDL) cholesterol binds to its receptor. The resultant endosome's LDLR adopts a hairpin shape when exposed to the acidic environment (Kataoka *et al*., 2021).

2.3.4. von Willebrand Factor

4. 2.3.1 . Von Willebrand factor

Von Willebrand Factor (vWF) is a glycoprotein ranging in size from 600,000 to 20 million Da that participates actively in platelet adhesion to either injured or disrupted vascular surfaces, activation and high-shear state platelet aggregation The vWF gene is located on the short arm p of chromosome 12 (12p13.2) with 52 exons that span 178 kbp (Ahmed & Abdalla, 2017).

vWF is synthesized in megakaryocytes and endothelial cells and follows several distinct pathways of secretion from vascular endothelial cells and platelets, the first represents a constitutive pathway linked directly to synthesis. The second is a regulated pathway involving storage of mature molecules for release following stimulation by one or more mediators, including histamine, leukotriene D4, platelet-activating factor, vascular permeability factor, the terminal component of complement, epinephrine, fluid mechanical forces, factor Vila, thrombin, and fibrin,In addition, Weibel–Palade bodies (containing vWF) are rapidly translocated to the cell surface of platelets following activation (Jakovljevic *et al*., 2011).

2.3.4.2.Von Willebrand factor: structure and function

The pre–pro-vWF molecule consists of a 22-amino acid signal peptide, a 741 amino acid pro-peptide, and a 2050 amino acid mature subunit. The pro-vWF monomer is composed of 4 distinct domains (A–D) as that following : NH2–D1–D2–D'–D3–A1–A2–A3–D4–B1–B2–B3–C1–C2–CK–COOH . vWF multimers are formed by C- and N-terminal intermolecular disulphide bonds, with the largest multimers exceeding 2 × 104 kDa and having the greatest adhesive activity. During synthesis, vWF undergoes extensive post-translational modification resulting in the addition of 12 N-linked and 10 O-linked glycosylation sites per mature monomer. Furthermore, the pro-peptide also contains four potential N-linked glycosylation sites whose function is not fully known. In total, carbohydrate accounts for approximately 20% of the molecular weight of vWF Mature vWF enters the plasma as a series of oligomers containing a variable number of subunits, ranging from a minimum of 2 to a maximum of ~ 40, with the largest HMWM having molecular weights, as previously mentioned, in excess of 20,000 kDa(Chapin, 2018) (Kozlov *et al*., 2022 ; Reardon *et al*., 2021)

2.3.4.3. Regulation of Von Willebrand factor

vWF is dynamically regulated by fluid shear stress which promotes aggregation of multiple vWF units and, at the same time, reduces multimer size using force-dependent cleavage by proteases, notably ADAMTS13, also known as vWF-cleaving protease (vWF-CP) , When vWF is exposed to tensile forces, it has a tendency to unfold and become elongated, asymmetric protein, unfolding the A2 domain in the process as well, which can then be cleaved by ADAMTS13 Shear stress above 60 dyne/cm2 is typically necessary to initiate vWF structural changes . Unlike the vWF-A1 and A3 domains, the vWF-A2 domain is more susceptible to conformation changes and cleavage by ADAMTS13 since the disulfide bridge in this domain is located between vicinal cysteines at the C-terminus compared to a disulfide bridge that lies between the N- and C-terminus of A1- and A3-domains making them more resilient to degradation(Kato *et al.*, 2018; Ozawa *et al*., 2023; Atiq et al., 2019).

2.3.4.4.Von Willebrand factor and platelet aggregate stability

The extracellular domain of glycoprotein (GP) Ibα serves as a primary vWF receptor that triggers shear stress-dependent platelet aggregation , its intracellular domain associates with actin-binding protein-280 (filamin 1a) that binds directly to filamentous actin, thereby linking the membrane skeleton to GPIbα.There is a significant increase in the amounts of actin that co-immunoprecipitate with GPIbα as platelets aggregate in response to shear stress. Monoclonal antibody blockade of vWF binding to GPIbα inhibits shear stress-induced platelet aggregation and actins association with GPIbα , pretreatment of platelets with CyD causes inhibition of actin binding to GPIbα in shear-activated platelets and increases the rate and magnitude of platelet disaggregation , these findings suggest that shear stress causes changes in the association between GPIbα and the actin-based membrane skeleton , the increased interaction between GPIbα and the actin-based membrane skeleton results from shear-induced vWF binding to GPIbα and is considered mechano-protective as it

controls shear-induced aggregation of activated platelets(Seaman *et al*., 2015;Mojzisch & Brehm, 2021)

2.3.4.5.Von Willebrand factor and angiogenesis

vWF is critical for normal vascular homeostasis and integrity. Quantitative and qualitative abnormalities that characterize VWD have shown both in vitro and in vivo to cause enhanced vascularization that increases inversely with the overall degree of residual vWF activity , the available evidence suggests that vWF binding to integrins and several components of Weibel-Palade bodies found within vascular endothelial cells, such as angiopoietin-2 and galectin-3, all converge to regulate vascular endothelial growth factor (VEGF) signaling. HMWMs of vWF are the most important regulators of angiogenesis (Mojzisch & Brehm, 2021 ; Kovacevic *et al*., 2019).

2.3.4.6. Prevalence of AVWS in Cardiovascular Disease

AVWS has been described is a number of cardiovascular diseases and conditions. In most instances, high blood shear rates and stress are present Congenital heart disease , the incidence of AVWS in patients with congenital heart disease ranges from 1–2% up to as high as 20–30% according to the complexity of disease and conditions that include high-shear blood flow as might be seen with Eisenmenger's syndrome (Jin et al., 2018) . In a study of children with either congenital pulmonic or aortic stenosis, there was a history of bleeding in 9% and 18%, respectively,seven of the 60 children (12%) had laboratory findings consistent with a diagnosis of AVWS, and two of these (28%) had a history of bleeding (Chapin, 2018;Seaman *et al*., 2015)

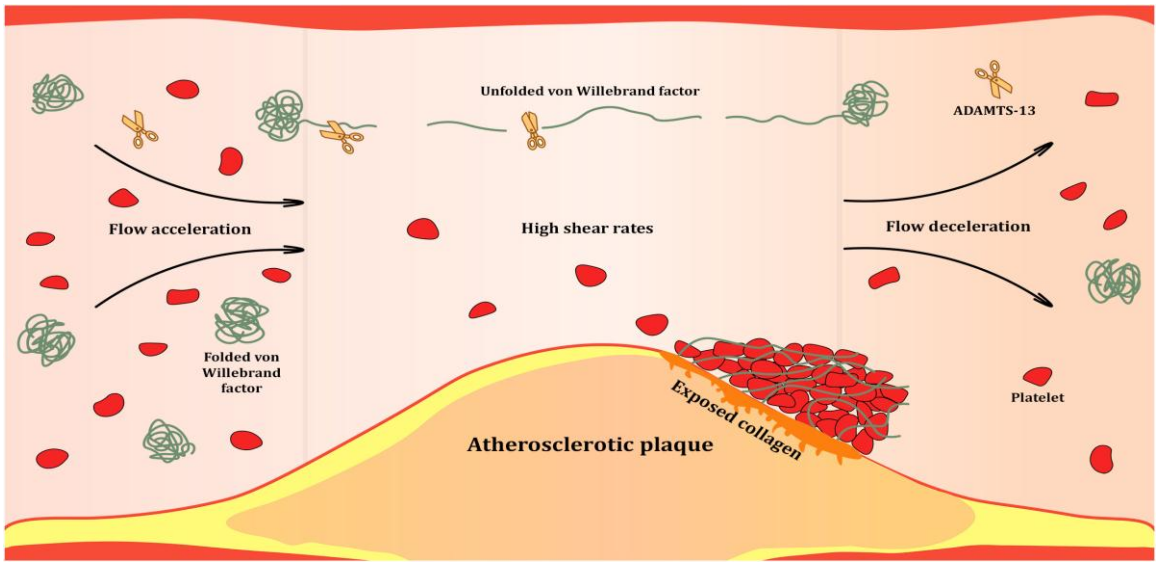
2.3.4.7. vWF and Cardiovascular

Diseases, disorders and conditions characterized by high blood shear stress and proteolysis of vWF can, in some circumstances, cause increased vWF and factor VIII levels, thereby paradoxically increasing the risk of venous and arterial

thromboembolic conditions , including myocardial infarction (MI) and ischemic stroke (IS), several potential mechanisms have been proposed, including a relative increase in vWF as compared to ADAMTS13 level , An elevated vWF level may confer as much as a three-to-four-fold higher relative risk of ischemic stroke as compared to patients with lower levels of vWF , Other studies have reported a concentration-dependent increased relative-risk of ischemic stroke, approaching seven-fold and for MI to be as high as four-to-five fold with increasing levels of vWF. Turbulent flow at sites of critical coronary stenosis may cause a local imbalance between vWF and ADAMTS13 (increased vWF/ADAMTS13 ratio in coronary flow) ، Heightened platelet activation stemming from platelet GPIbα may also contribute to a thrombotic phenotype. The incidence of MI or IS in patients with AVWS has not been well-defined and is likely under-recognized and diagnosed (Lenting *et al*., 2015 ;Patel *et al*., 2016) .

2.3.4.8. Von Willebrand factor and cardiovascular disease

Aortic stenosis Aortic stenosis (AS) is a valvular heart disease eventually resulting in the left ventricular outflow tract (LVOT) obstruction , The most common cause of AS is chronic (Lenting et al., 2015)



Figer (2-11)Shear-rate dependent activation of Von Willebrand factor (VWF) at the site of atherosclerotic stenosis. High shear rates occurring in the bloodstream at sites of significant atherosclerotic (Lenting et al., 2015)

2.3.4.9. Coronary artery disease and VWF

Coronary artery disease (CAD) is a chronic condition characterized by atherosclerotic plaque accumulation in the epicardial arteries, retention of atherogenic lipoproteins in arterial intima and low-grade vascular inflammation are recognized as the main drivers of atherosclerosis development retention and proinflammatory macrophage activation in the vessel wall , recently, the role of mitochondrial DNA mutations was suggested in induction of sterile vascular inflammation , atherosclerotic plaques in epicardial arteries can eventually rupture or erode, resulting in acute coronary syndrome (ACS) in a form of myocardial infarction MI Oxidation of low-density lipoproteins may link lipoprotein or unstable angina, Plaques can also gradually progress to significant narrowing of arterial lumen, causing angina pectoris heart failure, or remain asymptomatic. CAD usually follows a pattern of long stable periods intermitted by unstable episodes due to atherothrombotic events (Atiq *et al*., 2019) .

2.3.5. Genetic factors

Despite major advances in diagnosis and management, acute myocardial infarction (AMI)remains a serious healthcare burden worldwide, significantly increasing patients' morbidity and mortality , The severity of coronary artery disease (CAD) is a prognostic factor for major adverse cardiovascular outcomes in patients with AMI(Reardon *et al*., 2021) .

Several scoring systems areavailable for the quantitative evaluation of coronary artery lesions, and among them, the Gensini score is the most frequently used in clinical settings , the Gensini score assesses the quantity, location, and degree of stenosis of epicardial coronary artery lesions, providing a scientific evaluation

standard for CAD severity , in addition to environmental factors, genetic components have been revealed to be associated with the severity of CAD in AMI patients. Recent research has shown that CAD severity is influenced by variations in the angiotensin-converting enzyme (ACE) gene(Al-Gazally et al., 2016) .

In addition the severity of CAD in the previous studies was mostly based on the number of stenosed coronary arteries, rather than the Gensini score Thus, there is a lack of data on the association between ACE I/D genetic polymorphism and CAD severity evaluated using the Gensini score. The identification of factors affecting the severity of CAD can contribute to strategies for the primary and secondary prevention of AMI. Therefore, this study aimed to investigate the association between ACE I/D genotypes and the severity of CAD assessed using the Gensini score for Vietnamese patients with AMI (Al-Gazally *et al*., 2016) .

Ischemic stroke is death of brain tissue due to interruption of blood flow to a region of the brain, caused by occlusion of a carotid or vertebral artery or, less likely, a cerebral vein , The renin-angiotensin system (RAS) is a hormonal signaling mechanism implicated in the atherosclerosis and regulation of blood pressure (Lämmle, 2021).

Angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin system, plays important roles in vascular remodeling, atherosclerosis, and ischemic stroke2 , it catalyses the conversion of inactive angiotensin I to active angiotensin II, which is known to be involved in vascular hypertrophy, vasoconstriction, atherosclerotic processes3 , the association between ACE gene polymorphism with ischemic stroke risk is an interesting field(Tran *et al.*, 2023) .

The human ACE gene is located on chromosome 17q23, where an insertion/deletion polymorphism in intron 16 has been identified , this polymorphism is based on the presence (insertion, I) or absence (deletion, D) of a 300-bp DNA fragment , a co-dominant pattern of this polymorphism makes the DD genotype of ACE gene has been associated with highest serum ACE level and has been investigated as a potential susceptibility factor for ischemic stroke, a large number of studies have reported the association between the I/D polymorphism of ACE gene and the risk of ischemic stroke, but the results were inconclusive (Tran et al., 2023).

Previously published meta-analyses reported significant associations between ACE I/D and risk of ischemic stroke , There is substantial evidence suggesting a role of the rennin angiotensin system (RAS) in the development of hypertension and cardiovascular disease. Cerebrovascular endothelium has been shown to be rich in angiotensin-converting enzyme (ACE) by histochemical studies , Furthermore, in experimental stroke models in spontaneously hypertensive rats, ACE has been an important mediator of vascular changes, ACE has also been demonstrated in human studies to have an important role in the pathogenesis of white material lesions and lacunar infarcts , Homozygous presence of the deletion polymorphism has been associated with higher plasma ACE activity (Kovacevic *et al*., 2019) .

The ACE gene polymorphism has been investigated for its possible association with essential hypertension, coronary artery disease, atherosclerosis of the carotid artery and cerebral white matter lesions in patients with essential hypertension, and the findings seem to vary between populations of different genetic and environmental backgrounds. ACE gene polymorphism has also been associated with increased incidence of stroke in some populations, although contradictory results have been reported(Al-Gazally *et al*., 2016) .

3. Materials and Methods

3.1. Materials

3.1.1 Instruments

Instruments, tools and kit used in the present study ware showing in table (3.1) and table (3.2) .

Table (3.1) the Instruments and tools , used in the present study .

No.	Instrumental	Supplied Company	country
3	Catheterization device	Philips	Holand
4	Centrifuge	Sigma	Germany
6	Computed Tomogı Angiograph device	Toshiba	Japan
9	Distillator	Bibby science	England
10	Echocardiograph	Vivid E9	USA
11	Electrocardiograph	Philips	Holand
13	ELIZA	Biotec	USA
14	Freezer	Concord	Lebanon
15	Gel tubes	Sigma	Germany
16	Incubator	Fisher Scientific	USA
17	Microplate reader with 450nm wavelength filter	Elabscience	China
18	Micropipette 20,100 – 1000, 20 - 200, 5-50.	Slamed	Germany
19	Plane tube	Afma-Dispo	Jordan
20	Refregerator	ArGelik	Turkey

21	**Water path**	**SchutzartDin40050-IP memert,GMBH, Schwabach 20FRG**	**Germany**

Table (3.2) Chemical material study .

No.	Chemical material	Company	Country
1	**Proprotein convertase subtilisin / Ke (PCSKA) Kit**	**Elabscience**	**China**
2	**Lipocalin -2 Kit**	**Elabscience**	**China**
3	**Fibroblast growth factor 21**	**Elabscience**	**China**
4	**Von Willebrand protein factor**	**Elabscience**	**China**
3	**proprotein convertase subtilisin/kexin ty (PCSK9) gene**	**Elabscience**	**China**
4	**Angiotensin converting enzyme (ACE) gene**	**Elabscience**	**China**

3.2 . Subjects

3.2 .1. patients:

This is a case-control study that included ninety 90 patients divided into two groups 30 control and 60 patients with clinical evidence of coronary artery disease in the form of angina pectoris (60) This group divided into four groups as related diseases {smoker n = 36, non-smoker n = 24, hypertension blood = 40, mean blood pressure = 20} and subgroups divided into three groups by age { (40-49) n = 15, (50-59) n = 20, (60-69) n = 25} , This subgroup is divided into Three groups as related diseases {stable angina n = 15 and unstable n = 15 and myocardial infarction n = 30} . Body mass was measured as normal n = 20, obese n = 20 and high weight n = 20 , as well as measuring the percentage of fat for patients compared to control and Figure (3-1), diagnosed with typical chest pain, positive change in ECG, angiography and estimation of positive cardiac indices. From the coronary care unit (CCU) of the heart in Imam Hussein Medical City / Karbala Center for Diseases from for the period from November 2022 to 30,July 2023, where sufficient information was taken from The patient the study also included thirty men as a control group, while the men had no history of infection. Chest pain, no history of admission to the intensive care unit and normal resting ECG, control men are collected from relatives and outpatient clinic

Each patients groups of the study was subdivided into subgroups according to the age , body mass index (BMI) , lipid profile . The diagnosis of patients were based on clinical presentation , history and electrocardiogram (ECG) and patients report after the operation . Patients who have shown positive results for IHD patients with a recent history of atherosclerosis , angina

pectoris and myocardial infraction are diagnosed primarily on symptoms , such as chest pain on angiography and blood testing.

All participants are exposed to fluid about age, chest pain, history of admission to the intensive care unit, history of high blood pressure, and diabetes mellitus, while they were subjected to weight measurement, then blood samples are sent for analysis, and both male and female control patients are informed about the study and consent is guaranteed a plasma fraction for biochemical analyses was centrifugal separated and stored at −20°C, and another fraction for total genomic DNA analyses was maintained at 4°C until processing

3-2-2 - Control

samples included 30 healthy subjects with no heart problems , ages 30 to 69 years , for each control subject , a complete history was recorded . To be monitored , men should not have a history of heart disease , diabetes , or high blood pressure .

3-2-3 Blood sample

Blood samples were drawn by trained nurses or other health care professionals, at least after minimum of 12 hours of complete fasting and after minimum of 24 from onset of admission, centrifuged and freezing at -20C to keep serum stable for a few months. Five millimeters of venous blood was obtained by anti - arm venipuncture using a G10 needle withdrawn from IHD and control subjects , we put the sample in a tube gel and leave it for a quarter of an hour at room temperature until it settles , then put it in a centrifuge to separate the blood components and take the serum the serum were collected . , These samples were stored in a deep freeze (80 below zero) in the blood bank located at the heart center until they were used .

3-2-4 Excluded criteria

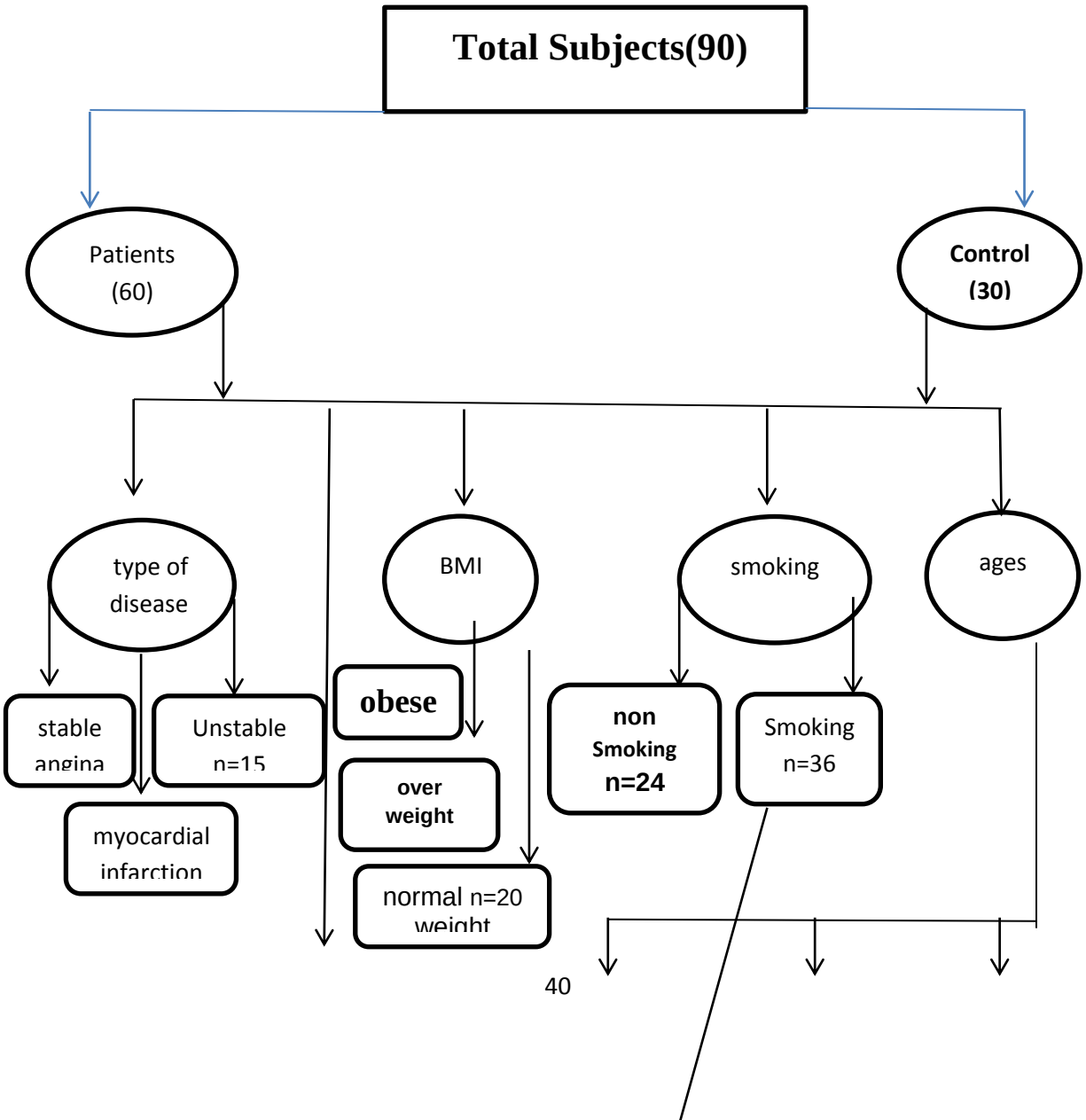
" Individuals who were suffering from systemic diseases associated with (all kinds of heart diseases , acute infections , rheumatic diseases , diabetes , hypertension , other chronic diseases as well as anemia and malignant diseases , as well as people suffering from infectious diseases and people who underwent operations surgery 3 months ago , all of them excluded from control samples .

3-2-5 Inclusion criteria

The patients criteria included in this study were ages , BMI , types of cardiovascular diseases , smoking and lipid profile .

3-2-6 Study Design

Each groups of samples was divided into subgroups according to the smoker and nonsmoker , types of disease , correlation , aged , and BMI figure (3-1)



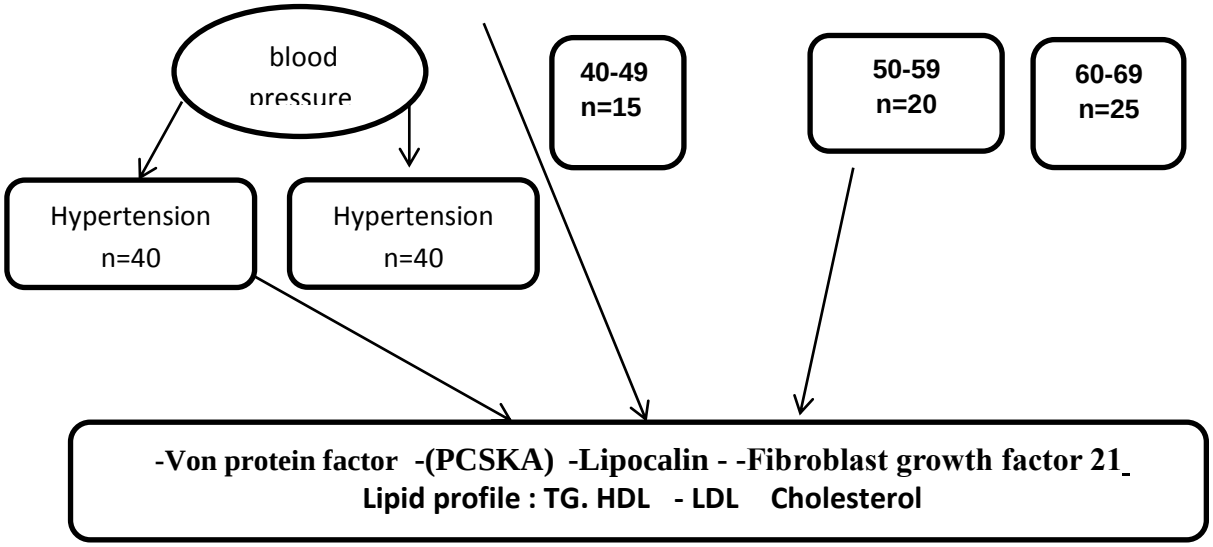


Figure (3-1) design current of study experiments

3.2.7. Medical History

All the patients and control groups were interviewed and examined by the specialists for the sake of the consistency of the data. A well structured questionnaire (figure 3.1) was developed for the study and was filled for every patient and control. The questionnaire cover the most important aspect of the medical history relevant to coronary artery disease including the residence ,age ,type and duration of the chest pain , admission to the coronary care unit ,smoking ,drug history ,etc. Also ask about history of previous conventional coronary angiography or coronary artery bypass grafting surgery and family history of premature coronary artery diseases.

3.2.8. Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃before centrifugation for 15min at 1000×g at 2~8℃. Collect the supernatant tocarry out the assay. Blood collection tubes should be disposable and be non-endotoxin. From the coronary care unit (CCU) of the heart in Imam Hussein Medical City / Karbala Center for Diseases from for the period from November 2022 to 30,July 2023, where sufficient information was taken from

The patient the study also included thirty men as a control group The brachial vein on the front of the elbow was employed, with 5-8 ml of blood was aspirated. The arm should be warm to improve blood circulation and distend the vein. A tourniquet was applied directly on the skin around the arm (usually from the left arm). The skin over the vein was sterilized with a small pad of cotton wool soaked with 70% ethyl alcohol, and the site was dried with clean gauze to prevent hemolysis, And using 18g *1 needle.

Gel tubes used without anti-coagulant, for blood to be used for preparing sera for subsequent biochemical tests. The blood was allowed to clot for 15 minutes, the clot shrinks and screw can be obtained by centrifugation and precautions were taken to avoid hemolysis. The serum samples were liquated in sterile test tubes using micropipette with sterile disposable tips. Each sample was labeled and given a serial number together with the patient name, the serum samples were frozen at (-20°C) for biochemical analysis (Flex et al., 2002).

3.3. Methods:

3.3.1. Measurement of Body mass index

Body mass index (BMI) is a measurement of a person's weight with respect to his or her height It is more of an indicator than a direct measurement of a person's total body fat , BMI , more often than not correlates with total body fats . This means that as the BMI score increases so does a person's total body fat , body mass index is a simple calculation using a person's height and weight . The formula is BMI = kg / m2 where kg is a person's weight in kilograms and m2 is their height in meters squared Overweight ranges(Sobhi Saqer, 2016).

3.4.1 . lipid profile

3.4.1.1 Measurements of total cholesterol (TC)

Specific kit for measuring human TC concentration in serum was supplied by Biolabo SA , china . (Appendix 3.1)

3.4.1.2 Measurements of high density lipoprotein (HDL)

Specific kit for measuring human HDL concentrations in serum was supplied by Biolabo SA , china . (Appendix 3.2)

3.4.1.3 Measurements of triglycerides (TG)

Specific Kit for measuring human TG concentrations in serum was supplied by Biolabo SA , china . (Appendix 3.3)

3.4.1.4 method of low density lipoprotein (LDL)

Low density lipoprotein was calculated by this formula : LDL (mg / ml) = TC (mg / ml) - VLDL (mg / dl) - HDL (mg / dl) .

3.4.3. ELIZA kits biomarkers

All kits were measured by ELIZA kits from company elabscience with code number and methods in appendix (A) , (B) and (C),(D) .

3.4.4. Molecular Study

DNA extraction

DNA was extracted from the blood samples included in the study for the purpose of conducting a molecular examination of the genes included in the study and control samples Components of the DNA Extraction Kit Components Components Amount ml / Amount

1- RBC Lysis Solution (360) ml

2- Cells Lysis Solution (100) ml

3- (Protein Removal Solution (40) ml

Method of extracting the Protocol of DNA Extraction DNA

DNA was extracted from the blood according to the kit instructions provided by Geneaid

1- microliters of blood were added to a volume of (1.5 ml) Eppendorf, then (300) (900) microliters of RBC lysis buffer was added to it, the mixture was gently .mixed, and left at room temperature for (5 minutes) to analyze blood cells

2- The tubes were then centrifuged at a speed of (3000 rpm) cycle/min for 5 .minutes

3- The clearing was gently discarded without damaging the white layer, with about (50) microliters of the clearing remaining for the purpose of mixing the .layer of white cells using the Vortex mixer

4- microliters of lysis buffer cell solution was added and the contents (300) were mixed with a Vortex device, then the tubes were incubated in the incubator for (10) minutes at a temperature of (60)ºC, with the tubes being shaken three .(times (every 3 minutes

5- microliters of Protein Removal Buffer was added to the tubes and (100) .mixed gently with a mixer, then placed on ice for 5 minutes

6- .Agitate the tubes for 3 minutes at a speed of 15000 rpm

7- Transfer the liquid to new tubes, discard the precipitate, and add (300) microliters of isopropanol to it, then mix the mixture gently to see the DNA .strands

8- The tubes were aspirated for 5 minutes at 15000 rpm

9- Isopropanol was gently discarded and (300) microliters of ethanol (70%) were added to the DNA concentrated on the walls of the tube, then the tubes .were shaken to wash the DNA

10- . The tubes were agitated for 3 minutes at 15000 rpm

11- Ethanol was gently removed by inverting the tubes onto a filter paper for .10 minutes

12- A solution of (TE Trice EDTA (100 µl) was added to the tubes, then the tubes were incubated in a water bath at a temperature of (60 °C) for (30-60) .minutes with shaking to dissolve the DNA

13- .The tubes were kept at a temperature (-20o) until use

Gel Electrophoresis

After extracting the DNA, the method (Sambrook et al., 1989) was used to ensure the presence of DNA extracted from blood Reagents of Gel Electrophoresis

1- Agarose

2- X TBE buffer solution Bromophenol blue dye

4- Ethidium bromide

5-.(Size parameters DNA ladder marker (100-1000 bp) and (100-2000 bp

Protocol of Gel Electrophoresis Steps •

Prepare the acarose jelly

1- Dissolve (0.8) grams of acarose in (100 ml) of 1 X TBE by heating the mixture to the boiling point using a water bath until all the gel particles have been dissolved, as the mixture appears clear without any suspended particles of .powder

2-.microliters of ethidium bromide was added to the agarose liquid (2)

3- Stir the agarose liquid to mix and avoid bubbles

4- Pour the mixture into the support plate, and after dipping the comb near one end of the plate

5- Leave the mixture to solidify at room temperature

6-Remove the comb gently as well as the plate supports after the mixture .hardens

7- The plate was placed in its support in the electrophoresis unit and then covered with 1X TBE transfer buffer as the gel was covered. *DNA loading, electrophoresis and DNA Loading

8-Mixing (10) microliters of DNA with (3) microliters of dye loading (Bromophenol Blue), as the samples were loaded into single wells of the gel. UV Light transillminator For the purpose of viewing the DNA bands, the bands .stained with ethidium bromide were photographed using a photo documentation system Molecular characterization of the studied genes .

A- Selection of primers

Primers were selected as shown in Table (1-3) for the purpose of conducting molecular detection and knowing the phenotypic polymorphism of genes and mutations (Kraus et al., 2022) .

Gene name	**sequencing**
proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene	**F- 5'-CAC GGTTGTGTCCCAAATGG-3(20mer)**
	R- 5'- GAGAGGGACAAGTCGGAACC-3'(20mer)
Angiotensin converting enzyme (*ACE*) gene	**F- 5'-CTG GAG ACCACT CCC ATC CTT TCT-3(24mer)**
	R- 5'-GAT GTGGCC ATC ACA TTC GTC AGA T-3'(25mer)

Table (3-3) The program used to identify the polymorphism of the gene (ACE) (Sun *et al*., 2009) (ACE) I / D gene Polymorphism

No.	Steps	Temperature	Time	No. of cycles

1	Initial Denaturation	94C°	2.5 min.	1
2	Denaturation	94C°	30 sec.	30
3	Annealing	60C°	105 sec.	
4	Extension	72C°	60 sec	
5	Final Extension	72C°	5 min	1
6	Final hold	4	-	

Table (3-4) The program used to identify the polymorphism of the gene (PCSK9) gene Polymorphism

No.	Steps	Temperature	Time	No. of cycles
1	Initial Denaturation	94C°	3 min.	3
2	Denaturation	94C°	30 sec.	35
3	Annealing	55C°	30 sec.	
4	Extension	72C°	30 sec	
5	Final Extension	72C°	5 min	1
6	Final hold	4	-	

Five µL of PCR products were added in 2 % agar gel and then conducted to a horizontal electrophoresis for 30 min under a condition of 100 V constant voltages . After that , the imaging was observed by gel imaging system and a fluorescent band polymorphism of PCSK9 gene of patients with coronary heart disease

3.5 . Statistical analysis

used a a program 23 of SPSS version to analysis data with significantly P-valu ($p \leq 0.05$) and t - test was used for a comparison between two groups , where test of one Anova was used to compare subdivided groups (Al-rawi, 2000)

4-The Results

4-1 .Comparison between Ischemic heart diseases patients and control .

The table(4-1)revealed a significantelevation($p<0.05$)in cholesterol concentration Triglyceride and LDL in patients of ischemic heart disease (321 + 512)(241 +222) and (30 + 594) respectively when compared with control group (136 + 466) (98 + 643) and (51 +411) respectively whereas significant decrease in HDL in patients (30 + 594) as compare with control group (51 +411) .

Table (4-1) lipid profile concentrations in Ischemic heart diseases patients and control group

parameters	Patients(60)	Control(30)
Cholesterol mg / dL	A 321 ± 512	B 136 ± 466
Triglyceride mg / dL	A 241 ±222	B 98 ± 643
HDL mg / dL	A 30 ± 594	B 51 ±411
LDL mg / dL	A 113 ± 816	B 70 ± 200

*** different letters refer to significant differences at *p < 0.05* level Mean + SE**

P - value ≤0.05

4.2 . Biochemical markers

4.2.1. Comparison between Biochemical markers and control .

Comparison between biomarkers in ischemic heart disease patients and control group the results listed in Figure (4-1) , (4-2) and (4-3) and (4-4) indicated a significant elevation $p < 0.05$ in Human Fibroblast Growth Factor 21 ،Proprotein Convertase Subtilisin/Kexin Type 9 ، Human Lipocalin 1 ، Human Von Willebrand Factor in patients (2.66) , (77.14) and (26.89) ،(2.62) respectively where compared with control group (0.5) , (27.34) (11.51) and (0.5) respectively .

