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Association of *Interleukin 32* Gene Polymorphism (rs 4786370) and Serum Level with Coronary Artery Disease

A Thesis

Submitted to the Council of the College of Medicine/ University of Kerbala in Partial Fulfillments of the Requirements for the Degree of Master in Medical Microbiology

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

(وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)

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Supervisor Certification

I certify that this thesis entitled (**Association of Interleukin 32 Gene Polymorphism (rs 4786370) and Serum Level with Coronary Artery Disease)** was prepared under my supervision at the College of Medicine/University of Karbala as a Partial Fulfillment of the requirements for the degree of Master in Medical Microbiology.

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Dedication

I dedicate this work

To....

To the great spirit that enveloped my existence with blessings and made it full of life... (Lord of the Worlds)
To the reasons that were not holes in my sails, but a gentle breeze guiding my ship towards safe shores... (My father and mother)
To the hands that were always present when life burdened me and came to my aid when paths became muddy... (My brothers and sister)
To all the scientific streams that nourished my intellectual oasis and became my reserve to lean on when the waves of need surprised me... (My teachers and supervisors)

_To all of these... I present to you the fruits of my journey...
with bouquets of wishes._

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Rasha
2025

Summary:

Coronary artery disease (CAD) is the most common cause of death in the general population. Marked by an inadequate supply of blood and oxygen to the myocardium. IL-32 is a proinflammatory cytokine, which enhances inflammation through inducing the secretion of different inflammatory cytokines. The single nucleotide polymorphism (SNP) in the promoter region of IL32 that seems to affect the expression of the isoforms in peripheral blood mononuclear cells, a mutation in the *IL32 gene* could contribute in different inflammatory diseases like CAD .

A total of 100 blood and serum samples were collected from both sex (48 males and 52 females), whose ages ranged from (40-70) years, attending to, Shaheed Al-Muhrab center for cardiac catheterization and surgery & Imam Al-Sadiq Hospital in Babylon city ,Iraq. during a period extended from October (2024) to January (2025). 100 blood samples were collected in an EDTA tube and gel tube, they divided into 50 samples from CAD patients and 50 samples from healthy individuals as control group. Blood and serum samples obtained from all participants to measure IL32 serum levels were confirmed by using Enzyme-Linked Immunosorbent Assay (ELISA) system, and *IL32 gene* polymorphism was measured using specific allele method by polymerase chain reaction technique.

The results of the current study showed a non-significant *($p>0.05$)* differences concerning age, age groups, and sex. The statistical analysis of economic status found a significant differences within patients and control groups respectively (*$p=0.0001$)* and (*$p=0.0015$).* About the family history of patients, there was a significant (*$p=0.0455$)* result regarding the patients without family history of cardiac disease. In addition, according to body mass index (BMI) categories the percentage of patients and controls were

74% and 86% respectively within the normal group, while 18% and 14% within overweight group and 8% of cases group were obese. Present study revealed that the highest percentage (50%) was found in age group of (61-71) years old concerning the patients with hypertension according to age group. On the other hand, this study found a significant result (*p=0.004*) in IL-32 serum level in cases group compared with control. Also, there were increased in the C-reactive protein (CRP) (7.202) and erythrocyte sedimentation rate (ESR) (17.06) levels in patients group.

Moreover, regarding the *IL32 gene* polymorphism (rs4786370), the genotypes frequency of this study with significant result (p= *0.0455*) between patients and control, the odds ratio of 2.2788, and a confidence interval (CI) (1.0165-5.1085). The CC genotypes were elevated in patients (54.0%) group compared with the control (34.0%). Whereas, TT genotypes were increased in control group (38.0%) compared with cases (16.0%). About the analysis of allele frequency there were a significant result (p= *0.0028*), with an odds ratio of 2.4113 and a confidence interval (1.3535 to 4.2959). The C allele was higher in patients (69%) compared with the control (48%), while, the T allele increased in the control (52%) compared with the patients group (31%). Current study also showed a significant outcomes concerning the Receiver Operative Characteristic Curve (ROC) analysis of IL-32, the area under curve (AUC) is 0.786, sensitivity is 0.60 and specificity is 0.88, with *(p= 0.000*).

The results of this study show an elevation occurred in IL-32 serum level in patients with CAD compared with the healthy individuals. Therefore, this interleukin may be used as a predictive marker for diagnosing of the CAD.As well as, the study has shown that the CC genotype was higher in patients compared to the control, while, TT genotype was higher in the healthy control compared to the patients. Therefore, the CC genotype may be related to the increase of the risk of

coronary artery diseases, while, TT genotype may have a protective role in this disease.

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List of Abbreviations

Code	Words
ACE	**Angiotensin converting enzyme**
ACS	**Acute Coronary Syndrome**
AUC	**Area Under Curve**
BMI	**Body Mass Index**
CABG	**Coronary artery by pass grafting**
CAD	**Coronary Artery Disease**
CCS	**Chronic Coronary Syndrome**
CCTA	**Coronary computed tomography**
CD	**Crohn's disease**
CHD	**Ischemic heart disease**
COPD	**Chronic Obstructive Pulmonary Disease**

CRP	**C-reactive protein**
CVD	**Coronary vascular disease**
ECG	**Electrocardiography**
ELISA	**Enzyme linked immunosorbents assay**
GD	**Graves disease**
GWAS	**Genome-wide association studies**
HDL	**High-Density Lipoprotein**
HRP	**Horseradish Peroxidase**
IBD	**Inflammatory Bowel Disease**
ICA	**invasive coronary angiography**
IL32	**Interleukin 32**
LDL	**Low-Density Lipoprotein**
MI	**Myocardial infarction**
mRNA	**messenger Ribonucleic Acid**
NSTEMI	**Non ST-Segments elevation**
PCI	**A percutaneous coronary intervention**
PCR	**Polymerase chain reaction**
RA	**Rheumatoid Arthritis**
ROC	**Receiver Operative Characteristic**

	Curve
SD	**Standard deviation**
SLE	**Systemic lupus erythematous**
SNP	**Single Nucleotide Polymorphisms**

Chapter One

Introduction

&

Literature Review

1.1- Introduction

Coronary artery disease (CAD) is a common heart condition that involves atherosclerotic plaque formation in the vessel lumen. This leads to impairment in blood flow and thus oxygen delivery to the myocardium. It is a cause of major morbidity and mortality in the worldwide (Komilovich ,2023). Heart disease is One of the dominant diseases in the world . According to the survey of each year, 18 Million people is dying in the around world due to cardiovascular disease (Chen and Hengjinda ,2021).

The major risk factors for CAD and some can be controlled but not others. The risk factors that can be controlled (modifiable) are: High Blood Pressure ; high blood cholesterol levels; smoking; diabetes; overweight or obesity; lack of physical activity; unhealthy diet and stress. Those that cannot be controlled (conventional) are: Age (simply getting older increases risk); sex (men are generally at greater risk of coronary artery disease); family history; and race. Hypertension is one of the risks in the development of coronary heart disease (CHD) (Hajar,2017).

Coronary artery disease is a chronic inflammatory disease usually caused by atherosclerosis, in which the coronary arteries become narrowed by atheromatous plaque. Plaques in atherosclerosis are formed through the accumulation of lipids and various immune cells. Both adaptive and innate immune systems are involved in the pathogenesis of atherosclerosis and facilitate plaque formation and disease progression (Mohmmad-Rezaei *et al* .,2021).

Interleukin-32 (IL-32) is a newly discovered inflammatory cytokine with eight isoforms in most mammals, including IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, IL-32η, IL-32θ, and IL-32 s . IL-32 is widely distributed throughout the body, It is a cytokine associated with

higher risk of cardiovascular diseases in inflammatory environments (Yang *et al*., 2019). IL-32 has single nucleotide polymorphisms (SNPs) and is found on human chromosome 16p13.3. The IL-32 promoter has the rs28372698 and rs4786370 polymorphisms, which have been linked to a number of illnesses, such as cancer, leishmaniasis, and systemic lupus erythematosus. (Wang *et al* .,2016 ;Yang *et al* .,2017). Recently, there has been a correlation between elevated cardiovascular mortality and the AA genotype of the rs28372698 polymorphism. (Alehagen *et al* .,2021) The functional impact of rs4786370 on rheumatoid arthritis patients' lipid profiles raises the possibility of a preventive role against cardiovascular disease (CVD). Thus, According to earlier research, IL 32 polymorphisms play a significant role in CAD. Cardiovascular disease incidence and progression are strongly correlated with IL-32 and its SNPs. The function of rs28372698 and rs4786370 in CAD susceptibility has not yet been documented, and research examining the connection between IL-32 levels and coronary stenosis in CAD patients are uncommon (Jin *et al* .,2022).

The aim of the Study:

The aim of the study is to investigate the relationship between *interleukin 32* gene Polymorphism and serum level in patients with coronary heart disease and healthy control and their association with progression of the disease, The following objectives are set to address the above aim.

1-To determine some demographic data in patients and control.
2-To estimate IL32 serum level in patients and control groups by ELISA test.
3-To evaluate some laboratory tests (CBC,CRP,ESR) in patients and control.
4-To detect *IL32* gene polymorphism by the allele specific method using PCR technique in patients and control groups.

5- To evaluate the relationship of *IL32* gene polymorphism (rs 4786370) with CAD.

1.2- Literature Review

1.2.1- Definition of Coronary Artery Diseases

CAD, also known as coronary heart disease (CHD) or ischemic heart disease, is when the coronary arteries that supply blood to the heart become narrowed or blocked, usually due to plaque buildup (Sebayang and Abdulgani , 2023) . The coronary arteries, which provide the heart with oxygenated blood, are the main organs affected. It is distinguished by atherosclerosis, a condition in which fatty deposits (plaques), cholesterol, and inflammatory cells build up inside the artery walls. (Malakar *et al* .,2019), These plaques cause the coronary arteries to progressively constrict, which lowers heart blood flow. The blood flow to the myocardium is insufficient to meet the oxygen and nutritional needs during elevated cardiac activity or stress because the coronary arteries constrict. Angina pectoris, another name for myocardial ischemia, is a condition marked by chest pain or discomfort that results from this imbalance between oxygen supply and demand. (Beck *et al*., 2021). CHD can lead to unstable angina, myocardial infarction (MI), and heart failure (Imes and Austin ,2013).

This disease is often caused by genetic, environmental, and lifestyle factors and account for many casualties worldwide. The risk factors of CAD include diabetes mellitus, hypertension, smoking, hyperlipidemia, obesity, homocystinuria, and psychosocial stress (Malakar *et al*.,2019).

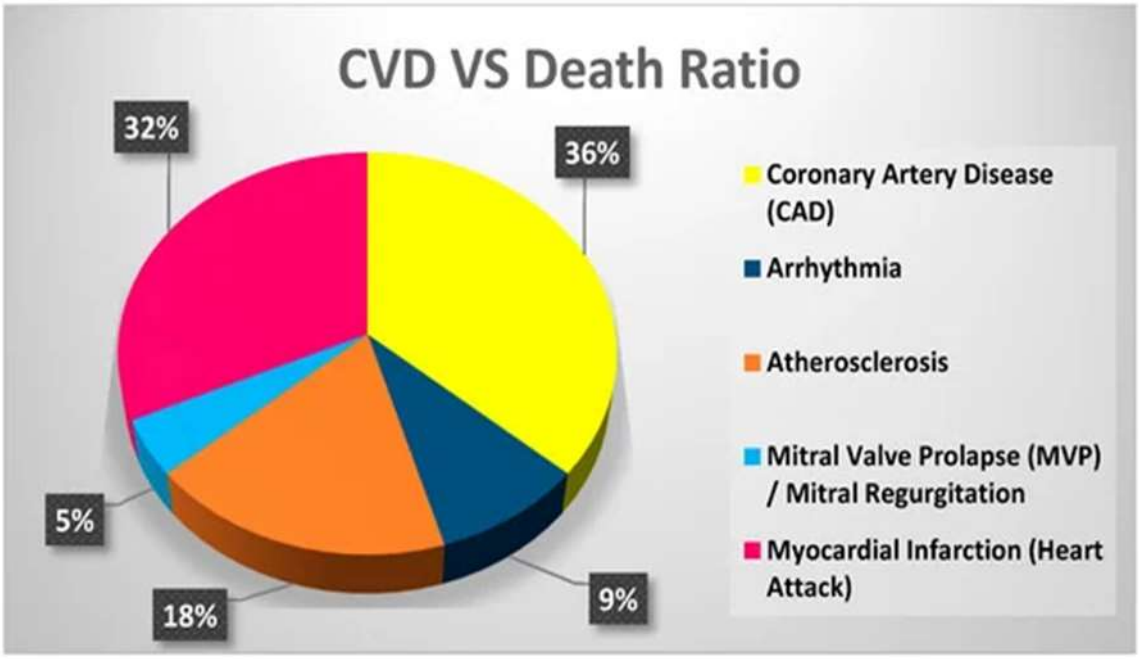
The most common symptoms of CAD are; shortness of breath result from little blood supply within blood vessels that cause hardly breathing or cannot getting enough air to breath. Chest discomfort ;Dizziness or light headedness ;Lake of energy or sudden fatigue might occur and angina (Prajapati *et al*.,2021) .

In addition, the person may feel lightheaded or faint, turn pale, get cold sweats, or experience nausea or vomiting. Women are more prone than men to experience back or jaw discomfort, nausea, vomiting, and shortness of breath (Nabel and Braunwald ,2012).

1.2.2- Epidemiology

Coronary artery disease is the leading cause of mortality and morbidity globally. This disease has been proved to be a major cause of death in developed and developing countries (Malakar *et al.*, 2019) . CAD is estimated to affect about 126 million individuals, corresponding to approximately 1.72% of the worldwide population (1655 per 100,000 individuals) (Abdul-Rahman *et al*.,2023), About 20.1 million adults age 20 and older have CAD (7.2%) and 2 in 10 deaths from CAD happen in adults less than 65 years old (Muthiah,2023). Also, It is a third leading cause of mortality worldwide and related with 17.8 million death annually (Roth *et al* ., 2017) .

In 2019, an estimated 17.9 million deaths worldwide were attributed to CVDs, accounting for 32% of all global deaths. Heart attacks and strokes accounted for 85% of these fatalities (World health organization, 2019). In the United States, CAD is estimated to affect 16.8 million people of these, 9.8 million have angina pectoris, and nearly 8 million have had a myocardial infarction (MI)(Bottardi *et al* .,2024). It costs over 200 billion dollars annually to the health care system in the United States (Komilovich ,2023).