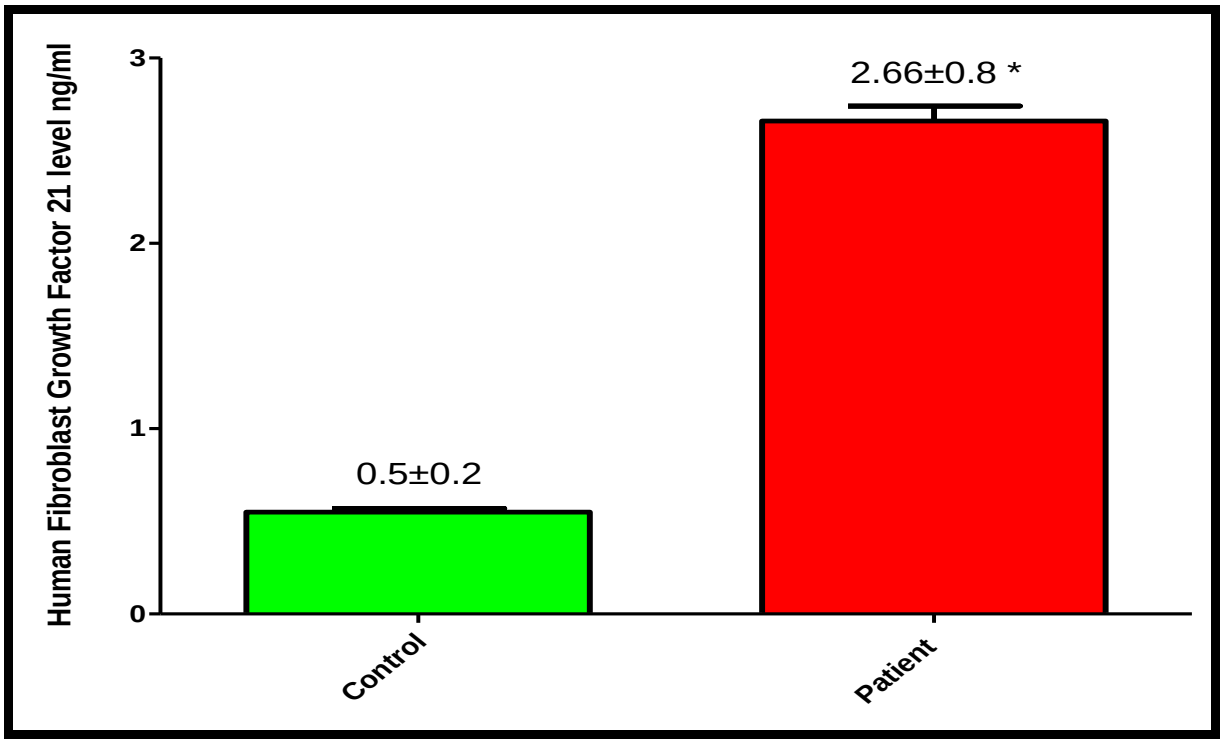


Figure (4-1) level of Human Fibroblast Growth Factor 21 in patient compare with control number of samples. * denotes significant (P<0.05 showed a significant increase (P< 0.05) in patient compare with control .

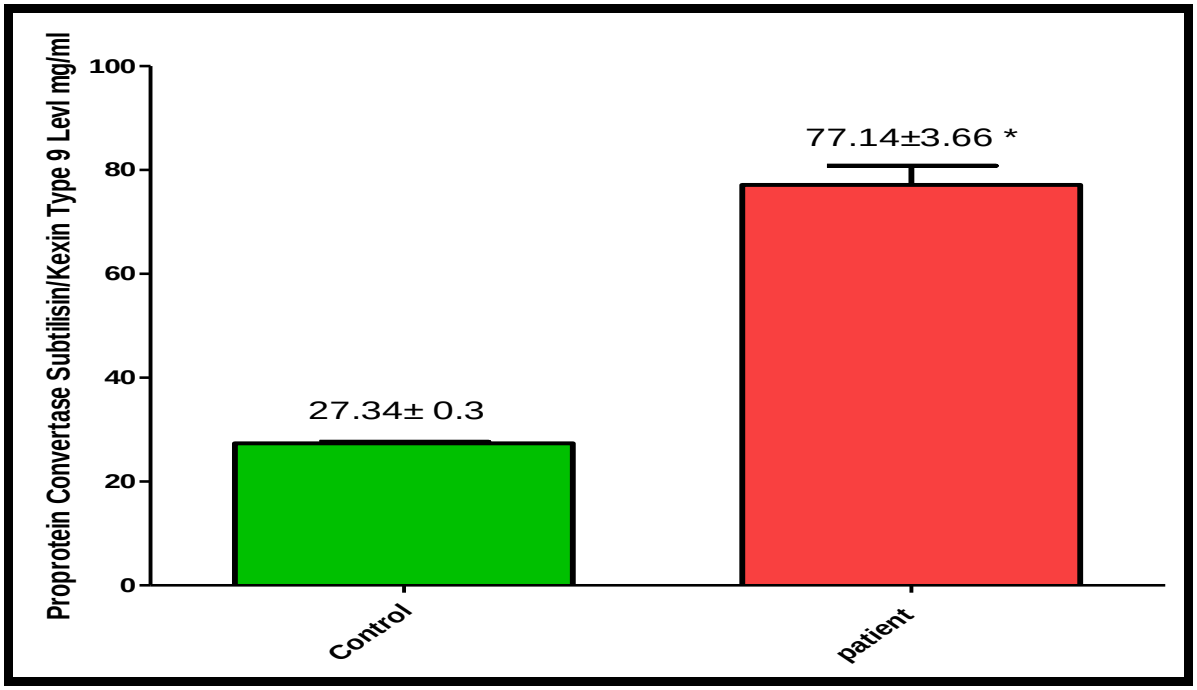


Figure (4-2) level of Proprotein Convertase Subtilisin/Kexin Type 9 in patient compare with control * denotes significant (P<0.05) showed a significant increase (P< 0.05) in patient compare with control

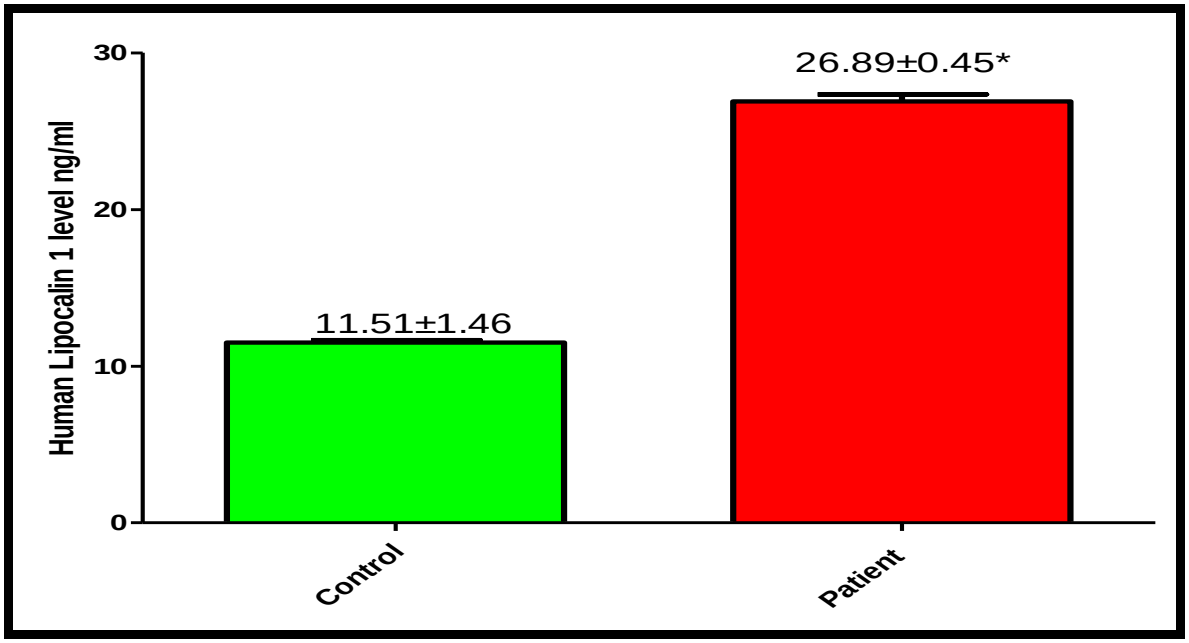


Figure (4-3) level of Human Lipocalin 1 in patient compare with control

*** denotes significant (P<0.05). showed a significant increase (P< 0.05) in patient compare with control**

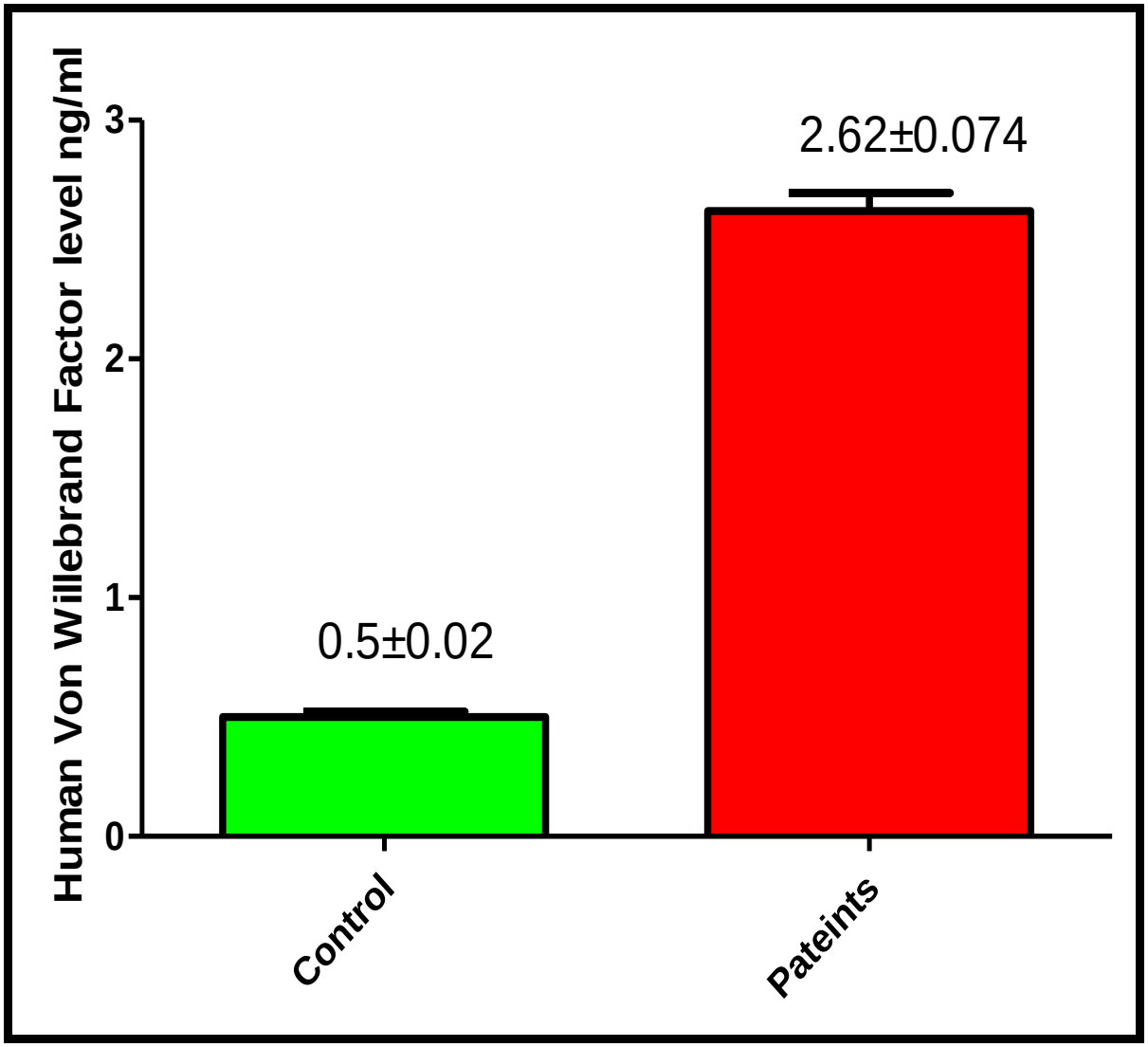


Figure (4-4) level of Human Von Willebrand Factor in patients compare with control * denotes significant (P<0.05) showed a significant increase (P< 0.05) in patient compare with control

4.2.2. comparison between biomarkers in ischemic heart disease patients according to smoking Figure (4-5) , (4-6) and (4-7) (4-8) revealed a significant elevation Proprotein Convertase Subtilisin/Kexin Type 9, levels ($p < 0.05$) in smoker patients in (2677.31 ± 124.53) , (1849.57 ± 76.29) and (2491.60 + 46.14) respectively as compare with non smoker patients (1757.31 ± 124.25) , (952.08 ± 44.47) and (1848.98 ± 20.5) respectively . 3000 Results 2677.315±

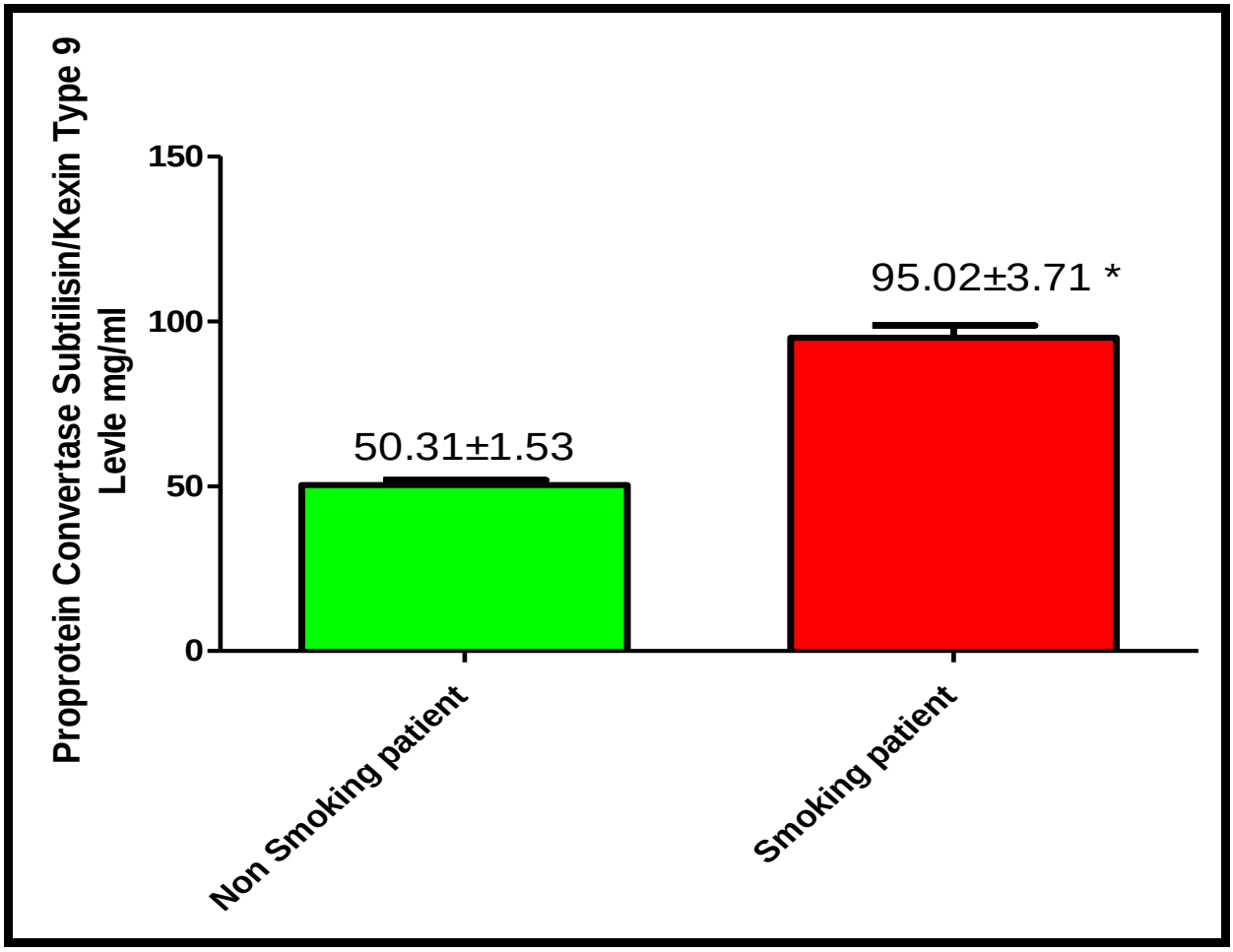


Figure (4-5) The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Non Smoking patient compare with Smoking patient.

Non Smoking patient = 24

Smoking patient = 36

*** denotes significant (P<0.05).**

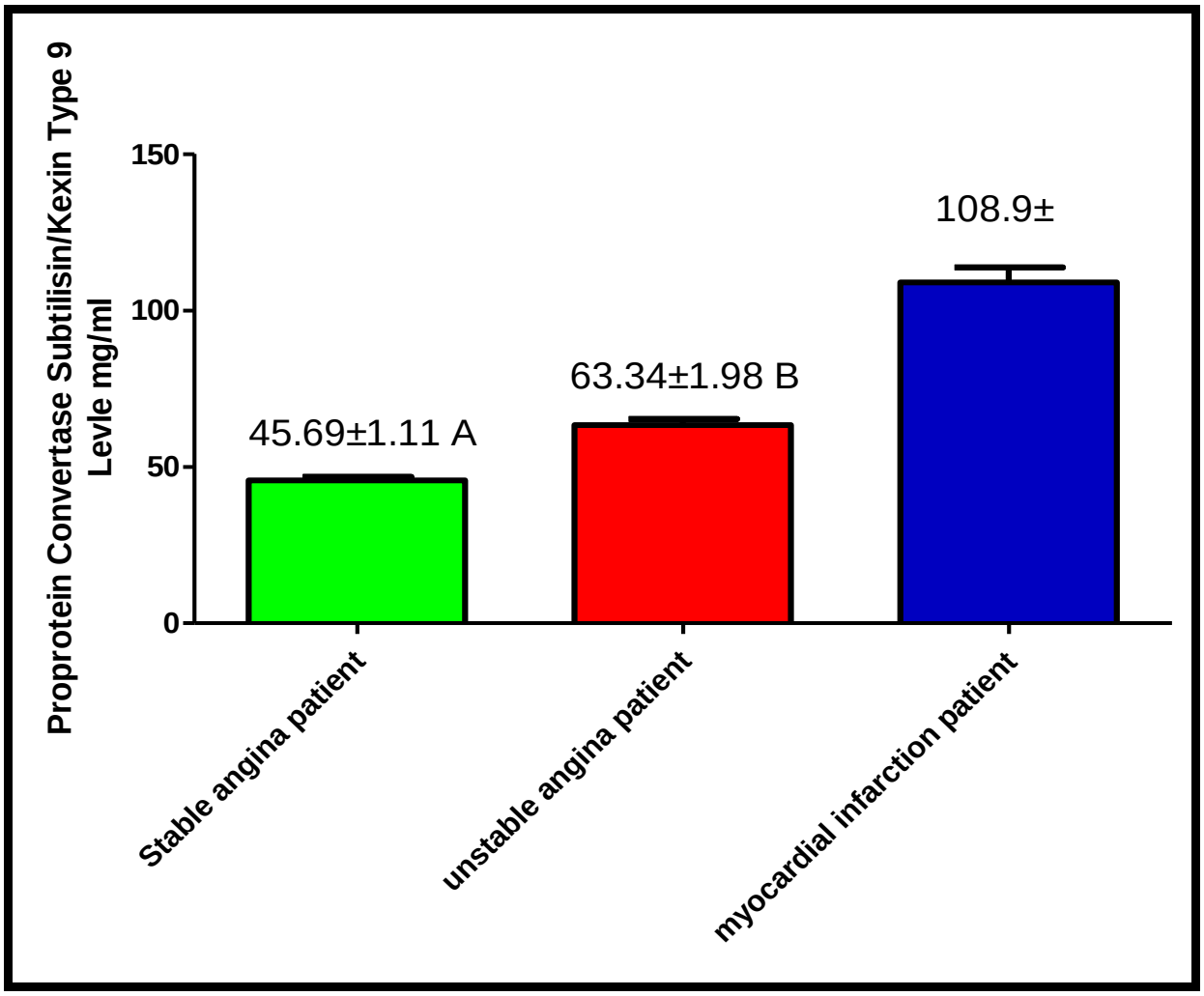


Figure (4-6) The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Stable angina patient compare with in Stable angina patient compare with unstable angina patient and myocardial infarction patienta significant Decrease (P< 0.05) Stable angina patient = 15 , unstable angina patient =15 myocardial infarction patient =30 , * Different letters denote significant (P<0.05).

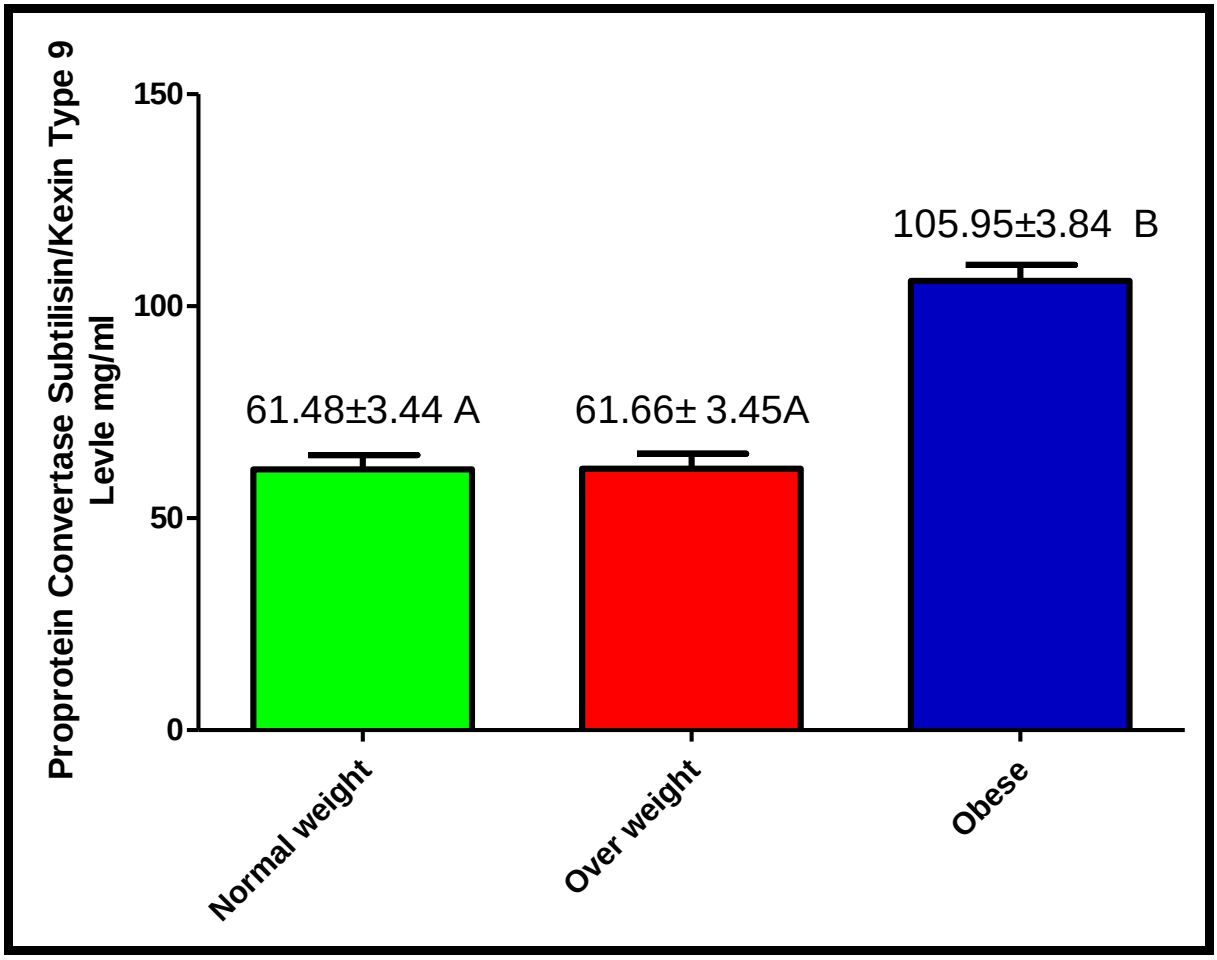


Figure (4-7) The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Normal weight patient compare with Overweight patient and Obese patient. The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient

Number of samples

Normal weight= 20

Over weight=20

Obese =20

*** Different letters denote significant (P<0.05).**

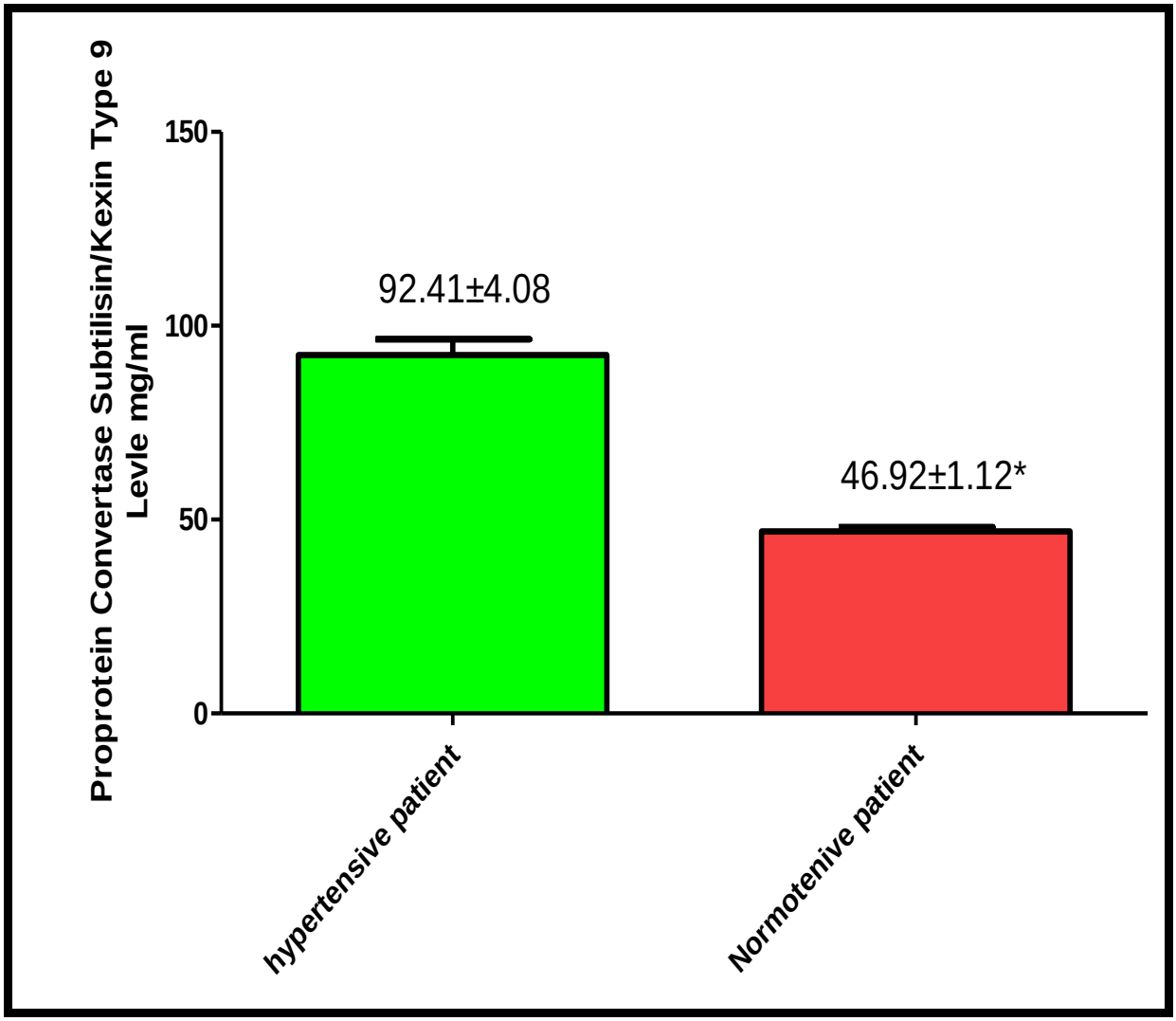


Figure (4-8) The level of Proprotein Convertase Subtilisin/Kexin Type 9 in hypertensive patient compare with Normotenive patient. Number of samples)The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient

hypertensive patient = 40

Normotenive patient =20

*** denotes significant (P<0.05**

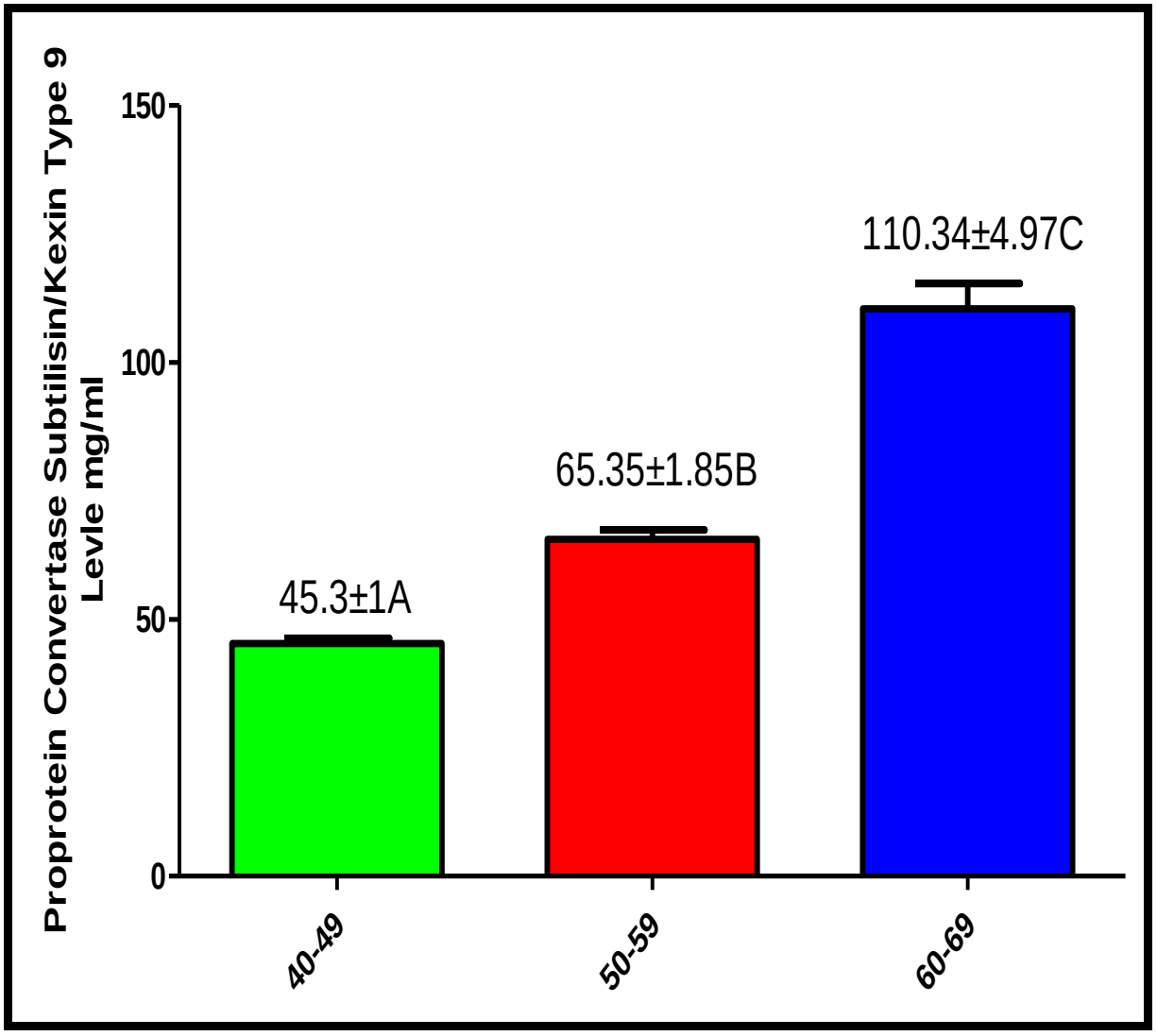


Figure (4-9)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Normal weight patient compare with Comparison between Biochemical marker Proprotein Convertase Subtilisin/Kexin Type 9 and different Age.

Number of samples

40-49 years n= 15

50-59 years n=20

60-69 yearsn =25

*** Different letters denote significant (P<0.05).**

4.2.3. comparison between biomarkers in ischemic heart disease patients according to smoking. Figure (4-10) , (4-11) and (4-12) (4-13) (4-14) revealed a significant elevation Human Fibroblast Growth Factor 21 , levels ($p < 0.05$) in smoker patients in (2677.31 ± 124.53) , (1849.57 ± 76.29) and (2491.60 + 46.14) respectively as compare with non smoker patients (1757.31 ± 124.25) , (952.08 + 44.47) and (1848.98 + 20.5) respectively . 3000 Results 2677.315+

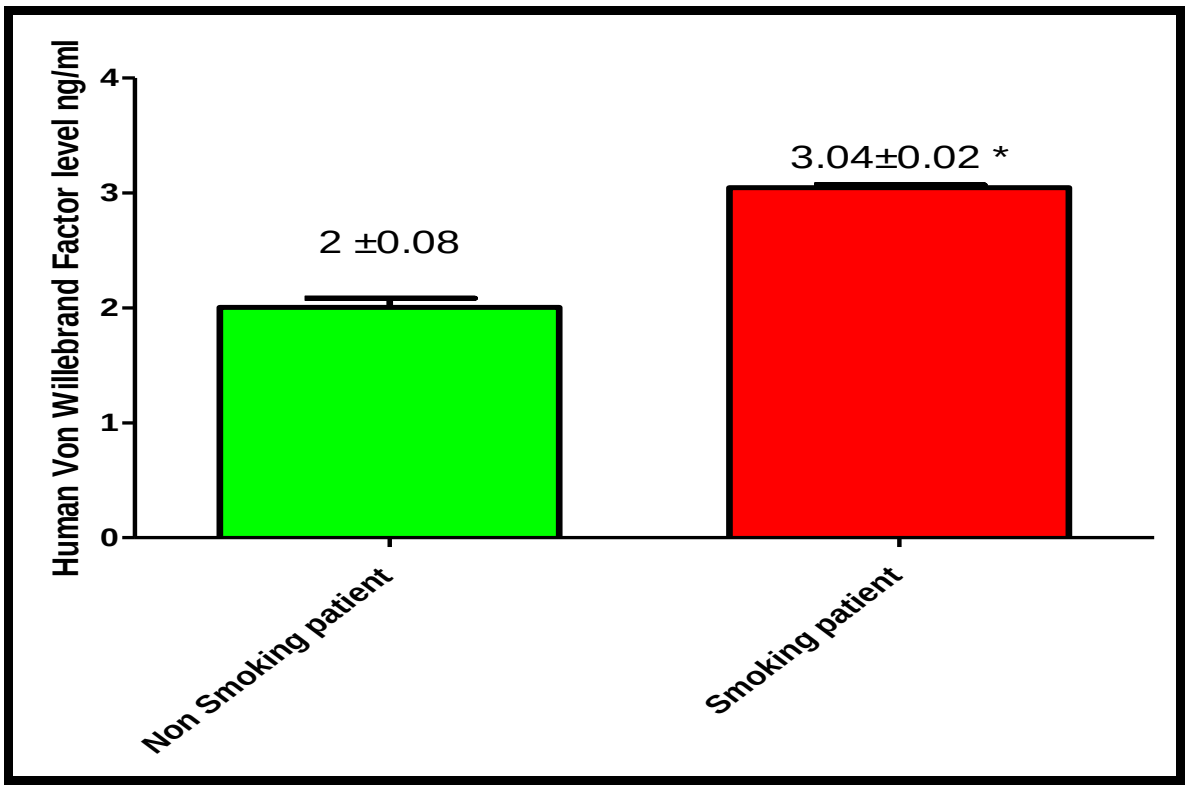


Figure (4-10) The level of Human Von Willebrand Factor in Non Smoking patient compare with Smoking patient The level of Human Von Willebrand Factor showed a significant Decrease (P< 0.05) in Smoking patient compare with Non Smoking patient . Number of samples

Non Smoking patient = 24

Smoking patient = 36

*** denotes significant (P<0.05).**

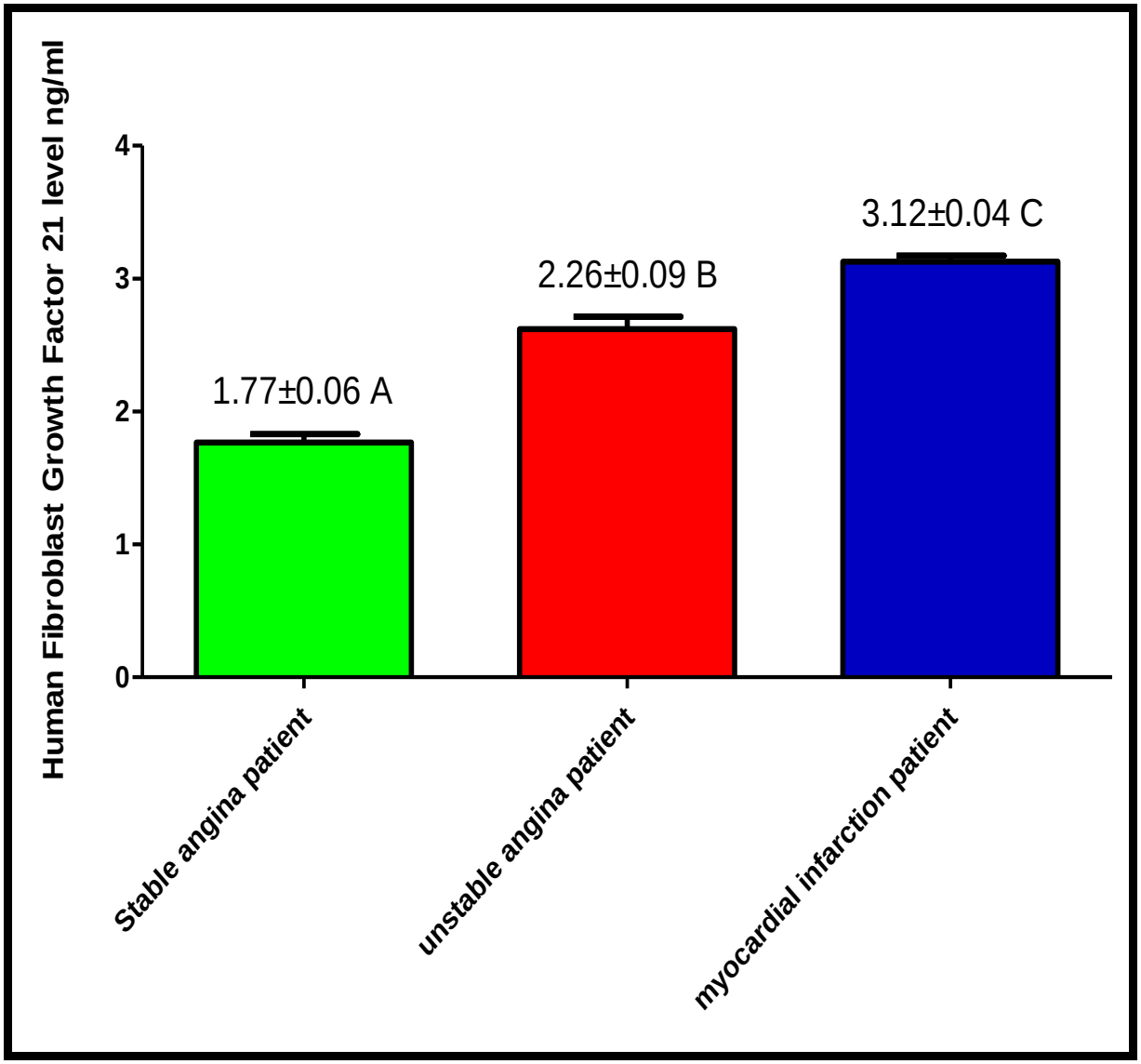


Figure (4-11) The level of Human Fibroblast Growth Factor 21 in Stable angina patient compare with unstable angina patient and myocardial infarction patient. The level of Human Fibroblast Growth Factor 21 showed a significant Decrease (P< 0.05) in Stable angina patient compare with unstable angina patient and myocardial infarction patient

Number of samples :

Stable angina patient = 15

unstable angina patient =15

myocardial infarction patient =30

*** Different letters denote significant (P<0.05).**

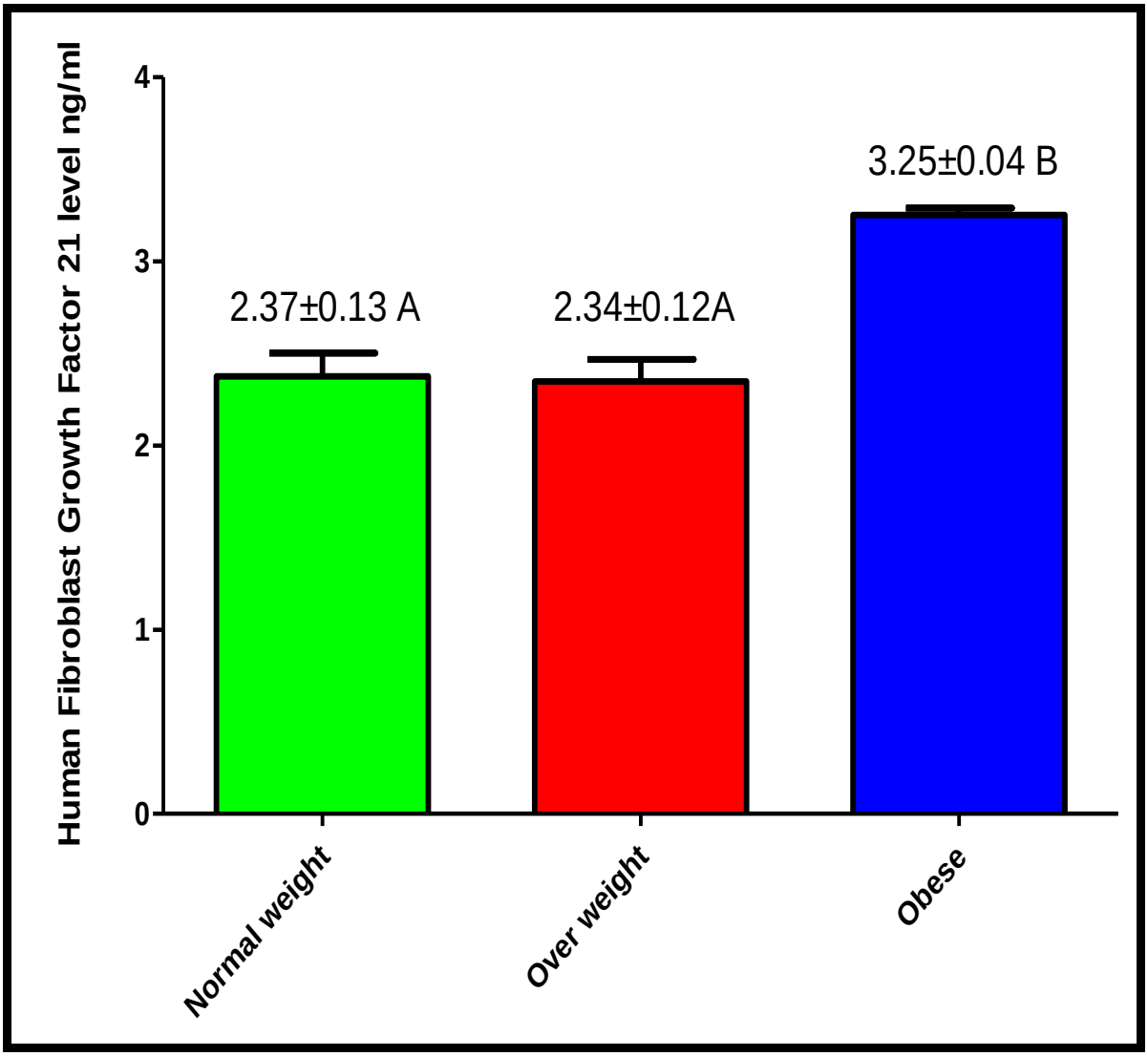


Figure (4-12) The level of Human Fibroblast Growth Factor 21 in Normal weight patient compare with Overweight patient and Obese patient. Number of samples, The level of Human Fibroblast Growth Factor 21 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-9).

Normal weight= 20

Over weight=20

Obese =20 ,

*** Different letters denote significant (P<0.05).**

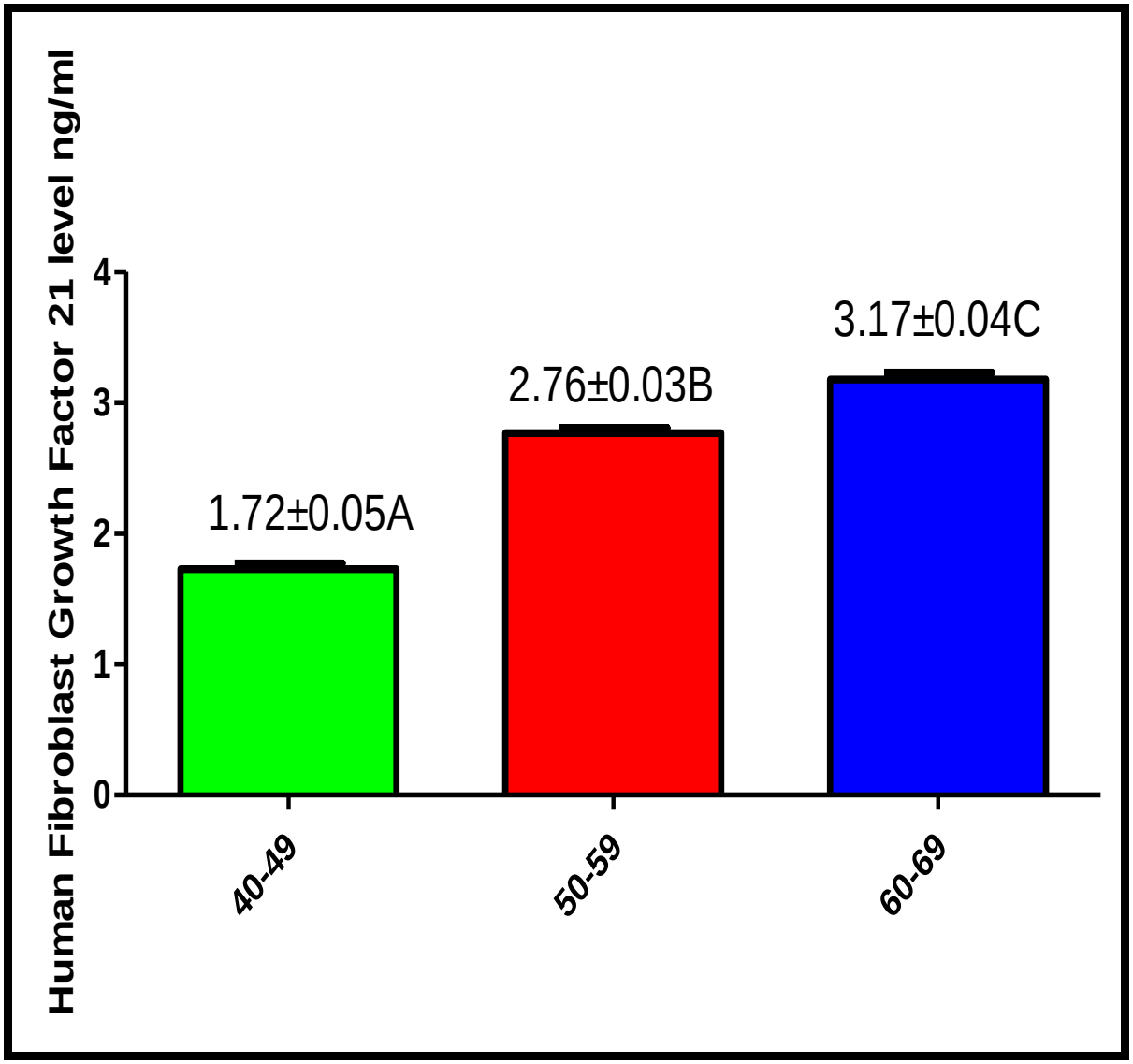


Figure (4-13) The level of Human Fibroblast Growth Factor 21 in different Age.

Number of samples :

40-49 years n= 15

50-59 years n =20

60-69 years =25

*** Different letters denote significant (P<0.05).**

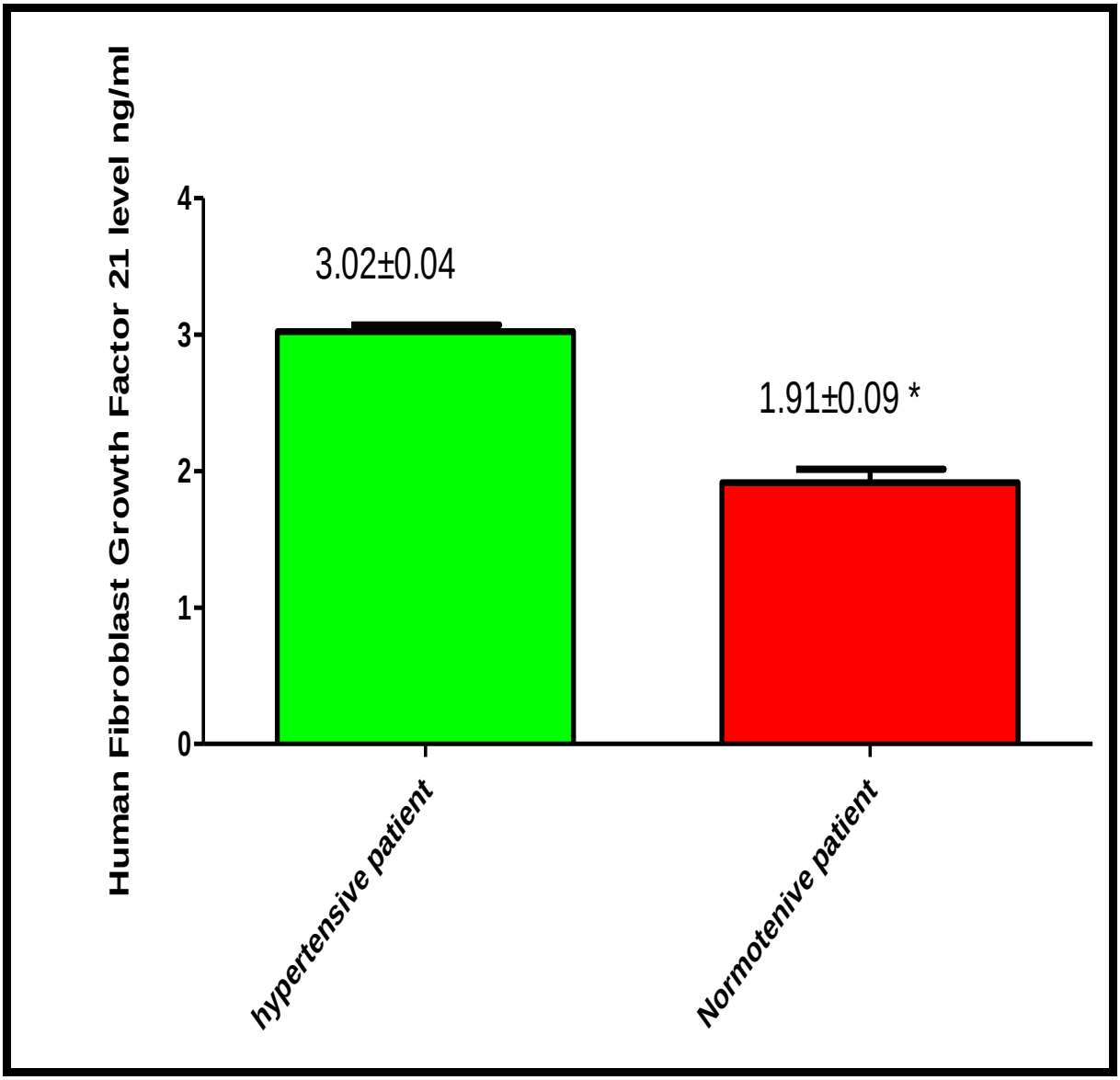


Figure (4-14) The level of Human Fibroblast Growth Factor 21 in hypertensive patient compare with Normotenive patient. The level of Human Fibroblast Growth Factor 21 showed a significant Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient .

Number of samples

hypertensive patient = 40

Normotenive patient =20

*** denotes significant (P<0.05).**

4.2.3.Comparison between Biochemical marker Human Lipocalin 1 Factor comparison between biomarkers in ischemic heart disease patients according to smoking . Figure (4-15) , (4-16) and (4-17) (4-18) (4-19) revealed a significant elevation Human Lipocalin 1 levels ($p < 0.05$) in smoker patients in (2677.31 ± 124.53) , (1849.57 ± 76.29) and (2491.60 + 46.14) respectively as compare with non smoker patients (1757.31 ± 124.25) , (952.08 ± 44.47) and (1848.98 ± 20.5) respectively . 3000 Results 2677.315± .

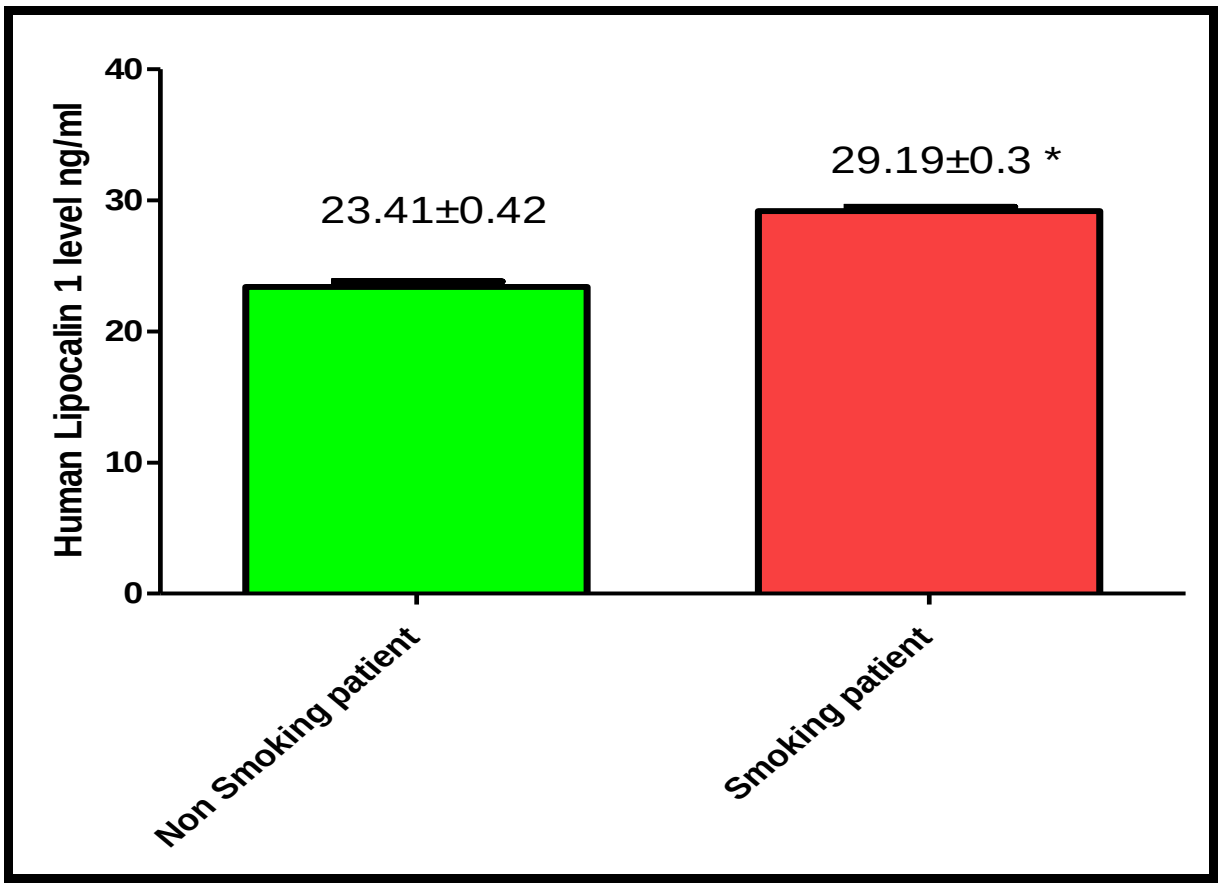


Figure (4-15) The level of Human Lipocalin 1 in Non Smoking patient compare with Smoking patient. Number of samples

Non Smoking patient = 24

Smoking patient = 36

*** denotes significant (P<0.05).**

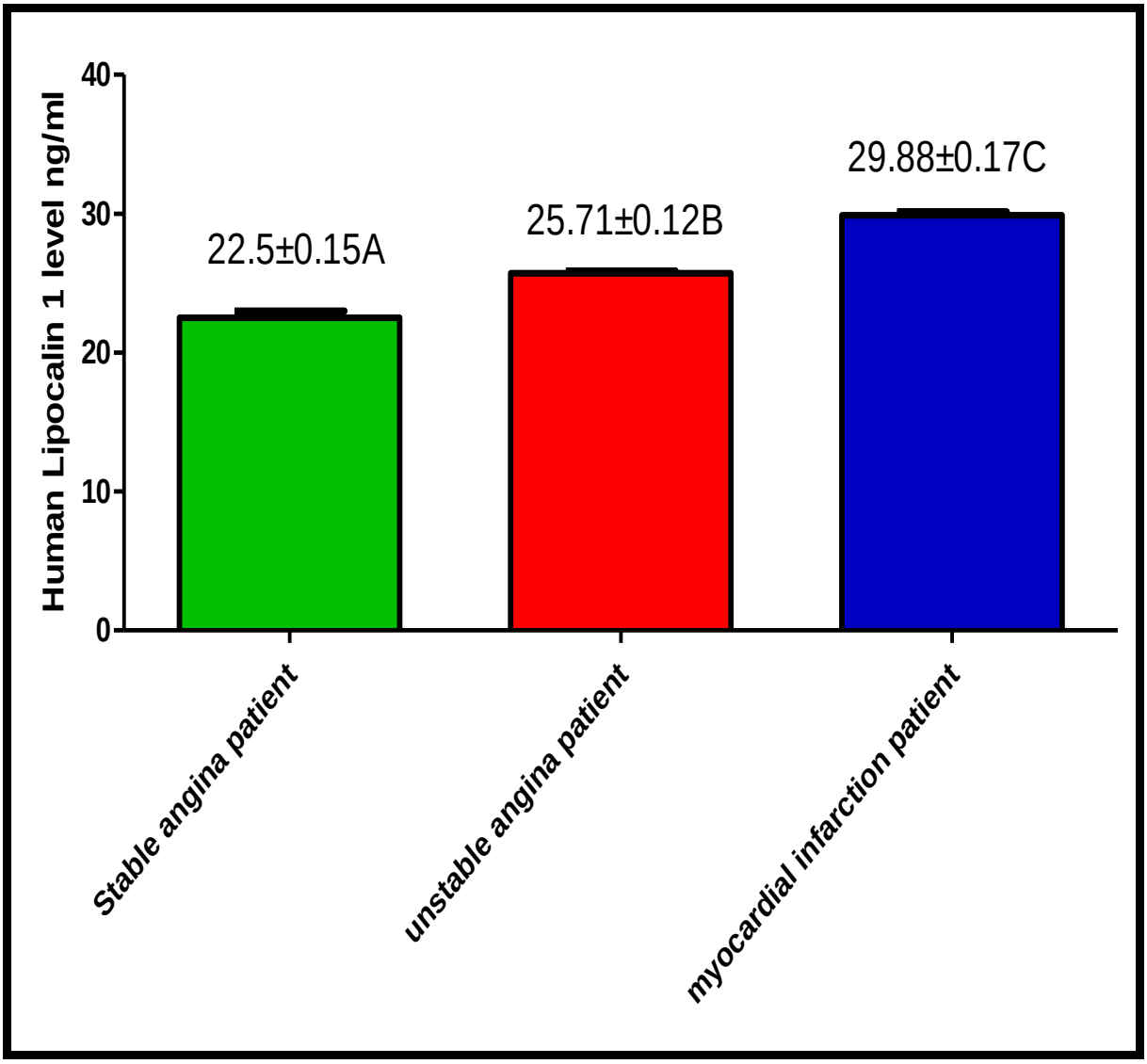


Figure (4-16) The level of Human Lipocalin 1 in Stable angina patient compare with unstable angina patient and myocardial infarction patient. The level of Human Lipocalin 1 showed a significant Decrease (P< 0.05) in Stable angina patient compare with unstable angina patient and myocardial infarction patient

Number of samples :

Stable angina patient = 15

unstable angina patient =15

myocardial infarction patient =30

*** Different letters denote significant (P<0.05**

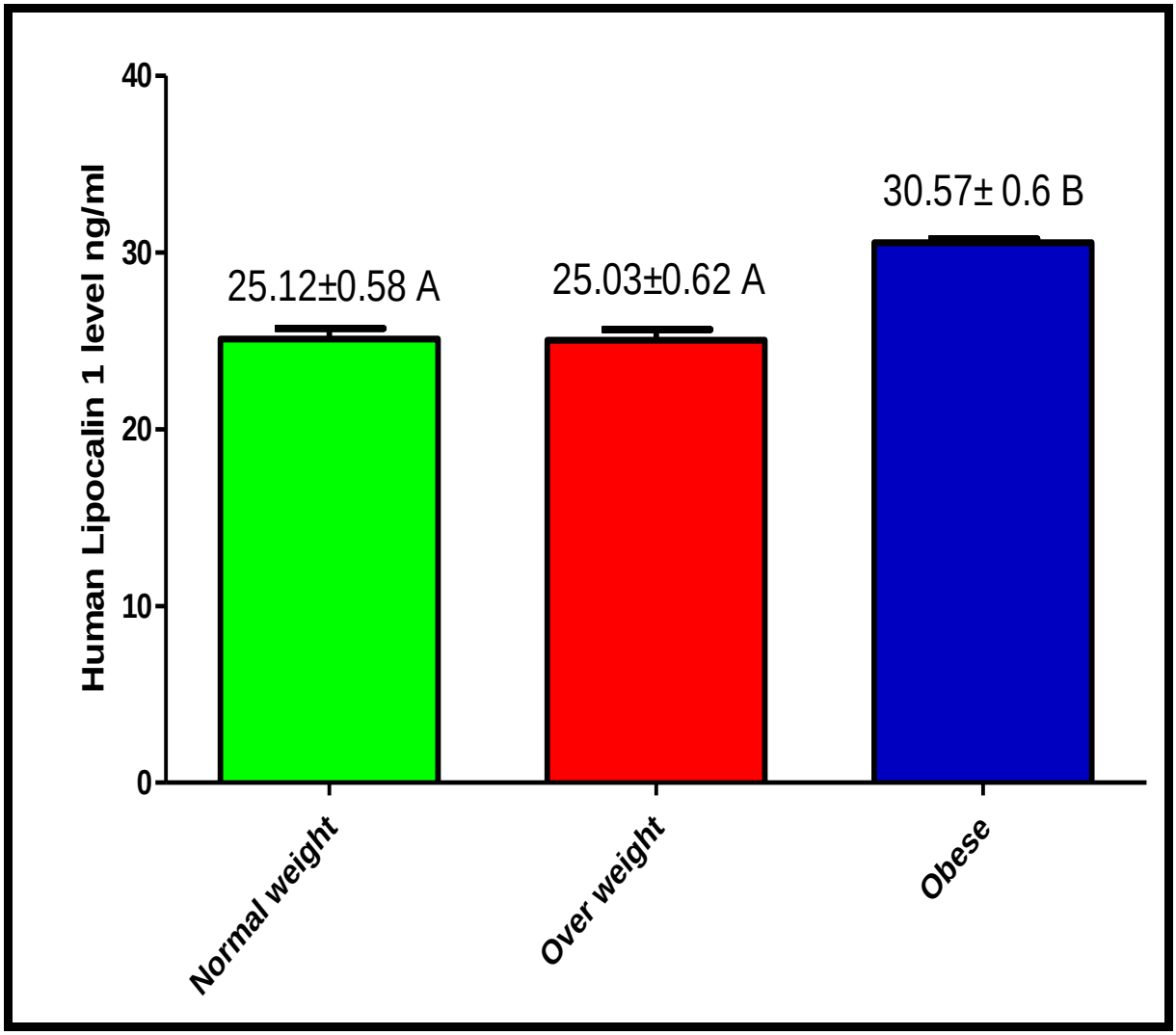


Figure (4-17)The level of Human Lipocalin 1 in Normal weight patient compare with Overweight patient and Obese patient. The level of Human Lipocalin 1 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient .

Number of samples :

Normal weight= 20

Over weight=20 Obese =20

*** Different letters denote significant (P<0.05)**

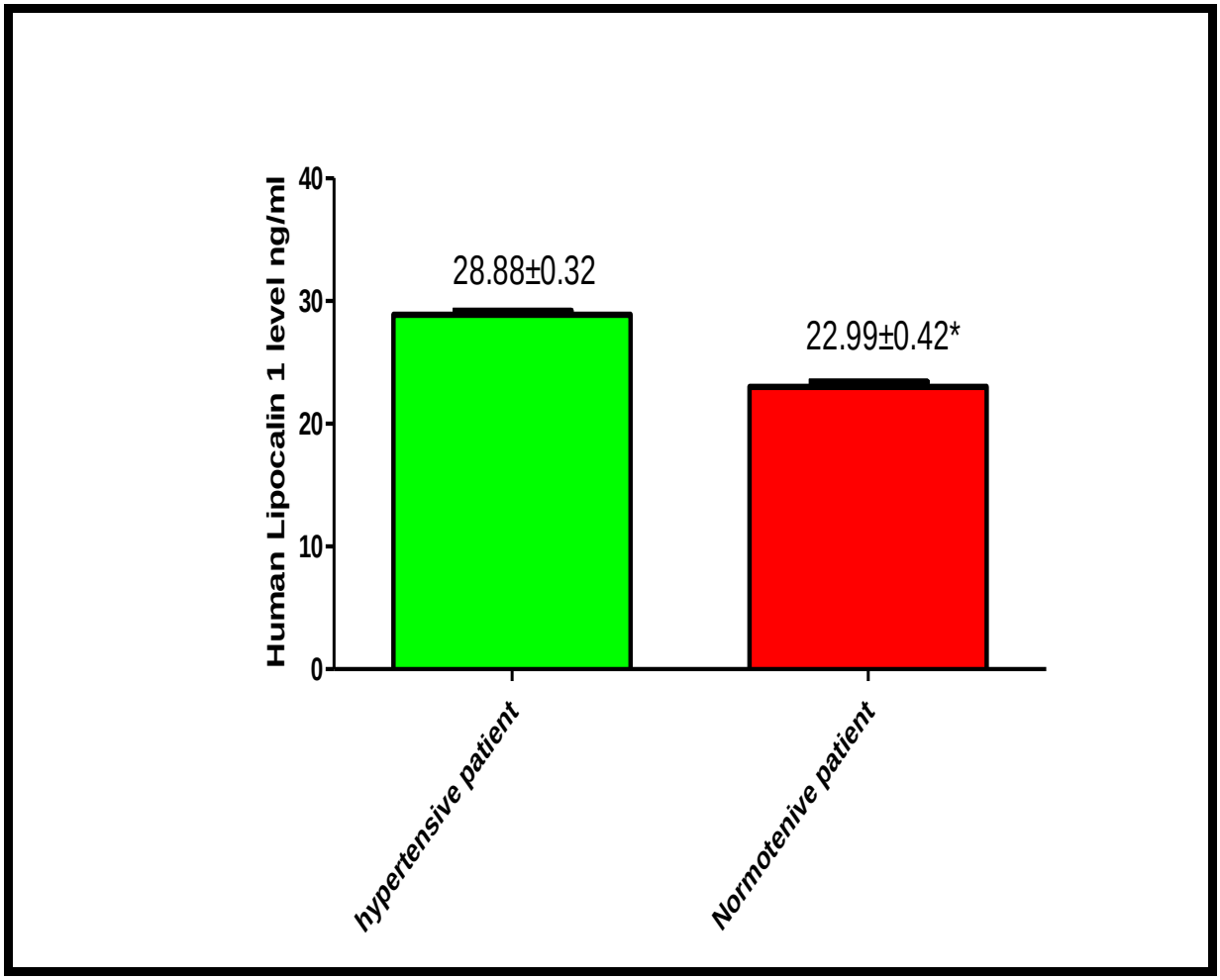


Figure (4-18) The level of Human Lipocalin 1 in hypertensive patient compare with Normotenive patient. The level of Human Lipocalin 1 showed a significant Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient

hypertensive patient = 40

Normotenive patient =20

*** denotes significant (P<0.05).**

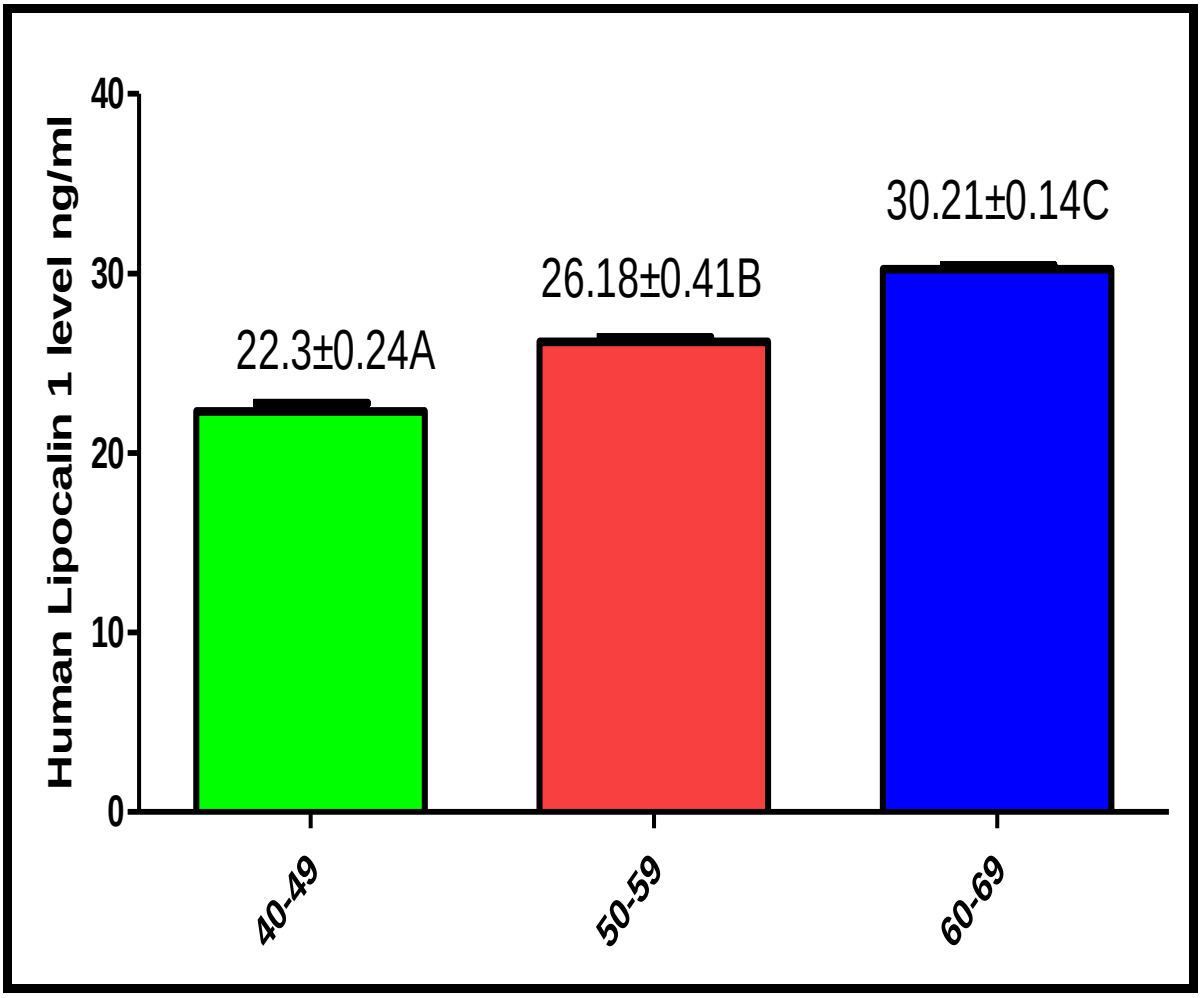


Figure (4-19) The level of Human Lipocalin 1 in The level of Human Lipocalin 1 showed a significant Decrease (P< 0.05) in different Age

Number of samples

40-49 years= 15

50-59 years n =20

60-69 yearsn =25

*** Different letters denote significant (P<0.05).**

4.2.4 . comparison between biomarkers in ischemic heart disease patients according to smoking . Figure (4-20) , (4-21) and (4-22) (4-23) (4-24) revealed a significant elevation Human Von Willebrand Factor levels ($p < 0.05$) in smoker patients in (2677.31 ± 124.53) , (1849.57 ± 76.29) and (2491.60 + 46.14) respectively as compare with non smoker patients (1757.31 ± 124.25) , (952.08 + 44.47) and (1848.98 + 20.5) respectively . 3000 Results 2677.315+

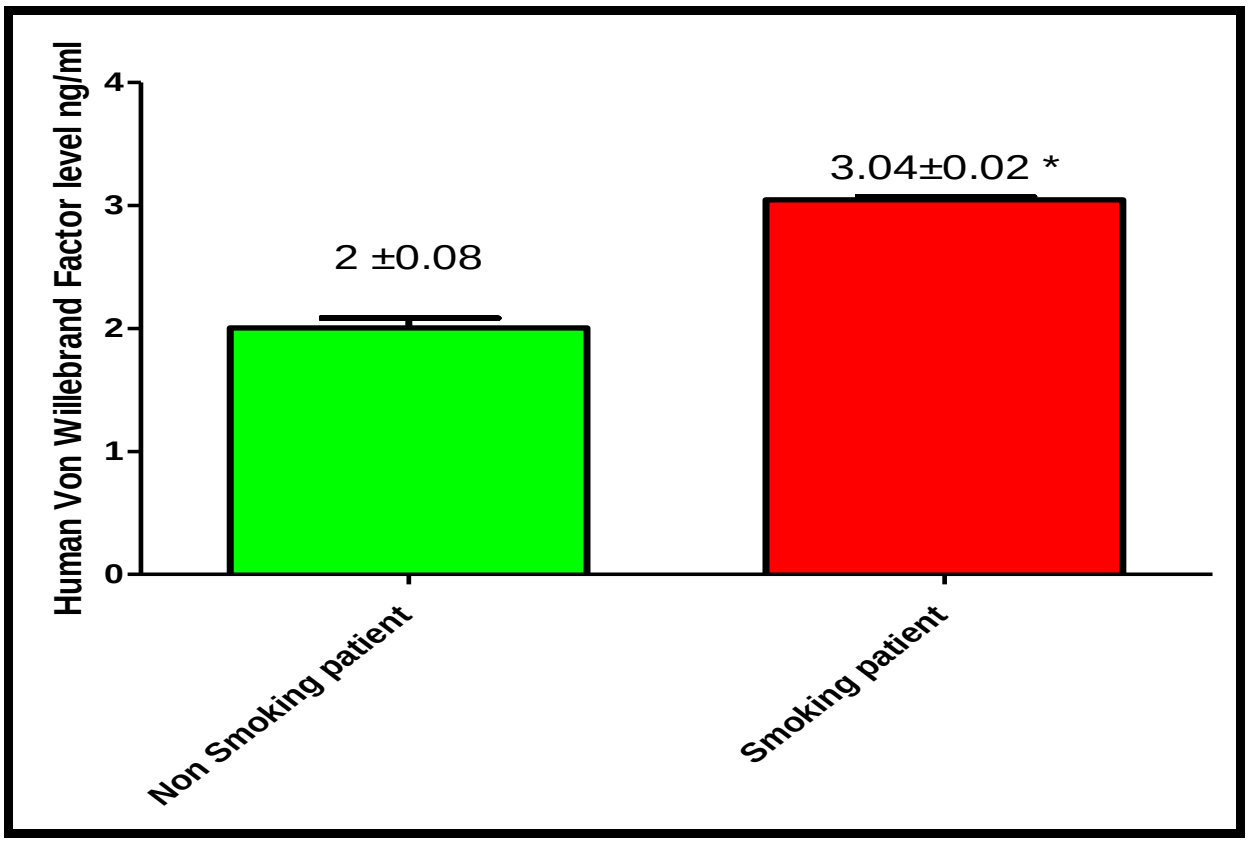


Figure (4-20) The level of Human Von Willebrand Factor in Non Smoking patient compare with Smoking patient.