(Figure 1-1): Percentage of death ratio from coronary artery disease compared to other heart diseases. (Khan *et al* .,2024)

In 2018, CAD was more prevalence among man (77%) than a women (4.6%). The study explained the difference in CAD was also observed by age (Lee *et al*.,2022). In 2022,CAD was included in 315 million cases globally. These numbers began to decrease by 18% since 1990. Central Europe, Eastern Europe and central asia had the highest prevalence ,while south Asia had lowest (stark *et al*.,2024). The mortality ratio from the disease is estimated to be 5 times higher in men than a women (Pathak *et al*.,2017). Numbers of mortality from Cardiovascular disease predicted to reach about 23.4 million in 2030 and more over Cardiovascular disease tends to affect younger age and thus could negatively affect the work force and economic productivity (Rashid and Hossain , 2022).

In the Middle East region, CVDs were responsible for 34.1% of all deaths in 2015 (Mokdad *et al* .,2018). The Middle East and North Africa region is one of the seven super regions of the Global Burden of Disease. It encompasses 21 countries, and it had an estimated population of

608.7 million in 2019, The three countries with the highest age-standardized incidence rates were Iran , Egypt, and Oman, while the three countries with the lowest age-standardized incidence rates were Turkey, Tunisia, and Algeria (Manla and Almahmeed ,2023) .

In Iraq, a study done in 2014, cardiovascular disease mortality was estimated to account for 33% in Iraq (Mohammad *et al*., 2015). Furthermore, in 2018, from World Health Organization (WHO) report about Iraq, there were (6.53%) deaths due to stroke and (18.92%) deaths due to coronary heart disease (Taher *et al* ., 2021).It was observed that among Iraqis, Acute Coronary Syndrome increased in prevalence over the last two decades, and has become the leading cause of death in this population (Allami ,2024).

The epidemiological data on the incidence and prevalence of coronary artery disease (CAD) as evidence of awareness are limited due to the unavailability of evidence-based national guidelines for the management of cardiovascular disease and surveillance studies as compared to other Eastern Mediterranean countries (Traina *et al*., 2017).

1.2.3. Etiology

Coronary artery disease is a multiple causes disease that is caused by genetic and environmental factors include constant and modifiable risk factors (Abdul-Rahman *et al*.,2023) .

1.2.3.1 Constant Risk Factors :

-Genetic factors

Genetic predisposition for CAD is a key contributor. Studies have identified multiple genetic variants associated with an increased risk of CAD, including those affecting lipid metabolism, inflammation, and vascular function. Family history remains one of the strongest risk indicators, suggesting a hereditary component. Advances in genomic research may lead to better risk prediction and personalized treatment

strategies for individuals with a genetic predisposition to CAD (Khera and Kathiresan ,2017)

-Age

Cardiovascular Diseases (CVDs) are the main age-related diseases, as the risk increases significantly with advancing years. Over time, arteries undergo structural and functional changes, including endothelial dysfunction, arterial stiffening, and plaque accumulation, which contribute to a higher likelihood of atherosclerosis. Older individuals also have a greater prevalence of other risk factors such as hypertension and diabetes, further increasing CAD risk (Corella and Ordovás ,2014).

- Gender

Gender plays a significant role in the prevalence, presentation, and outcomes of coronary artery disease (CAD). Men generally develop CAD at a younger age compared to women, likely due to hormonal and genetic factors. Estrogen provides a protective effect in premenopausal women by improving lipid profiles and vascular function, but this advantage diminishes after menopause, leading to an increased CAD risk (Maas and Appelman , 2010).

- Family history

Family history for CAD is an established cardiovascular risk factor and it is progressively acquiring importance in patients' cardiovascular risk stratification. Numerous studies have demonstrated that individuals with a first-degree relative affected by CAD have a significantly higher risk of developing the condition themselves (Di Lenarda *et al*.,2024).

1.2.3.2.Non constant (Modifiable) Risk Factors

-Smoking

Passive tobacco smoking is the most important modifiable risk factor accounting about 35% - 40% of all smoking related death. The prevalence of CAD is a higher among smokers as the incidence of myocardial infarction (Dahlgren,2023).

- Obesity

Obesity and overweight play a key role in the development of cardiovascular disease or myocardial ischemia. In addition, obesity is an independent risk factor for cardiovascular disease and usually leads to the development of multiple atherosclerotic cardiovascular disease risk factors—including hypertension, hyperlipidemia, and diabetes (Battineni *et al.*,2021).

-Hypertension

Hypertension is a worldwide chronic non-communicable disease, a major disease that endangers human health. The studies have shown that people with hypertension have a higher tendency to develop coronary atherosclerosis than those with normal blood pressure (Wei *et al* .,2024).

- Lipid levels

Hyperlipidemias, which includes hypertriglyceridemia, hypercholesterolemia, increase concentration of LDL and decrease concentration of HDL , Hypercholesterolemia remains an important modifiable risk factor for CAD. Increased low-density lipoproteins (LDL) increased the risk for CAD and elevated high-density lipoproteins (HDL) decrease the incidence of CAD (Komilovich ,2023) .

-Psychosocial stress

Psychological factors may contribute to the physio pathological process of CADs. In women, angina is related to the phenomenon of mental stress ischemia , a transient myocardial ischemic response to mental

stress. Mental stress tends to further affect the cardiovascular system in women compared to their male counterparts (Gilabert-Garcia *et al*., 2023)

-Excessive alcohol consumption

One of the most significant mortality risk factors is alcohol consumption. The amount of alcoholic beverage ingested is directly correlated with the chance of developing a disease. Globally, the amount of pure alcohol used by adults has been steadily increasing. From 5.9 L in 1990 to 6.5 L in 2017, the amount is predicted to rise to 7.6 L by 2030. Between one and two servings of alcohol per day were associated with the lowest risk of death for both sexes (Chudzińska *et al*.,2022).

- Diabetes mellitus

The American heart association considers diabetes mellitus and it s precursor condition – abnormal glucose metabolism due to Insulin resistance

to be a major factor for cardiac and vascular disease as well as kidney disease (Weir ,2024)

1.2.4. Types of disease

1.2.4.1Chronic Coronary Syndrome (CCS)

Chronic or stable angina is the initial manifestation of CAD in approximately 50 % of all incidence. usually caused by obstructive of at least 1 large epicardial coronary artery atheromatous plaque . Angina occurs due to mismatching between myocardial oxygen demand and supply leading to myocardial ischemia (Saraste and Knuuti,2020).

Stable angina

This term refers to chest pain ,typically results from imbalance between heart oxygen demened and coronary blood supply. It is termed "chronic" and "stable" when symptoms persist for at least two months without changes in severity or triggers. Stable angina is a common manifestation of Ischemic heart disease and it is prevalence increases with age and is more common in estimated to affect 16.8 million people in the united state . nearly 9.8 million patiens have angina pectoris and 8 million have a myocardial infarction (MI) (Cassar *et al* .,2009). Chronic or stable angina is the initial manifestation of CAD in approximately 50 %men than a women . Symptoms typically include constrictive chest discomfort ,though some patients ,especially the elderly and diabetes ,may have a silent ischemia without obvious symptoms (Lloyd-Jones, 2022) .

1.2.4.2 Acute Coronary Syndrome (ACS)

Coronary artery disease can range from being a symptomatic to causing a cute coronary syndrome (ACS) or sudden cardiac death .This term has been use to describe all sudden reduced blood flow to heart muscle , therefore , it does not has ability to work well or leading to die (Amesterdam *et al* ., 2014). Its often presenting with a prolong stable phase . But it can suddenly become unstable. Leads to acute phase. Therefore the term" stable" coronary artery disease can be misleading as a risk of acute even with pharmacological therapies. Recent advancements have shifted the focus from assessing ischemia to evaluating the anatomical burden of the disease , including non-obstructive plaque that may not be detectable with traditional tests .Coronary computed Tomographic angiography has become a key diagnostic tool for detecting of both obstructive and non-obstructive plaques , improving risk assessment and guiding preventive treatments (Ueng *et al* ., 2023) .

Unstable angina

Its caused when atherosclerosis plaque rupture or injury .Red blood cells responsible for clotting , follow by collecting on the surface of the plaque causing a large blood clots to form . This clot can block off the coronary artery and ,then trigger the symptoms of unstable angina ,which they are similar to those of stable angina but, can last for 20 to 30 minutes or even longer. Its gradually gets worse with time and it may not respond to treatment ,and this is a medical emergency case that could progress to heart attack (Ketterer *et al*., 2004).

1.2.4.3 Heart attack

It is a medical term for myocardial infarction when the coronary arteries that supply the blood to heart muscle become narrowing due to atherosclerosis plaque when the plaque rupture causing a blood clot to form ,this then, totally or partially block off suddenly the blood supply to heart starving of oxygen .

This can cause irreversible damage to the heart muscle if left untreated (Jevon .,2012).

There are two main types of heart attack that causing damage to the heart muscle:

- An ST-elevation myocardial infarction (STEMI) , It is a greatest sever type of myocardial infarction usually results from prolonged thrombotic occlusion of the coronary artery as a thrombus formation followed by rupture of plaque. In hyper reactive state , circulation platelet is enhanced following acute myocardial infarction and associated with the extent of myocardial damage (Hartopo *et al* ., 2016).

- Non ST Segments elevation (NSTEMI) ;Less severity than (STEMI)

When the blood clot partially occludes the coronary artery ,and , result only a portion of the heart muscle being supplied by the affected artery dies(Morrow and Braunwald ,2003) .

Myocardial infarction has been classified in to five sub types :

Type 1:- Spontaneous myocardial infarction related to ischemia caused by a primary coronary event such as plaque destruction or rupture, or dissection .

Type 2:- Myocardial infarction secondary to ischemia resulting from an imbalance in oxygen needs and supply such as coronary spasm ,arrhythmias hypertension , hypotension ,and anemia .

Type 3:- Unexpected cardiac death (Sudden) with symptoms myocardial ischemia which ,death occur before blood samples could be collected .

Type 4:- Myocardial infarction associated with percutaneous coronary intervention (PCI) .

Type 5 :- Myocardial infarction related to coronary artery bypass graft surgery .(Bengtsson , 2011; Samuelsson *et al*., 2021).

1.2.5. Pathogenesis

An accumulation of lipids, fibrous elements, and inflammatory chemicals in the walls of the major arteries is the hallmark of atherosclerosis, a silent, gradual disease (Wang and Butany, 2017 ; Buja, 2023).

The process initiates with the efflux of low-density lipoprotein (LDL) cholesterol to the sub endothelial space, which can then be modified and oxidized by various agents (Sameem *et al.*,2018) This particles are potent Chemotactic molecules cause monocyte adherence and migration to the sub-endothelium space by causing the endothelial surface to express

vascular cell adhesion and intercellular adhesion molecules (Gianopoulos and Daskalopoulou, 2024) . Monocyte differentiates to macrophage in intima media. Different subsets of monocyte have been identified, and their role appear to be different according to the phase of atherosclerosis in which they involved (Hristov and Weber, 2024).

Macrophage links oxidized LDL by scavenger receptor to become foam cells .And also have proinflammatory function ,including the release of cytokines ,such as interleukin and tumor necrosis factor . The end result of this process is formation of the first atherosclerosis lesion (Rajamaki *et al* .,2010)

Other types of leukocyte ,such as lymphocyte and mast cell ,also accumulate in the sub endothelial space. The cross reaction between monocyte ,macrophage , foam cells ,and Tcells result in cellular and humoral immune response , ultimately in a chronic inflammatory state with the production of several proinflammatory molecules (Filatova *et al*.,2023).

This process keeps on as smooth muscle cells migrate into the intima from the artery's medial layer, followed by the transition from a fatty streak to a more complex lesion, when smooth muscle cells are in the intima media ,They produce more cellular matrix molecules, creating a fibrous cap that covers the original fatty streak Foam cells inside the fibrous cap die , and release lipids that accumulate in the extracellular space (Brown *et al*., 2016). The final result of this process is formation of the second atherosclerosis lesion "the fibrous plaque". Once the thickness of the fibrous cap is the key for maintaining integrity of the atherosclerosis plaque (Sakakura *et al*., 2014). Two types of plaque can be defined according to balance between formation and degradation of the fibrous cap, stable and unstable or vulnerable.

Stable plaques have an intact thick fibrous cap contain smooth muscle cells in a rich matrix with collagen (Mori *et al* ., 2017). This type of plaque has a protrusion in to the lumen of the artery cause flow – limiting stenosis that is leading to ischemia ,and often stable angina, while vulnerable plaque has a thin fibrous cap made from mostly type 1 collagen and few or no smooth muscle cells ,but rich with macrophage and pro inflammatory and prothrombotic molecules (Bentzon *et al*.,2014).

These plaques are tends to erosion or rupture ,due to exposing the plaque to the circulatory coagulation proteins which causing thrombosis , and sudden lock on of the artery lumen and usually an acute coronary syndrome (Libby *et al* ., 2005 ; Sakakura ,*et al*., 2014) .An intra plaque hemorrhage may also
contribute in progression and development of atherosclerosis (Torii *et al*., 2024).

1.2.6 Diagnosis

An early and accurate diagnosis of CAD allows timely administration of appropriate treatment and helps to reduce the risk of complication and death Multiple imaging are available to evaluate patients suspected of having coronary artery diseases (Woods *et al* ., 2024).

1.2.6.1Clinical diagnosis of CAD

- Chest pain (angina pectoris) :- is the primary symptoms of the disease, characterized by discomfort in the chest , jaw , arm, and other sites. The pain often occurs in the middle and can radiate to the left arm. This condition has a tendency toward sudden fatal outcomes (Komilovich ,2023).

- Shortness of breath :- result from reduction in blood flow supply that bears oxygen to the heart muscle , when heart cannot pump enough blood

to enriches bod needs which may develop in shortness in breath (Pelter, 2012) .

-Heart attack or acute myocardial infarction :- occurs when coronary artery completely blocked and common signs and symptoms are breathlessness on exertion at rest on lying flat and at night and fluid retention leading to peripheral oedema (ankle swelling)fatigue , decreased exercise tolerance or increased recovery time after exercise (Smit *et al* .,2020)

1.2.6.2 The Electrocardiography (ECG)

It is a simple recording corresponds to the electrical activity of the heart. It provides information about both the physiology and anatomy of the heart (komilovich ,2023), This signal has been analyzed and utilized for various purposes , such as , measuring the heart rate , examine the rhythm of

heart beats and heart abnormalities emotion recognition and biometric identification (Saini *et al* ., 2022).

1.2.6.3 Echocardiography

Echocardiography is a type of cardiac ultrasonography. It is an effective, non-invasive testing method used for both acute and chronic conditions. It can provide a wealth of helpful information ,including the size and shape of heart (internal chamber size quantification), pumping capacity ,and the location and extent of any tissue damage (Lang *et al* .,2015) . An echocardiogram can also give physician other estimates of heart function, such as a calculation of the cardiac output , ejection fraction, and diastolic function (how well the heart relaxes) .Echocardiography can help detect cardiomyopathy, and many others (Agrawal *et al* .,2022).