Number of samples

Non Smoking patient = 24

Smoking patient = 36

*** denotes significant (P<0.05).**

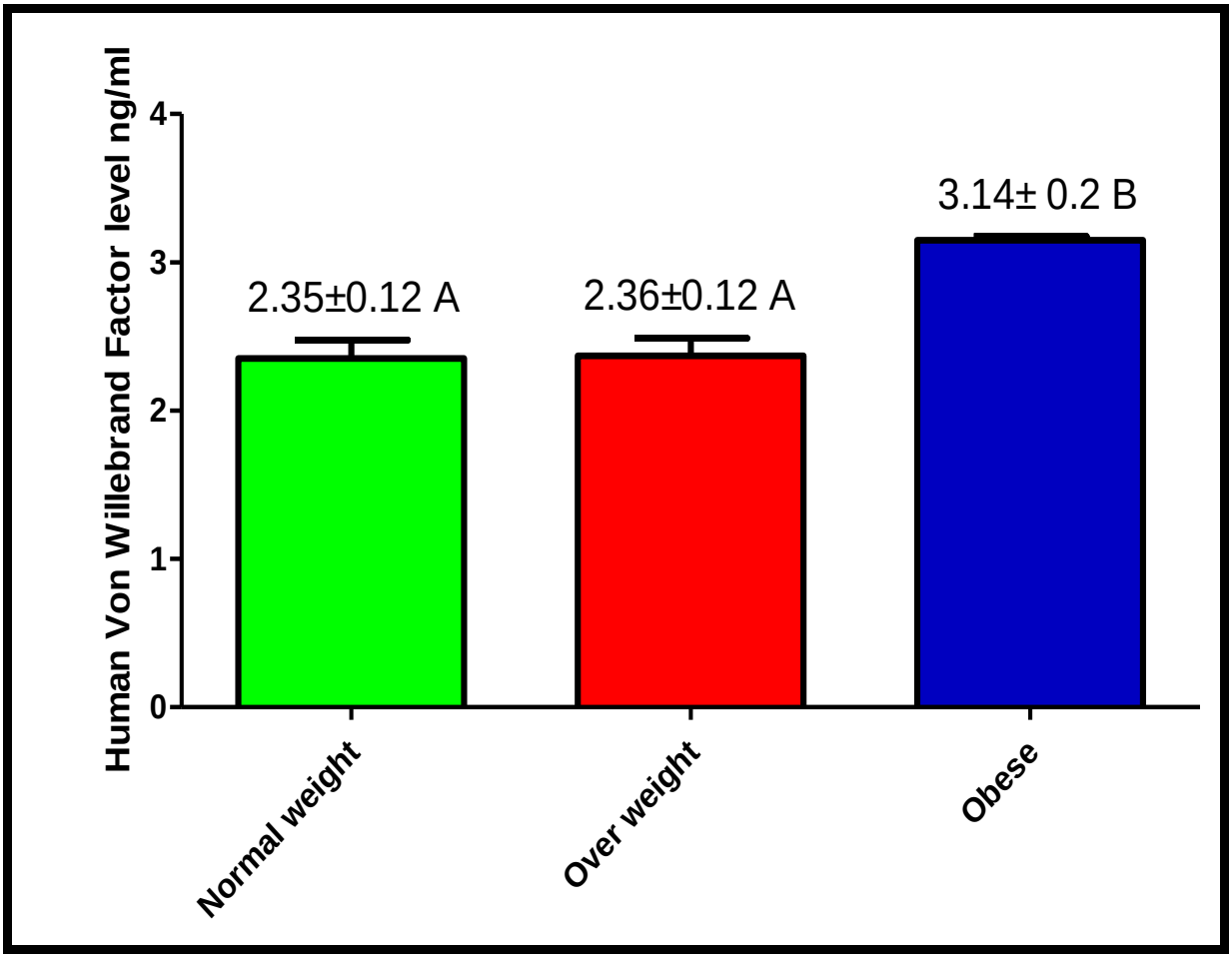


Figure (4-21) The level of Human Von Willebrand Factor in Normal weight patient compare with Overweight patient and Obese patient. The level of Human Von Willebrand Factor showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient

Number of samples

Normal weight= 20

Over weight=20

Obese =20

*** Different letters denote significant (P<0.05).**

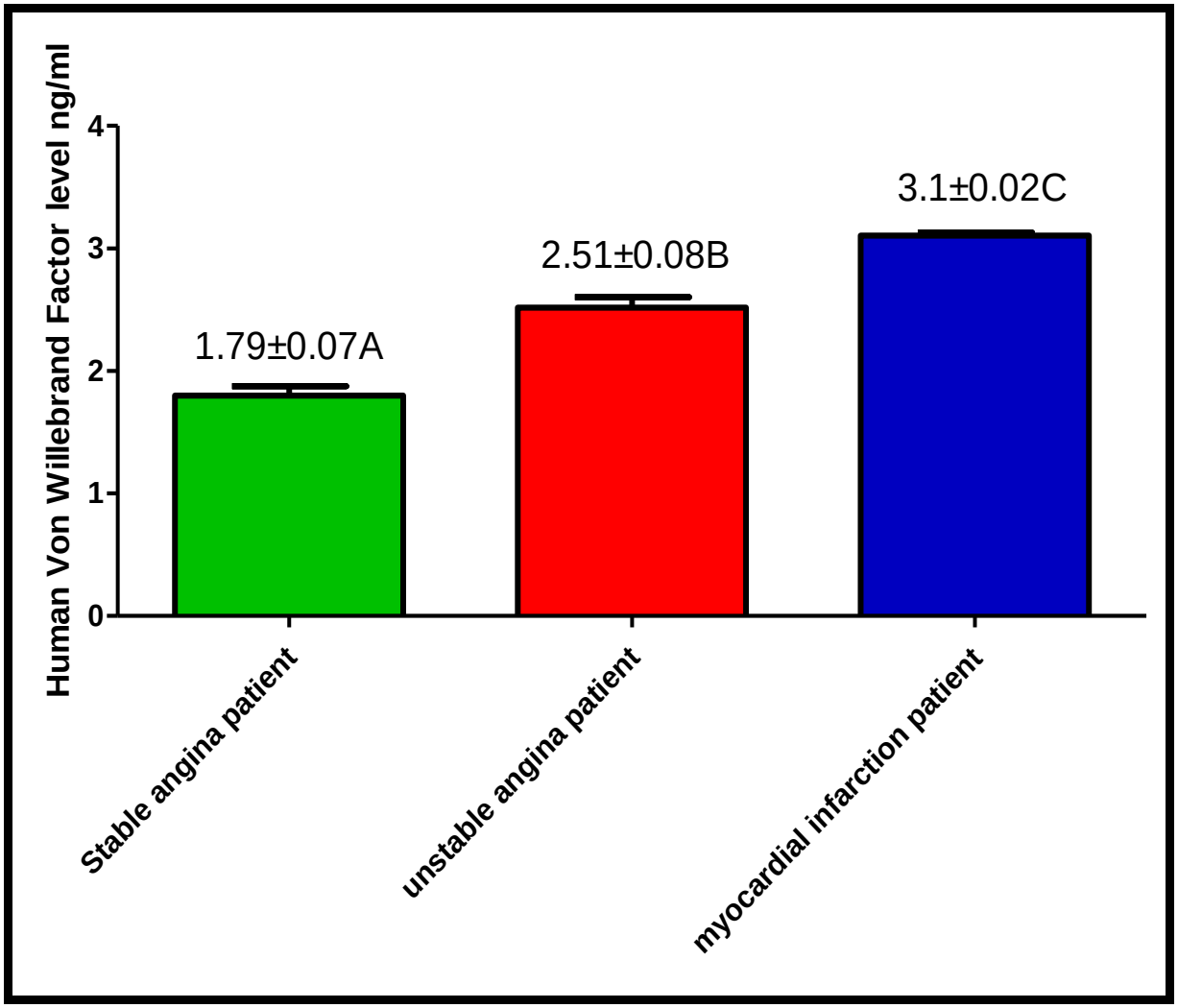


Figure (4-22) The level of Human Von Willebrand Factor in Stable angina patient compare with unstable angina patient and myocardial infarction patient. The level of Human Von Willebrand Factor showed a significant Decrease (P< 0.05) in Stable angina patient compare with unstable angina patient and myocardial infarction patient (figure 4-41).

Number of sample

Stable angina patient = 15

unstable angina patient =15

myocardial infarction patient =30

*** Different letters denote significant (P<0.05).**

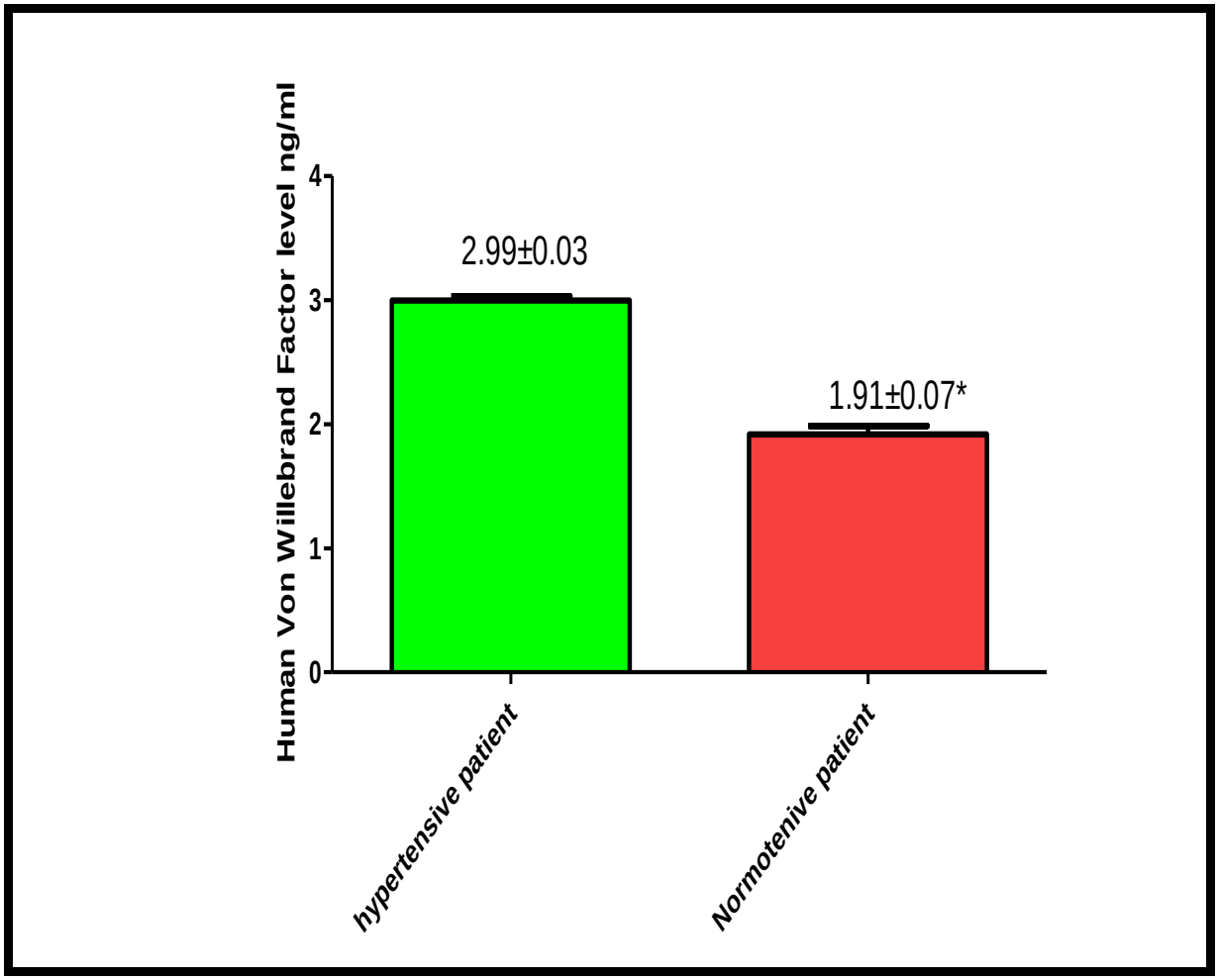


Figure (4-23) The level of Human Von Willebrand Factor in hypertensive patient compare with Normotenive patient. The level of Human Von Willebrand Factor showed a significant Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient (figure 4-45).

Number of samples :

hypertensive patient = 40

Normotenive patient =20

*** denotes significant (P<0.05).**

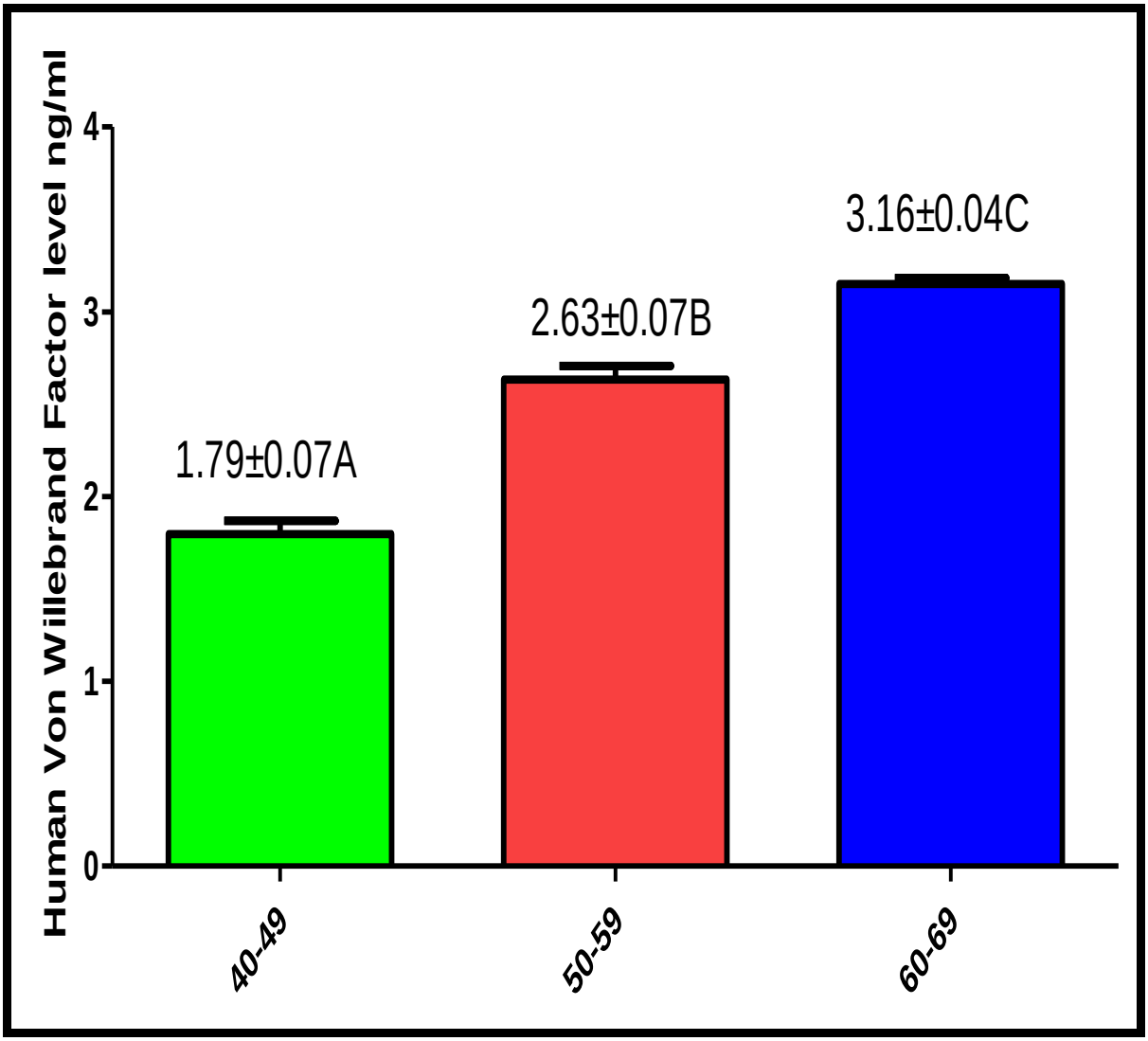


Figure (4-24) The level of Human Von Willebrand Factor in Normal weight patient compare with Overweight patient and Obese patient. The level of Human Von Willebrand Factor showed a significant Decrease (P< 0.05) in different Age.

Number of samples :

40-49 years= 15

50-59 years =20

60-69 years n =25

*** Different letters denote significant (P<0.05)**

4-3 .The correlation

4.3.1 .Correlation between biomarkers and Lipid profile .

The results listef in figure (4-25) , (4-26) and (4-27) referd to significant expulsion correlation between DGF15 and cholesterol , LDL and triglyceride with square r = (0.9583) , (0.8789) and (0.0026) respectively whereas figure (4_28) showed a reverse correlation between GDF15 and HDL - C level with square r = 0.7439 . The figure (4_29) , (4_30) and (4_31) indicated also expulsion significant correlation between tryptase and cholesterol , LDL and TG in value of square r = (0.8981) , (0.8197) and (0.0015) respectively whereas reverse significant correlation between trptase level and HDL - C as square r = (0.6671) Results The same results in figure (4-32) , (4-33) and (4-35) a expulsion correlation significantly TG between IL - 1 beta and cholesterol , LDL and TG as square r- (0.9195) , (0.8163) and (0,0019) respectively and reversely with HDL - C as square r = (0.6683

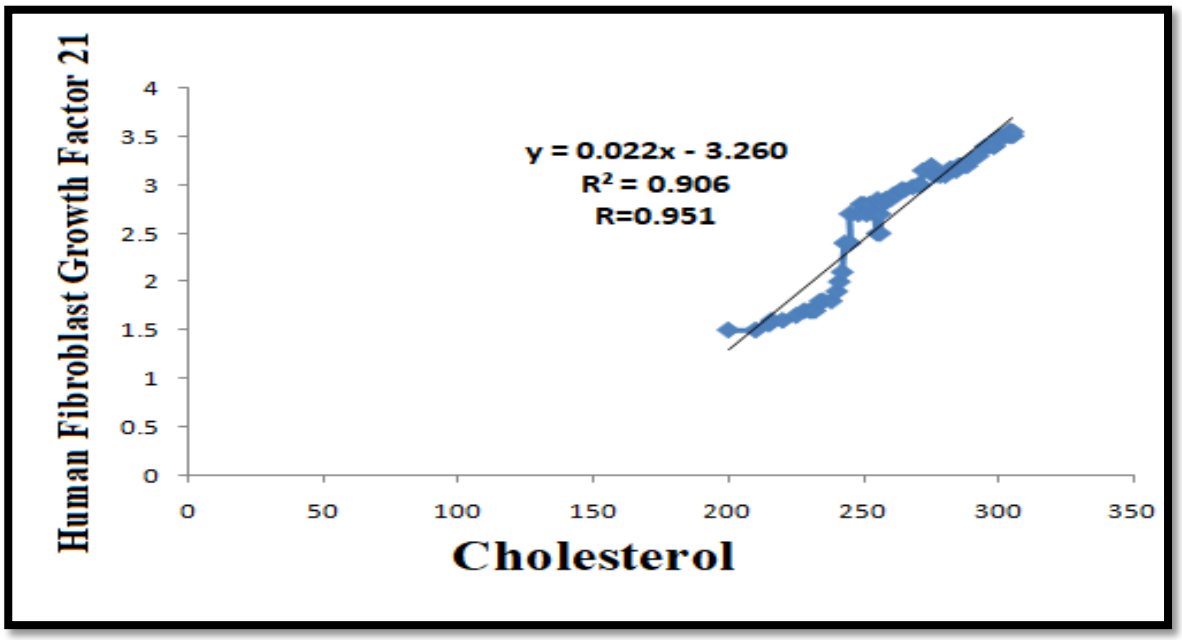


Figure (4-25) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Cholesterol, The results of the current study showed that there is a positive relationship

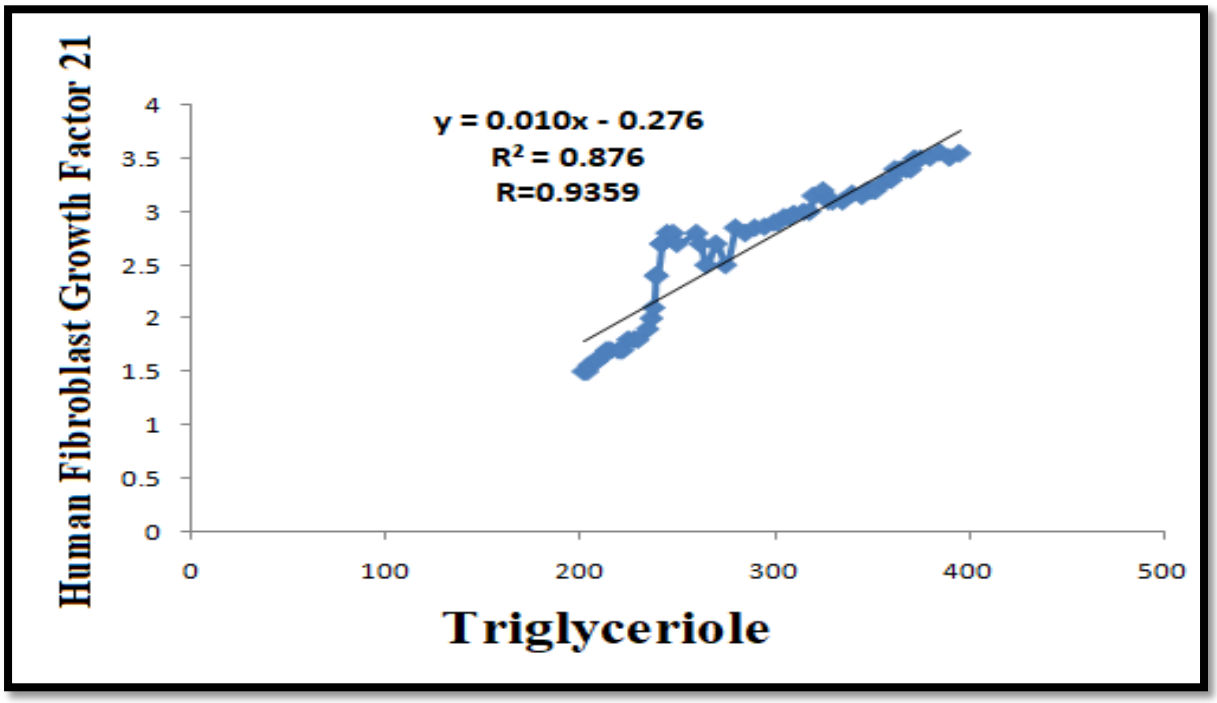


Figure (4-26) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Triglyceriole The results of the current study showed that there is a positive relationship

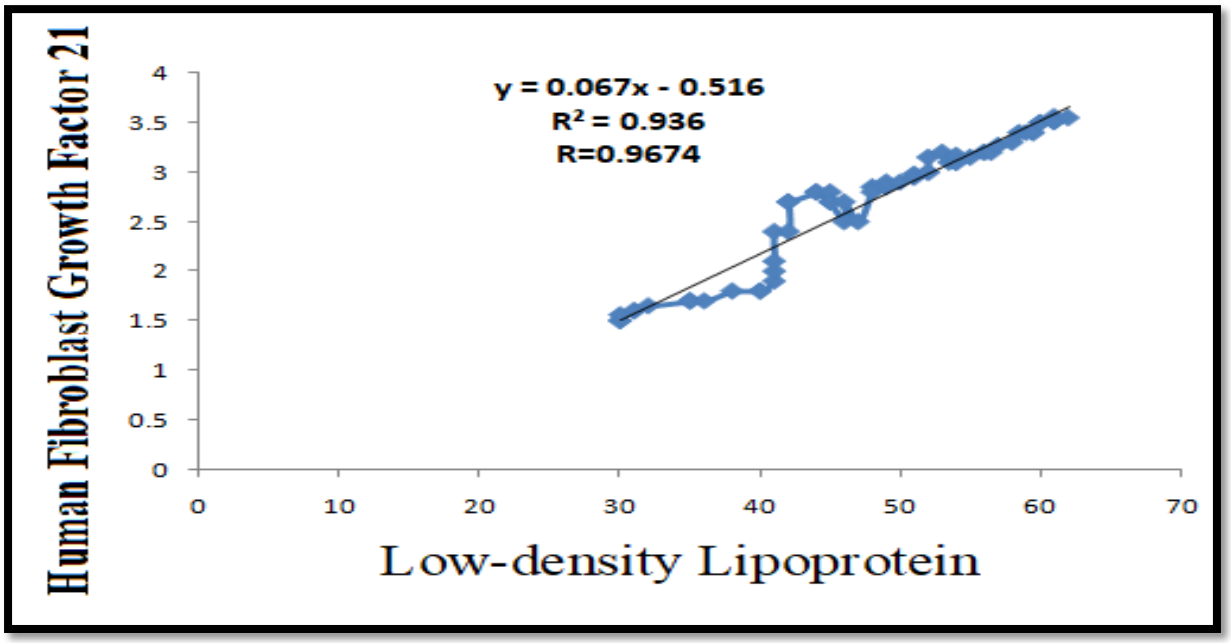


Figure (4-27) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Low-density Lipoprotein The results of the current study showed that there is a positive relationship

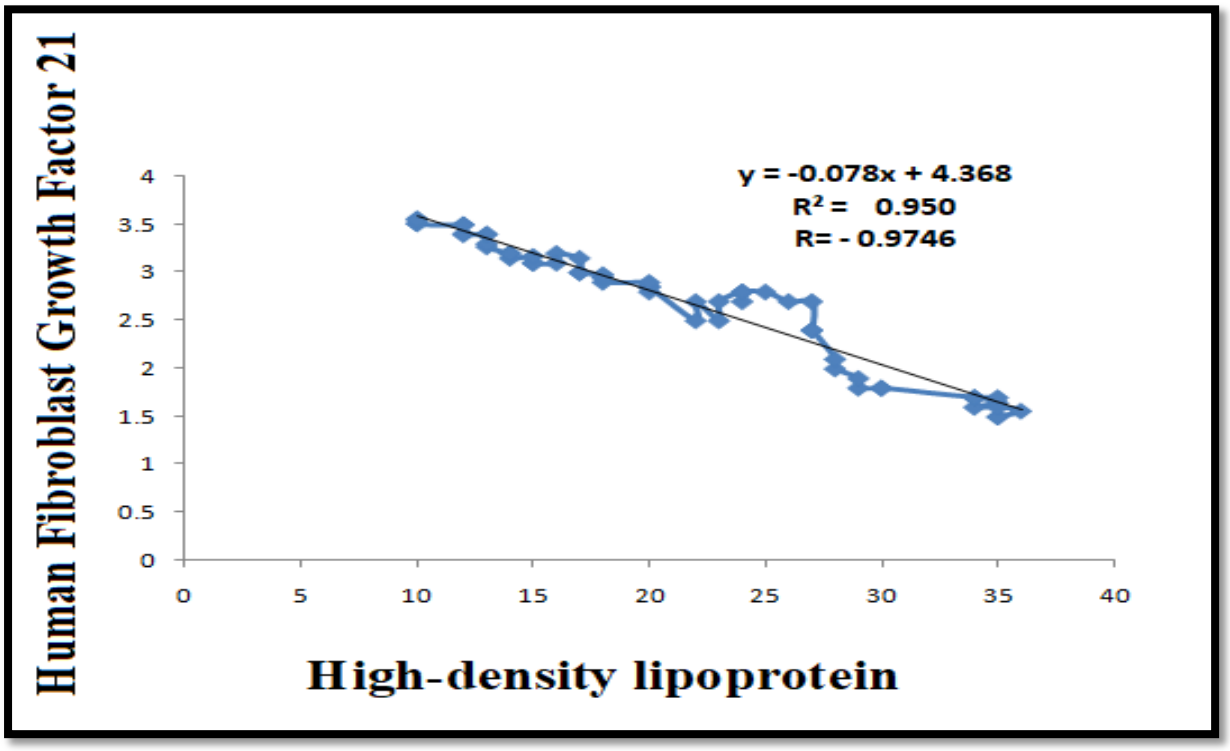


Figure (4-28) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and High-density lipoprotein, The results of the current study showed that there is a negative relationship

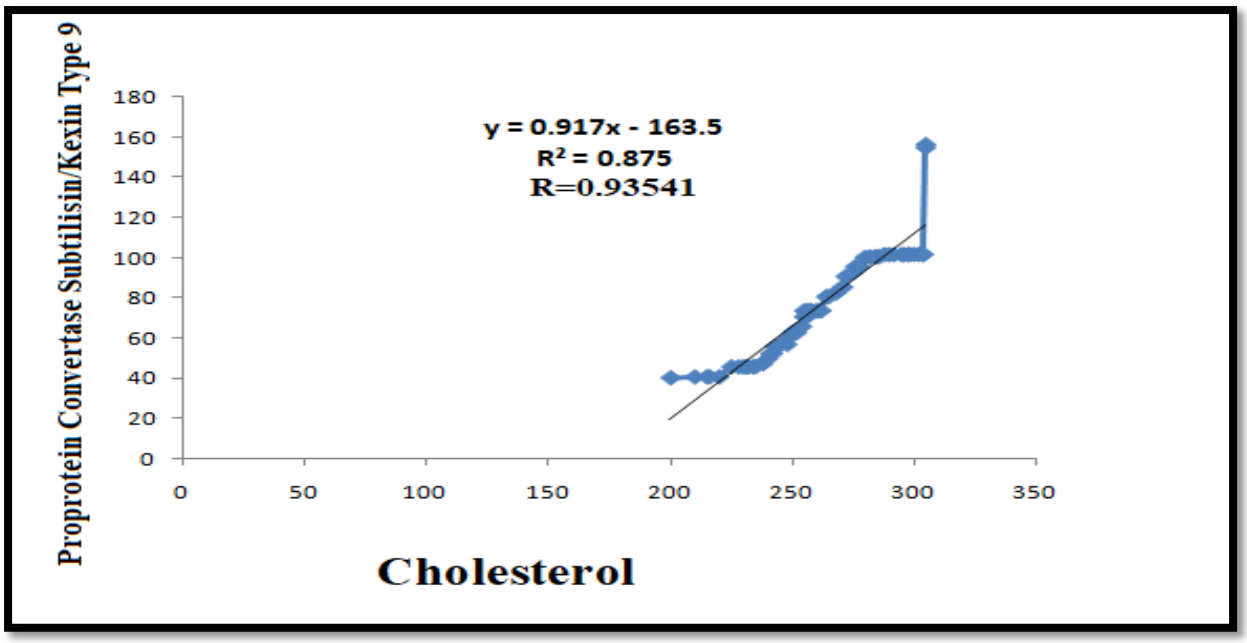


Figure (4-29) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Cholesterol ,The results of the current study showed that there is a positive relationship

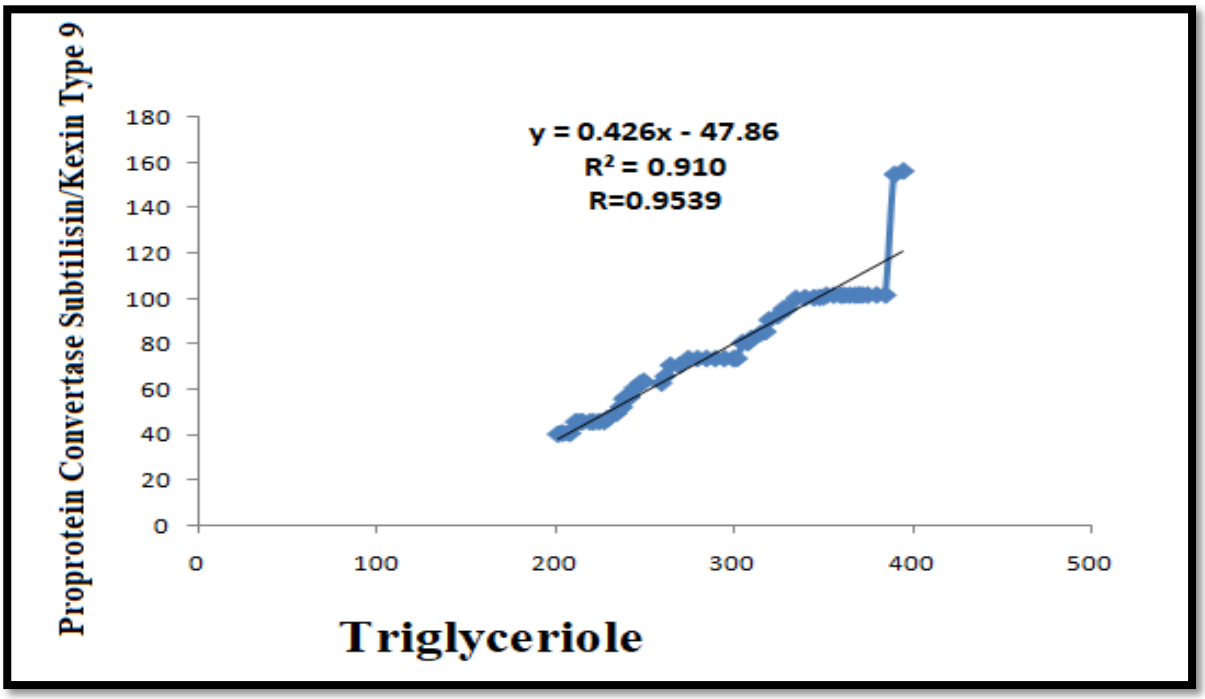


Figure (4-30) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Triglyceriole ,The results of the current study showed that there is a positive relationship