1.2.6.4 Coronary Computed Tomography Angiography

An accurate noninvasive substitute for invasive coronary angiography (ICA) for assessing the presence and anatomic extent of coronary artery disease (CAD) in individuals at moderate to high risk is coronary computed tomography (CCTA) (Trost *et al* .,2022). Routine invasive coronary angiography (ICA) without intravascular imaging does not allow for the visualization of plaque, whereas coronary computed tomography angiography does. It has already been acknowledged that high-risk plaque characteristics on CCTA, such as the Napkin ring sing (NRS), positive remodeling (PR), and low-attenuation plaque (LAP), may be useful in identifying patients who are more likely to experience cardiovascular events (Meloni *et al* .,2024)

1.2.6.5 Cardiac Catheterization

It is the most reliable, suitable, and gold standard method for assessing ischemic coronary heart disease. It is an intrusive process that has related risks. The procedure is not appropriate for everyone. Patients with an intermediate pretest probability for CAD are typically the best candidates for it in non-ACS settings. All STEMI patients and a subset of NSTEMI patients receive an urgent cardiac catheterization in the ACS situation. This operation is performed under moderate sedation. Serious complications, such as allergic reactions and renal damage, could result from the contrast exposure (Shahjehan *et al*.,2024).

1.2.6.6 Biochemical Marker

Many biomarkers, including adhesion molecules, cytokines, and C-reactive protein, have been linked in recent years to a poor cardiovascular prognosis. Numerous investigations revealed a correlation between cardiovascular disorders and inflammatory biomarkers, indicating their utility to identify the risk of an acute ischemic event and the detection

of vulnerable plaques. The emerging inflammatory markers are well divided for diagnosis and prognosis and plaque instability of coronary artery disease (Borun ,2022).

Among these, the lectin-like oxidized low density lipoprotein receptor-1 may play a significant role in the assessment of plaque instability as well as in diagnosis. Myeloperoxidase, myeloid-related protein 8/14, and pregnancy-associated plasma protein-A are known indicators of plaque instability, whereas amyloid A, fibrinogen, and pentraxin 3 are developing inflammatory markers in the acute phase. Finally, some research showed that circulating miRNAs play a role in heart failure, acute myocardial infarction, and coronary artery disease, but before the initiation of coronary ischemia, albumin is released. Troponin, myoglobin, and creatine kinase-MB are time-dependent release components associated with myocardial necrosis (Morrow and Braunwald ,2003). The extent of these events influences the circulating troponin level. Therefore, it is important to identify the fundamental steps leading to atherosclerotic plaque rupture (Lubrano and Balzan,2015).

1.2.7 Treatment

Treatment generally involves life style mode, medication , and intervention or surgical procedure if necessary.

1.2.7.1 Lifestyle mode

-Diet and nutrient: a healthy diet includes lowering in saturated fats, cholesterol, salts, and rich with fruits ,vegetables ,and grains (Gaudel *et al*., 2021).

-Exercises: when physical activity is regular may help to reduce risk of blood pressure, and mange the body weight, then it improves cardiovascular health (Winzer *et al*., 2018)

-Smoking cessation: limiting smoking is one of the most important recommendation to prevent progression of CAD.

-Psychological health: stress reduction therapy and exercise can be beneficial in management of CAD .

(Eckle *et al* ., 2014 ; Elkoustaf *et al* .,2019)

1.2.7.2 Medication

-Anti platelets agents: Anticoagulants, or blood thinners that reduce blood clotting, are used to treat certain blood vessel, heart, and heart rhythm conditions. These medications aid in the prevention of harmful blood clots from forming in the blood vessels or heart, as well as the prevention of clots from growing larger and causing more serious problems (Volpe and Gallo, 2020).

-statin: (atorvastatin ,rosuvastatin) lowering LDL cholesterol and having activity of anti inflammatory reduce plaque formation in arteries ,moreover, reduce CAD progression (Diamantis, 2017)

-Beta –blockers: these medications reduce palpitation, and blood pressure, decreasing hearts oxygen demand that is may qualify chest pain (angina) (Mancia *et al*.,2022).

-Calcium channel blockers: are medication that prevent calcium from entering the cells of the arteries and heart .This medication has a potential to reduce the heart pumping strength while also relaxing the blood vessels (Cholack *et al* .,2021).

-Angiotensin converting enzyme (ACE) inhibitors: work by decreasing levels of hormones that regulate blood pressure. This allows blood to flow more easily throughout the body (Maghiar *et al*., 2020).

1.2.7.3 Interventional procedures

A percutaneous coronary intervention (PCI) is a minimally invasive procedure to open blocked coronary arteries (Batra *et al*., 2022). In cardiology, they have been in use for more than a decade for surgeries like mitral valve repair ,coronary artery bypass graft and septal defect closure (Kandaswamy and Zuo ,2017).

Coronary artery by pass grafting (CABG): is a surgical procedures used to treat coronary heart disease when medication and percutaneous coronary intervention fail to provide optimal results (Ohmes *et al.,* 2017).

Two techniques for performing CABG

- On-pump CABG :- performed using a cardiopulmonary bypass machine that temporarily takes over the function of heart and lung during the surgery.
- Off – pump CABG :- performed without using a cardiopulmonary bypass machine ,allowing the heart to continue beating during the procedure (Sebayang ,2023).

1.2.8 Role of IL32 as immune marker in coronary artery diseases

Interleukin-32 (IL-32) is a pro-inflammatory cytokine that was first identified in 1992 as a novel human gene encoding a 27-KD protein expressed in activated natural killer (NK) cells and T cells in humans and was named NK4 (Dahl *et al* .,1992). In 2005, Kim et al found that the product of NK4 possesses the characteristics of a pro-inflammatory cytokine, and accordingly, the name was changed to IL-32. Formerly named natural killer cell transcript 4 (NK4), is constitutively produced by peripheral blood mononuclear (PBMC), epithelial and endothelial cells. It is also detected in many nonimmune cells, including epithelial cells,

endothelial cells, mesenchymal stromal cells, fibroblasts, and hepatocyte (Zhang *et al* .,2016).

Additionally, melanoma cells, colon cancer cells, pancreatic and thyroid cancer tissues and cell lines, and other malignant cells have IL-32 expression. Studies using single cell sequencing have shown that Tregs are crucial IL-32 suppliers in a number of diseases (Zhavidij *et al* .,2020). IL-32 consists of eight splice variants, however, Interleukin-32 (IL-32) is a newly discovered inflammatory cytokine with eight isoforms in most mammals, including IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, IL-32η, IL-32θ, and IL-32 s .It is widely distributed throughout the body, and its expression can be observed both in immune and non-immune cells (Yang *et al*.,2019).

An anti-inflammatory responses and protective roles of IL32 were also observed in liver fibrosis and , lipopolysaccharide-induced arthritis and airway inflammation in a mouse model of asthma, It modulates the immune response against diverse pathogens like Leishmania (Santos *et al*., 2019). IL32 has been associated with cancers of inflammatory nature too. It also plays an important role in chronic inflammatory diseases like RA, lung and airway disease like COPD (Gautam and Pandit ,2021).

Only the IL-32α, IL-32β and IL32γ isoforms have been extensively studied (Damen *et al*., 2017). An abundance of IL-32α is found in hematopoietic cells; whereas IL-32β and IL-32γ are the major isoform in endothelial cells and are the most active isoforms, respectively (Kim *et al*., 2005). The production of IL-32 is triggered in response to viral infections such HIV, influenza A, EBV, human papillomavirus (HPV), and HSV as well as bacterial illnesses brought on by *Helicobacter pylori* and

Mycobacterium tuberculosis (MTB). (Smith *et al.*, 2011; Lia *et al*., 2015; Alehagen *et al*., 2020).

Overexpression of IL-32 has been reported in rheumatoid arthritis (RA) and Crohn's disease , as well as, in human symptomatic atherosclerotic plaques, compared to asymptomatic individuals (Heinhuis *et al*., 2015 ; Albuquerque *et al*., 2021). Although the precise explanation for this apparent discrepancy in the activity of IL-32 remains unknown, it may be due to differences in inflammatory regulators between species and/or diseases. It has been detected in human endothelial cells of atherosclerotic plaques, and different isoforms have been demonstrated to exhibit distinct functional roles (Yang *et al*., 2019).

Furthermore, through IL-1β and other pro-inflammatory cytokines, IL-32 controls the activity of endothelial cells in the aortic, coronary, and pulmonary circulations (Xu *et al* .,2017).

On the other handSARS-COV-2 viral challenge may cause IL-32 to be activated in local macro- and micro-vessels through the ACE2-spike protein pathway. IL-32 might help reduce inflammation locally as well as systemically (Linder *et al* .,2020), which probably fails in patients with severe COVID-19 but may work in those with moderate COVID-19. Particularly in the more vulnerable COVID-19 patients, this would lead to the infarction of key organs, such as the kidney, lung, and heart (Law *et al* .,2021).

The possible protective effect of interleukin-32 (IL-32) in *Mycobacterium tuberculosis* infection has been indicated. However, few studies have been focused on IL-32 in tuberculosis patients. Additionally, the production, regulation, and role of IL-32 in tuberculous pleurisy were investigated. It is found that the content of IL-32 in tuberculous pleural

effusion was higher than the level in the malignant pleural effusion and transudative pleural effusion (Guatam *et al* ., 2024).

Recent data points to IL-32 as being essential for both the pathophysiology of chronic inflammation and the host's defense against infections. Numerous autoimmune conditions, including rheumatoid arthritis and inflammatory bowel disorders, have been associated with abnormal IL-32 expression. Additionally, a recent study revealed the role of IL-32 in the etiology of type 1 diabetes. (Albuquerque *et al* ., 2020).

1.2.9. Role of *IL32* gene polymorphism in coronary artery diseases

Immune responses demand the rapid and precise regulation of gene protein expression. Splicing is a crucial step in this process; ~95% of protein-coding gene transcripts are spliced during mRNA maturation. Alternative splicing allows for distinct functional regulation, as it can affect transcript degradation and can lead to alternative functional protein isoforms (Van Haaren *et al* .,2024). There is increasing evidence that splicing can directly regulate immune responses. For several genes, immune cells display dramatic changes in isoform-level transcript expression patterns upon activation (Su and Huang, 2021).

Interleukin-32 is a novel cytokine whose presence has been confirmed in most of the mammals except rodents (Kim *et al*.,2005). The IL-32 gene was identified on human chromosome 16 p13.3.The gene has eight exons and nine splice variants, namely, IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, IL-32η, IL-32θ, and IL-32s (Kang *et al* .,2014). The biological activity of IL-32 varies according to the isoforms with IL-32 γ and IL-32 θ having the most potent biological activity (Choi *et al* .,2009 ; Shim *et al* .,2022).

IL-32γ, IL-32α, and IL-32β are the most researched variants. Despite having fewer studies, the most prevalent variation is IL-32β. The lack of a particular antibody to detect IL-32 variants makes it rather difficult to identify them at the protein level. Using variant-specific primers instead of protein-level analysis, the expression pattern and regulation of IL-32 variants may be determined (Sohn *et al* .,2019).

The second shortest version, IL-32ε, has a 148 amino acid sequence that includes protein domains 2, 7, and 10. Compared to the protein domain 1 of other versions, the first domain of IL-32ε contains four more amino acid residues. IL-32ε has a different translation beginning codon because of mRNA splicing, and its protein domain 7 is the shortest, with four amino acid residues. It also has domain 8, just like IL-32β. Thus, IL-32δ's biological action may resemble that of the IL-32β version. The shortest IL-32α variation is most similar to the IL-32γ version. However, The range of IL-32β's biological activity is between IL-32α and IL-32γ (Choi *et al* .,2009).

IL-32θ is the third shortest form, consisting of 168 amino acid residues. In IL-32θ, protein domains 1, 5, and 10 are present. The presence of domain 10 in IL-32ε suggests that IL-32θ and IL-32ε share a similar biological action. Within cells, the isoforms can interact with one another to regulate their individual functions (Kang *et al* .,2014).

Three single nucleotide polymorphisms (SNPs) (rs28372698, rs12934561, rs4786370) of the IL32 gene have been proposed as modifiers for different diseases (Shamoun *et al* .,2018). Damen *et al* ,reported a possible protective role against cardiovascular disease by the variant rs4786370 in the gene of IL-32. Thus, previous reports indicate that polymorphisms in the gene of IL-32 are important in disease development (Damen *et al* .,2017). It was found to induce the expression of various inflammatory cytokines including TNF-α, IL-6, and IL-1β as well as

macrophage inflammatory protein-2 (MIP-2) and has been reported previously to be involved in the pathogenesis and progression of a number of inflammatory disorders, namely, inflammatory bowel disease (IBD), gastric inflammation and cancer, rheumatoid arthritis, and chronic obstructive pulmonary disease (COPD)(Khawar *et al* .,2016).

Since IL-32 transcripts were originally found in activated T-cells or NK cells it has important functions in pathogen infections-associated with Th1-mediated immune response (Dahl *et al* .,1992). The roles of IL-32 against viral and intracellular bacterial infections have been studied by different research groups (Bai *et al* ., 2010; Bai *et al* .,2011; Schenk *et al* .,2012; Semango *et al* .,2018; Akusum *et al* .,2018). IL-32 is involved in immune cell differentiation and proliferation during pathogen infections. Th1-mediated immune responses after viral and intracellular bacterial pathogen infections activate T or NK cells producing IL-32 variants. The immune cells-produced IL-32 variants mainly act on myeloid type immune cells (Jeong ,*et al* .,2014), due to expression of proteinase 3 (PR3), whereas lymphoid cells do not respond to IL-32 .PR3 is known as one of neutrophil serine proteinase expressed in monocyte, but not expressed in lymphoid cells. PR3 binds to IL-32 and modulate IL-32 activity. PR3 is the only molecule that was confirmed as an IL-32 interacting cell surface molecule with biochemical data. This data support that IL-32 exhibits biological activity with only myeloid cells in inducing inflammatory cytokines ((Lee *et al* .,2017).

Several studies have shown an association between IL32 gene polymorphisms and several diseases. The intronic SNP rs12934561 has been reported as a susceptibility gene for acute lung injury (Arcaroli *et al.*,2011) and endometrial cancer (Yu *et al*.,2015). An association of IL32 promoter SNP rs28372698, giving a higher expression of IL32γ has been

found with thyroid carcinoma (Plantinga *et al* .,2013). Furthermore, another study demonstrated that a genetic variant of this SNP is associated with an increased risk of gastric cancer (Gonzalez *et al* .,2014). IL32 promoter SNP rs4786370 has been suggested to have a possible protective role against cardiovascular disease (Damen *et al* .,2017).

Individuals who suffer from rheumatoid arthritis (RA) are more likely to acquire cardiovascular diseases (CVD). Atherosclerosis development may be related to interleukin (IL)-32, which has been demonstrated to be implicated in the pathophysiology of RA (Kerola *et al* .,2021). However, the In people at higher risk for CVD (plaque positive), the CC-genotype was linked to higher levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDLc).A functional effect of a promoter single-nucleotide polymorphism (SNP) in IL32 on lipid profiles in RA patients and individuals, suggesting a possible protective role of this SNP against CVD (Damen *et al* .,2017).

Genome-wide association studies (GWAS) documented that variations in multiple genes especially inflammatory-related factors including PPARα, interleukin 18 (IL-18), IL-1β, SIRT2, and CD14 receptor were closely related to the susceptibility of CAD (Luo *et al* .,2021).

Chapter Two

Materials

&

Methods

2. Subjects, Materials and Methods

2.1. Subjects

The sample size includes (100) participants , were previously clinically diagnosed by doctors, (50) as a patient group with coronary artery diseases , and (50) as a healthy control. The samples were taken from both sexs (male & female), they were (28) females patients ,and the males patients were (22). whose ages range from (40 – 70) years old .attending to Shaheed al muhrab center for cardiac catheterization and surgery & imam al-sadiq hospital in Babylon city /Iraq. During the period extending from October (2024) to January (2025).A healthy control includes (24) females ,and (26) males with their ages ranging between (40-70). Case information sheets involving age ,sex ,body mass ,and others were carried out from each patient. Each patient and healthy control was questioned about demographic parameters (Appendix-1).

Inclusion criteria include :

All patients with coronary artery disease were diagnosed based on clinical symptoms and other investigations .

Exclusion criteria include :

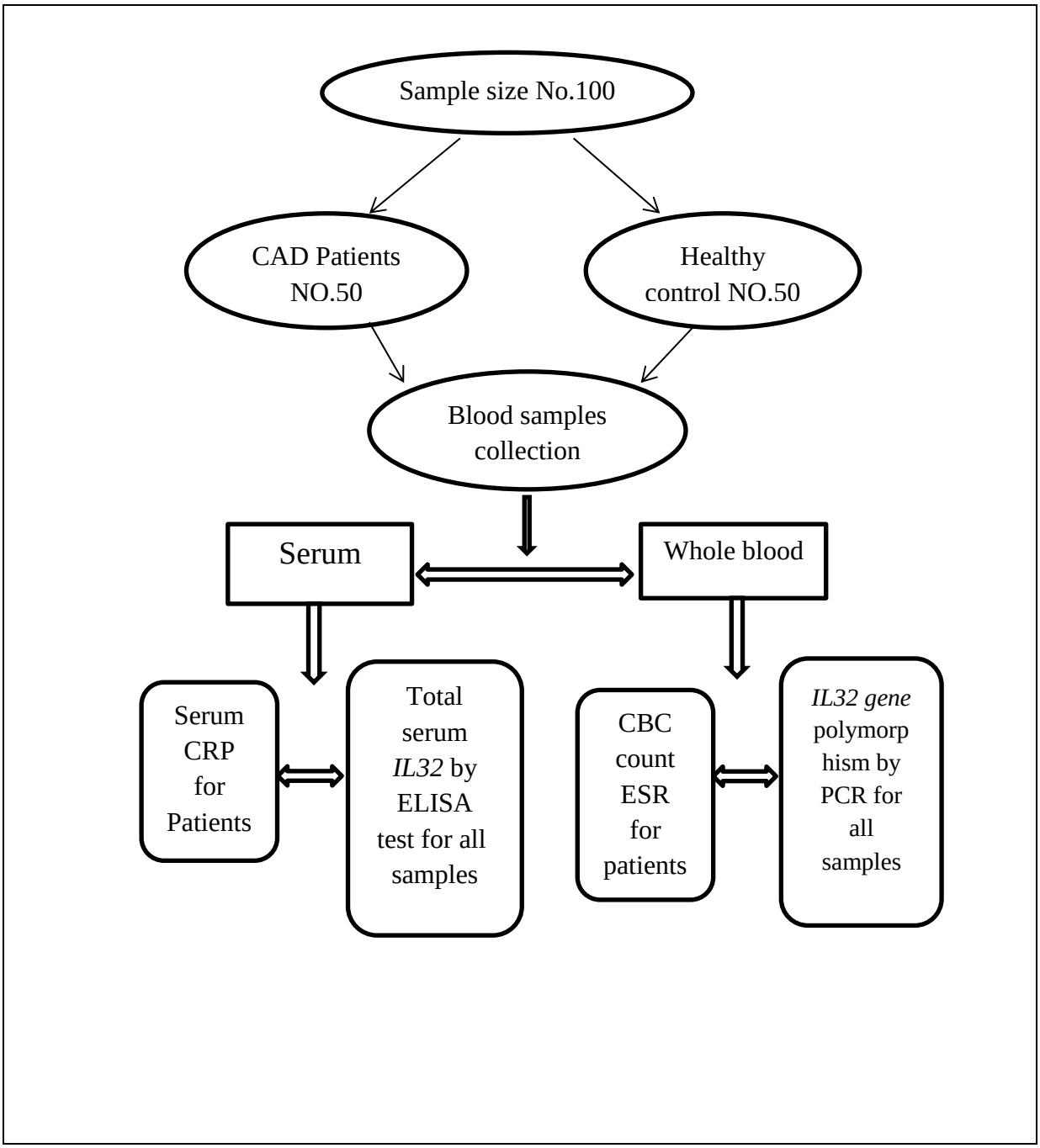
Patients who have pneumonia ,cancer ,parasitic infection , kidney disease, hepatic disease and autoimmune diseases were excluded.

2.1.1. Ethical approval

An ethical certificate was obtained from the relevant committees at the University of Kerbala, No. 24-46, dated (2024/9/29). Approval was obtained from the Babylon Health Directorate to complete the study, No. 107, dated (2024/12/29). Additionally, verbal approval was taken from all the patients or any family members, before taking the sample. During sample collection, health measures and safety were taken.

2.1.2 Study design

Case- control study.



Figure(2-1): Scheme of study design

2.2. Materials

2.2.1. Laboratory Equipment and instrument

The main instruments and Equipment that used in this study were displayed in table (2-1).

Table (2-1): Laboratory Equipment and Instrument.

NO.	Laboratory Equipment and Instrument	Company	Source
1	Autoclave	Hirayama	Japan
2	Biological safety cabinet	EuroClone Safemate	Italy
3	Centrifuge	Bio base	China
4	Conventional PCR system	Bio base	China
5	Cool box	Alfowz	Iran
6	Cooling centrifuge	Bio base	China
7	Deep freezer	Hitachi	Japan
8	EDTA tube	ALS	China
9	Electric oven	Samsung	Korea
10	ELISA Devices (washer & reader)	Biotic	USA
11	ELISA printer	Epson	Japan
12	Eppendorf tube	ALS	China
13	Flasks (different size)	Jlassco	India
14	Gel electrophoresis	Bio base	China
15	Incubator	Bio base	China
16	Micropipette set	SLAMED	Germany
17	Multichannel micropipette set	SLAMED	Germany
18	Pipette tip	ALS	China
19	Refrigerator	Panasonic	Korea

20	UV Transilluminator	Bio base	China
21	Vortex	Bio base	China

2.2.2. Chemical and Biological Material

The main chemicals that are used in this study were given in Table (2-2).

Table (2-2): Chemical and Biological Materials with their Manufacturing Company and Country of Origin .

NO.	Chemicals and Biological Materials	Company	country
1	Absolute Ethanol	Bioneer	Korea
2	Agarose	Conda	Aspain
3	Ethanol 70%	MIRNIA	Iraq
4	Ethidium bromide	Bioneer	Korea
5	distiller water	GFL	Germany

2.2.3. ELISA Kit Used in the Study

ELISA kit that used in this study was demonstrated in Table (2-3).

Table (2-3): ELISA Kits used in The Study

ELISA Kit	Manufacturing company	Country
Human IL32 ELISA Kit Catalogue number:SL0994Hu	SUNLONG®	China

2.2.3.1. ELISA Kit Content of Human IL32

The kit of ELISA contents are presented in Table (2-4).

Table (2-4): Kit Materials provided and Storage of IL32

NO.	Materials	96 determinations	storage
1	Microelisa strip plate	1	2-8 C
2	Standard: 270 pg/ml	0.5 ml x 1 bottle	2-8 C
3	Standard diluent	1.5 ml x 1 bottle	2-8 C
4	HRP-conjugate reagent	6 ml x 1 bottle	2-8 C
5	Sample diluent	6 ml x 1 bottle	2-8 C
6	Chromogen solution A	6 ml x 1 bottle	2-8 C
7	Chromogen solution B	6 ml x 1 bottle	2-8 C
8	Stop solution	6 ml x 1 bottle	2-8 C
9	Wash solution	20 ml(30X) x 1 bottle	2-8 C

2.2.4. DNA Amplifications Materials

2.2.4.1. DNA Extraction Kit

Table (2-5) showed contents of DNA extraction kit.

Table (2-5): Kit Components of DNA Extraction

NO.	DNA Extraction Kit	Company	Country
1	Proteinase K(20mg/ml)	Add bio	Korea
2	Spin column (100pcs)	Add bio	Korea
3	Binding buffer (25ml)	Add bio	Korea
4	Washing buffer 1	Add bio	Korea
5	Washing buffer 2	Add bio	Korea
6	Elution(25ml)	Add bio	Korea
7	2 ml collection tube 100 pcs	Add bio	Korea

2.2.4.2. Polymerase Chain Reaction Materials

The contents of PCR materials were listed in Table (2-6).

Table (2-6): The PCR Materials

NO.	PCR Materials	Company	Country
1	PCR PreMix	Bioneer	Korea
2	100-1500 bp DNA ladder	Bioneer	Korea
3	Free nuclease water	Bioneer	Korea
4	TBE (Tris-Borate EDTA) Buffer	Bioneer	Korea

2.2.4.3. AccuPower PreMix

The contents of the PreMix used in PCR are given in Table (2-7).

Table (2-7): The contents of the PreMix used in PCR with their manufacturing company and country of origin.

NO.	PCR PreMix compositions	Company	Country
1	Taq DNA polymerase	Bioneer	Korea
2	dNTPs (dATP, dCTP, dGTP dTTP)	Bioneer	Korea
3	Reaction buffer with 1.5mM MgCL2	Bioneer	Korea
4	Stabilizer and tracking dye	Bioneer	Korea

2.2.4.4. Primer

Primer design according to nucleotide sequence gene bank website by using primer blast https://www.ncbi.nlm.nih.gov/tools/primer-blast/. The following primers were used in this study to identify the *IL32 gene* polymorphism listed in Table (2-8).

Table (2-8) primers sequences of *IL32 gene* polymorphism used in this study.

NO.	Primer name	Primer sequences (5´-3´)	Product size (bp)
1	Common F (reverse)	5'-TCAGGAGAATTGCTTGAACCCAGG -3'	414
2	R-T allele(T)	5'-CAGCTAAAAGTCATGCTTTAGGCTTCA -3'	
3	R-C allele (C)	5'-CAGCTAAAAGTCATGCTTTAGGCTTCG -3'	

2.3.Methods

2.3.1. Sample Collection

Blood samples of 5 ml were drawn from the veins of 100 subjects (50 patients and 50 controls) using a disposable syringe and sterile technique. 3 ml of the blood was distributed into two EDTA tubes (each tube containing 1.5 ml of blood) for hematological and molecular testing and stored at -20°C for PCR to determine the presence or absence of *IL32 gene* polymorphism in the blood samples studied.

The 2 ml of the remaining blood was allowed to clot after that serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum was collected in Eppendorf tube and stored at -20°C for ELISA test to determine the concentration of IL32 in the samples.

2.3.2 Complete Blood Count

The blood specimen in EDTA tube was shaken up then was examined as soon as possible in Sysmex XN-350 five differential automated hematology analyzers (Sysmex, Japan) to count white blood cells.

2.3.3. Sterilizing Method

2.3.3.1. Ethanol 70%

The outer surface of the workbench and some study tools were treated with 70% ethanol.

2.3.3.2. Autoclave (moist heating)

The tools was sterilized using moist heat sterilization at a temperature of 121 ^{o}C and under pressure of 1.5 bar for 20 minutes .

2.3.4. Preparation of Tris-Borate-EDTA Buffer

This solution was prepared according to the manufacturing company (MarLiJu/Korea) by Dilution of the solution from 10x to 1x by adding 100 ml of tris-borate-EDTA buffer (10x TBE) prepared by MarLiJu/Korea to 900 ml of sterile distilled water and kept at room temperature .

2.3.5. Estimation of IL32 Kit

2.3.5.1. Test Principle of IL32

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to IL32. Standards or samples are added to the appropriate Microelisa stripplate wells were combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody

specific for IL32 is added to each Microelisa stripplate well and incubated. Free components are washed away.

The TMB substrate solution is added to each well, only those wells that contain IL32 and HRP conjugated IL32 antibody appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of IL32. The concentration of IL32 in the samples can be calculated by comparing the OD of the samples to the standard curve.

2.3.5.2. Reagent Preparation

Before using, all reagents were brought to room temperature, to make a 180 pg/ml standard stock solution, 300ul of the standard was combined with 150ul of standard diluent. Before producing dilutions, the standard was let set for 15 minutes with gentle agitation. Duplicate standard points were prepared by diluting the standard stock solution (270ng/ml) 1:5 with standard diluent to obtain solutions of 180pg/ml, 120 pg/ml, 60 pg/ml, and 30 pg/ml. The final standard was standard diluent (15pg/ml). Any leftover solution should be frozen at -20°C and used within one month of freezing . table(2-9) were suggested dilutions of standard solutions:

Table (2-9): steps dilution method for ELISA kit.

180 pg/ml	Standard No.1	300ul original standard +150ul standard diluents
120 pg/ml	Standard No.2	300ul original standard +150ul standard diluents
60 pg/ml	Standard No.3	150ul original standard +150ul standard diluents
30 pg/ml	Standard No.4	50ul original standard +150ul standard diluents
15 pg/ml	Standard No.5	50ul original standard +150ul standard diluents

Dilution method:

Five tubes were taken and each filled with 150 µl of the standard diluent, then 300 µl of the standard solution added to the first tube and

mixed it with vortex to make a working solution at a dilution rate 1:5, then the information in the attached user manual was followed by withdrew 300 µl from the first tube and transferred it to the second tube and mixed it as well to make a 2 working solution, then withdrew 150 µl from the second tube and pumped it into the third tube and mixed it as well to make a working solution at a dilution rate of 1.5 and so on… to the fifth tube and kept another tube to be considered the blank.