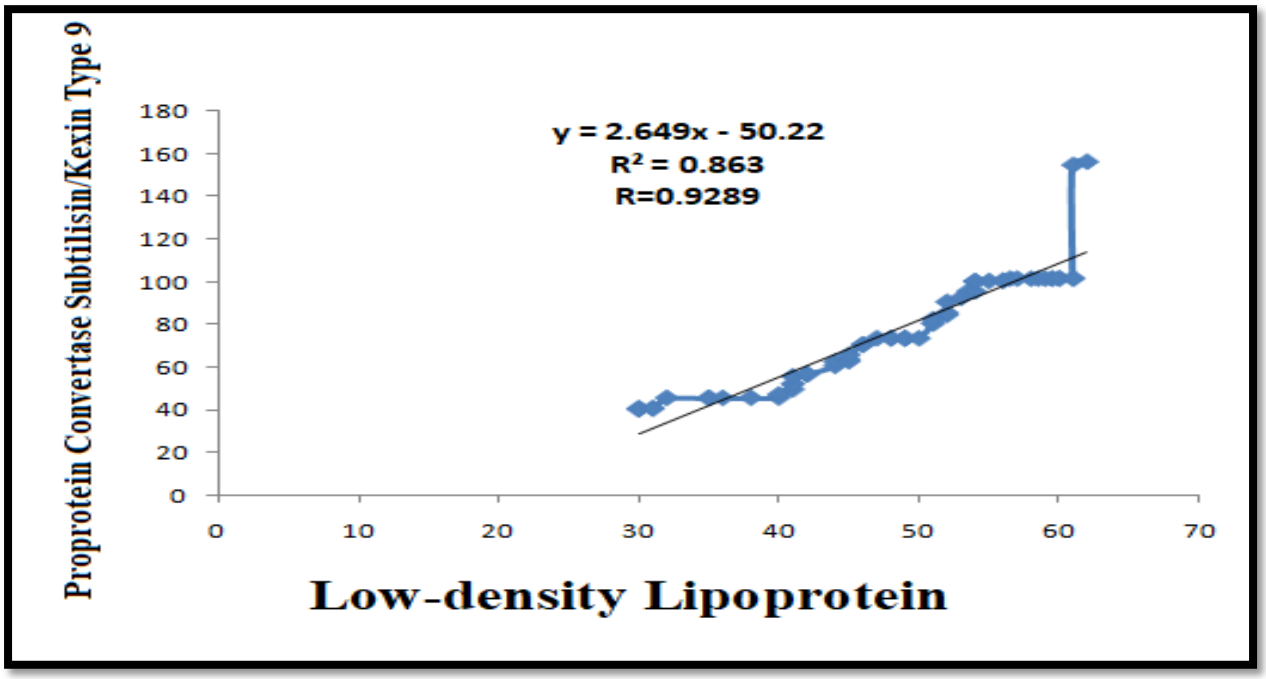


Figure (4-31) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Low-density Lipoprotein ,The results of the current study showed that there is a positive relationship

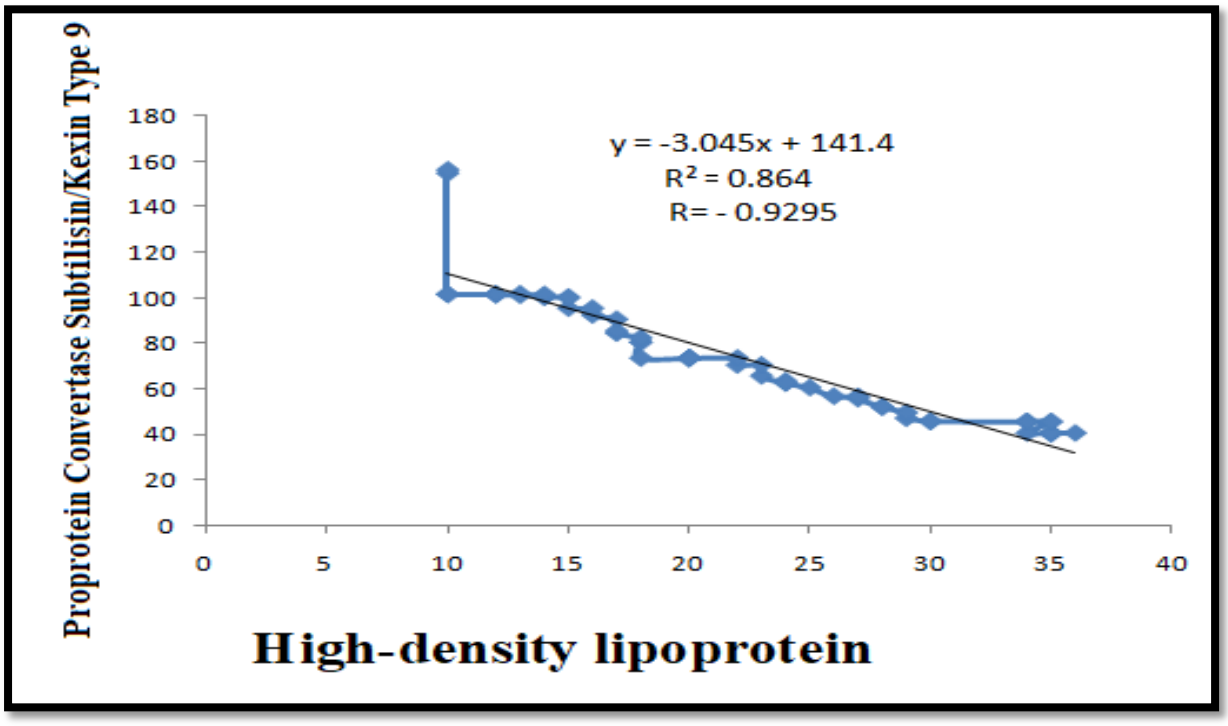


Figure (4-32) The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and High-density lipoprotein, The results of the current study showed that there is a negative relationship

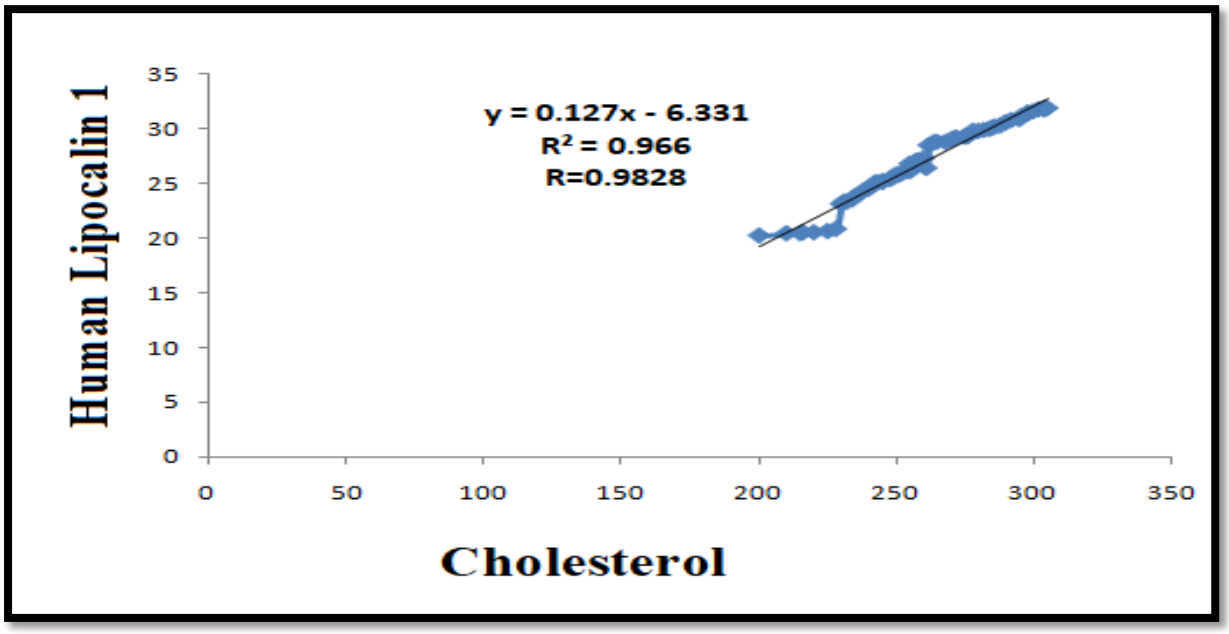


Figure (4-33) The correlation coefficient relationship between Human Lipocalin 1and Cholesterol The results of the current study showed that there is a positive relationship

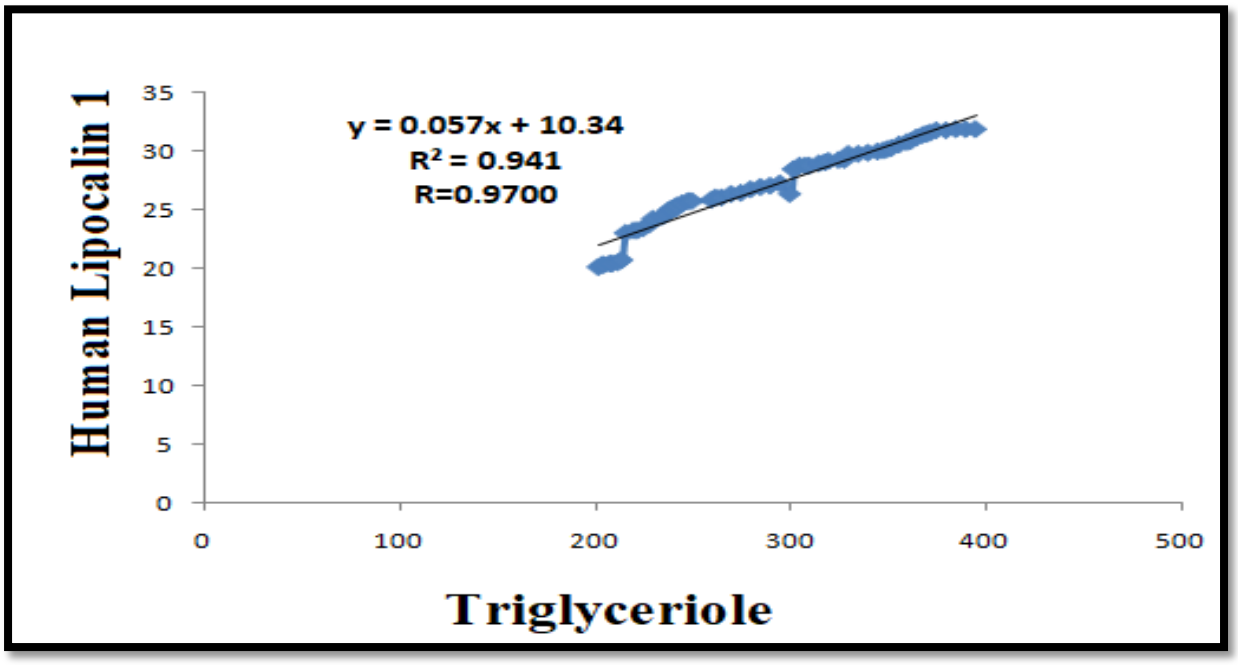


Figure (4-34) The correlation coefficient relationship between Human Lipocalin 1and Triglyceriole The results of the current study showed that there is a positive relationship

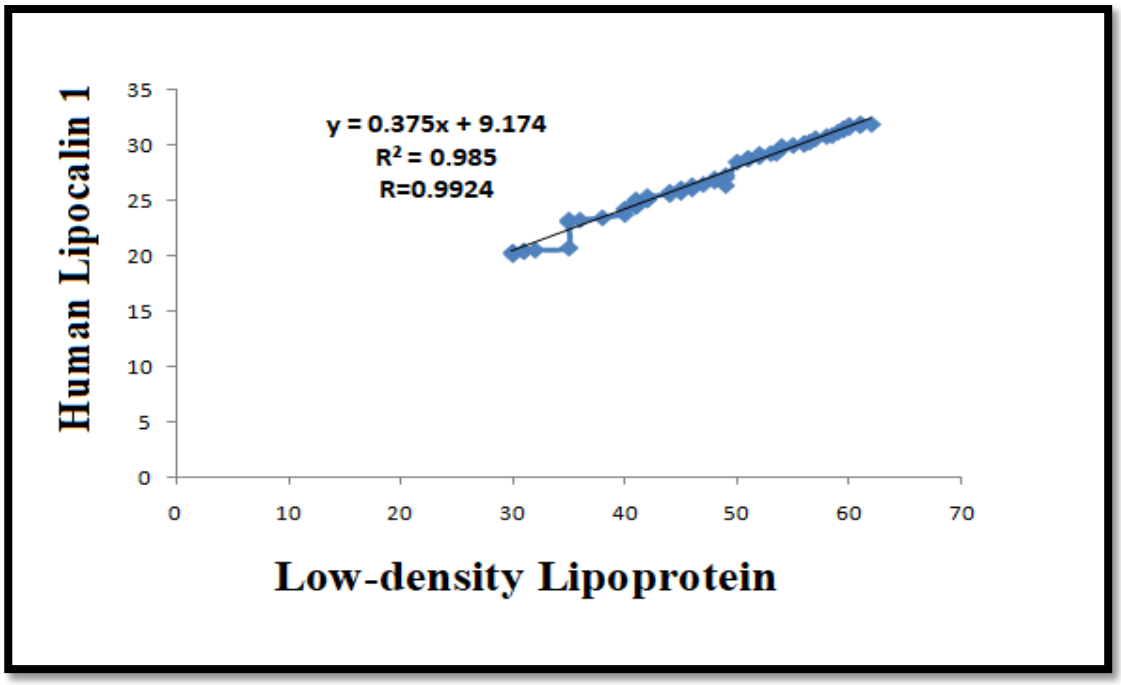


Figure (4-35) The correlation coefficient relationship between Human Lipocalin 1and Low-density Lipoprotein The results of the current study showed that there is a positive relationship

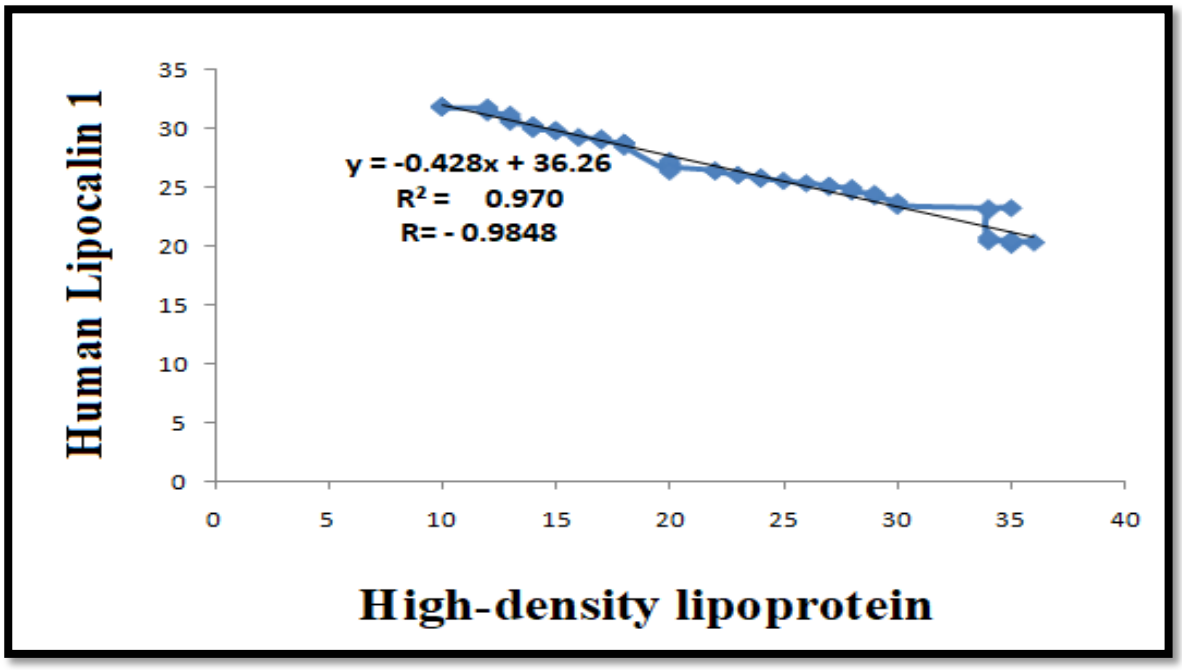


Figure (4-36) The correlation coefficient relationship between Human Lipocalin 1and High-density lipoprotein ,The results of the current study showed that there is a negative relationship

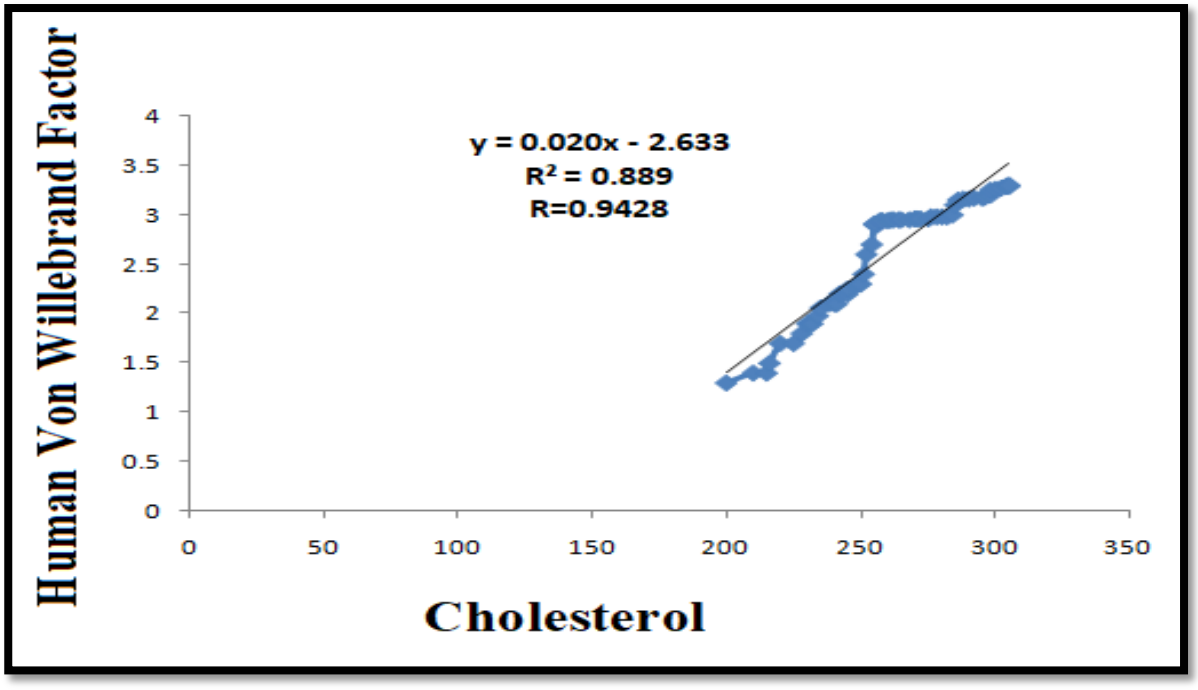


Figure (4-37) The correlation coefficient relationship between Human Von Willebrand Factor and Cholesterol ,The results of the current study showed that there is a positive relationship

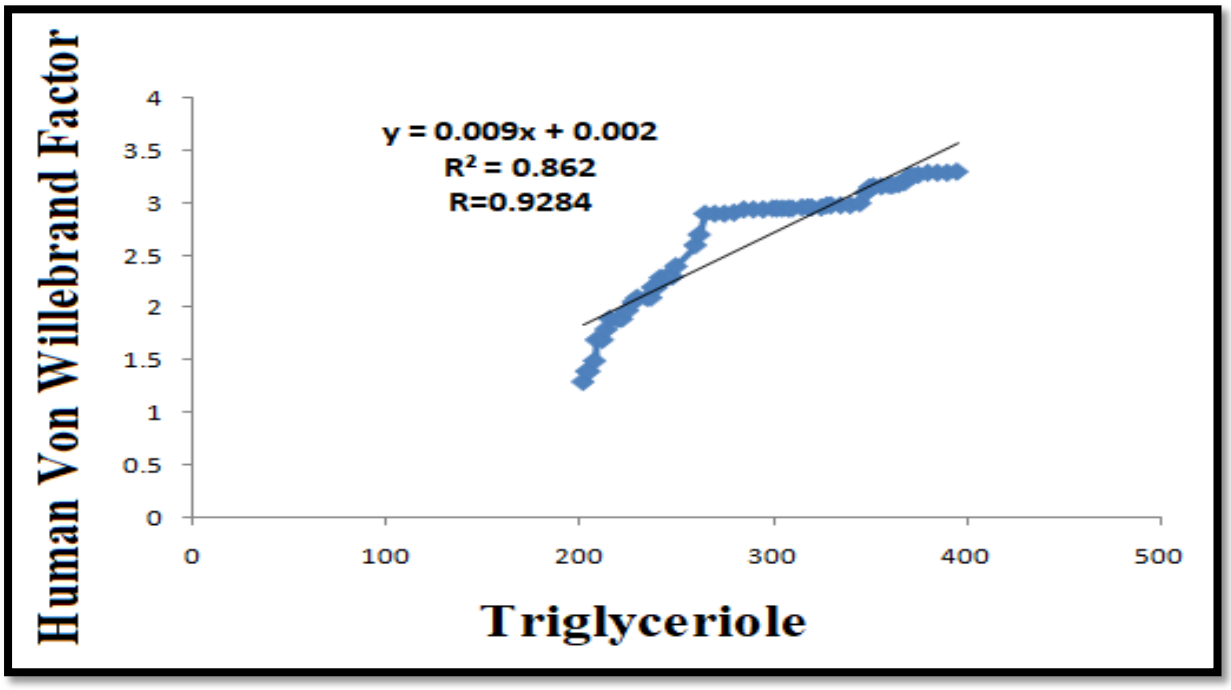


Figure (4-38) The correlation coefficient relationship between Human Von Willebrand Factor and Triglyceriole, The results of the current study showed that there is a positive relationship

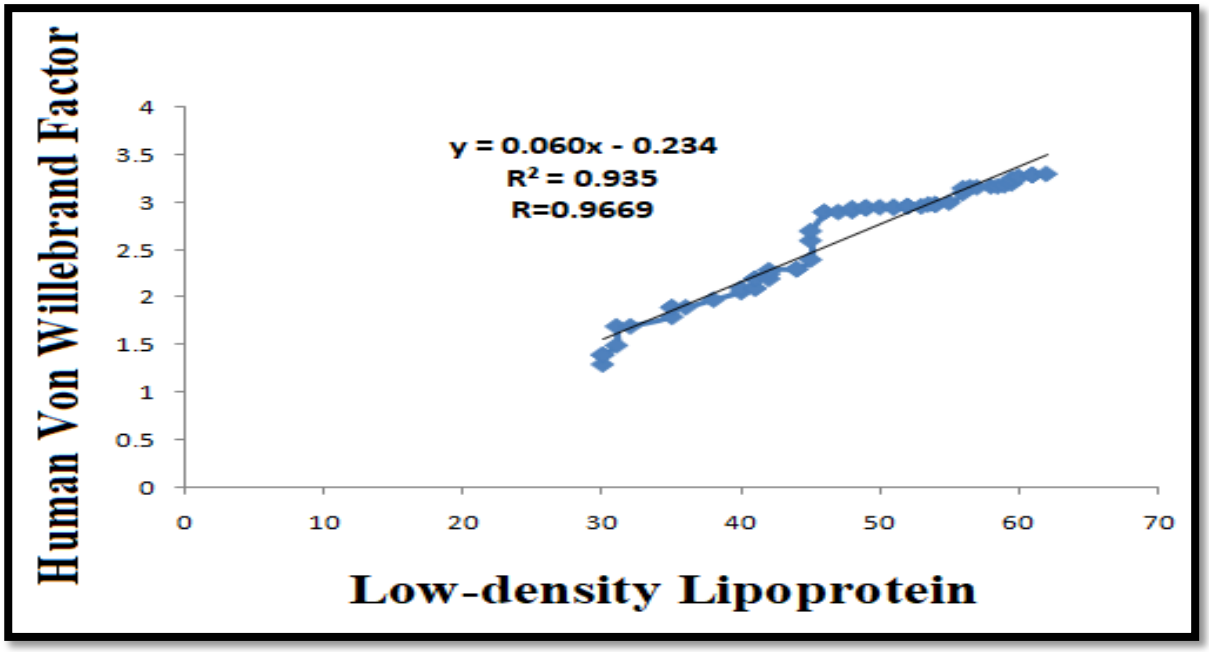


Figure (4-39) The correlation coefficient relationship between Human Von Willebrand Factor and Low-density Lipoprotein The results of the current study showed that there is a positive relationship

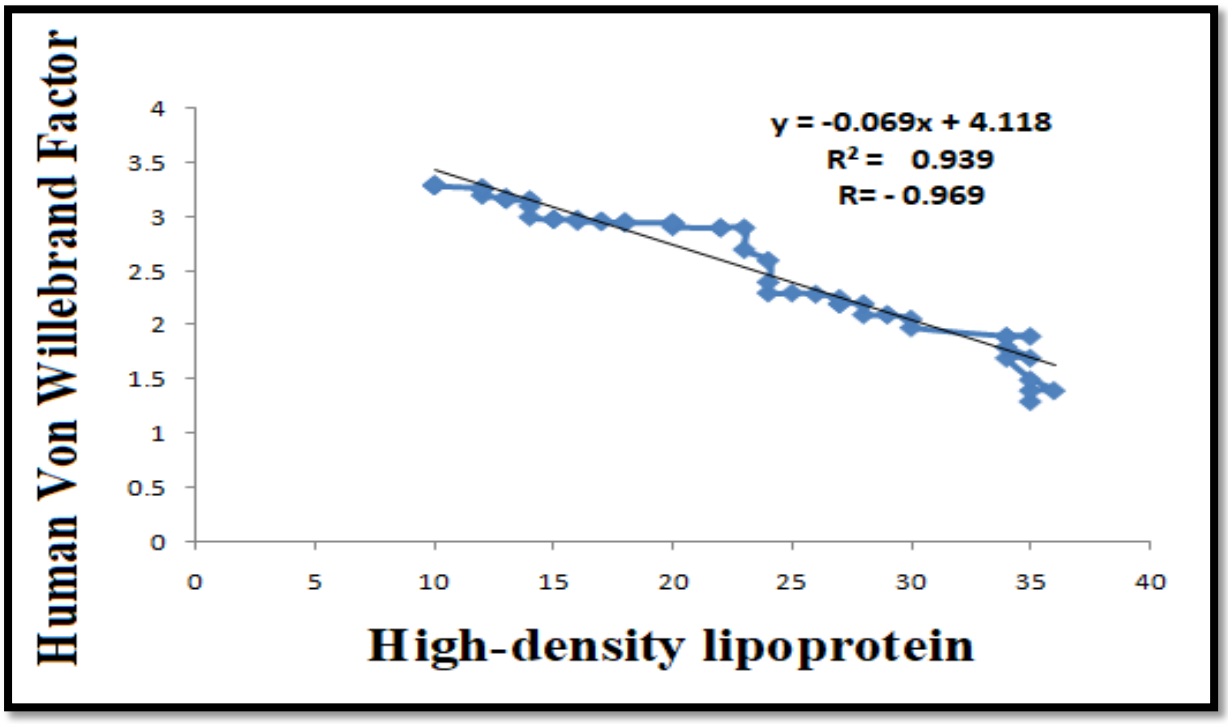


Figure (4-41) The correlation coefficient relationship between Human Von Willebrand Factor and High-density lipoprotein ,The results of the current study showed that there is a negative relationship

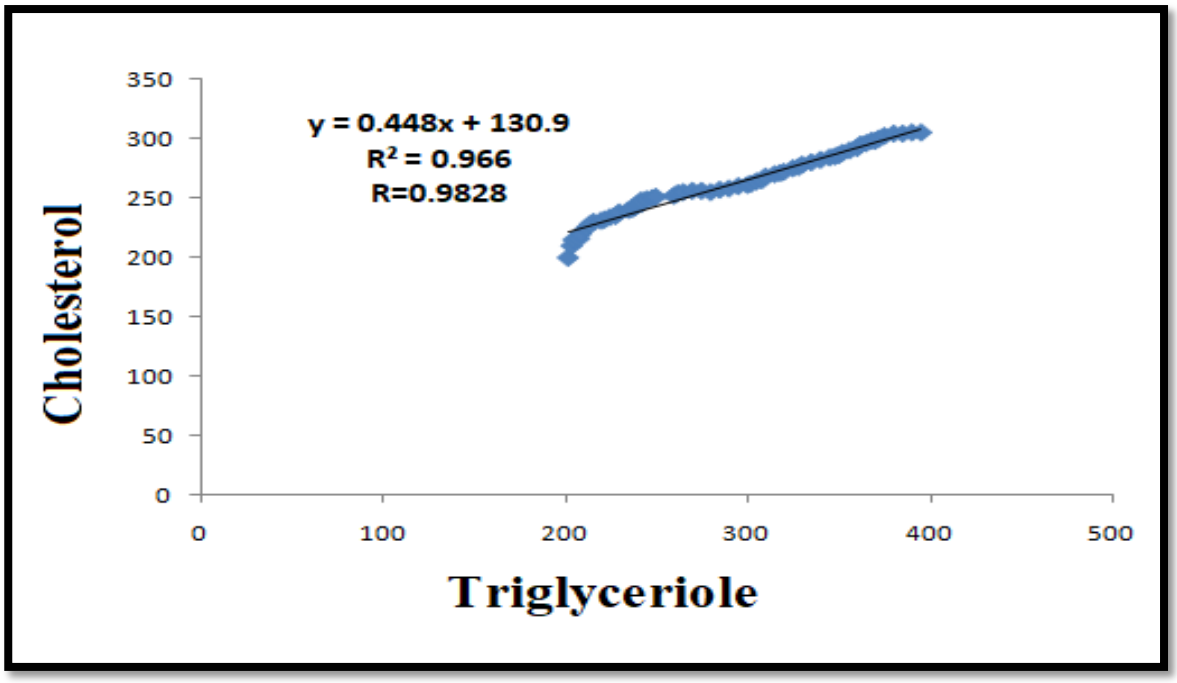


Figure (4-42) The correlation coefficient relationship between Cholesterol and Triglyceriole The results of the current study showed that there is a positive relationship

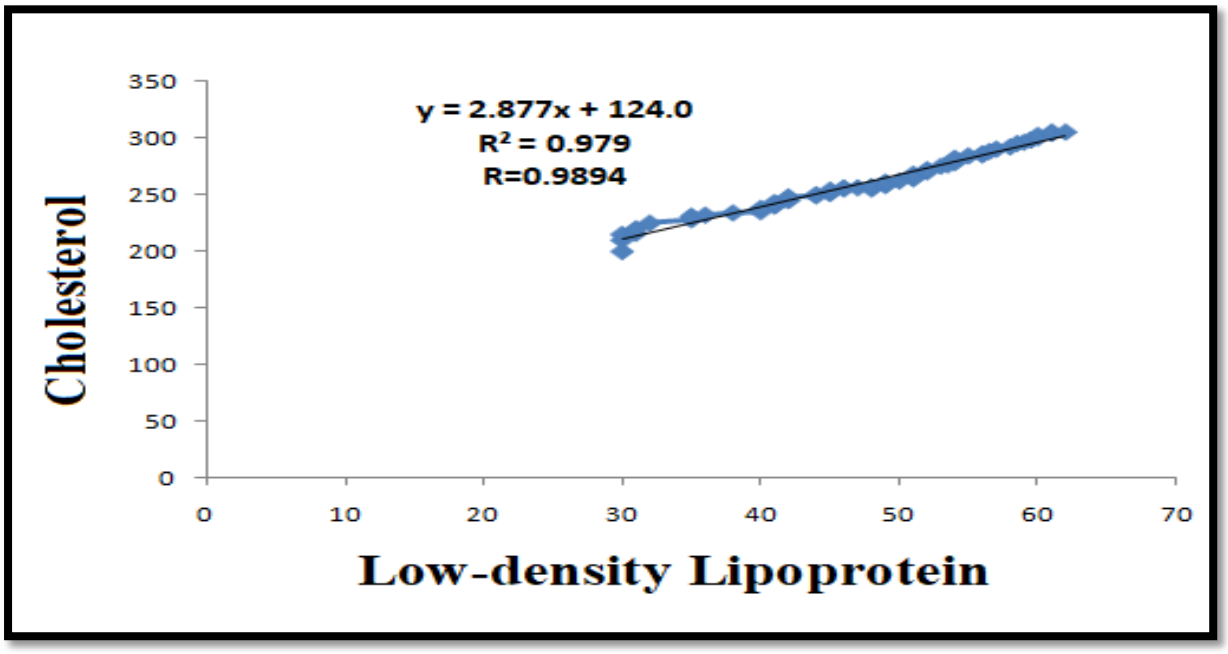


Figure (4-43) The correlation coefficient relationship between Cholesterol and Low-density Lipoprotein The results of the current study showed that there is a positive relationship

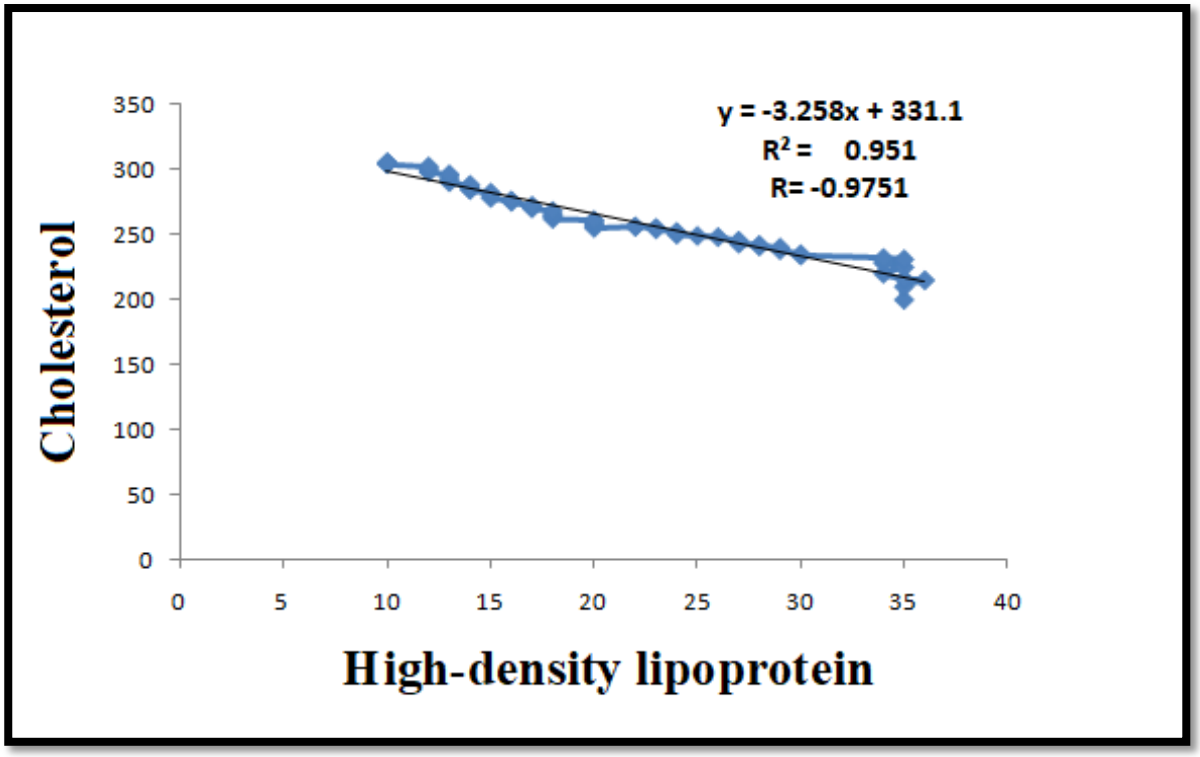


Figure (4-44) The correlation coefficient relationship between Cholesterol and High-density lipoprotein The results of the current study showed that there is a negative relationship

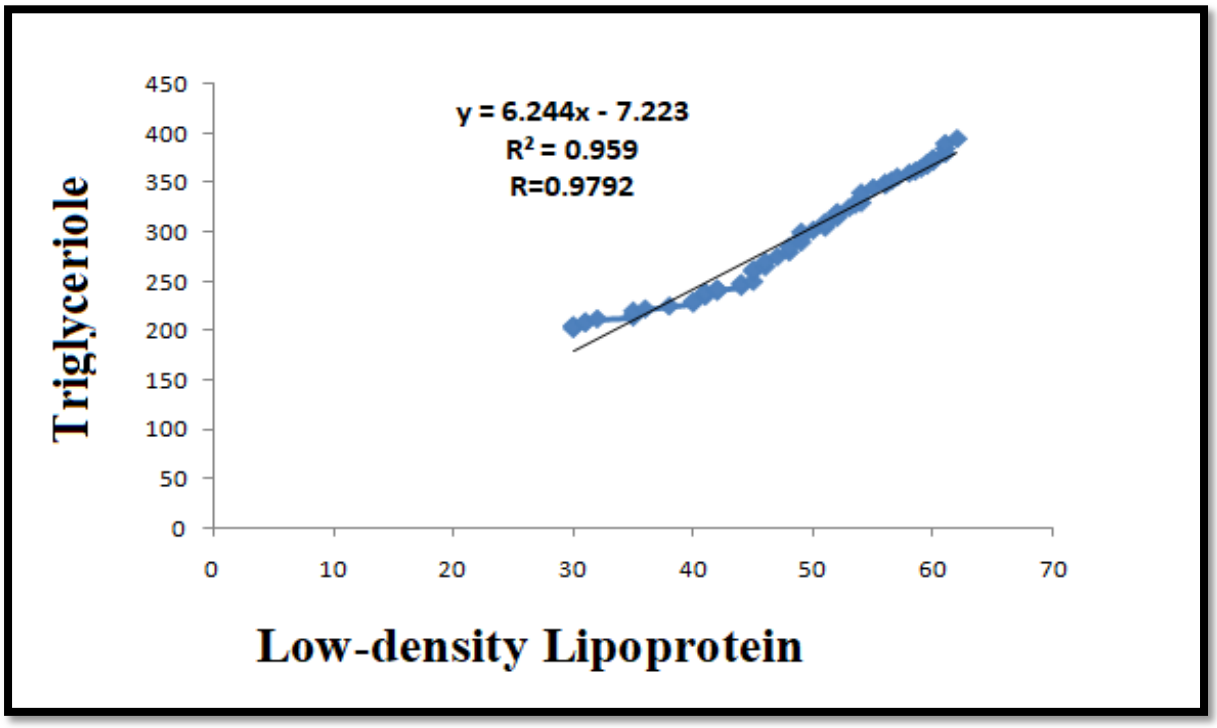


Figure (4-45) The correlation coefficient relationship between Triglyceriole and Low-density Lipoprotein ,The results of the current study showed that there is a positive relationship

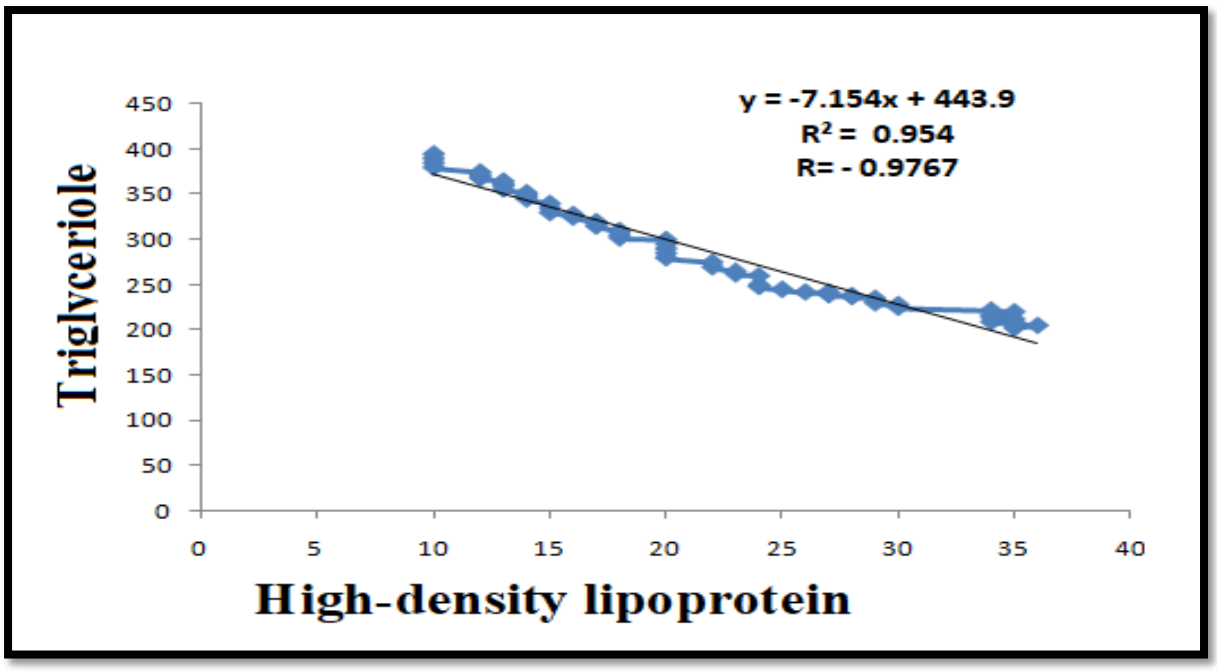


Figure (4-46) The correlation coefficient relationship between Triglyceriole and High-density lipoprotein ,The results of the current study showed that there is a negative relationship

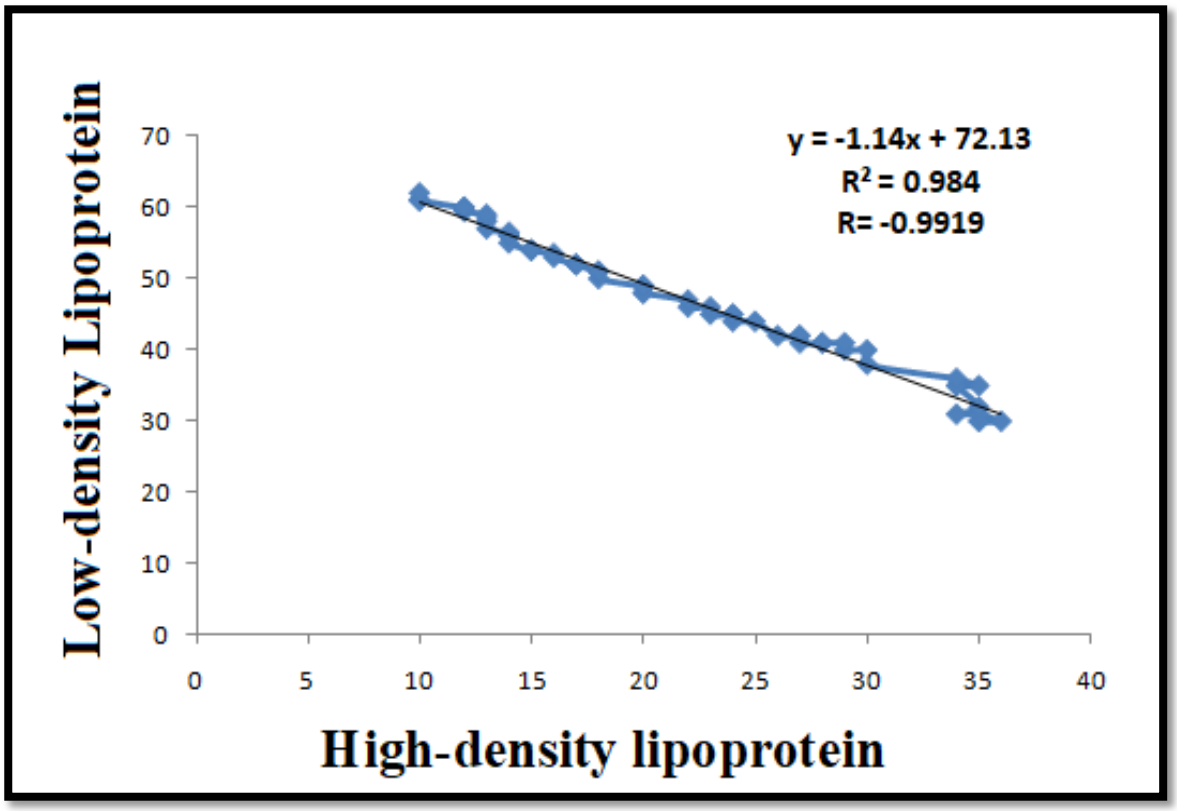


Figure (4-47) The correlation coefficient relationship between Low-density Lipoprotein and High-density lipoprotein ,The results of the current study showed that there is a negative relationship

4-4- Molecular Detection

4-4-1 .Molecular characterization of the ACE gene polymorphism

The results of electrophoresis of the PCR product showed the presence of three types of genotypes for the two groups: a homozygous (Deletion) genotype (DD), represented by the band with a molecular size of 190 bp, a homozygous (Insertion (II) genotype) represented by the 490 bp band, and an asymmetric genotype (ID). Heterozygous is represented by two bands (bp490, bp190)(Figure 4-48)

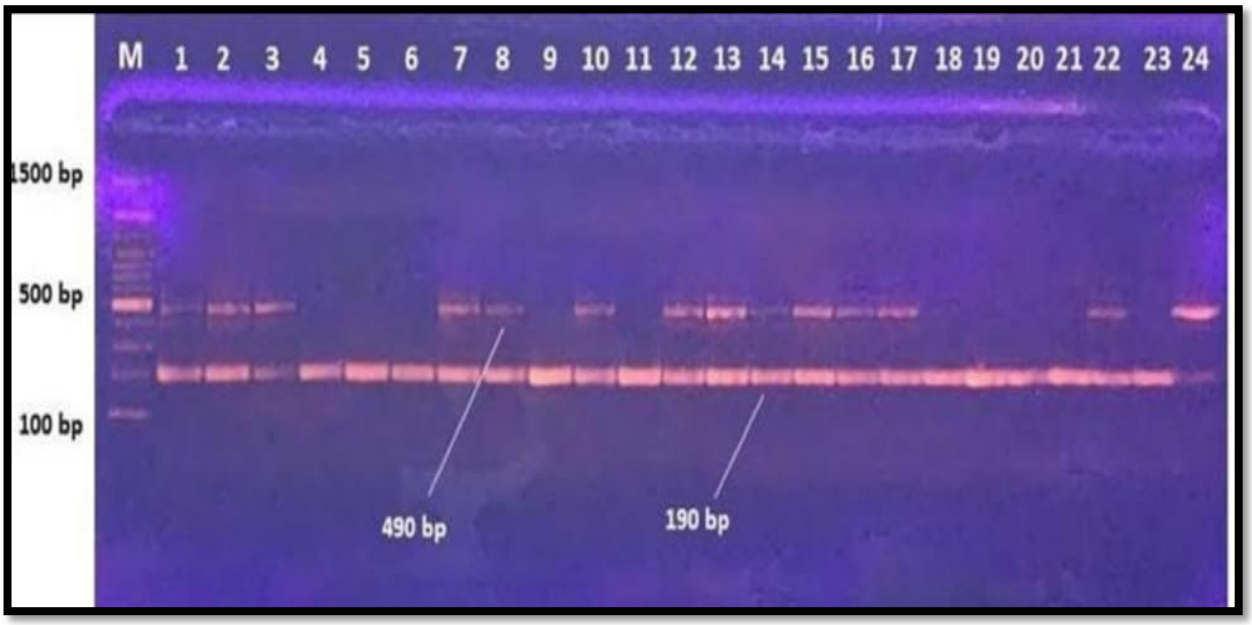


Figure (4-48) Electrophoresis of the PCR product for the polymorphism Angiotensin Converting Enzyme (ACE) gene to characterize phenotypic polymorphism on an agarose gel prepared at a concentration of 2%, with a DNA Ladder (bp voltage of 70 V/cm and a current of 40 mA, for two hours 100-1500

Deletion 190) (bp (DD) 2, 3, 5, 6, 7

Deletion Insertion(190- 490) bp (DI) Domain (1, 4, 10)

(II) Insertion 490 bp

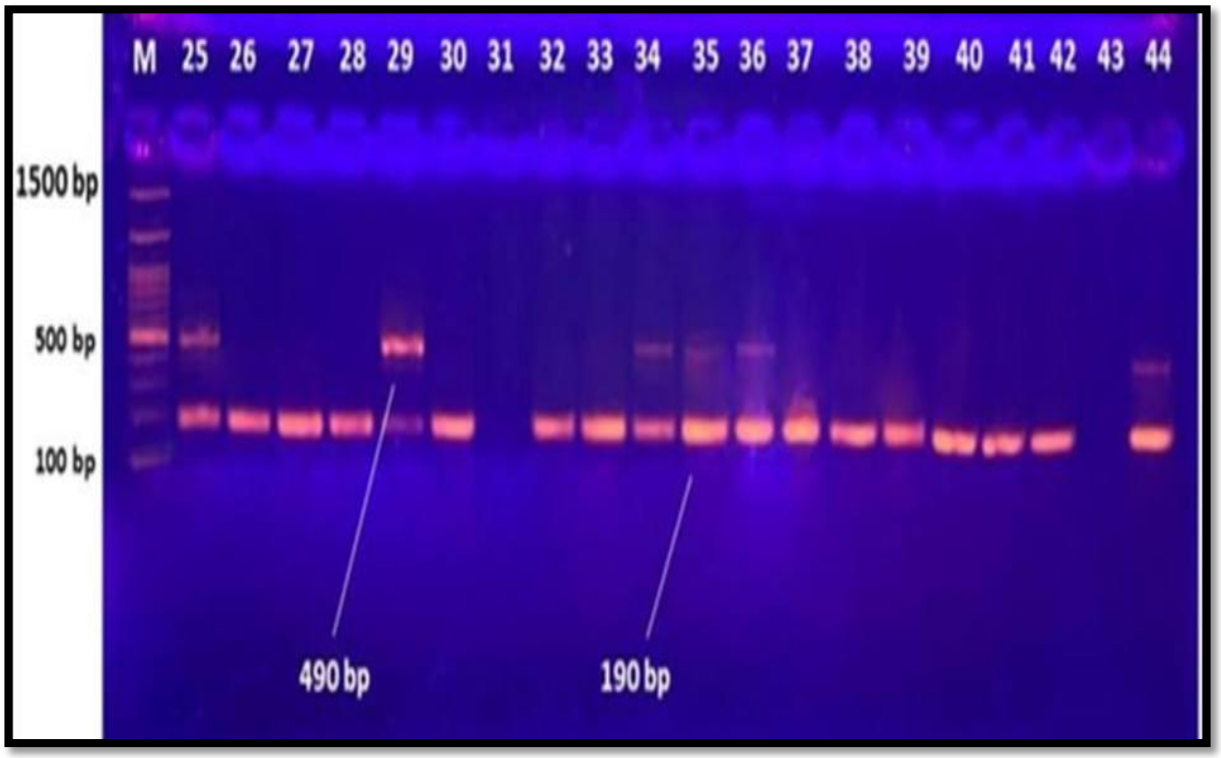


Figure (4-49) Electrophoresis of the PCR product for the Angiotensin Converting Enzyme (ACE) gene to characterize the phenotypic polymorphism and dominance of the allelic form I) / (D - control, on an agarose gel prepared at a concentration of 2%, with a voltage difference of 70 V/cm and a current of 40 mA, for two hours .

Domain allelic form (DI) (bp 190, 490. Domain (1) DNA Ladder 29,34,36,35,44 (bp 100-1500).

Table (4-1) Genotyping of *ACE* gene polymorphism with allele frequency. ACE gene polymorphism characterization in ischemic stroke patients and control group

Genotype *ACE*	**DD**	**DI**	**II**	**Total**	**Allele frequency D - I**	
Control *ACE* (N) %	8 % 26.66	17 % 56.68	5 % 16.66	30	%54 35.5	%46
***ACE* Patient (N) %**	30 % 50	25 % 41.67	5 % 8.33	60	%67	%31
Total	38%	42%	11%	**90**		

P≤	**0.001**	**0.001**	**Genotype**	**Control**	**Patient**
D allele (Total)	(8) %	(17) %	**DD**	(8) %	(30) %
			DI	(17) %	(25) %
I allele (Total)	(5) %44	(43) %	**II**	(5) %5	(5) %
			D	%30	%60
P≤	0.001	0.001	**I**	%52	%29

4.4.2 .Molecular characterization of the PCSK9 gene polymorphism

The results of electrophoresis of the PCR product showed the presence of three types of genotypes for the two groups:

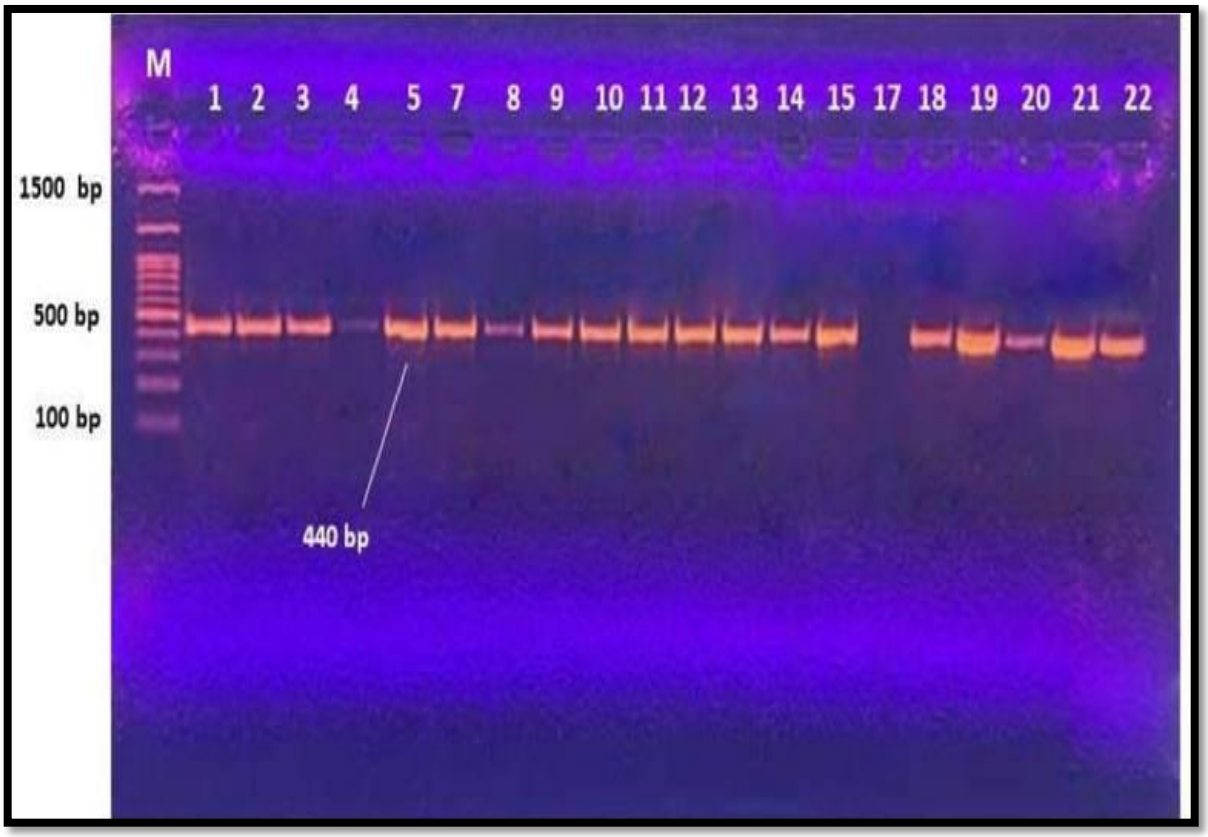


Figure (4-50) Electrophoresis of the PCR product for the (PCSK9) gene to characterize phenotypic polymorphism on an agarose gel prepared at a concentration of 2%, with a voltage of 70 V/cm and a current of 40 mA, for two hours DNA Ladder 100-1500 bp (bp 440)

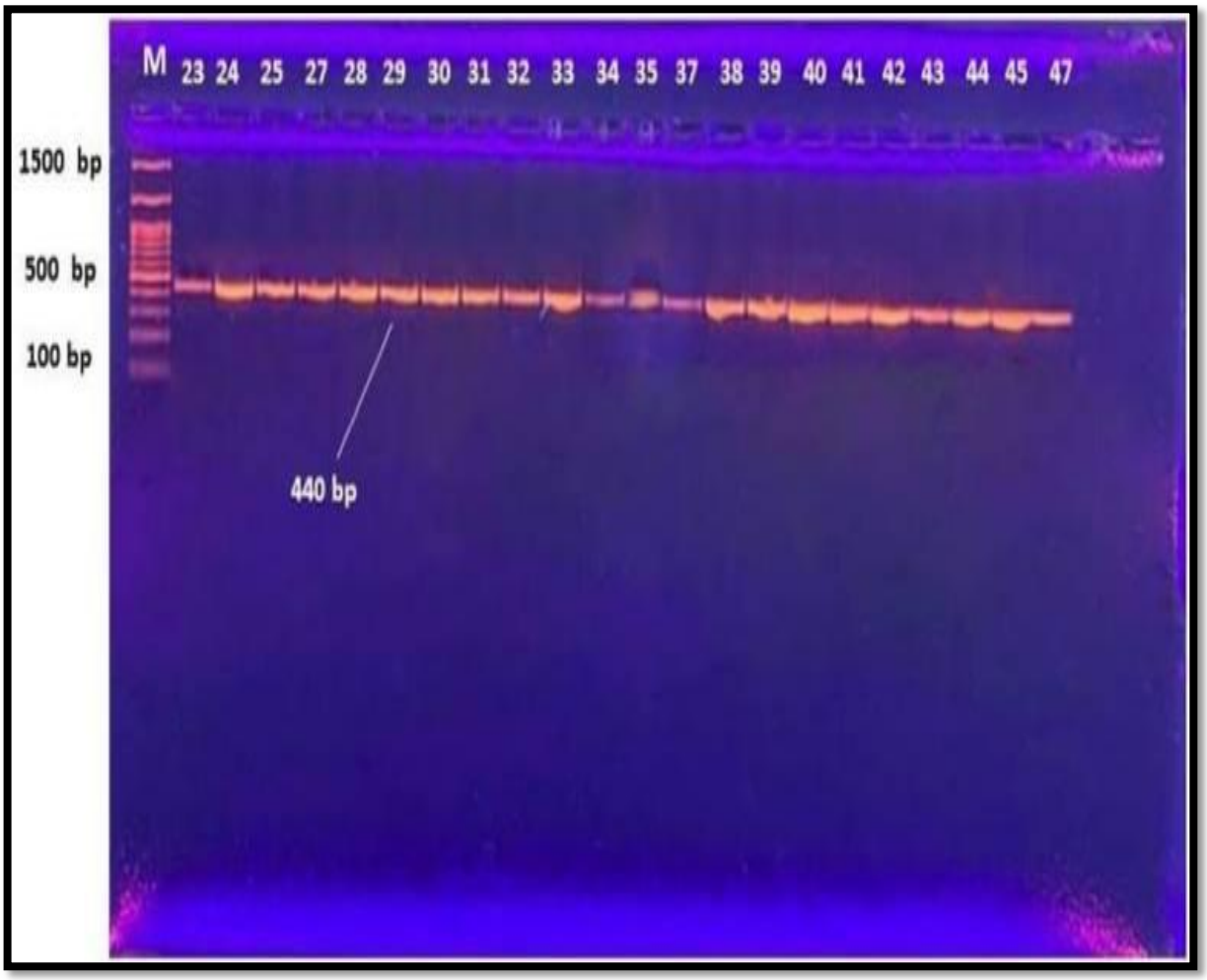


Figure (4-51) Electrophoresis of the PCR product for the (PCSK9) gene to characterize phenotypic polymorphism on an agarose gel prepared at a concentration of 2%, with a voltage of 70 V/cm and a current of 40 mA, for two hours DNA Ladder 100-1500 bp (bp 440)

5.1. Lipid profile

The Table (4-1) showed a significant increase in cholesterol level , triglyceride and LDL - C level in patients significantly in compare with control whereas figure (4-1) revealed significant decrease in HDL - C level in patients in compare with control The present results agree with recent study Shabana *et.al* (2020) That showed a hypercholesterolemia , hypertriglyceridemia and low HDL level with high LDL level associated with obesity in Pakistani subject

Another studies has been documented the roles of high cholesterol triglyceride and LDL level as a risk factors for cardiovascular disease and frequency of coronary events enhance in relation to lower HDL and these observations explained the lower HDL due to transfer of cholesterol ester from HDL - C to VLD and conversion of hepatic lipase into smaller particles in which tacked by arterial wall especially by macrophages lead to atherosclerosis so that the increase level of VLDL related with triglyceride positively because the main component of triglyceride is VLDL (Years, 2022)

Previous studies has been indicated that hydrophobic nature of triglyceride , cholesterol and cholesterol ester form a lipoprotein due transport to many tissues also excess fatty acids in liver and converted to free and esterified cholesterol and package in several tissues lead to atherosclerosis (Chen *et.al.* , 2009) .

Another studies has been documented the roles is High-risk patients who are unable to maintain use of moderate LDL-C target or who are unable to achieve their LDL-C target goal, are at increased risk of recurrent myocardial infarction and coronary heart disease events compared with those demonstrating high adherence to moderate- or high- regimens who reach their LDL-C goals(Kataoka *et al*., 2021)

. Our studies suggested that 5 other PCSK9 variants of unknown significance, where we were able to perform cascade screening, did not have a large impact on LDL-c metabolism in families. The exact underlying mechanism by which some of

the earlier described PCSK9 variants result in increased LDL-c levels *(Musumeci et al.*, 2023)

5.2. Biochemical markers

5.2.1.Comparison between biomarkers in ischemic heart disease patients and control

group the results listed in Figure (4-1) , (4-2) and (4-3) and (4-4) indicated a significant elevation $p < 0.05$ Figure (4-1)show level of Human Fibroblast Growth Factor 21 in patient compare with control Our study explores the association between FGF21 levels and ischemia , the subject of conflicting results in recent years. Several cross-sectional study showed that serum FGF21 levels were significantly elevated in patients with compared The observed increase serum levels of FGF-21 in the ischemia group, as compared to the control group, may be attributed to the phosphatidylinositol 3-kinase/Akt (PI3K/Akt), ERK1/2 (extracellular signal-regulated kinase)(Crudele *et al*., 2023) .

AMPK (AMP-activated protein kinase) pathways may all be triggered in heart tissue by the FGF21 signaling pathway (Patel et al.,2014). Furthermore, despite the fact that FGF21 is an endocrine FGF, the heart also seems to be a focus of locally produced FGF21 therfore according to a prior research, after myocardial injury, adipose tissue releases FGF21, which acts via the FGFR1/b-Klotho-PI3K-Akt1-BAD signaling network to protect the heart (Liu SQ et al.,2012). This supports a prior study's conclusion that the level of FGF21 can predict morbidity and mortality in coronary heart disease (Lenart *et al*.,2013).

An earlier investigation revealed that cardiomyocytes made and secreted FGF21 (Planavila et al.,2013). Cardiac FGF21 was secreted with high level in reaction to cardiac ischemic stress, and it prevented isoproterenol-induced cardiac hypertrophic injury.

Research findings has been indicated that individuals who experience ischemia or myocardial injury are at a higher risk of both short-term and long-term mortality (Smilowitz et al., 2018) The and variations in the prevalence of ischemia across studies can plausibly be attributed to dissimilarities in the gender of patients who underwent coronary intervention, which may have that FGF21 has a greater prognostic significance than those who did not undergo such intervention (Nestelberger *et al*., 2017, Chapman *et al*., 2018.

The study examines consecutive cases with elevated FGF levels and their correlation to ischemia incidence among individuals has a significant risk rates model incorporating duration, CAD, stroke, and vascular complication also with age and gendar (Chapman et al., 2018). These results are supported by other study that indicated that gender agreement and several clinical factors had minimal influence on the prevalence of FGF associated with subsequent ischemia (Cediel et al., 2017). Contrary, an analysis of the SWEDEHEART registry (Swedish Web- system for improvement and development of medical care in Heart Disease estimated According to Recommended catheterization) revealed a decrease in the risk related to ischemia due to the implementation of stabilizing characteristics and treatments (Baron *et al*., 2015) .

Myocardial ischemia injury induce cell apoptosis and MI, leading to an impairment in cardiac function growing evidence from both in vitro and in vivo studies demonstrate that exogenous FGF21 protected the cardiomyocytes and demonstrates that elevated serum FGF21 occurs in in patients, with risk factors Baseline serum FGF21 level is an predictor of future CVD events in the general population and is a promising novel a biomarker CVD of the discuss of high level of FGF21.

Figure (4-2) show high level of Proprotein Convertase Subtilisin/Kexin Type 9 in patient compare with control The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant increase ($P< 0.05$) in patient compare with control (figure 4-2) The has been shown that previous observation high in serum levels of

PCSK9 in the ischemia group, as compared to the control group, may be attributed to the significant incidence of hyperlipidemia in the population, which is known to be related to elevated levels of low-density lipoprotein cholesterol (LDL-C) and an increased risk of cardiovascular complications(Receptor et al., 2012). According to the previous Dysfunctional calcium signaling may also be a contributing factor to certain myopathies that result in mitochondrial depolarization and subsequent calcium release, leading to the occurrence of calcium swells and post-calcium release by the sarcoplasmic reticulum. This is a suggested pathway for the activation of caspases and the subsequent induction of apoptosis and the elevation of calcium levels could potentially enhance the activity of phospholipid-dependent PKC, leading to the suppression of **CLC** channels and consequent hyperexcitability of the cytoplasmic membrane and this is associated with high level of PCSK9 (Apostolopoulou *et al*., 2015; Pierno *et al*., 2009

The PCSK9 protein has been identified as a significant factor in determining the limit of serum LDL-C levels, as well as the development of coronary and intracranial atherosclerosis Therefore, it has the potential to serve as a useful predictor of large-vessel atherosclerosis (Tavori et al., 2013). The present reset disagrees with The PCSK9 I474V variant has not demonstrated a significant correlation with lipid levels or the risk of CHD in males without pre-existing health conditions (Kotlega et al., 2016). The study found that there was a rise in serum concentration of PCSK9 that was proportional to the extent of LDL-C reduction which contradicts the expected outcome of elevated levels of PCSK9 (Roman *et al*., 2022).

The current study findings are consistent with a previous investigation, which demonstrated that PCSK9 inhibitors did not induce myalgia in patients and did not result in any adverse cardiovascular outcomes when compared to a control group receiving standard doses (Roman et al., 2022). The objective of this study was to assess the potential of PCSK9 as a viable therapeutic agent for enhancing cardiovascular health following catheterization , Anthor stady showed that the

PCSK-9 protein exhibits an affinity towards the LDL receptors (LDL-R) and facilitates the process of endocytosis, thereby leading to a reduction in cholesterol levels by promoting the reduction of LDL-R. Consequently, there is a subsequent reduction in lipoproteins and serum LDL-C levels (Watts et al., 2017). Research has indicated that the utilization of PCSK-9 treatments as a supplementary therapy to statin monotherapy leads to a significant reduction in LDL-C levels. Several studies have indicated that the utilization of PCSK-9 leads to a significant reduction in the incidence of treatments associated with cardiac adverse events (Turgeon *et al*., 2018).

In a study conducted in 2022 was a follow-up it analyzed eight years' worth of follow-up data (O'Donoghue et al., 2019; O'Donoghue et al., 2022) and the study compared cases with a history of cardiovascular events (CVEs) to control groups a after analyzed and arrest and results indicate that there were significant decreases in both LDL-C degrees and CVEs. The demonstrated a sustained decrease in LDL-C and CVE in patients for a period exceeding eight years subsequent to the original trial. Therefore concluded potential for the enduring safety and effectiveness of PCSK9 over time , as a result, there is a decrease in the incidence of CVEs, as evidenced by various studies (AlTurki *et al*., 2019).

The study found that there was statistically significant difference in serum levels of PCSK9 between patient ischemia groups. However, there was a significant high in serum levels of PCSK9 patient patient ischemia groups. Studies have demonstrated that PCSK9 inhibitors have the specific capability of decreasing lipoprotein(a) levels by 20-30%. This holds significant importance as research has demonstrated that lipoprotein(a) is the predominant contributor to cardiovascular ailments and exhibits minimal susceptibility to lifestyle modifications or gender. The ongoing CARUSO study has also reported comparable assertions (Aranzula *et al*., 2021).

Figure (4-3)show level of Human Lipocalin 1 in patient compare with control significant ($P<0.05$) The level of Human Lipocalin 1 showed a significant increase ($P< 0.05$) in patient compare with control (figure 4-3) .

That showed present results absence of a statistically significant difference in the serum concentrations of neutrophil gelatinase-associated lipocalin (NGAL) between the ischemic heart patient group suggests that NGAL is unlikely to have any myocardial impact in the absence of structural heart disease. Nonetheless, the same with multiple fixed flow-limiting coronary stenosis and MI . Studies indicated that this particular substance might not play a crucial role in cardio protection, both innate and adaptive immune responses in mucosal organs, maintaining adipose tissue cells, and as a biomarker in diseases of inflammatory and non-inflammatory origin (Stefan *et al*., 2021)

Parameters such as NGAL, KIM-1 (Wang 2017), or combinations of the urinary insulin-like growth factor-binding protein (IGFBP-7) and tissue inhibitor of metalloproteinase (TIMP-2) (Fan et al, (2019; Nalesso et al , (2020) have been proven their efficacy as dependable diagnostic and prognostic tools. This is consistent with (Stefan et al., 2021), wherein an analysis was conducted on serum samples obtained from both healthy individuals and those afflicted with MI or other autoimmune-mediated conditions, with a view to determining the concentrations of specific pro- and anti-inflammatory mediators MI or myocarditis, which could potentially be identified by more advanced high-sensitivity assessments (Pichery *et al.*, 2012).

The study results are consistent with the perspectives of individuals diagnosed with chronic kidney disease (CKD) (Gungor et al., 2017). Serum IL-33 and ST were found to be related to CVEs and parameters of endothelial dysfunction in patients with CKD. The two analytes exhibited increased levels as the estimated glomerular filtration rate decreased, and heightened concentrations were linked to impaired FMD and cardiovascular risk. Furthermore, there is concurrence with the findings of Bakr et al.'s (Bakr et al., 2021) investigation, which demonstrated elevated cytokine levels in the serum of patients compared to those of healthy individuals. Furthermore, there was a significant correlation observed between the activity of the disease and serum

levels Nevertheless, Bao et al., 2012 have demonstrated through experimental studies on participants with CKD that the knockout of TGF-β enhances IL-33. Although the inflammatory regulation of IL-33 varies depending on the disease, it has been observed that it provides protection against pathological remodeling after ischemi, myocyte hypoxia, pressure overload, and increased Ca2+ release in the heart (Seidlmayer et al., 2016) .

In a study conducted by Chen et al ,(2021), multivariate regression analysis was utilized to identify IL-33 as an independent predictor of HT, the study found that lower serum levels of IL-33 were related to an increased risk of developing HT. Elevated levels of IL-33 were found to be positively correlated with a greater risk of HT and an increase in NGAL. Conversely, elevated levels of cytokines were correlated with a favorable prognosis in affected individuals. Segiet *et al*., (2019) reported that the mean serum content of patients was lower in comparison to the control group On the other hand, the study finding agree with indicating that NGAL had no effect on infarct size or prognosis in patients as compared to control (Kitakaze *et al*., 2007)..

Figure (4-4) show level of Human Von Willebrand Factor in patient compare with control The level of Human Von Willebrand Factor showed a significant increase ($P< 0.05$) in patient compare with control (figure 4-4)

significantly associated The main finding of this study was that higher VWF levels were with a higher coronary plaque burden in CAD patients who had already received statin treatment activation but not actual endothelial denudation, is the initial step in plaque formation and this process is inhibited by the inactivation of VWF in a rabbit model also the formation of fatty streaks in VWF-deficient mice is delayed compared with that in control mice(Cheng *et al*., 2018).

VWF and/or the state of endothelial activation reflected by increased VWF levels may contribute to the pathogenesis of early atherosclerotic plaques Endothelial

dysfunction, which is characterized by impaired bioavailability of endothelial-derived nitric oxide (NO), is involved in the development of atherosclerosis. Endothelial NO plays a crucial role in vasodilation, platelet aggregation vascular smooth muscle proliferation, and endothelial-leukocyte interactions and It has been the resean of high level of VWF may be related with blockade of NO production increased VWF levels in humans,NO may be an inhibitor of endothelial VWF secretion*(Elkhidir et al*., 2017).