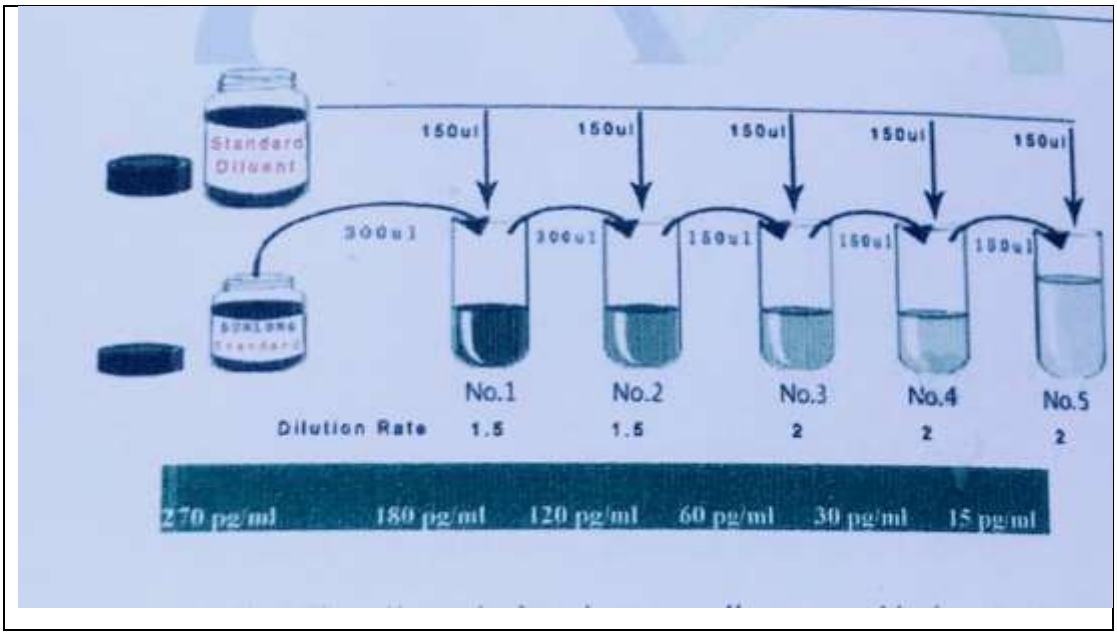


Figure (2-2): steps of the Duplication Dilution

2.3.5.3. Assay Procedure :

1. All reagents, standard solutions, and samples had been prepared as instructed. The reagents had been brought to room temperature before use, and the assay had been performed at room temperature.
2. The required number of strips for the assay had been determined. The strips had been inserted into the frames for use, while the unused strips had been stored at 2-8°C.
3. Dilution of Standards: The standard solutions had been diluted using small tubes first, after which a volume of 50 µl from each tube had been pipetted into the microplate wells.

4. In the Microelisa strip plate, a well had been left empty as a blank control. In the sample wells, 40 µl of sample dilution buffer and 10 µl of the sample had been added (dilution factor of 5). The samples had been loaded onto the bottom of the wells without touching the well walls and had been mixed well with gentle shaking.
5. Incubation: The plate had been incubated for 30 minutes at 37°C after being sealed with a Closure plate membrane.
6. Dilution: The concentrated washing buffer had been diluted with distilled water (30 times for 96T and 20 times for 48T). Specifically, 10 mL of wash buffer had been diluted with 300 mL of deionized or distilled water to prepare 310 mL of Wash Buffer.
7. Washing: The Closure plate membrane had been carefully peeled off, and the wells had been aspirated and refilled with the wash solution. The wash solution had been discarded after resting for 30 seconds, and the washing procedure had been repeated five times.
8. A volume of 50 µl HRP-Conjugate reagent had been added to each well except for the blank control well.
9. Incubation: The plate had been incubated again for 30 minutes at 37°C after being sealed with a Closure plate membrane.
10. Washing: The Closure plate membrane had been carefully peeled off, and the wells had been aspirated and refilled with the wash solution. The wash solution had been discarded after resting for 30 seconds, and the washing procedure had been repeated five times.
11. Coloring: A volume of 50 µl of Chromogen Solution A and 50 µl of Chromogen Solution B had been added to each well, followed by gentle shaking. The plate had then been incubated at 37°C for 15 minutes while being protected from light.

12. Termination: A volume of 50 µl stop solution had been added to each well to terminate the reaction, causing the color in the wells to change from blue to yellow.
13. The absorbance O.D. at 450 nm had been measured using a Microtiter Plate Reader. The OD value of the blank control well had been set to zero. The assay had been carried out within 15 minutes after adding the stop solution.

2.3.5.4. Calculation of Results

Known concentrations of Human IL32 Standard and its corresponding OD reading had been plotted on the log scale (x-axis) and the log scale (y-axis), respectively. The concentration of IL32 in the sample had been determined by plotting the sample's O.D. on the Y-axis. The original concentration had been calculated by multiplying the dilution factor.

2.3.6. PCR Procedure

2.3.6.1. DNA Extraction Procedure

The DNA extraction included the following steps

1. 20 µl of Proteinase K buffer (20 mg/ml) have been added to a 1.5 ml micro-centrifuge tube.
2. 200 µl of whole blood sample have been transferred to the 1.5 ml micro-centrifuge tube with Proteinase K Solution: If the sample volume had been less than 200 µl, the appropriate volume of PBS should be added.
3. 200 µl of Binding Buffer have been added to the sample tube, and mixed well by pulse-vortexing for 15 sec.
4. Then incubated at 56°C for 10 min: Longer incubation times had had no effect on the yield or quality of the purified DNA.

5. 200 µl of absolute ethanol added and had mixed well by pulse-vortexing for 15 sec: After this step, a brief spin-down had been performed to get the drops clinging under the lid.
6. Carefully the lysate transferred into the upper reservoir of the spin column with a 2.0 ml collection tube without wetting the rim.
7. Then centrifuged at 13,000 rpm for 1 min: The flow-through have been poured off, and the spin column had been assembled with the 2.0 ml collection tube.
8. 500 µl of Washing Buffer 1 have been added to the spin column with the collection tube and had centrifuged at 13,000 rpm for 1 min: The flow-through had been poured off, and the spin column had been assembled with the 2.0 ml collection tube.
9. 500 µl of Washing Buffer 2 have been added to the spin column with the collection tube and had centrifuged at 13,000 rpm for 1 min: The flow-through had been poured off, and the spin column had been assembled with the 2.0 ml collection tube.
10. The spin column had dried by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in the spin column.
11. The spin column have been transferred to a new 1.5 ml micro-centrifuge tube.
12. 120 µl of Elution Buffer have been added to the spin column with the micro-centrifuge tube, and had let it stand for at least 1 min.
13. The genomic DNA had eluted by centrifugation at 13,000 rpm for 1 min. The eluted DNA had been stored at -20 °C until used for PCR.

2.3.6.2. Primer Preparation

The primers were prepared according to the manufacturer's instructions to form a stock solution of the desired concentration of 100 pmol/µl by dissolving of The lyophilized primers with 250 ml of deionized water. Then the working solution was prepared by dissolved 10 µl of 100

pmol/µl with 90 µl of deionized distilled water to form 100 µl of 10 pmol/ µl .

2.3.6.3. Polymerase Chain Reaction (PCR) Mixture

Polymerase Chain Reaction Mixture were list in Table (2-10).

Table (2-10) Polymerase Chain Reaction Mixture.

NO.	PCR Mixture	Volume (µl)
1	PreMix	5
2	Common F (reverse)	2
3	R-T allele(T)	1
4	R-C allele (C)	1
5	Template DNA	3
6	Nuclease _free water	13
7	Total volume	25µl

2.3.6.4. Polymerase Chain Reaction Conditions by Allele specific PCR

To amplify a target DNA, specific primer pairs and the conventional PCR were used. The Allele specific PCR method used for producing a PCR product. Denaturation, annealing, and elongation were the three stages that comprise the process typical and they repeated cycle after cycle (amplicon). The conditions for the PCR were listed in Table (2-11).

Table(2-11):PCR condition for Allele specific for Amplification of *IL32*gene

NO.	Step	Temperature	Time	Number of cycles
1	Initial Denaturation	95°C	5 min	1
2	Denaturation	95°C	Sec 20	30-35 cycles
3	Annealing	60°C	Sec 20	30-35 cycles

4	Extension	72°C	0.5-1 min	30-35 cycles
5	Final extension	72°C	3-5 min	1
6	Hold	4 °C	∞	

2.3.6.5. Agarose Gel Electrophoresis

2.3.6.5.1. Preparation of Agarose Gel:

Agarose gel was prepared by adding agarose powder (2%) to 1X TBE buffer previously prepared in percent specific for each PCR products. The mixture was placed in boiling water bath until it become clear, allowed to cool to 50°C, and 2 µl ethidium bromide at concentration of 0.5 mg/ml was added. The agarose poured kindly in equilibrated gel tray earlier set with its comb. The agarose allowed to solidify at room temperature for 30 minutes. The comb made wells used for loading DNA samples.

2.3.6.5.2. Agarose Gel Electrophoresis:

The amplified PCR products were detected by agarose gel electrophoresis and visualized by staining with ethidium bromide. PCR products were loaded to the agarose gel wells. 5µl from single product to single well in known sequence, followed by suitable ladder to one of the wells in the row. The gel tray was fixed in electrophoresis chamber. 1X TBE buffer was added to the chamber until covered the surface of the gel. The electric current was performed at 50 volt for 30 minute . Ultraviolet transilluminator was used for the observation of DNA bands

2.3.6.5.3. Electrophoresis Results:

The electrophoresis results were identified using gel documentation system. The base pairs of DNA bands were measured according to the ladder. The positive results were distinguished when there was DNA band equal to the target product size. Finally, the gel was photographed using gel documentation saving picture.

2.4. Statistical analysis

The statistical analysis for current study was done using the Statistical Package for the Social Sciences software, version 26 (IBM, SPSS, Chicago, Illinois, USA), and Microsoft Excel 2010 program. Descriptive statistics has been done to all participants of each group. Data was analyzed for means, and the standard deviation was computed for the continuous variables, whereas frequency was used for computing the qualitative data. The mean of the investigated parameters were compared between the two groups using t-Test; Chi-Square test was applied to compare between percentages; Differences among groups were analyzed using one-way ANOVA analysis of variance. To detect the specific groups that differ, the LSD test has been performed. The statistical analysis of Logistic Regression has been applied to provide the p-value, odds ratio, and confidence interval in the context of analyze genotypes frequency. A receiver operating characteristic (ROC) curve has been applied to assess the diagnostic ability of IL-32 as a biomarker to distinguish between CAD cases and control, and to determine whether IL-32 can serve as a predictive factor for CAD. The Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant (Duncan *et al*., 1983; Basher, 2003).

Chapter Three

Results

3. Results

3.1 Demographic data for the study population

Demographic data for the study population are illustrated in Table (3-1). The results of statistical analysis showed non-significant ($p>0.05$) differences regarding age, age groups, sex, and economic status. Regarding economic status, the statistical analysis revealed a significant differences within-patients ($p=0.0001$) and within-control ($p=0.0015$) groups comparison, where the higher percent of both groups were found in the Middle class. With respect to habitat status, the higher percent (46%) of patients were resided in urban region, while higher (54%) percent of controls were resided in sub-urban region, with significant differences ($p=0.0128$ and 0.0001, respectively) within and between patients and controls groups. As for Education status, the results of the statistical analysis showed that the percentages of patients and controls were significantly ($p= 0.0002$) lower in Graduate or above group. Regarding to distribution of patients according to family history, it is found that 40% of CAD patients had a family history with cardiac disease, with significant ($p=0.0455$) differences. In the context of smoking status, the majority (70%) of CAD patients were non smokers ($p=0.0001$).

Table (3-1): Demographic data for study population

Parameters	Study population No. (%)		*P value*	
	Patients (n=50)	**Control (n=50)**		
Age (year) Mean ±S.D	56.00 ±9.750	54.84 ± 8.938	.413^{NS}	
Age group	**Patients (n=50)**	**Control (n=50)**	**Total (n=100)**	***P value***
39-49 y	15 (30%)	16 (32%)	31 (31%)	0.7995^{NS}
50-60 y	17 (34%)	17 (34%)	34 (34%)	1.0000^{NS}
61-71 y	18 (36%)	17 (34%)	35 (35%)	0.9042^{NS}
P value	0.7558^{NS}	0.9608^{NS}		
Sex				
Sex	**Patients (n=50)**	**Control (n=50)**	**Total (n=100)**	***P value***	**ODD (CI95%)**
Male	22 (44%)	26 (52%)	48 (48%)	0.2611^{NS}	.725 (.330-1.594)
Female	28 (56%)	24 (48%)	52 (52%)		
Economic status				
Economic status	**Patients (n=50)**	**Control (n=50)**	**Total (n=100)**	***P value***
Higher class	12 (24%)	14 (28%)	26 (26%)	0.5791^{NS}
Middle class	30 (60%)	25 (50%)	55 (55%)	0.3404^{NS}
Lower class	8 (16%)	11 (22%)	19 (19%)	0.3304^{NS}
P value	0.0001*	0.0015*		
Habitat status				
Urban	23 (46%)	11 (22%)	34 (34%)	0.0036*
Sub-urban	16 (32%)	27 (54%)	43 (43%)	0.0177*
Rural	11 (22%)	12 (24%)	23 (23%)	0.7681^{NS}
P value	0.0128*	0.0001*		
Education status				
Graduate or above	7 (14%)	7 (14%)	14 (14%)	1.0000^{NS}
Secondary school or less	22 (44%)	22 (44%)	44 (44%)	1.0000^{NS}
Illiterate	21 (42%)	21 (42%)	42 (42%)	1.0000^{NS}
P value	0.0002*	0.0002*		
Family history of CVD for Patients				
Yes	20 (40%)		50 (100%)	0.0455*
No	30 (60%)			
Smoking status for Patients				
Smoker	**15(30%)**		50 (100%)	0.0001*
Non-smoker	**35(70%)**			

*Significant difference at the 0.05 level by chi-square test, T-test, and Odds ratio.
NS: Non-significant difference

3.2 Distributions of CAD patients and control according to different factors:

3.2.1 Distributions of CAD patients and controls according to BMI categories

Figure (3-1) depicts the means and SD for BMI in patients and controls. The figure shows that BMI was significantly (*p=0.022*) higher in CAD patients (23.08±3.19) compared to controls (22.44±2.17); while the Figure (3-2) displays the distribution of patients and controls according to BMI categories. The percentage of patients and controls was 74% and 86% within normal category, 18% and 14% within overweight category and 8% of patients were obese, while no control individual found in obesity category.

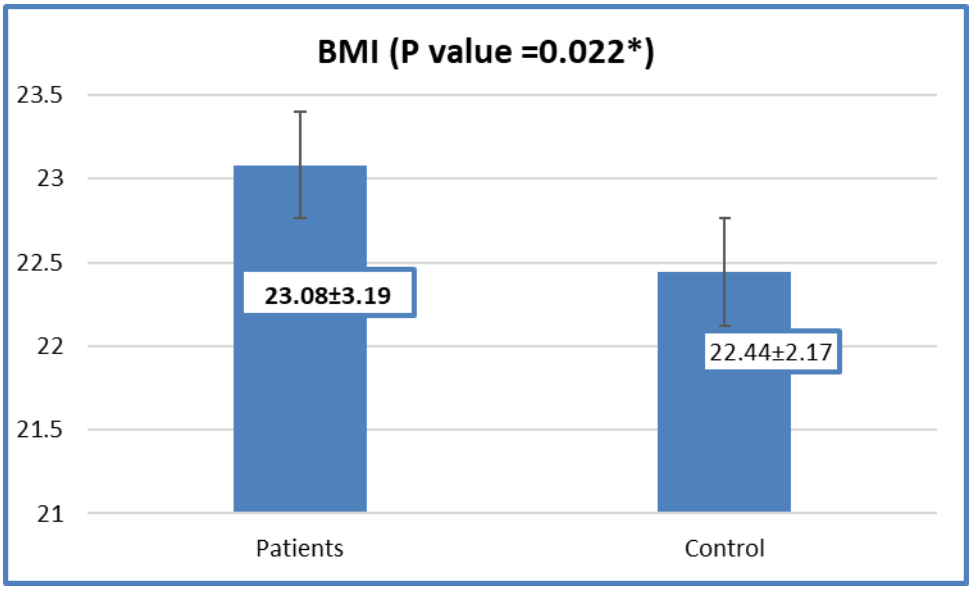


Figure (3-1): BMI value in study population

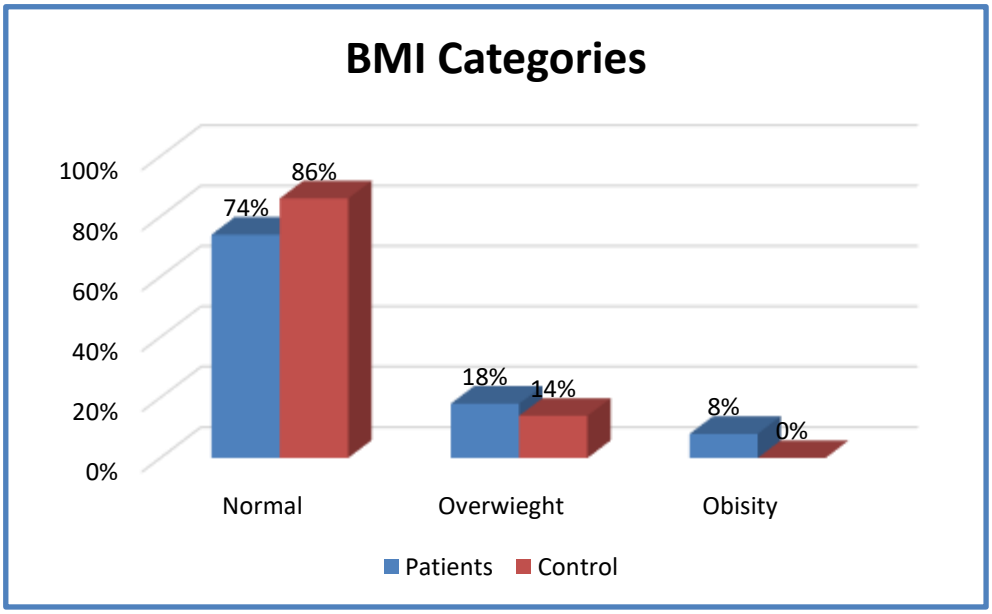


Figure (3-2): Percentage of BMI categories in study population

3.2.2 Distributions of CAD patients according to diet status

Figure (3-3) shows the distribution of patients according to the nature of their diet into two groups: vegetarians and non-vegetarians; where 42% were vegetarians and 52% were non-vegetarians, without significant (p=0.6892) differences between these groups.

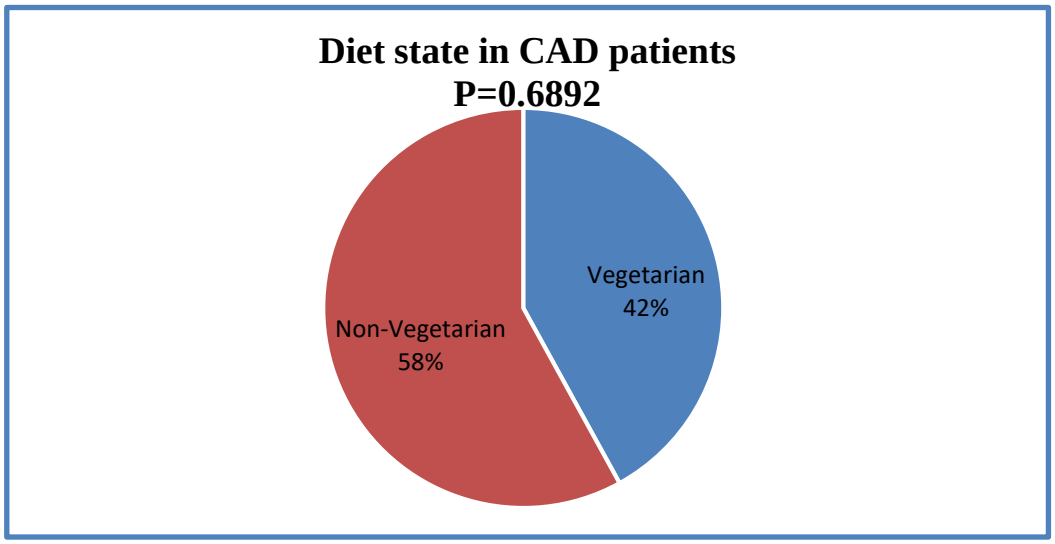
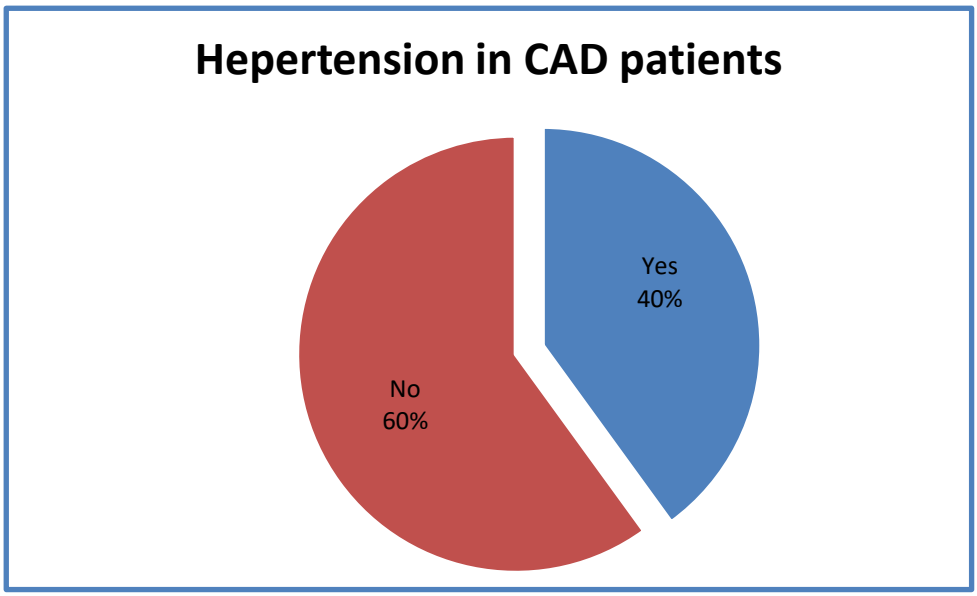


Figure (3-3): Distribution of diet state in CAD patients

3.2.3 Classification of CAD patients according to hypertension Status

Figure (3-4) depicts the distribution of CAD patients depend on the presence or absence of hypertension. The figure exhibited that 40% of patients have hypertension, while 60% of patients don't have hypertension.



Figure(3-4): Hypertension state in patients

3.3 Association between hypertension and some demographic factors

3.3.1 Distribution of hypertension in CAD patients according to age categories

Among hypertensive patients, 40% were in age category of 39-49 year, 29.4% of the hypertensive patients fell within age category of 50-60year. Notably, the highest percentage was found in age category of 61-71 year, with 50% of CAD patients experiencing hypertension. Despite non-significant (*p=0.1486*) differences, these results highlight the varying percentage of hypertension across different age categories, as displayed in Figure (3-5).

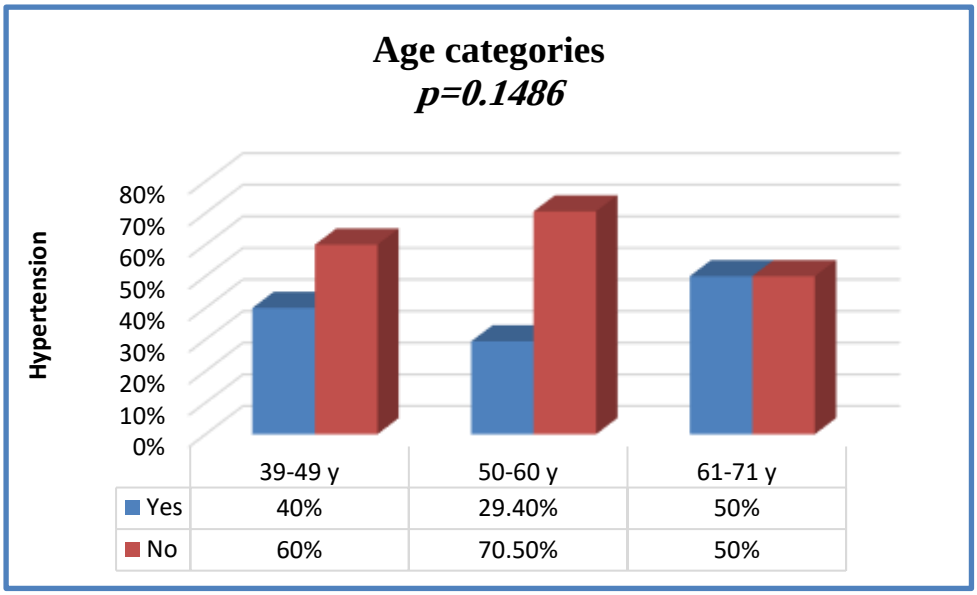


Figure (3-5): Distribution of hypertension condition in patients according to age categories

3.3.2 Distribution of hypertension in CAD patients according to BMI categories

Among CAD patients, 45.90% were in BMI category of normal weight, 22.20% of patients fell within BMI category of overweight. 25% of CAD patients experiencing obesity, with statistical significant (*p= 0.0018)* differences as shown in Figure (3-6).

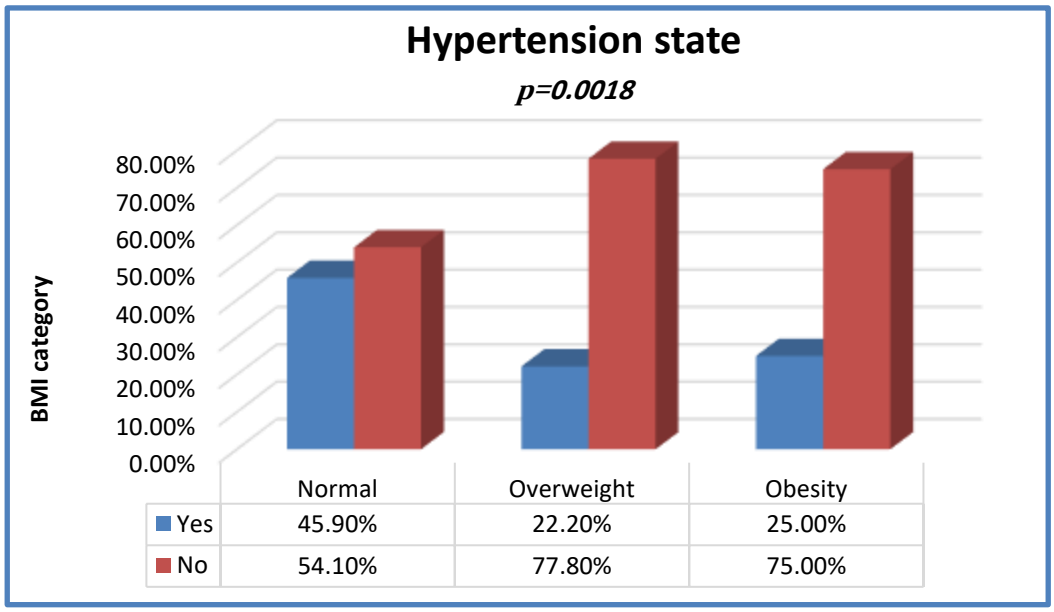


Figure (3-6): Distribution of hypertension condition in patients according to BMI categories

3.3.3 Distribution of hypertension in CAD patients according to diet state

The present study also deals with the distribution of hypertensive CAD patients according to diet state; where 45% of hypertensive were vegetarian, while 55% were non-vegetarian, no significant (*p=0.4756*) differences were recorded as shown in figure (3-7).

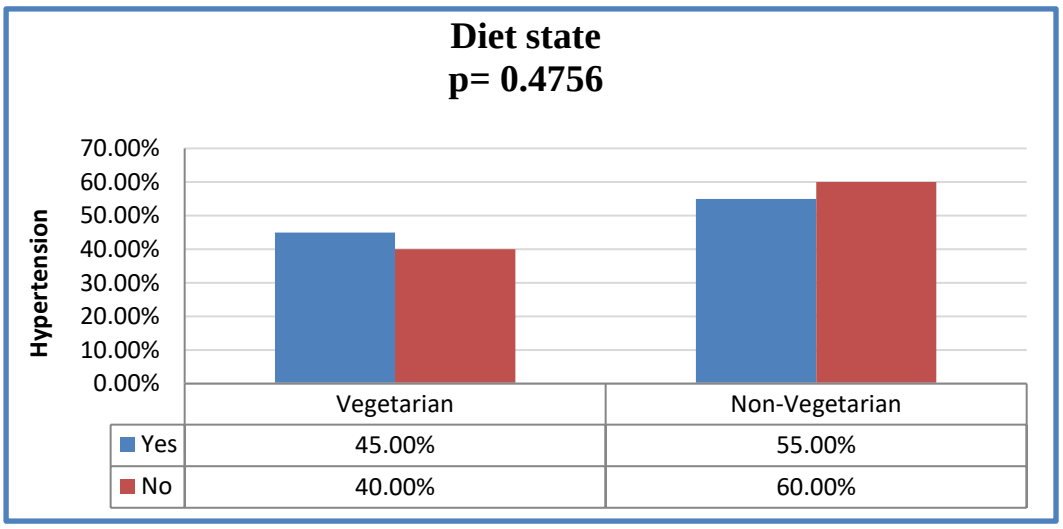


Figure (3-7): Distribution of hypertension state in patients according to diet

3.3.4 Distribution of hypertension in CAD patients according to smoking habit

Figure (3-8) shows the relationship between smoking and pressure in heart patients, where the percentage of smokers, those with pressure and those without was 30%, so no significant differences appeared in this relationship.