Several human studies have reported a positive association between VWF levels and the risk of CAD Furthermore, a strong association between the extent of atherosclerosis in both the aortic arch and the carotid arteries and VWF levels in patients with transient ischemic attack or ischemic stroke has been reported also recent study using IVUS also showed that higher VWF levels were associated with a higher coronary plaque burden in CAD patients Similarly, a positive association between the VWF level and coronary plaque burden in addition elevated VWF levels may reflect impaired endothelial NO generation in vascular endothelial dysfunction and advanced atherosclerosis in patients with atherosclerotic cardiovascular diseases(Ehsan *et al*., 2022)

This meta-analysis summarizes evidence for association between high-circulation vWF levels and clinically adverse outcomes in patients with CAD and these dta on plasma vWF at three time points was included therefore results indicated that the plasma vWF was significantly increased in the adverse event group on 24 h and 48 h after primary CAD , Subgroup analyses revealed that the association of increased vWF level with short-term MACEs is stronger(Fan *et al*., 2020).

In addition, increased vWF level displays a positive association on MACEs in ACS and MI other than stable CAD plasma level of vWF is an indicator for the risk of MACEs among patients with CAD vWF is mainly synthesized in endothelial cells upon endothelial cell injury, vWF is released into the blood circulation. In as well as , vWF binds to platelet receptors GPIb-IX-V, GPIIb/IIIa and GPIb to promote

thrombosis so that combination of vWF and collagen causes a conformational change in the site of vWF binding to factor VIII, which promotes fibrin agglutinatio VWF also mediates platelet adhesion on activated (Lenting *et al*., 2015).

endothelial cells, enhancing thrombus formation even in the absence of endothelial denudation and The role of vWF may be associated with endothelial dysfunction and inflammation , which contribute to the cardiovascular risks. In addition vWF involves in the pathogenesis of atherosclerosis myocardial infarction, nonfatal stroke, or target vessel revascularization, are more likely to occur in patients with severe CAD(Kozlov *et al*., 2022) .

The increased risk of vWF for MACEs in CAD may be caused by prothrombotic or hypercoagulable conditions, which promote the formation of occlusive thrombus VWF promotes thrombus formation by mediating platelet adhesion and aggregation . lead to inflammatory response involved in the progression of atherosclerotic plaques may also promote an increased secretion of vWF Therefore, vWF may be considered as a potential clinical biomarker. Previous studies reported that PCI leads to a significant increase of vWF levels compared with the pre-procedural levels , PCI itself causes endothelial cell damage due to mechanical injury by catheter manipulations . In addition, hemodynamic effects of transient myocardial injury during PCI contributes to the increased vWF levels The prognostic role of vWF in patients with CAD is even more convinced than other acute phase-reactive proteins such as his-CRP and fibrinogen(Kandeel *et al*., 2018)(Kafkas *et al.*, 2012)

Studies have shown that elevated early vWF levels in patients with CAD are an predictor of adverse events over the next 2 weeks to 1 month, whereas other acute phase response proteins CRP are not, Compared with reactive proteins, vWF is released locally during vascular injury without new synthesis of proteins(Fan *et al*., 2020). Recent case-control studies also demonstrated that the higher plasma vWF or lower ADAMTS13 levels were closely associated with the risk of MI , ischaemic stroke . However, the present study is the first meta analysis that highlights the long-

term prognostic value of plasma vWF levels in patients with ischemia Our vWF is a promised indicator of the clinical outcome in patients with coronary artery disease. The dramatic increase of plasma vWF implies its potential roles in the diagnosis of CAD. Plasma vWF levels of CAD patients (Ziv-Baran *et al*., 2023)

5.2.2. Proprotein Convertase Subtilisin/Kexin Type 9

The Result of the current study has shown elevated serum Study The effective of Smoking on the level of Proprotein Convertase Subtilisin/Kexin Type 9 in Non Smoking patient compare with Smoking patient. The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease($P< 0.05$) in Smoking patient compare with Non Smoking patient (figure 4-5) (figure 4-6) (figure 4-7) (figure 4-8) (figure 4-9).

The present study showed that smokers induced PCSK9 expression and decreased LDLR expression in HepG2 cells Epidemiological studies have shown that dyslipidemia is associated with CS exposure. Smokers have higher blood cholesterol levels, higher plasma triglyceride concentrations and lower HDL C concentrations than non-smokers, and stopping smoking for more than 6 years can reduce the risk of dyslipidemia. Animal experiments have also shown that exposure to smokers can cause dyslipidemia. LDL C is an undoubted causal factor in atherosclerosis. Long-term exposure to smokers has been shown to increase serum LDL C levels in experimental animals(Ma *et al*., 2021).

A previous study indicated that decreased expression of LDLR on liver cells caused by smokers' exposure may be the reason behind the increase in LDL C levels in the blood. The prevent study CSE stimulated the expression of PCSK9 mRNA and protein, which may explain the mechanism by which CSE inhibits LDLR expression PCSK9 plays a critical role in regulating cholesterol homeostasis . It binds LDLR on the surface of hepatocytes, and activates endosomal and lysosomal degradation of LDLR in the liver, leading to increased serum LDL C levels (Paciullo *et al*., 2019)

In resent study, the smoker induced PCSK9 expression in HepG2 cells in a time- and concentration-dependent manner, consistent with decreased LDLR expression. However, in addition to reducing LDLR on the surface of hepatocytes, another important role of PCSK9 is the regulation of inflammation (Julius *et al*., 2019) .

The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-3). Obesity is widely spread around the world, especially among males due to its relationship with obesity Our study also confirmed that obesity represents a major burden on public health. Studies contain data on the effects of obesity and the degree of apnea on the development of dyslipidemia *(Huijgen et al*., 2021)

The resent study The level of Proprotein Convertase Subtilisin/Kexin Type 9 has been showed a significant decrease in Normal weight patient and Overweight patient compare with Obese patient Various mechanisms have been proposed for how the presence of obesity causes disturbances in metabolic homeostasis. The most important of them is CIH which promotes oxidative stress, systemic inflammation, and dyslipidemia through the induction of hypoxia-inducible factor-1 (HIF-1) synthesis in the liver, which activates the SREBP-1C transcription factor and increases PCSK9(Tao *et al*., 2013)

In a studies other reactive oxygen species appeared to induce PCSK9 expression through activation of nuclear factor kappa B (NF-κB) in hepatocytes and suggested the presence of obesity affect PCSK9 they were positively correlated for elevated plasma PCSK9 also a positive association between circulating PCSK9 levels and LDL cholesterol levels in patients obese patients not receiving lipid-lowering therapy (Zhang et al., 2015)(Astinchap et al., 2021)

In recent SREBP-2 activity in patients leads to an increase in circulating PCSK9 protein Furthermore, patients with PCSK9 levels above average showed a higher

proportion of CM and stable angina than patients with unstable angina and myocardial infarction and a lower proportion of NCM(Astinchap *et al*., 2021).

Recent studies have examined the expression of the PCSK9 gene and whether its role in lipid metabolism can be affected by various pathophysiological conditions such as diabetes, coronary artery disease, and obesity The expression of furin, its inhibitor serpinB8, and MT1-MMP was studied in monocytes derived from obese patients. In addition, resistin, which is known to be elevated in obese patients, was identified (Kappert *et al*., 2013)

and recent study groups on the basis of BMI for systolic blood pressure, but not for sex, age, height, diastolic blood pressure, heart rate, diagnosis, concomitant medications, or serum parameters analyzed . Cardiovascular risk increases with BMI The finding of cytoplasmic localization of serpinB8 in monocytes and its downregulation in macrophages does not in itself exclude its function as a furin inhibitor. BFA triggers the fusion of microtubules of the trans-Golgi network (TGN) and early endosomes, leading to their structural and functional dissection from the Golgi complex(Pekkarinen *et al*., 2021),

The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Stable angina patient compare with unstable angina patient and myocardial infarction patient Recent study have PCSK9 is a well-established target for treating hypercholesterolemia and atherosclerosis progression Stable angina patient with unstable angina patient and myocardial infarction patient. Although the major source of PCSK9 is the liver, in a recent experimental study in mice and explanted human hearts Ding et al. reported that PCSK9 is up-regulated in the zone bordering the infarct area and determines, at least in part, infarct size, cardiac function, and autophagy Stable angina patient with unstable angina patient and myocardial infarction patient anther has been reported that PCSK9 is highly expressed in vascular smooth muscle cells, and its expression and development of autophagy are regulated by well-known inflammation mediators, such as lipopolysaccharide,

tumour necrosis factor α (TNFα), and reactive oxygen species in infract area (Schreckenberg *et al*., 2022)

Recent studies have also been reported by other authors to associate the inflammatory response with PCSK9 and recent report showed that recombinant human PCSK9 induces an inflammatory response on macrophages by inducing the pro-inflammatory cytokines TNFα, interleukin-1, interleukin-6, monocyte chemoattractant protein-1 and C-X-C Motif Chemokine Ligand 2 (CXCL2 ,It suggests that the inflammatory action of PCSK9 on macrophages is mainly dependent on the LDL receptor Furthermore, a role for PCSK9 in regulating the clearance of pathogenic lipids in infract area such as lipopolysaccharide (Krychtiuk *et al*., 2021)

The present study PCSK9 is a well-established target for the treatment of hypercholesterolemia and atherosclerosis progression in stable angina patient along with unstable angina patient and myocardial infarction patient. Although the main source of PCSK9 is the liver, in a recent experimental study in mice and transplanted human hearts, Ding et al. reported that PCSK9 is upregulated in the region adjacent to the infarct area , at least in part, infarct size, cardiac function, and autophagy in a stable angina patient with an unstable angina patient and a myocardial infarction patient (Planavila *et al*., 2015)

The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease in hypertensive patient compare with Normotenive patient (figure 4-5).
In the current study, found that PCSK9 level was positively associated with hypertension but such a positive association was absent after adjustment for To be different from the study conducted by Participants in were not treated with lipid-lowering medications that could increase the level of PCSK9 Moreover, hypertensive patients were younger in present study compared to those in the study by Lee et al while correlates with age and hypertension. All of this could explain the difference in results Moreover, a significantly positive association between PCSK9 and was found in male hypertensive patients, while in female hypertensive patients there was

a positive tendency for PCSK9 to be associated with Which may result from a smaller sample of females Interestingly, the positive association between PCSK9 and male hypertension was absent after adjustment for age. Likewise, the positive association between PCSK9 and hypertensive patients with blood pressure control after adjustment for age(Yang *et al*., 2016)

Similar to our findings, in a study conducted in the United Arab Emirates, hypertension and male gender were predictive factors for dyslipidemia Android fat distribution could explain the increased prevalence of dyslipidemia among males. Furthermore, 68.1% of male participants had high blood pressure and were overweight or obese. This may explain the high prevalence of dyslipidemia in our sample(Milojević *et al*., 2022) .

Another study showed a high percentage of patients with hyperlipidemia and high blood pressure among the Lebanese, a percentage that has increased in the past decades. Age and BMI were the main factors associated with abnormal lipid status and blood pressure, with age and body mass being the main causes of cardiovascular disease and atherosclerosis, with Lp(a) being higher in women and associated with higher blood pressure. Age, body mass. Index, not fat. PCSK9 was associated only with age, and HDL-C, and TG in both men and women was inversely associated with HDL-C in men. Because PCSK9 and Lp(a) levels are markers of coronary artery calcification in asymptomatic patients with familial hypercholesterolemia(Julius *et al.*, 2019)

In previous study, found that plasma PCSK9 levels were associated with BP in patients with and without PCSK9 Hypertension. Moreover, we also found that PCSK9 level was independently associated with hypertensive patients investigating the relationship between PCSK9 level and BP and hypertensive and normotensive patients (Yang *et al*., 2016).

Furthermore, a positive association was found between PCSK9 concentrations and systolic blood pressure but reduces epithelial Na+ uptake by reducing epithelial Na+ channel expression, and research speculated that PCSK9 could contribute to blood

pressure control and that a level of PCSK9 would increase the risk of hypertension However, that PCSK9 deficiency did not alter blood pressure and sodium balance in of hypertension, supporting our finding thst indicated association between PCSK9 level and blood pressure in patients with coronary artery disease.

The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-6) The current study, we found that PCSK9 level was positively associated with but such a positive association was absent after adjustment for age. To be different from the study conducted Participants in our study were not treated with lipid-lowering medications that could increase the level of PCSK9 Moreover, age patients were younger in our study compared to those in the study while with age.(Yang *et al*., 2016)

All of this could explain the difference in results. positive association between PCSK9 and age was found in male hypertensive patients, while in female hypertensive patients there was a positive tendency for PCSK9 to be associated with age Which may for age. the positive association between PCSK9 and cIMT disappeared in hypertensiver patients with blood pressure control after adjustment for age(Yang et al., 2016) Similar to our findings, in a study conducted in the United Arab Emirates, hypertension and male gender and age were predictive factors for dyslipidemia Android fat distribution could explain the increased prevalence of dyslipidemia among males (Milojević *et al*., 2022)

Another study showed a high percentage of patients with hyperlipidemia and high blood pressure among the Lebanese, a percentage that has increased in the past decades. Age and BMI were the main factors associated with abnormal lipid status and blood pressure, with age and body mass being the main causes of cardiovascular disease and atherosclerosis, with Lp(a) being higher in women and associated with higher blood pressure. Age, body mass. Index, not fat. PCSK9 was associated only with age, and HDL-C, and TG in men was inversely associated with HDL-C in

men. Because PCSK9 and Lp(a) levels are markers of coronary artery calcification in asymptomatic patients

5.2.3 . Human Fibroblast Growth Factor 21

The level of Human Fibroblast Growth Factor 21 showed a significant decreasein Non Smoking patient compare with Smoking patient (figure 4- 10) (figure 4-11) (figure 4-12) (figure 4-13) (figure 4-14).

Recent study indicate that smoking is the leading cause of preventable deaths and diseases in modern society. It is known that smoking is a pivotal risk factor for various diseases, such as cardiovascular diseases and metabolic diseases. The anti-inflammatory property of FGF21 therefore smoking is known to promote inflammation by stimulating the release of proinflammatory cytokines such as interleukin(Eilenberg *et al*., 2016).

Im anther of FGF21 recent studies, as also shown in meta-analysis, have shown that smoking significantly upregulates cFGF21 levels, suggesting that it may be a compensatory response to smoking-induced inflammatory stress as well as serum FGF21 levels are closely related to lifestyle interventions. It can be elevated by smoking, excessive caloric intake, a high-carbohydrate diet, and a high-calorie, high-fat diet. The amino acid FGF21 has multiple metabolic regulatory effects, including stimulating hepatic oxidation of fatty acids, reducing hepatic triglyceride accumulation, and increasing Insulin sensitivity and preference suppression. Smokers ingest sugar through the central (Domouzoglou *et al*., 2015)

Although long-acting FGF21 analogues did not reduce plasma glucose levels in humans, they successfully lowered blood lipids, increased serum adiponectin levels, and showed several effects on weight loss(Nakano *et al*., 2019) The current study suggests that elevated cFGF21 may benefit regulation of metabolism and is considered a useful biomarker to evaluate the effects of metabolic reactions. The current systematic review of the literature and meta-analysis showed significant

effects of different lifestyles on cFGF21 level. Elevated cFGF21 level may be an excellent indicator of global metabolic and cardiovascular systems induced by lifestyle intervention and smoked and may be a protective and/or compensatory response to inflammation and stress under stressful conditions. The level of Human Fibroblast Growth Factor 21 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 5-8).

Recent studies indicate that obesity is associated with elevated levels of cFGF21, and that the expected beneficial effects of FGF21 to improve glucose tolerance and reduce plasma glucose and triglyceride levels are attenuated or even lost in obesity, suggesting an FGF21-resistant state. CRIWL at the cFGF21 level has been inconsistent, with many factors involved, such as the number and type of participants, the type of time frame for the dietary intervention following the intervention type and outcome measure, and the amount of weight loss , that can influence CRIWL-induced cFGF21(Gillum *et al*., 2020) .

Anther explanation has been with this further indicated that FGF21 could serve as a nutritional marker for control while very high protein content could compensate for obesity is associated deficiency, fat loss as the dominant factor driving this. cFGF21 downregulation (Domouzoglou *et al*., 2015)

At the same time, it rescued VLCD-induced protein deficiency and attenuated the protein deficiency-induced upregulation of cFGF21, resulting in an overall decrease in cFGF21 level, even with higher total caloric intake(Domouzoglou *et al*., 2015)

Interestingly, obesity were significantly associated with FGF-21 concentrations in our suggesting a relevant association. In fact, FFA have been described as physiological stimulators of FGF-21 secretion in both animals and obese humans (Lin *et al*., 2010)

Other recent study FGF-21 to regulate lipolysis in WAT, increase insulin-independent glucose uptake in 3T3-L1 adipocytes, improve insulin resistance and in

other words, improve the underlying pathological mechanisms of MetS and NAFLD. It is a surprising finding that FGF-21 was increased in Obesity is frequently associated with MetS and NAFLD Moreover, all obesity and NAFLD parameters as well as HOMA insulin resistance were not associated with FGF-21 (Villarroya *et al*., 2018)

The explanation of FGF-21 in obese patient and liver fat content are associated with elevated levels of cFGF21; Thus, decreased cFGF21 levels could reflect decreased body fat after without malnutrition On the other hand, protein restriction regulates cFGF21 level. Thus, with protein deficiency can affect the level of cFGF21 in two ways: downregulation of cFGF21 via CR-induced fat loss and upregulation of cFGF21 protein deficiency

The level of Human Fibroblast Growth Factor 21 showed a significant Decrease ($P< 0.05$) in Stable angina patient compare with unstable angina patient and myocardial infarction patient (figure 4-9).

Recent study has been suggest that FGF21 is an endocrine FGF21, and the heart also appears to be a hub for locally produced FGF21. After myocardial injury, adipose tissue releases FGF21, which acts via FGFR1 or stable angina patient or unstable angina patient and network to protect the heart myocardial infarction signaling (Villarroya *et al*., 2018)

This supports the conclusion of a previous study that FGF21 level can predict morbidity and mortality in patients with stable angina or patients with unstable angina, myocardial infarction, and coronary heart disease (Lenart *et al*., 2013).

A previous investigation revealed that cardiomyocytes synthesized and secreted FGF21 Cardiac FGF21 was secreted in response to cardiac ischemic stress in stable angina patient with unstable angina patient and myocardial infarction and prevent isoproterenol-induced cardiac hypertrophy. (Planavila *et al*., 2013)

This finding contrasts with other who found that patients with AMI after PCI had significantly higher serum FGF21 levels Further investigation revealed a link between FGF21 and AMI. Chen et al. found that FGF21 was associated with AMI and coronary heart disease for myocardial infarction which is consistent with present findings (Chen, Lu & Zheng, 2018)

In Myocardial ischemia and I/R injury induce apoptosis and myocardial infarction, leading to impaired cardiac function. Increasing evidence from In vitro and In vivo studies demonstrates that exogenous FGF21 protects cardiac myocytes from apoptosis and myocardial infarction, and improved cardiac function through activation of the PI3K-Akt1-BAD pathway in FGF21-KO mice (Liu *et al*. 2013)

and Akt-GSK3b-caspase apoptosis. It has been suggested that activation of these pathways would lead to a reduction in myocardial infarct area and increased cardiac function Evidence regarding the effects of FGF21 on inhibiting cardiovascular cell apoptosis in in vitro models is FGF21 protects H9c2 cells from I/R injury in a dose-dependent manner by enhancing energy supply and reducing inflammation and apoptosis leading to its suppression. of caspase 3-induced dependent pathways in H9c2 cell lines through the Akt-GSK3b pathway (Cong *et al*. 2013)

A previous study found that activation of peroxisome proliferator-activated receptor alpha (PPARa) led to the synthesis and release of FGF21. Released into culture media, FGF21 protected CMECs from Ox-LDL-induced lipotoxicity by reducing DNA fragmentation in an autocrine manner In an ex vivo model of global cardiac ischemia, it was shown that injection of recombinant rat FGF21 10 minutes before ischemia can protect the heart from I/R injury by reducing MI and increasing cardiac function through activation of the MAPK-PI3k-Akt signaling pathway (Patel *et al*. 2014) .

The study also demonstrated that the Sirt1-PPARa pathway plays an important role in the control of FGF21 expression in the heart also evidence from in vivo studies

demonstrate that continuous administration infusion for days in FGF21-KO mice induced cardiomyopathy and led to MI, impaired cardiac metabolism and loss of cardiac function in the rat heart (Planavila *et al*. 2013) (Joki *et al*. 2015).

it Planavila et al , (2013) has been shown that FGF21 attenuated cardiac hypertrophy by decreasing hypertrophic markers including atrial natriuretic factor (ANF) and a skeletal actin(aSKA) Moreover, FGF21 decreased the heart weight/body weight ratio and cardiomyocytes area, and also improved cardiac function

All of these findings indicate that exogenous and endogenous FGF21 play an important role in protecting the heart from apoptosis via several pathways including PI3K-Akt1-BAD and Akt-GSK3b-caspase 3 dependent mechanisms, leading to decreased infarction and increased left ventricular function under I/R injury lipotoxic infarct area and increase cardiac function and MI conditions, Moreover, FGF21 prevented oxidative stress As well as increasing energy supply to cardiomyocyte lines under injury conditions Myocardial ischemia resulting from coronary artery disease (CAD) is the leading cause of myocardial infarction that can impair cardiac function by reducing ejection fraction EF), which leads to an insufficient amount of oxygen reaching the body tissues and this contributes to the development of cardiac hypertrophy and heart failure due to compensatory mechanisms of the heart

The level of Human Fibroblast Growth Factor 21 showed a significant Decrease ($P< 0.05$) in hypertensive patient compare with Normotenive patient (figure 4-10). The plasma FGF21 The study showed that serum FGF21 levels had a strong relationship with waist circumference systolic blood pressure, lower extremity atherosclerotic disease and carotid artery intima-media thickness in patients FGF21 level was also increased in atrial fibrillation (AF) patients and was shown to be an independent risk factor for AF (Han *et al*. 2015).

In cases of non-alcoholic fatty liver disease (NAFLD) and CAD, serum FGF21 was associated with an adverse steatotic profile and also showed a positive association

with total cholesterol (TC) and triglycerides (TG) and hypertensive (Shen *et al*. 2013) .

Recent studies have shown that FGF21 levels in the blood are associated with the metabolic status of patients. Elevated serum FGF21 levels in systolic blood pressure Many pathological conditions of the heart under metabolic dysregulation can be explained by conditions of FGF21 resistance, which have been observed in ex vivo experiments with obese rat hearts and in in vivo experiments with DIO rat livers and white adipose tissue (Fisher *et al*. 2010) (Patel *et al*. 2014).

They found that the hearts of hypertensive obese mice had increased expression and secretion levels of the protein FGF21 mRNA, FGF21. Despite the high level of FGF21, disruption of FGF21-FGFR1-b-Klotho signaling and decreased ERK1/2, Akt, and AMPK have been observed under this condition (Patel *et al*. 2014).

These results suggest that the obese state with related with hypertensive caused an impairment of the FGF21 signaling cascade, and that a feedback mechanism allowed increased FGF21 production to overcome the FGF21 receptor signaling defect (Lenart-Lipinska *et al*. 2013).

FGF-21 contributes to regulating WAT lipolysis, increasing insulin-independent glucose uptake in 3T3-L1 adipocytes, improving insulin resistance and in other words, improving the underlying pathological mechanisms of MetS and NAFLD. Surprisingly, FGF-21 was an increase in blood pressure. Obesity-induced MetS and NAFLD are frequently associated with MetS and NAFLD. Moreover, all parameters of MetS and NAFLD as well as HOMA insulin resistance were not associated with FGF-21 However, these findings fit the hypothesis that obesity and blood pressure are a serious problem FGF-21 resistance status Thepresent data observed level of Human Fibroblast Growth Factor 21 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 5

Recent studies have shown that not only does body composition change with age, but also many of the hormones and factors involved in growth, maturation, and maintenance of systemic physiological requirements. PPARs, which influence the contribution of liver and adipose tissue to the appearance of circulating FGF21, are key regulators in various age-related pathophysiological processes related to energy metabolism and oxidative stress, and PPARs are also associated with bone resorption (catabolic) processes (Li *et al*., 2022)

Several studies have demonstrated that PPARa and PPARg inhibit the expression of inflammatory genes, such as cytokines, metalloproteinases, and acute phase proteins In future studies, management of FGF21 expression and secretion will be determined by Certain hormones and transcription factors are worth considering. Age may have a greater influence on FGF21 levels in younger individuals, which may be explained by differential underlying regulatory factors and pathways that are also subject to aging Differences. Whether maintaining bone mass in individuals (Panahi *et al*., 2016).

‘ previous study has been foand in healthy presentation is an age-related increase in circulating FGF21, highlighting a potential differential age effect in response to metabolic demand over the life course. However, at low levels of FGF21, bone density may explain the age-related association. Given the role of FGF21 in cellular energy metabolism as a regulator of lipid and glucose utilization in animal models and at this point it is age-related increases in FGF21 are a consequence of body composition (Hanks *et al*., 2015).

Examine several notable strengths. In conclusion, in healthy presentation is an age-related increase in circulating FGF21, highlighting a potential differential age effect in response to metabolic demand over the life course. However, at low levels of FGF21, bone density may explain the age-related association , Given the role of FGF21 in cellular energy metabolism as a regulator of lipid and glucose utilization in animal models and our findings presented here, at this point it is whether age-related increases in FGF21 are a consequence of body composition or, alternatively,

causative , It is known that muscle contraction has effects like insulin on glucose uptake in skeletal muscle protein isolate, and skeletal muscle is the main place of glucose uptake in normal mode Therefore, it is wise to assume that increasing muscle mass is an effective method for improving insulin sensitivity , In addition, it has been shown that fatty deposits in the muscle triglyceride levels are an important aspect of body combined exercise training and are associated with insulin resistance. Therefore, changes in fat mass inside the muscle may affect insulin sensitivity(Eilenberg *et al*., 2016) .

5.2.4 . Human Lipocalin 2

The level of Human Lipocalin 1 showed a significant Decrease ($P< 0.05$) in Smoking patient compare with Non Smoking patient (figure 4-15) (figure 4-16) (figure 4-17) (figure 4-18) (figure 4-19).

Recent study have indicated that this protein may be as smoked at the level of Human Lipocalin 2 in non-smoking patients as used in patients with cardiovascular disease events. NGAL has been reported to be independently associated with an increased risk of cardiovascular disease in adult patients. Those suffering from chronic diseases. Smoking kidney disease. However, they recommended further work to determine the benefit of smoking to improve risk prediction of adverse CVD outcomes (Zylka et al., 2016; Park et al., 2017).

Other previous Study As in adults, children with CKD have a higher prevalence of traditional cardiovascular risk factors associated with uremia (Mitsnefes, 2012) In this population, to our knowledge, NGAL has not been evaluated as an early marker of CVD. Here, blood NGAL levels were significantly higher in smoking patients with cardiovascular disease compared to patients without cardiovascular disease and healthy controls. It has good predictive ability for cardiovascular disease, with good sensitivity, specificity, positive predictive value, negative predictive value, and 80% accuracy, also results from human histology and animal studies also showed that smoking NGAL is highly expressed in the heart and is also expressed in

Atherosclerotic plaques and both in myocarditis and myocardial failure (Cruz et al., 2012)

Several clinical studies have reported the role of NGAL in various CVD events. Compared with control subjects, chronic heart failure patients have been reported to have significantly elevated levels of both urine and serum NGAL Serum and urine NGAL levels correlate with various renal function indices including GFR, urinary albumin, creatinine, blood urea nitrogen, and cystatin C Similar to findings observed in chronic heart failure, serum NGAL also appears to be associated with renal function in acute heart failure and coronary heart disease. cardiovascular disease in present result and these observation agree with study of (NGAL) that showed a high level of NGAL in serum and urine in patient with card and especially heart failure patient The explanation of of high level NGAL may high exposure to smoking due to associated NGAL and high prevalence (Helanova et al., 2014).

The level of Human Lipocalin 1 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-13).

This study was designed to find the effect of control and obesity on lipocalin-2 in patients with .Obesity, determined by BMI, was insignificantly correlated with LCN2 This was against to Wang study, which found a strong positive correlation between LCN2 and BMI Moreover, we found no significant difference in LCN2 between obese and non obese patients with diabetes. This was comparable to El-mesallamy findings We found that BMI was significantly elevated in female patients with diabetes compared to males in the same group. In a study done at the Shanghai diabetic institute, LCN2 was significantly correlated to metabolic syndrome indicated by waist circumference (Reinehr et al., 2012).

Another study agreed with the earlier one done by Lee in Seoul, South Korea Moreover, there was strong negative correlation between HDL and LCN2 in Wang study, but it was not significant in this study , LCN2 was found as a biomarker of

diabetic kidney diseases in urine as reported in studies done in Ohio and Brazil (Milojević *et al.*, 2022)

Previous study, significant correlation between LCN2 and obese has been found That obese subjects were found to be suffering from NAFLD depending on abdominal ultrasonography examination this was an expected finding due to several reasons that were confirmed by our investigations and is in agreement (Lenzi et al. 2015).

This study showed that NAFLD is well known to Our obese group adolescents suffered from visceral adiposity (also called central or abdominal adiposity). This was elicited through their WC, HC, and WHR. All measurements were significantly much higher than those of their control mates. In accordance with us, many researchers have emphasized that the visceral adiposity is much more crucial than mere subcutaneous adiposity concerning the risk of NAFLD occurrence (Panahi et al., 2016)

In obesity, there are high levels of lipopolysaccharides which not only act as inflammatory stimuli but are also one of the reasons why IR occurs. Accordingly, we can consider that lipopolysaccharides are one single common factor or explanation between NGAL high levels and IR state elevation. Another explanation is that NGAL antagonizes the effects of TNF-α in adipocytes and macrophages where TNF-α is well known to promote IR (Martins *et al*. 2014) .

high NGAL level may been present resent abundantly expressed in the liver neutrophil granules and has been declared to be implicated in innate immunity, inflammation, and apoptosis therefore, maybe NGAL too acts as a "double-edged sword" suggested that chronic inflammation causes "pre-conditioning" against excessive acute hyper-inflammation in obesity.

The level of Human Lipocalin 1 showed a significant decrease ($P< 0.05$) in Stable angina patient compare with unstable angina patient and myocardial infarction patient (figure 5-14).

Recent study has been found that plasma NGAL levels in STEMI patients were higher than those in control and non-STEMI patients, as inflammation and dyslipidemia play a role in the pathogenesis of atherosclerosis and plaque destabilization. The primary cause of coronary thrombosis and myocardial infarction (STEMI) is the rupture of atherosclerotic plaques and Plaque rupture does not always cause an acute event either because insufficient thrombus formation or lumen area that does not adequately restrict flow. Alternatively, healing of plaque rupture leads to the development of stenosis, and Hong et al. It was reported that 69% Of patients with myocardial infarction in whom rupture is caused by a plaque lesion, asymptomatic lesion progression to milder plaques may result from subclinical cycles of rupture and healing(Buso *et al*., 2023)

Recent studies have shown that plaques develop from It is essentially mild to obstructive at the time of myocardial infarction, and plaque development occurs before it actually occurs Plaque rupture in addation found that total cholesterol, LDL-C, and some circulating Inflammatory cells with NGAL were higher in patients with STEMI compared with patients and control groups. Previous studies have attributed an important role to neutrophils in development Atherosclerosis and acute coronary syndromes (ACSs)(Julius *et al*., 2019) .

previous study found that plasma NGAL Plasma NGAL levels were significantly higher in CVD than in the control group. in Another study, found that plasma NGAL levels were higher in patients with AMI of patients with stable coronary artery disease(Miñana et al., 2020)

A previous study also confirmed that plasma NGAL levels were elevated in patients with myocardial infarction In significantly and positively correlated with plasma NGAL levels. Previous studies have confirmed that NGAL strongly stimulates

NGAL expression in isolated neonatal cardiomyocytes, suggesting a role for IL-1β in Regulation of NGAL expression in MI (Eilenberg *et al*., 2016)

A previous study by Soylu et al. also found that plasma NGALlevels were associated with the SYNTAX score Increased plasma NGAL levels after pPCI in STEMI patients were a better predictive marker of 30-day mortality than NGAL levels before Some studies suggest that NGAL may have prognostic value in HF patients because high levels of plasma or urinary NGAL are associated with more renal complications or mortality i level of NGAL was associated with an increase in the proportion of adverse cardiac events and overall mortality from all causes and these high levels of NGAL may be explained in part by the renal failure observed in a large number of HF patients (Le *et al*., 2023) .

However, several studies have showed that NGAL was a predictor of CV incident even in the absence of renal dysfunction therefore circulating NGAL levels have also been described as predictors of CV complications in patients with CKD

The level of Human Lipocalin 1 showed a significant Decrease ($P< 0.05$) in hypertensive patient compare with Normotenive patient (figure 4-15).

In study In patients with essential hypertension, plasma NGAL level were higher than in healthy subjects and correlated with blood pressure In clinical studies, polymorphisms in the promoter of NGAL have been associated with changes in blood pressure(Sirois *et al.*, 2017) .

The role of NGAL in hypertensive mechanisms was also demonstrated in animal models of obesity. NGAL KO mice were protected against hypertension, inflammation and cardiometabolic dysfunction induced by a high fat diet The direct role of NGAL in blood pressure control was further demonstrated by the combined administration of recombinant NGAL and linoleic acid in mice, which induced an increase in mouse blood pressure (Pekkarinen *et al*., 2021)

a study from our demonstrated the crucial role of NGAL in the setting of aldosterone-mediated hypertension. Indeed, the global genetic inactivation of NGAL

in prevented the increase in blood pressure induced show The level of Human Lipocalin 1 in hypertensive patient compare with Normotenive patient *(Eilenberg et al*., 2016).

The level of Human Lipocalin 1 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-16). In study, Aside from the role of NGAL in inflammation and studies It suggests that NGAL is involved in iron homeostasis. on condition Iron overload and its deficiencies have been linked Impaired heart function and hypercoagulability, may which associcefed with eldwage in cardiovascular diseases patient plays a major role in the development of some heart muscle diseases In addition, NGAL may play a role in regulation Aldosterone-induced hypertension, with genetic inactivation of NGAL in mice prevented mineralocorticoid-induced anemia the pressure increases (Eilenberg *et al*., 2016) .

Moreover, laboratory studies indicate this NGAL may affect fibroblast proliferation. In view of The major role of proliferation, especially by myofibroblasts, in remodeling mechanisms and proliferative effects of NGAL It may also be involved in pathological cardiac remodeling associated With the aging of the population, the proportion of elderly patients Percutaneous coronary intervention has increased to a greater extent, Older adults are also more susceptible to procedure-related complications and peri-interventional mortality After percutaneous coronary intervention, though It seems to bring great benefits in terms of quality life*(Miñana et al*., 2020).

Furthermore, screening investigations for coronary heart disease such as Coronary computed tomography angiography and exercise stress tests are less frequent Useful in this elderly population due to poor exercise Capacity and coronary artery calcification related artifact therefore NGAL levels may be in addition to cTnI levels in elderly patients It represents a prospective method for selecting elderly patients

with Atypical symptoms who will benefit most from invasive Coronary angiography(Years, 2022)(Liu *et al.*, 2021)

Serum NGAL levels are elevated and have a significant association with CV death in patients with CHD. The significant predictive value of elevated NGAL levels may be linked to a higher degree of inflammatory reactions in CHD patients because NGAL expression in a healthy general population was linked with all inflammatory markers.

5.2.5. Human Von Willebrand Factor

The level of Human Von Willebrand Factor showed a significant increase ($P< 0.05$) in Smoking patient compare with Non Smoking patient(figure 4-20) (figure 4-21) (figure 4-22) (figure 4-23) (figure 4-24).