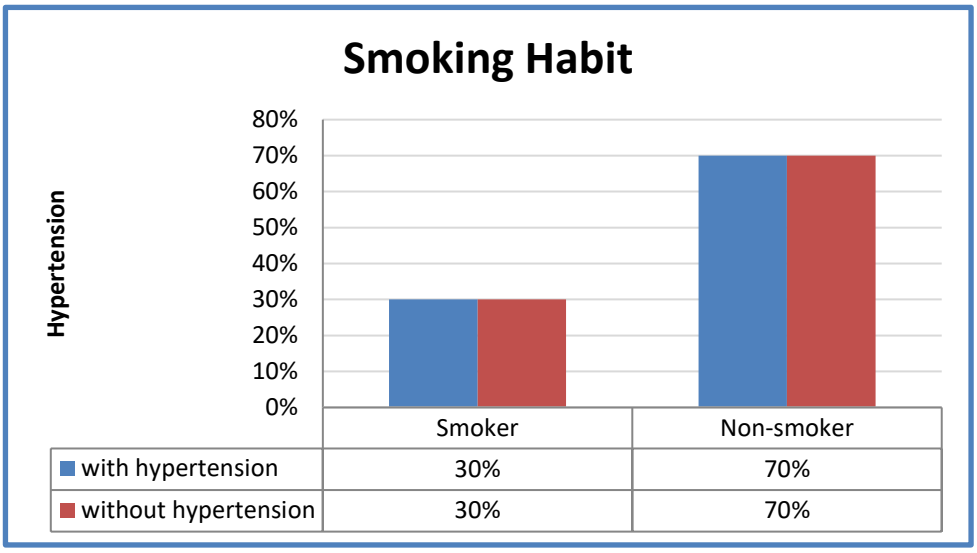


Figure (3-8): Distribution of hypertension state in patients according to smoking habit

3.4 Evaluation of immunological and laboratory markers in study population

3.4.1 Evaluation of Serum levels IL-32 for Study Population

Table (3-2) showed the levels of IL-32 in both CAD patients and control, where the result of statistical analysis revealed a significant (*p=0.004*) elevation in IL-32 levels in CAD patients compared with control individuals.

Table (3-2): Serum levels of IL-32 for study population

Parameters	**Study population Mean ± Sd.**		***P value***
	Patients (n=50)	**Control (n=50)**	
IL-32 Pg/ml	23.232 ± 7.855	15.984 ± 5.168	0.004*
*Significant difference at the 0.05 level by T-test.			

3.4.2 Evaluation of laboratory Parameters in CAD patients

Figure (3-9) illustrates the levels of some laboratory markers in CAD patients; where the mean of eosinophil was 0.128, neutrophil was 5.1478, monocytes was 0.5156, basophil 0.0744, lymphocytes was 3.0062, CRP was 7.202, and ESR was 17.06.

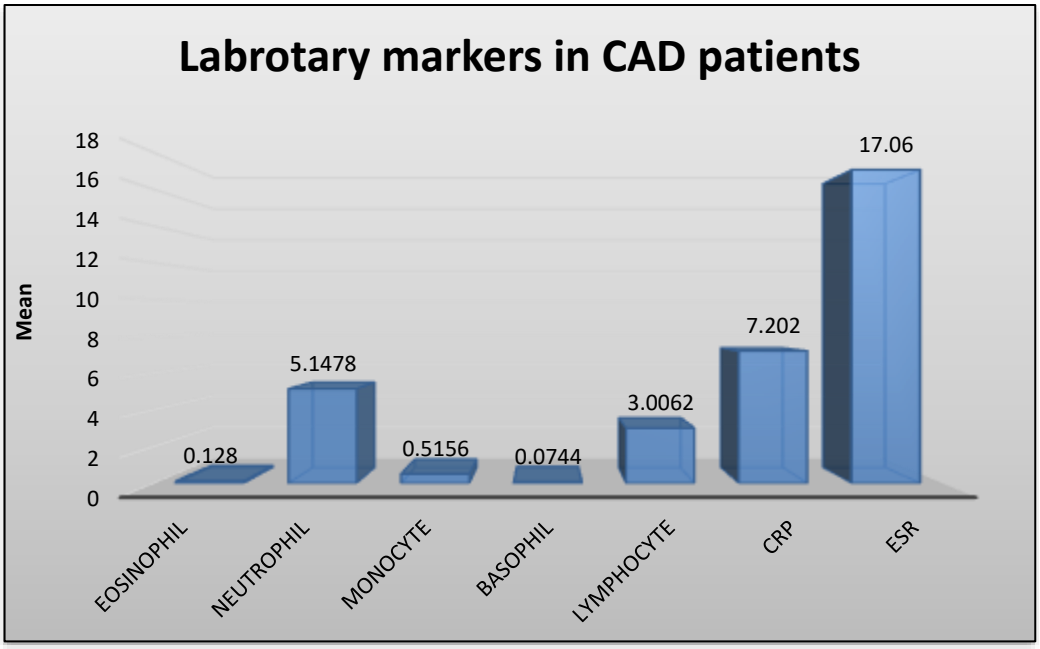


Figure (3-9): Laboratory markers in CAD patient

3.5 Multivariable analysis for immunological and laboratory markers in CAD patients

3.5.1 Impact of age categories on studied markers in CAD patients

The impact of age categories on studied markers is displayed in Table (3-3). The statistics analysis revealed non-significant differences in the distribution of the studied markers according to age groups, except for CRP and ESR, which showed a significant (p=0.002 and *0.000*, respectively) increase in the age category of 61-71 y.

Table (3-3): Study markers for CAD patients according to Age categories

Laboratory markers	Age group (year) Mean ± Std. Deviation			P value
	39-49 y (n=15)	50-60 y (n= 17)	61-71 y (n=18)	
IL-32	21.148±9.03	23.1826±7.2885	25.0151±7.3153	0.379^{NS}
Eosinophil	0.1020 ±0.061	0.1449 ± 0.2784	0.1337 ±0.1133	0.786^{NS}
Neutrophil	4.697±1.52	4.5871±1.28309	6.0528±3.2047	0.108^{NS}
Monocyte	0.4780 ±0.232	0.5306 ±.33284	0.5328 ±.2693	0.828^{NS}
Basophil	0.0833±0.010	0.0728 ±0.028	0.0684 ±0.037	0.793^{NS}
Lymphocyte	3.0473 ±0.95904	2.6547 ±0.5604	3.3039 ±1.644	0.264^{NS}
CRP	6.1000 ±2.06885 ^{b}	5.3471 ±2.05706 ^{b}	9.8722 ±1.21035^{a}	0.002*
ESR	12.600 ±5.3426 ^{b}	13.529 ±6.5299 ^{b}	24.111±12.0190 ^{a}	0.000*

NS: Non- significant difference, *Significant difference under p ≤ 0.05 by One way – ANOVA test, Different small letters refer to significant among groups. Least significant Difference (LSD) values for CRP=2.59, for ESR=6.046

3.5.2 Impact of BMI on studied markers in CAD patients

The impact of BMI categories on studied markers is showed in Table (3-4). The statistics analysis demonstrated non-significant (*p>0.05*) differences in the distribution of the studied markers according to BMI categories.

Table (3-4): Study markers for CAD patients according to BMI

Laboratory markers	BMI score Mean ± Std. Deviation			P value
	Normal (n=37)	Overweight (n= 9)	Obesity (n=4)	
IL-32	23.35±8.137	24.395±7.645	19.523±5.901	0.587^{NS}
Eosinophil	0.136 ±0.202	0.1147 ±0.06679	0.077 ±0.068	0.798^{NS}
Neutrophil	5.503±2.461	4.178 ±1.463	4.0375 ±1.025	0.179^{NS}
Monocyte	0.526 ±.284	0.474 ±.299	0.5100 ±0.223	0.886^{NS}

Basophil	0.081 ±0.069	0.062 ±.039	0.0425 ±0.034	0.429^{NS}
Lymphocyte	3.071 ±1.190	2.784 ±0.969	2.9100 ±1.679	0.802^{NS}
CRP	7.500 ±3.388	6.566 ±1.3566	5.8750 ±2.136	0.679^{NS}
ESR	17.51 ±1.74	16.333 ±3.411	14.500 ±3.316	0.832^{NS}
NS: Non- significant difference under p ≤ 0.05 by One way – ANOVA test				

3.5.3 Impact of Diet on studied markers in CAD patients

The impact of diet on studied markers is showed in Table (3-5). The statistics analysis demonstrated non-significant (*p>0.05*) differences in the distribution of the studied markers according to diet types.

Table (3-5): Study markers for CAD patients according to Diet

Laboratory markers	**Diet state Mean ± Std. Deviation**		**P value**
	Vegetarian (n=21)	**Non-vegetarian (n= 29)**	
IL-32	24.404±8.992	22.383±6.961	0.375^{NS}
Eosinophil	0.103 ±0.024	0.1458 ±.0394	0.408^{NS}
Neutrophil	5.047 ±2.095	5.221 ±2.246	0.794^{NS}
Monocyte	0.5171 ±.069	0.5145 ± 1.250	0.974^{NS}
Basophil	0.0695 ± .0395	0.077± .014	0.648^{NS}
Lymphocyte	2.868 ±1.203	3.105 ± 1.164	0.486^{NS}
CRP	7.466 ± 1.001	7.01 ±0.727	0.707^{NS}
ESR	18.333 ± 2.647	16.138 ±1.546	0.451^{NS}
NS: Non- significant difference under p ≤ 0.05 by T-test			

3.5.4 Impact of Hypertension on studied markers in CAD patients

Regarding the impact of hypertension on IL-32 and some laboratory markers, only a significant increase in the levels of Eosinophil was recorded in hypertensive patients, a trend toward significant (*p=0.064*) was found in IL-32, their levels were increased in hypertensive patients,

while the reminder markers showed non-significant differences as illustrated in Table (3-6).

Table (3-6): Study markers for CAD patients according to Hypertension

Laboratory markers with normal range	Hypertension state Mean ± Std. Deviation		P value
	Hypertensive patients (n=20)	Non- Hypertensive patients (n= 30)	
IL-32	25.717 ±9.046	21.576±6.599	0.064^{NS}
Eosinophil =0.02-0.5*10^9 /L	0.2078 ±0.06	0.075 ± 0.01	0.008*
Neutrophil = 2.0-7.5*10^9/L	5.252 ±2.403	5.078 ± 2.246	0.795^{NS}
Monocyte=0.2-0.8*10^9/L	0.4870 ±.05122	2.535 ±.0564	0.558^{NS}
Basophil=0.01-0.1*10^9/L	0.0936 ± 0.0196	2.062 ± 0.0064	0.077^{NS}
Lymphocyte=1.0-4.0*10^9/L	3.1670 ± 1.259	2.89 ± 1.123	0.435^{NS}
CRP <5	7.9600 ±1.029	6.697 ± 2.261	0.299^{NS}
ESR 0-20 mm/hr	18.700 ± 2.405	15.967 ± 1.747	0.351^{NS}

NS: Non- significant difference, *Significant difference under p ≤ 0.05 by T- test

3.6 Molecular analysis

3.6.1 *IL-32* gene genotypes and allele frequency in study population

The current study investigated the effect of genetic polymorphism of the *IL-32* gene on CAD patients versus healthy control individuals, The frequency of genotypes in CAD patients was found to be as follows: 27 patients carried CC genotype, 8 patients had TT genotype, while 15 had CT genotype. For the control individuals, the distribution was as follows: 17 individuals carried CC genotype, 19 individuals carried TT genotype, and 14 individuals had CT genotype.

The analysis of genotypes frequency yielded p-value of *0.0455*, with an odds ratio of 2.2788, and a confidence interval (CI) ranging from 1.0165-5.1085. This finding indicated a statistically significant association

between this genetic polymorphism and CAD condition, and means that individuals with CC are 2.2788 times more likely to develop CAD compared with those don't have CC genotype.

As well, the analysis of allele frequency yielded p-value of *0.0028*, with an odds ratio of 2.4113 and a confidence interval of 1.3535 to 4.2959; this findings indicate a significant association between alleles and progression of CAD within studied patients, as present in table (3-7) and Figure (3-10).