In arecent study has been suggested the effect of vwF level and the study of cigarette smoking on the study included to smokers and from th study both tout level and total white blood cells were measured and significad increase has been noticed associated with CVD in both VWF and white blood cell and considered as an indicator for atherosclerosis and cardiovascular disease It has been suggested that the level of von Willebrand factor (VWF) increases between In smokers, the cause may be due to the cytotoxic effects of oxygen-forming lipid peroxidase Free radicals and the effects of nicotine and carbon monoxide which increased both WBC count and vWF levels were significantly associated with the number of cigarettes smoked daily, and the duration of smoking, indicating The association of smoking with increased WBC counts and vWF levels is dose-dependent(Ma et al., 2021)

In the recent, of coronary artery disease (CAD significantly studies in healthy subjects were initially described as somewhat weak The relationship between VWF levels and the risk of CAD that did not It always reaches statistical significance. Even more encouraging is that recent nested case-control studies Prospective epidemiological study of myocardial infarction PRIME), showed a 3-fold increased risk of severe coronary heart disease fatal or non-fatal MI) in individuals with low

plasma VWF levels in the highest quartile compared to those in the lowest quartile Quarter. The difference persisted even after modification Multiple markers of inflammation.60 In addition, showed a significant increase in CAD risk relative to Highest lush VWF levels that persisted even after modification traditional risk factors and further conducted a meta-analysis For all relevant population-based prospective studies Which additionally confirmed this relationship(Monocytes, the cellular hall-mark of CAD, 2022) , (Planavila *et al.*, 2015)

other study have been proven So it is. MI almost always occurs afterwards arterial rupture Laser painting of a proliferating fat nucleus composed of Textile and functional agent. Living up to the name This occurs when VWF binds to the exposed artefact of the formation Which leads to the sea voyage and the formation of a Gabes water lakes. Hemostasis occurs simultaneously With the filter chain activated, Poland is informed In thrombosis hyper congealable state may be resulted from heavy smoking to that associated with narrowing of blood vessels (hypertension) with a high platelets adhesive molecule and Chemo attractant factor which is important in immune response (Qin *et al*., 2021)

other study VWF, important element In this way, the risk is greatly reduced Blood clots when its level changes. the fourth The NIS database prevents us from answering important questions Questions, such as whether or not it is a VWF score Highlights the deficiency or comprehensiveness of VWD and suffers from protection Against cardiovascular diseases. GN The risk of developing disease due to VWD compared to non-VWD times At Christmas, our study is the first to report a Reduced prevalence of cardiovascular disease among a group of Patients with VWD in Imam Hussein Medical City patients admitted to the Cardiology Center, with evidence That VWD provides protection against cardiovascular disease Events. Moreover, he supports the idea of it VWF may be a therapeutic target in CVD Certainly these results are essential and orders New clinical trial(Bian *et al.*, 2022)

In recent research has been indicated the VWD MI patient may be bind exposed artifact lead to form a sea voyage and Gabe s water lacks and thrombosis occur- in MI patient the UKF in MI patient .

The level of Human Von Willebrand Factor showed a significant Decrease($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-18).In study has This cross that the prevalence Of cardiovascular disease in VWD patients more than in Non-VWD patients. Moreover, after modification to Age, gender, high blood pressure, high blood fats Obesity and smoking increase the risk of developing cardiovascular disease by The results provide further evidence that VWD and its associated pathology are common Hypocoagulable state provides protection against Cardiovascular diseases(Eilenberg *et al*., 2016).

The level of Human Von Willebrand Factor showed a significant increase($P< 0.05$) in Stable angina patient compare with unstable angina patient and myocardial infarction patient (figure 5-19).

No previous study has been suggested the relation between VWF and anging also MI so that the explanation of the high level of VWF in MI in compare with unstable or stable angina may be discussed as arole of VWF as an important coagulative factor of increase with a degree of CVD and count platelets in addition to inflammation represented infiltration of macrophage nutrophile and Low density lipoprotein therefore infraction area codard as as positively related with VWF markers

In summary, in the setting of reperfused MI, elevated plasma LDL-C worsens postinfarct thromboinflammation vis-a-vis VWF-mediated platelet adhesion, leading to less microvascular reflow, larger infarct size and greater degree of adverse remodeling. All of these detrimental effects of elevated LDL-C appear to be mitigated by therapies that prevent endothelialassociated VWF

The level of Human Von Willebrand Factor showed a significant Decrease ($P< 0.05$) in hypertensive patient compare with Normotenive patient (figure 4-20). In

previous has been indicated study has prevalence Of cardiovascular disease in VWD patients more than in Non-VWD patients. Moreover, after modification to Age, gender, high blood pressure, high blood fats Obesity and smoking increase the risk of developing cardiovascular disease by with VWD than in patients without VWD. these The results provide further evidence that VWD and its associated pathology are common Hypocoagulable state provides protection against Cardiovascular diseases(Eilenberg *et al.*, 2016).

hypertension leed to narrowing the blood vesseles accompanying by accumulation of platelets ; Low density lipoprotein , macrophage with inflammatory factor such as cytokines all all these factors positively related with high level of VWD ,we also did directly measure the effects of Lipid leve on VWF self-association because of the lack of techniques capable of measuring this phenomenon With respect to the beneficial effects of hypertensive patient on LV function, it is possible to distinguish the relativecontributions of infarct size reduction and anti-inflammatory effects(Jakovljevic *et al.*, 2011).

In summary, in the setting of MI a hypertensive patient , elevated plasma Lipid leve worsens postinfarct thromboinflammation vis-a-vis VWF-mediated platelet adhesion, leading to less microvascular reflow, larger infarct size and greater degree of adverse remodeling. All of these detrimental effects of elevated LDL-C a hypertensive patient appear to be mitigated by therapies that prevent endothelialassociated VWF.

The level of Human Von Willebrand Factor showed a significant Decrease ($P< 0.05$) in Age Figure (4-21)

The CVD of VWD in severe incresease forms has been associated with a in annual rates. Chronic conditions that increase over an individual's lifespan also alter bleeding and thrombotic risk, as well as the risk for different types of trauma, surgery, and medications. VWD patients are not entirely protected from thrombotic conditions or atherosclerosis, and wherever possible standards of care for

cardiovascular disorders, malignancies, and other diseases should be followed , in previous study has This cross that the prevalence Of cardiovascular disease in VWD patients more than in non -VWD patients. Moreover, after modification to Age, high blood fats Obesity and smoking increase the risk of developing cardiovascular disease by in patients with VWD than in patients without VWD these The results provide further evidence that VWD and its associated pathology are common Hypocoagulable state provides protection against Cardiovascular diseases(Eilenberg *et al*., 2016).

In summary, in the setting of MI endothucial dysfunction with high level these LDL-C high inflammatory status with high thrombosis process may be associated with lewdly age and mediated by high level of VWF

5.3.The correlation

5.3.1.The correlation relationship between Human Fibroblast Growth Factor 21 and Triglyceriole- Cholesterol - Low-density Lipoprotein -High-density lipoprotein

The results of the current study showed that there is a positive relationship Figure (5-21)show The correlation coefficient relationship between Human Fibroblast Growth Factor 21 and Triglyceriole

Fibroblast growth factor 21 (FGF21) is an endocrine hormone that is a critical regulator of energy homoeostasis and a potential therapeutic target for treating diabetes and obesity. Pharmacological administration of FGF21 to obese animal models markedly improves insulin sensitivity and causes weight loss (Gillum et al., 2020). positive correlation between administration of FGF21 analogues to obese human . improve metabolic Lipid profile such as triglyceride & cholesterol (Qin *et al*., 2021)

Circulating levels of FGF21 are primarily produced by the liver, but can also be produced by other tissues during stress. FGF21 plays a critical role in a number of physiological processes including the adaptive fasting response, enhancing insulin sensitivity during refeeding and overfeeding, and regulating food preferences. Macronutrients. Upon entering the circulation, FGF21 acts by signaling to specific tissues that express the classical FGF receptor, fibroblast growth factor receptor 1 (FGFR1), and the co-receptor β-Klotho. Although FGFR1 has a broad tissue expression pattern, β-Klotho is expressed in a limited number of metabolic tissues and provides specificity for FGF21 signaling that interacts with FGFR1 through its N terminus and with β-Klotho through its C terminus. This C of FGF21 is essential for activation of the receptor complex to initiate signaling (Nakano *et al.*, 2019)

In this study, we provide new mechanistic insights Which indicates that the well-established TG lowering effect of FGF21 can be explained By increasing the elimination of TRLs as well as decreased production Pronounced elimination of TRL in adipose tissue, in particular WAT in lean mice and BAT in obese mice are consistent with Previously reported role of adipose tissue in mediating metabolism Effects of FGF21, as shown in experiments using FGFR1 and fat-specific KLB knockout mice (Ding *et al*., 2012).

In interaction with Hepatic insulin action (Emanuelli et al., 2014), and the effects of FGF21 on Adipose tissue could also explain the observed fat suppression VLDL-TG secretion, i.e. through plasma suppression NEFAs via insulin sensitization or direct FGF21 action on WAT (Kambouris *et al*., 2013; Li *et al*., 2014)

Critical factor for FGF21-dependent TRL elimination. previous job Proved that in the postprandial state, adipose tissue LPL It is activated by insulin (Wang and Eckel, 2009; Kersten, 2014 FGF21 administration confers systemic insulin sensitivity including WAT (Camporez *et al*., 2013; Lee *et al*., 2014), indicating It improves adipose tissue-induced insulin signaling FGF21 is involved in LPL activation. However, cross talk FGF21 and insulin signaling pathways are still under

investigation Clearly, more work is needed to understand the interaction Insulin and FGF21 signaling in the complex regulation of LPL Production and maturity (Kirsten, 2014). suggesting a possible independent mechanism Through which FGF21 can trigger this process. We noticed a Strong effect of a single dose of FGF21 on TG and oligomer metabolism This view is supported , by a recent publication Equal decreases in plasma TG and NEFA concentrations have been reported In UCP1-null mice and WT controls after treatment with a long-acting FGF21 analogue (Ve´ niant *et al*., 2015).

Another study reported that under DIO conditions, FGF21 Induced TG lowering was attenuated in Ucp1-deficient mice (Sams et al., 2015) which is consistent with our results BAT is shown to be important for TRL elimination in DIO mice. at any Event, FGF21-dependent browning of WAT can be dispensed with FGF21-dependent TRL shedding can be considered a As a result of the increased sympathetic flow caused by FGF21 (Owen *et al*., 2014).

Fibroblast growth factor - 21 and lipid profile Many studies has been indicated apostive relation between FGF - 21 and cardiovascular disease and with risk factor of lipid profile such as LDL - C Triglyceridy and BMI , cholesterol and insulin and show three fold of FGF - 21 in CVD

Also previous study has been found negative correlation between FGF 21 and Apo - A_1 which considered as a main Sourch of HDL - C and promote - efflux of cholesterol from tissues to liven Further studies has been determined a strong relation between FGF – 21 and dyslipidemia Avecent study has been showed that FGF - 21 drugs improve LDL - c and cholesterol and lipid metabolism by modulation of lipid synthesis also FGF21 may be stimulate fatty acid d oxidation and VLD secretion Another study FGF21 has an important role in the metabolism of fats and carbohydrates and energy balance. FGF21 can increase lipolysis through the interaction of beta-3 adrenergic receptors on epinephrine in adipose tissue. It is also capable of decreasing blood glucose levels through the action of insulin. This

function is performed through the AMPK signaling pathway and causes the transfer of GLUT4 to the cell membrane and muscle glucose uptake. Still, its performance in humans has not been fully understood in physiological conditions FGF21 has been shown to increase catecholamine and decrease serum insulin, so it increases lipolysis and metabolic changes As has been said above, FGF21 can affect lipid metabolism, lipid profile, glucose, insulin (insulin resistance).

5.3.2.The correlation relationship between Proprotein Convertase Subtilisin/Kexin Type 9 and Triglyceriole- Cholesterol - Low-density Lipoprotein -High-density lipoprotein

positive correlation between high level pcsk9 and Low density Lipoprotein may discuss as arole by ability of pask9 to been freely with atherogenic Lipoprotein sach as LP(A) and LDL and when bending occure on LDL receptors (LDLR) lead to increment in LDL level in circulation therefore administrate -on of pcsk9 inhibitor reduce the CVD risk by reduce the expression of LDL(Julius et al., 2019).

also many studies has been shown the relation between pcsk9 with TG is is very high risk because its contain Lipoprotein rich especially VLDL and free fatty acid therfore possible mechanism the role has postulated involved of of pask9 in activation of proinflammatory cytokines lead to excessive release fatty acid and coagulative factors positively correlated with high risk of cardiovascular disease (Schlein et al., 2016) .

the relation between pcsk9 and VLDL , has been closely related that pcsk9 induced a cholesterol contents and Apo-B containing lipoprotein which lead to induce therefore administration of pask9inhibitor lead to regulation both VLDL - c and LDL - C of in patients with high cardiovascular disence with high risk of cardiovascular disease , A negative correlation between pask9 and HDL - C may be by reduce of uptake a molecule called Apo-E which containing HDL particles (Al-Kraity & Al-Dujaili, 2017).

In the current study, however, we unequivocally showed that the G516V variant translates to a proprotein with increased affinity for the LDLR, and thus likely prevents the dissociation of the LDLR from LDL-c particles following internalization of the LDLR-LDL complex in the hepatocyte. This variant at position 516 is located within the M1 module of PCSK9 C terminal domain. Hypothetically, PCSK9-G516V may result in an increased affinity for cyclase-associated protein 1, the protein that, once bound to PCSK9 mediates caveolae-dependent endocytosis and lysosomal degradation of the LDLR. This particular characteristic has been shown for the PCSK9-A514T variant, which, by virtue of this effect, is considered a gain of function (GOF) variant(Schreckenberg *et al*., 2022).

In patients with hypercholesterolemia, adherence to guideline-based therapy has been associated with lower direct medical costs and hospitalization rates Although the costs associated with PCSK9 inhibitors represent another potential barrier to prescription of these therapies, they may be offset by cost savings made in cardiovascular events avoided. At the time of this study, results of the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk trial(Amput *et al*., 2019).

Our studies suggested that other PCSK9 variants of unknown significance, where we were able to perform cascade screening, did not have a large impact on LDL-c metabolism in families. The exact underlying mechanism by which some of the earlier described PCSK9 variants result in increased LDL-c levels (Abboud *et al*., 2007) .

As a result, currently PCSK9 inhibitors, can reduce the LDL-C, and are associated with a reduction in the risk of cardiovascular events There are three ongoing studies in clinical trials which aim to investigate the effects of PCSK9 inhibitors on cardiovascular function (Julius *et al*., 2019).

several clinical studies showed that PCSK9 inhibitors increased plasma glucose level, but not increased the incidence of diabetes In contrast, only one study mentions that PCSK9 inhibitors decreased LDL-C and mortality rate but increased the risk of diabetes In that study, they found that PCSK9 mice had reduced plasma insulin level and impaired glucose tolerance (Wu *et al.,* 2022) .

In the human body, LDL-C levels are regulated primarily through LDL receptors found on hepatocytes. As mentioned above, PCSK9 is a proteolytic enzyme that destroys LDL receptors and hence indirectly modulates serum LDL-C. Blocking or binding circulating PCSK9 using alirocumab or evolocumab leads to more LDL receptors and a decrease in serum LDL-C. Extrahepatic organs such as the kidney, gut, and central nervous system contribute to PCSK9 production and, presumably, local modulation of LDL-R expression, despite its hepatic origin. If PCSK9 attaches to the LDL-R before the LDL particle, the entire complex enters the hepatocyte and gets destroyed by the lysosome. This implies that lowering the amount of free PCSK9 available to attach to the LDL-R will reduce the destruction of the receptor, therefore, resulting in less LDL-C in the plasma(Julius *et al*., 2019) .

Our studies suggested that other PCSK9 variants of unknown significance, where we were able to perform cascade screening, did not have a large impact on LDL-c metabolism in families. The exact underlying mechanism by which some of the earlier described PCSK9 variants result in increased LDL-c levels is not fully elucidated(Musumeci *et al*., 2023) .

In the current study, however, we unequivocally showed that the G516V variant translates to a proprotein with increased affinity for the LDLR, and thus likely prevents the dissociation of the LDLR from LDL-c particles following internalization of the LDLR-LDL complex in the hepatocyte. This variant at position 516 is located within the M1 module of PCSK9 C terminal domain. Hypothetically, PCSK9-G516V may result in an increased affinity for cyclase-associated protein 1, the protein that, once bound to PCSK9 mediates caveolae-dependent endocytosis and lysosomal

degradation of the LDLR. This particular characteristic has been shown for the PCSK9-A514T variant, which, by virtue of this effect, is considered a gain of function (GOF) variant(Schreckenberg *et al.*, 2022).

5.3.3.The correlation relationship between Human Lipocalin 1and Triglyceriole-Cholesterol - Low-density Lipoprotein -High-density lipoprotein

CAD has been suggested many common pathological and physiological characteristics. Typical cardiovascular risk factors such as dyslipidemia, hypertension and obesity increase the risk of dyslipidemia is the main link between NGAL and elevated risk of CAD . In this work, univariate analysis showed that the higher LDL-C, HbA1c, and ApoB and lower ApoA-I and HDL-C were significantly associated with an increased CAD risk of elderly T2DM patients, suggesting that dyslipidemia and inflammatory disorders were important risk factors of CAD During the development of CAD, the NGAL level is elevated in addition to chronic inflammation, which is a well-established feature and it is positively associated with serum NGAL level even after adjustment for several confounders (Kovacevic *et al*., 2019).

Some study exploring the pathogenic molecular mechanisms of NGAL in atherosclerosis and CAD have shown that the NGAL/MMP-9 complexes destabilize the artery plaque, which could be detected in lipid centers and on the side facing the lumen area detected in clinical plaques. also it suggests that NGAL/MMP-9 complexes are involved in vascular inflammation and reconstruction in atherosclerosis . sdlDL-C is well known to cause arteriosclerosis, which may be because that the sdLDL-C is small and deposited in the lining of the arteries and bound to glycoproteins In addition, sdLDL-C is not easy to be cleared due to low affinity with LDL-C receptor, so it is easy to be oxidized and modified, which stimulates the macrophages to absorb lipid, forming foam cells A study has showed that the sdLDL-C level is significantly associated with NGAL and the severity of CAD(Years, 2022) .

In addition, NGAL has been showed was closely correlated with BMI, TG, hsCRP, and neutrophils; and sdlDL-C level was positively correlated with LDL-c, TG and ApoB, and negatively correlated with HDL-C correlated with gender and BMI, but not with TG hsCRP, and neutrophils. Although NGAL is positively correlated with TG, hsCRP, and neutrophils(Eilenberg *et al.*, 2016)

The sdLDL-C concentration may be a challenge risk factor in healthy people Regardless of typical lipid tests such as TC, TG, LDL-C, and HDL-C, higher level of sdLDL-C has been detected among men and older individuals, leading to an association with greater mean carotid artery thickness confirmed that NGAL was positively in recently, studies have found that the arterial stiffness progression in normotensive subjects could be predicted independently based on the sdLDL-C concentration. Normotensive participants with high quantiles of sdLDL-C were more likely to develop progressive arterial (Years, 2022)

5.3.4. The correlation relationship between Human Von Willebrand Factor and Triglyceriole- Cholesterol - Low-density Lipoprotein-High-density lipoprotein

The present study agreed with the earlier one done there was strong negative correlation between HDL and LCN2 in Wang study, but it was not significant in this study . LCN2 was found as a biomarker of diabetic kidney diseases in urine as reported in studies done in Ohio and Brazil . models of reperfused MI have indicated that VWF-mediated microvascular adhesion of platelets occurs in coronary ischemia-reperfusion injury and can influence microvascular reflow and infarct size so that investigated chronically elevated LDL-C common risk factor for atherothrombotic eventspredisposes to post-MI VWF-mediated microvascular platelet adhesion and secondary sequelae including impaired reflow and thromboinflammation (Jakovljevic *et al*., 2011).

In patients with acute MI, LDL-C at the time of presentation has been paradoxically associated with better clinical outcomes. Mechanistically, adverse effects of LDL-C

in MI involve recognized pro-thrombotic and proinflammatory effects on cells that regulate innate immunity, platelets, and endothelial cells LDL-C also promotes the self-association of VWF fibers, rendering them more resistant to proteolytic cleavage.VWF multimers that are secreted by endothelial cells, particularly upon endothelial cell activation can remain attached to the cell surface where they are exposed to shear from blood flow. Shear exposes the VWF A1 domain that interacts with the platelet GP Ib IX/V complex and the A2 domain where ADAMTS13 cleavage occurs.Lateral self-association of VWF multimers mediated by Cys-Cys disulfide bonds at the C2 domain, impairs the ability of ADAMTS13 to cleave VWF and promotes thrombosis(Atiq *et al*., 2019).

In acute MI, any VWF self-association that occurs from high LDL-C may be further worsened by reperfusion injury–related oxidative stress, which also increases self-association These studies revealed that after myocardial ischemia-reperfusion, elevated LDL-C had greater microvascular endothelial-associated VWF

larger infarct size than inPlatelet adhesion in the microcirculationand platelet adhesion in the microcirculation can not only impair reflow but also promote intravascular recruitment of inflammatory cells(Reardon et al., 2021)

5-4- DNA genotyping

The DD genotype is known as an independent risk factor in several cardiovascular diseases such as hypertrophic cardiomyopathy, myocardial infarction14and ventricular hypertrophy, as well as chronic renal diseases such as IgA nephropathy16, diabetic nephropathy, renal scarring and congenital urological anomalies. The results of the present study suggest an association between ischemic stroke and the presence of DD genotype and D allele in patient population(Krychtiuk *et al*., 2021).

The results of prior studies of ACE polymorphism in CVD patients have been consistent with some groups reporting a positive association between the DD genotype and/or D allele and stroke, while others reported to the opposite. Doiet al. reported a significant association between the polymorphism of ACE gene and the

incidence and mortality rate of ischemic CVD in patients age 30-60 years or younger in apatients . Also Kostulaset al. reported a positive correlation between ACE gene polymorphism and ischaemic cerebrovascular disease (Tran *et al*., 2023).

ACE could be involved in the pathogenesis of CVD disease by several biological mechanisms, including activation of angiotensin I and inactivation of bradykinin, resulting in decreased tissue perfusion, and stimulation of plasminogen activator inhibitor type I , Howeve, the role of ACE either as a direct mediator of or secondary following an acute CVD not fully understood. The plasma ACE concentration is an important factor in increasing the risk of cardiovascular and cerebrovascular diseases, since long exposure to high levels of plasma ACE may result in vascular wall thickness and stiffness (Krychtiuk et al., 2021). Early studies demonstrated a strong correlation between the D allele and levels of circulating, intracellular, and tissue activity of ACE , since both alleles have co-dominant effects on ACE levels, homozygous DD genotype result in the highest levels of the enzyme, while homozygous II genotype result in the lowest, and heterozygous DI genotype result in an intermediate level.In present study, we found that variant genotypes of ACE I/D polymorphism were associated with higher CVD in Iraqi population *(Al-Darraji et al*., 2022).

we demonstrate that WAT culture supernatants containing significant increases in MCP-1 gene expression strongly facilitated macrophage migration compared to lean littermates. Obesity-promoted macrophage migration by WAT was prevented by both PCSK 9 and inhibition, confirming the concept that the PCSK-driven MMP activation cascade is important for WAT inflammation. Among the PCSK9 family, mutations causing partial deficiency in PCSK9 have been linked to obesity, and mutations in PCSK9 are associated with hypercholesterolemia and coronary heart disease(Sobhi Saqer, 2016).

increased in CVD , its did impact on adipogenesis in paint on a high-fat diet The importance of PCSK9 in MT1-MMP activation in cardiometabolic syndrome is

further supported by our patients' data Here we demonstrate increased MT1-MMP and its cognate convertase PCSK9 in monocytes from CVD patients compared to CVD individuals Circulating monocytes are characterized by a pro-inflammatory/remodeling phenotype in obese patients in our study, transcript levels of correlated with the patients' BMI, and MT1-MMP correlated . this suggests for the first time a concordant regulation of in monocytes in CVD patients towards the pro-migratory/pro-inflammatory status characterizing CVD (Sobhi Saqer, 2016).

Furthermore, while PCSK9 was increased, levels of were not significantly altered, supporting a disruption in the balance of LDL and its inhibitor in CVD and adipose patients. This suggests that PCSK9 may be a contributor to MNC furin/MT1-MMP regulation. Interestingly, regulation of hepatic LDL receptors by resistin involves the related PCSK family member PCSK9, recently identified as a novel target in patients suffering from severe dyslipidemia and CVD (Gai *et al*., 2021) .

Conclusions and Recommendation

6.1. Conclusions

1. The significant increase in all Biomarker Proprotein Convertase Subtilisin/Kexin Type 9 Human , Fibroblast Growth Factor 21 , Human Lipocalin 1 Factor , Human Von Willebrand Factor associated with elder age ,obesity and smoking .
2. the Severity of disease repented by myocardial infarction related with high level of biomarkers in compare with stable and unstable angina.
3. The hyperlipidemia repented by high level of lipid profile Cholesterol , Triglyceride , LDL associated positively with all biomarkers.
4. Low HDL.C level as a risk factor for cardio Vascular disease negative related with high biomarkers level .
5. All biomarkers in present result consider as prognostic markers for early predictors of cardio Vascular disease
6. Molecular characterization of the ACE gene polymorphism The results of electrophoresis of the PCR product showed the presence of three types of genotypes for the two groups: a homozygous (Deletion) genotype (DD), represented by the band with a molecular size of 190 bp, a homozygous (Insertion (II) genotype) represented by the 490 bp band, and an asymmetric genotype (ID). Heterozygous is represented by two bands (bp490, bp190)
7. High levels of PCSK9 were positively but modestly associated with risk of MI in age- and sex-adjusted analysis, but the association was largely attenuated after adjustment for LDL-C. Our results are consistent with the biological understanding of PCSK9 and of its effect on atherosclerosis being mainly mediated by changes in LDL-receptor function. Whereas PCSK9 has emerged as a novel target for preventing and limiting the development of coronary artery disease our findings suggest that blood levels of PCSK9 do not contribute

additional useful information in cardiovascular risk assessment beyond the information provided by lipid measurements.

8. Molecular characterization of the PCSK9 gene polymorphism the results of electrophoresis of the PCR product showed the presence of three types of genotypes for the two groups: a homozygous (Deletion) genotype (DD), represented by the band with a molecular size of 440 bp, a homozygous (Insertion (II) genotype) represented by the 440 bp band, and an asymmetric genotype (ID). Heterozygous is represented by two bands (bp440)

6-2-*Recommendations*

1- The present study recommend a ministry of health to use all biomarkers as early prediction of cardiovascular disease .
2- Future study for the using asame biomarkers Coronary artery calcification disease .
3- Another molecular study must be take of familial history for (CVD) patents and in relation to the Same biomarkers present study .
4- Study a same biomarkers in autoimmune disease such as (diabetes type1 and systemic lupus erythromatosis)
5- Future study for the same biomarkers in Women after menopause in CVD patient.
6- Anther study related a changes in sex hormones testosterone and estrogen in female with Same biomarkers in (CVD) patients .
7- The study recommended to use a marker of PCSK9 and Human Von Willebrand Factor as a good biomarkers in Middle east hospitals in Iraqi early predictions of (CVD) .

CHAPTER SEVEN

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الخلاصة

في جميع بلدان العالم، لا يزال احتشاء عضلة القلب أحد أسباب المرض والوفيات. قد يحدث ذلك عندما يتجاوز نقص تروية عضلة القلب الكامل، وهو ما يسمى انخفاض تدفق الدم إلى عضلة القلب، آليات الشفاء التي تهدف إلى الحفاظ على توازن الجسم ووظائفه المنتظمة ، وأدى ذلك إلى تحسين القدرة على تحديد الأفراد المعرضين لخطر كبير، من أجل التنبؤ بدقة والتشخيص لدى الأفراد الذين يعانون من احتشاء عضلة القلب، من الضروري فحص المؤشرات البيوكيميائية المختلفة ، ترتبط بعض المؤشرات البيوكيميائية، مثل عامل نمو الخلايا الليفية - 21 (FGF-21)، باحتشاء عضلة القلب، والليبوكالين المرتبط بالجيلاتيناز (NGAL)، والبروتين المحول سبتيليسين/كيكسين 9 (PCSK9) لدى مرضى القلب.

تم تقييم الهدف من الدراسة الحالية لتقدير بعض الواسمات البيو كيميائية المرتبطة بأمراض القلب الافقارية للتنبؤ واعتبارها علامة تنبؤيه لهذا المرض، وتقييم الدراسة لعامل بروتين فون - عامل نمو الخلايا الليفية -21 (FGF-21)، باحتشاء عضلة القلب، والليبوكالين المرتبط بالجيلاتيناز (NGAL)،والبروتين المحول سبتيليسين/كيكسين 9 (PCSK9) في ستين مريضا(60) يعانون من امراض القلب الافقارية التي تم جمع العينات من مركز كربلاء لأمراض القلب في مدينة الامام الحسين الطبية في مدينة كربلاء المقدسة من فترة 1نوفمبر 2022 إلى 30 يوليو 2023 من الذكور المصابين بمرض نقص التروية ومجموعة السيطرة(مجموعة صحية ظاهريا) ثلاثون (30) وهذه المجموعة تمت مطابقة اعمارهم من (30) - (69) كمجموعة مرضى .

تضمنت معايير الدراسة الحالية صورة تعريف الدهون و اربع علامات بيوكيميائية . تم تقسيم المرضى إلى أربع مجموعات فرعية حسب الأعمار ونوع المرض والتدخين ومؤشر كتلة الجسم. أظهرت نتائج النتائج الحالية وجود زيادة معنوية في مستوى الكولسترول والدهون الثلاثية ومستوى LDL وانخفاض معنوي في مستوى HDL لدى مرضى القلب الإقفاري مقارنة مع مجموعة السيطرة. كما كانت العلامات البيوكيميائية مرتفعة أيضًا (عامل النمو التفاضلي 15، التربتاز والإنترلوكين 1 بيتا) في المرضى مقارنة بالمجموعة الضابطة. وبحسب الأعمار فإن الأعمار الأكبر (60-69) سنة كانت لها زيادة معنوية عالية في جميع العلامات مقارنة مع (50-59) و(40-49) و(30-39) سنة. في أنواع من مرضى أمراض القلب الإقفارية أظهرت نتائج الدراسة الحالية زيادة كبيرة في جميع العلامات الحيوية في احتشاء عضلة القلب بالمقارنة مع مرضى الذبحة الصدرية غير المستقرة والذبحة الصدرية المستقرة، وكانت هناك اختلافات كبيرة بين احتشاء عضلة القلب والذبحة

الصدرية غير المستقرة ومرضى الذبحة الصدرية المستقرة. جميع المؤشرات الحيوية زادت بشكل ملحوظ في المرضى المدخنين مقارنة مع المرضى غير المدخنين.

في المرضى الذين يعانون من السمنة المفرطة، كانت جميع العلامات البيوكيميائية مرتفعة بشكل ملحوظ بالمقارنة مع المرضى الذين يعانون من زيادة الوزن والوزن الطبيعي. أشارت دراسة الارتباط الحالية إلى وجود علاقة معنوية موجبة بين (عامل بروتين Lipocalin- (PCSKA)- Von -عامل نمو الخلايا الليفية 21) مع مستوى الكوليسترول و C - LDL و TG بينما توجد علاقة سلبية معنوية بين جميع المؤشرات الحيوية ومستوى HDL. وخلصت الدراسة الحالية إلى أن جميع المؤشرات الحيوية (عامل بروتين فون -(PCSKA) - ليبوكالين -عامل نمو الخلايا الليفية 21) تعتبر بمثابة خطر للتنبؤ والتنبؤ بتصلب الشرايين المرتبط بأمراض القلب الإقفارية.

كانت نتائج دراسة تعدد أشكال الإنزيم المحول للأنجيوتنسين لدى مرضى الأمراض القلبية الوعائية متسقة مع بعض المجموعات التي أبلغت عن وجود ارتباط إيجابي بين النمط الجيني DD و/أو أليل D والسكتة الدماغية، في حين أبلغ آخرون عن عكس ذلك. دويتو آل. تم الإبلاغ عن وجود ارتباط كبير بين تعدد أشكال جينات الإنزيم المحول للأنجيوتنسين وحدوث ووفيات أمراض القلب والأوعية الدموية الإقفارية لدى المرضى الذين تتراوح أعمارهم بين 30 إلى 60 عامًا أو أقل في المرضى غير المصابين . ذكرت وجود علاقة إيجابية بين تعدد أشكال الجينات ACE والأمراض الدماغية الوعائية. أظهرت الدراسات المبكرة وجود علاقة قوية بين الأليل D ومستويات نشاط الدورة الدموية داخل الخلايا والأنسجة للإنزيم ACE. نظرًا لأن كلا الأليلين لهما تأثيرات سائدة مشتركة على مستويات الإنزيم المحول للأنجيوتنسين، فإن النمط الجيني DD المتماثل يؤدي إلى أعلى مستويات الإنزيم، في حين أن النمط الجيني المتماثل II يؤدي إلى المستوى الأدنى، والنمط الجيني المتخالف DI يؤدي إلى مستوى متوسط. في الدراسة الحالية، وجدنا أن الأنماط الجينية المتغيرة لأشكال ACE I/D ارتبطت بارتفاع أمراض القلب والأوعية الدموية لدى المرضى.

لقد أثبتنا أن المرضى تحتوي على كمية كبيرة من البروتين PCSK9، المسببة لنقص جزئي في PCSK9 بالسمنة، ووجود تراتبط بين PCSK9 وفرط كوليستيرول الدم وأمراض القلب التاجية.

زيادة في الأمراض القلبية الوعائية، وتأثيرها على تكوين الشحوم في الطلاء في نظام غذائي عالي الدهون. إن أهمية جين PCSK9 في تنشيط MT1-MMP في متلازمة التمثيل الغذائي للقلب يتم دعمها بشكل أكبر من خلال بيانات . يشير هذا لأول مرة إلى زيادة التنظيم المتوافق للخلايا الوحيدة في مرضى الأمراض القلبية

الوعائية تجاه الحالة المؤيدة للهجرة/المؤيدة للالتهابات التي تميز CVD PCSK9، ولم تتغير مستوياتها بشكل كبير، مما يدعم حدوث اضطراب في توازن LDL ومثبطه في مرضى القلب والأوعية الدموية ومرضى السمنة. قد يكون PCSK9 هذا مساهمًا في التنظيم. ومن المثير للاهتمام أن تنظيم مستقبلات LDL الكبدية عن طريق الريسيستين يشمل عضو عائلة PCSK ذو الصلة PCSK9، والذي تم تحديده مؤخرًا كهدف جديد في المرضى الذين يعانون من اضطراب شحوم الدم الشديد والأمراض القلبية الوعائية.

أظهرت نتائج النتائج الحالية زيادة معنوية في الكوليسترول والدهون الثلاثية ومستوى LDL وانخفاض معنوي في مستوى HDL في مرضى القلب الإقفاري مقارنة مع مجموعة السيطرة . كما تم رفع الواسمات البيوكيميائية (عامل) تمايز النمو 15 ، التربتاز والإنترلوكين 1 بيتا) في المرضى بالمقارنة مع مجموعة السيطرة . حسب الأعمار ، كان كبار السن (60_69) سنة زيادة معنوية عالية في جميع العلامات بالمقارنة مع (50_59) ، (4940) و (30_39) سنة . في أنواع مرضى القلب الإقفاري ، أظهرت نتائج الدراسة الحالية زيادة معنوية في جميع المؤشرات الحيوية في احتشاء عضلة القلب مقارنة بالذبحة الصدرية غير المستقرة ومرضى الذبحة الصدرية المستقرة باستثناء L - 1betal لم تكن هناك فروق ذات دلالة إحصائية بين احتشاء عضلة القلب والذبحة الصدرية غير المستقرة والذبحة الصدرية المستقرة المرضى . زادت جميع المؤشرات الحيوية بشكل ملحوظ في المرضى المدخنين مقارنة مع المرضى غير المدخنين . في المرضى الذين يعانون من زيادة مؤشر كتلة الجسم ، ارتفعت جميع الواسمان البيو كيميائية بشكل ملحوظ بالمقارنة مع المرضى الذين يعانون من زيادة الوزن والوزن الطبيعي .

أشارت دراسة الارتباط الحالية إلى وجود علاقة معنوية موجبة بين (لعامل بروتين فون - (PCSK9) - ليبوكالين -عامل نمو الخلايا الليفية 21) مع مستوى الكوليسترول ، LDL - C و TG بينما هناك علاقة معنوية سلبية بين جميع المؤشرات الحيوية ومستوى HDL - C .

لخصت الدراسة الحالية إلى أن جميع المؤشرات الحيوية عامل بروتين فون - (PCSK9) - ليبوكالين -عامل نمو الخلايا الليفية 21) تعتبر خطرًا للتشخيص والتنبؤ بتصلب الشرايين المرتبط بأمراض القلب الإقفارية و يمكن استخدام مستويات FGF-21، NGAL، و PCSK9 لتحديد وتشخيص احتشاء عضلة القلب .

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قسم علوم الحياة

دراسة بعض المؤشرات الحيوية الفسيولوجية و الأشكال الوراثية لدى مرضى أمراض القلب الإقفارية في محافظة كربلاء

بكالوريوس كلية التربية العلوم الصرفة 2011

ماجستير كلية التربية العلوم الصرفة 2013

أطروحة مقدمة إلى مجلس كلية التربية للعلوم الصرفة - جامعة كربلاء وهي جزء من متطلبات نيل درجة دكتوراه في علوم الحياة - علم الحيوان

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