Table (3-7): *IL-32* gene genotypes and allele frequency in study population

Genotypes	**CVD patients (50)**		**Healthy control (50)**		***P value***	**ODD (CI95%)**
	n	%	n	%		
CC	27	54.0%	17	34.0%	0.0455*	2.2788 (1.0165 – 5.1085)
TT	8	16.0%	19	38.0%		
CT	15	30.0%	14	28.0%		
Alleles frequency						
C	69	69%	48	48%	0.0028*	2.4113 (1.3535 to 4.2959)
T	31	31%	52	52%		
ODD: odds ratio, CI: confidence interval						

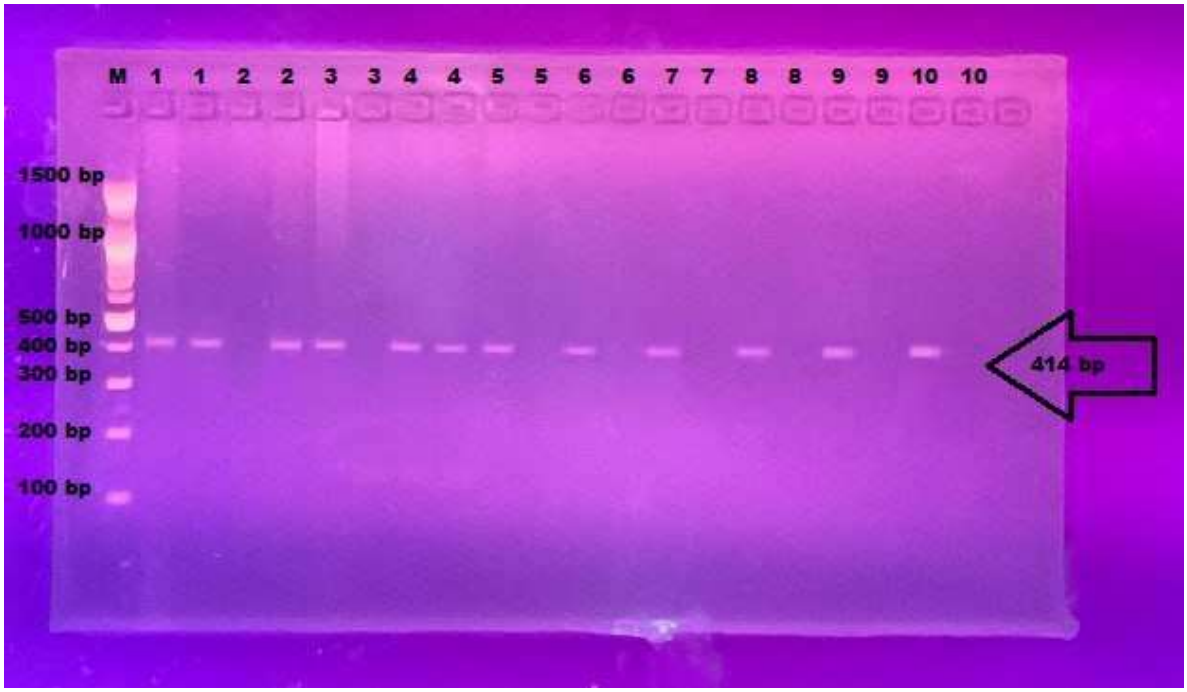


Figure (3-10): Allele-specific PCR genotyping of SNP rs4786370 (T/C) in 10 patients . M: DNA ladder; Lanes 1-20: Each patient was loaded in duplicate (two wells per patient), with the first well representing the T allele and the second well representing the C allele. PCR product band size: 414 bp. And showed TT genotype (lanes 3, 5,6,7,8,9,10) ,CC genotype (lanes 2). And TC genotype (lanes 1,4).

3.6.2 The distribution of serum levels of IL-32 in study population according to genotypes of *IL-32* gene

Table (3-8) displays the distribution of serum levels of IL-32 according to genotypes of *IL-32* gene, The results of statistical analysis revealed non-significant (*p=0.454*) differences between levels of IL-32 that distributed according genotypes in CAD patients; on the other hand, A significant (*p=0.005*) increase was recorded in healthy control carried TT genotype compared with those carried CT and CC genotypes.

Table (3-8): Serum levels of IL-32 in study population according to Genotypes of *IL-32* gene

Genotypes	Concentration of IL-32 pg./ml Mean ± Std. Deviation			
	No.	Patients	Control	No.
CC	27	24.535883 ± 8.8588120	12.842154 ±2.7545976 [b]	17
TT	8	21.699746 ± 6.6404855	17.219553 ± 5.6172715 [a]	19
CT	15	21.702384 ± 6.4072785	18.125033 ± 5.2535470 [a]	14
P value	0.454[NS]		0.005*	
LSD	5.49		3.29	

*** Significant difference under p ≤ 0.05 by One way – ANOVA table NS: Non-significant difference; Different small letters refer to significant among groups.**

3.7 Percentage Distribution for Genotypes of *IL-32* gene by sex in study population

Table (3-9) shows the relative distribution of *IL-32* genotypes by sex in CAD patients. The results of the statistical analysis indicated that the CC genotype was significantly (*p=0.0001*) increased in females, while the TT genotypes were significantly (*p=0.0001*) increased in the male group. On the other hand, CT genotype did not show significant differences between male and female patients.

Table (3-9): Percentage Distribution for Genotypes of IL-32 gene by sex in patients group.

Genotypes	Sex No. (%)		P value
	Male (22)	Female (28)	
CC	8 (29.62%)	19 (70.37%)	0.0001*
TT	6 (75%)	2 (25%)	0.0001*
CT	8 (53.3%)	7 (46.7%)	0.4817[NS]

***Significant difference under p ≤ 0.05 by chi-square test. NS: Non- significant difference**

3.8 Receiver Operative Characteristic Curve Analysis

The Receiver Operative Characteristic Curve (ROC) analysis of IL-32 showed significant outcomes, as revealed in Table (3-10) and Figure (3-11). Area under curve (AUC) to IL-32 is 0.786, reflecting good diagnostic ability, sensitivity is 0.60, while the specificity is 0.88, and the cut-off value is 20.71. The p-value is 0.000, indicating that the results were statistically significant.

Table (3-10): ROC curve of IL-32 in patients

Markers	**AUC**	**Sensitivity**	**Specificity**	**Cut-off**	**P-value**
IL-32	0.786	0.60	0.88	20.71	0.000*
AUC: area under curve; *Significant difference at the 0.05 level by ROC analysis					

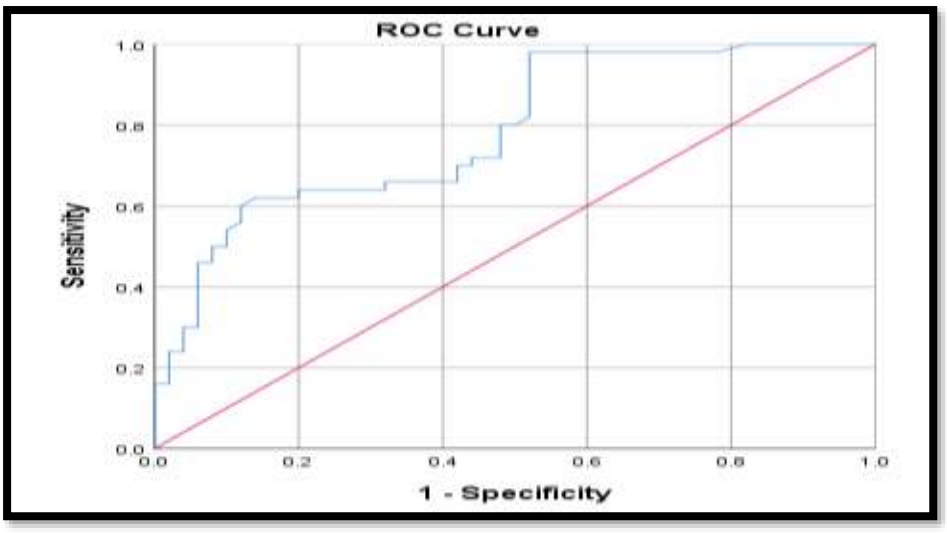


Figure (3-11): ROC curve for IL-32 in CAD patients

Chapter Four

Discussion

4. Discussion

4.1. Demographic Data for Patients and Control

Coronary artery disease (CAD) is a prevalent heart condition characterized by the buildup of atherosclerotic plaque within the arterial lumen. Blood flow impairment reduces oxygen delivery to the myocardium. CAD is the most common cause of major morbidity and mortality in the worldwide (Shahjehan *et al* .,2024) .

The results of statistical analysis regarding age within the study groups as in table (3-1) showed non-significant difference. Despite non-significant relationship regard age mean . The study had shown higher frequency of CAD occurs in the age mean 60-70 years. This results were in the same line with a study conducted by (Madhavan *et al* .,2018) who reported a greater prevalence of coronary artery disease in patients more than 80 years of age. Similar finding were reported by Amen *et al* in (2020) who showed the incidence of myocardial infarction was higher in patients 65 to 84 years of age. Komilovich had done a study in (2023) , observed rising in the incidence of CAD with age ,and increased to about 4% as the age group reached 75 to 84 years. This result might be due to the accumulation of risk factor and fatty acid deposition additional to the lifestyle modification.

Regarding the sex , this study had shown non-significant differences (P >0.05) between study group regarding sex, as found in table (3-1) . This finding was compatible to a study had done by Yang *et al*., (2019) who found no significant difference in the sex and age . Mohammad-Rezaei *et al*.(2021) also had shown the same finding ,No significant differences regarding the sex and age). In addition, a study achieved by Guo *et al* in (2018) have proven

that women present with CAD at a later age and with a greater number of comorbidities, especially high blood pressure, diabetes mellitus, renal failure, and prior congestive heart failure, while men have shown a higher smoking habit, being overweight, prior myocardial infarction , and going through a previous percutaneous coronary intervention. In Iraq Allami (2024) had mentioned males were more likely to developing CAD at younger age than females. This result may attributed to the frequency of regular smoking ,family history of coronary heart disease and being overweight. while, female were older an less likely to be smoker.

Concerning the economic status ,this study had shown a significant difference between study groups in middle income participants ,and that was agreement with the result of World Health Organization (2019), which shown at least three-quarters of the world deaths from CAD occur in low and middle income countries. People living in low-middle income countries often, do not have the benefit of primary health care programmers for early detection and treatment of people with risk factors for this disease. Additionally, have less access to effective and equitable health care service, which respond to their needs for many people in these countries detection often late.

Regard to habitat status, the study reported that a higher percentage (46%) of patients were resided in urban region, while (54%) of controls were resided in sub-urban region, with significant differences ($p=0.0128$ and 0.0001) within and between patients and controls . These results are due to the place of sample collection, where, it joined in the centers of large cities which are dense with urban population, few studies indicated that the greatest incidence occurs in rural area. Where a health care and health awareness are

lacking . Although, urban areas, in general, are characterized by a relatively high availability of (fast-)food outlets and are conducive to the adoption of more western diets, rich in salt, sugar and saturated fat, potentially contributing to the unfavourable blood lipids observed. On the other hand, life style in rural area characterized by diet, as well as occupational activities due to agricultural area. This study was consistent with Groot *et al* .,2019. which reported a higher lipidemia (which increase the risk of CAD) in urban area compared to rural area due to the same reasons.

Education was the socioeconomic indicator most consistently associated with outcomes, and people with low levels of education in low-income and middle-income countries had a markedly higher risk of major cardiovascular events compared with those with higher levels of education. Those with low levels of education in low-income countries receiving very poor secondary prevention, and markedly poorer diabetes and hypertension treatment compared with all other groups . Borkowski *et al* in (2024) indicate that individuals with lower educational levels experience higher rates of incidence and mortality due to CAD. Liu *et al.* (2024) observed an inverse relationship between education level and CAD risk factors; notably, reduced educational attainment is associated with increased levels of hypertension, body weight, smoking prevalence, and ECG abnormalities These results were compatible to the finding in this study that's revealed a significant different was increasing the risk of CAD within secondary school or less ,and illiterate people compared to graduate or highly educational people.

According to the family history to CAD of the patients, there was a significant difference within patients group , in this study ,there were 40% of

patients have a family history to CAD ,where 60% of them did not have a family history to CAD . This result was in line with a study accomplished by Di Lenarda *et al* ,(2024) who shown a positive family history of CAD is closely associated with a higher risk of developing CAD. In contract, Teragawa *et al* , (2023) ,revealed that family history to CAD was not indicator of CAD severity and prognosis. This result was due to the following reasons: first, it was conducted using a small sample size, second, family history of CAD lacks a standard definition, and finally, The definition of family history of CAD often uses cases occurring in the first degree of consanguinity, but the study included cases in the second degree of consanguinity due to the potential for heritability.

Smoking is strongly associated with both the development and severity of CAD, the present study showed that 30% of patients were smoking , while, 70% of them weren't . This finding was incompatible with a study had done by Salehi *et al* ,(2021),that revealed an association occured between CAD and cigarettes smoking. Cigarettes and Nicotine can result in harm to the cardiovascular system by increasing the level of free radicals and other toxic substances, which may be related to an increased risk of CAD . That led to reduced blood flow to the heart. Nicotine also induced myocardial necrosis, vascular inflammation , and calcium deposition in arteries (atheroma) development of atherosclerosis. Moreover ,smoking alters lipid profile by increasing LDL cholesterol and triglyceride, contrary ,decreasing HDL cholesterol .These alteration lead to CAD progression (Chen *et al* .,2023). Similar studies supported this finding like (Morovatdar *et al* .,2021) who found an association between smoking and increased risk of CAD.

4.2 Distributions of Study population according to different factors:

4.2.1 Distributions of cases and controls according to BMI categories

According to the BMI, the distribution of CAD was higher in patients (23.08±3.19), compared to control (22.44±2.17) as shown in figure (3-1). and this may attributed to increase the comorbidities risk factor of CAD like hypercholesterolemia, hypertriglyceridemia, hypertension and diabetes mellites. Zhao *et al*.,(2021) who found an association between BMI and risk of CVD incidence , Figure (3-2) shown the percentage distribution of the disease, as the estimation of patients and control were higher within normal weight 74% and 86%, overweight category were 18% and 14% , finally 8% of patients were obese, while no control individual found in obesity category. A study conducted by Dikaiou *et al* .,(2021) found as a higher estimation was in normal weight (22.5–<25 kg/m2) and the lower among the obese and severely obese category. while, patients with low–normal BMI at about 22 kg/m^2 had the lowest risk.

4.2.2 Distributions of cases according to diet status

study has shown non-significant relationship within patients group regard nature of their dietary state as shown in figure (3-3) where the vegetarian were 42% .On the other hand, non-vegetarian were 58%. Similar result had mentioned by Szczepańska *et al* ., (2023) found 42% of patients consumed vegetables several times in their weekly meals. However, 57% of them consumed both red and white meat. Recently, Sapala *et al* .,(2025). declared that unusual dietary patterns were apparent despite the fact that there was no elevated risk of malnutrition utilizing, especially with regard to inadequate consumption of fruits, vegetables, legumes, whole grains,

nuts, dairy products, and seafood. An unbalanced dietary status might increase the risk of body weight, hypertension and body fats, which increased the risk of cardiovascular disease

4.2.3 Classification of cases according to hypertension Status

According to the hypertension status among coronary artery disease patients, there were (40%) of patients suffering from high blood pressure, while , (60%) of them were not suffering as in figure (3-4). This finding agrees with a study achieved by Vrsalovic *et al*. (2024) which reported that blood pressure value <130/80 mm Hg was associated with lowest risk of CAD . In addition the study showed that patients with high blood pressure with CAD had increased risk of myocardial infarction (MI) ,stroke, and cardiovascular death comparing with health control. Additionally, Berge *et al*., (2022) found an association between hypertension and non-obstructive CAD. Hypertension effects both obstructive and non-obstructive forms of CAD by contributing to microvascular damage, which effects small blood vessels in the heart, causing myocardial ischemia ,and raises the risk of cardiovascular events.

4.3 Relationship between hypertension and some demographic factors

4.3.1. Distribution of hypertension in cases according to age categories

The current study showed non-significant differences (*p=0.1486*) among hypertensive patients regard age category, as in figure (3-5). The highest prevalence were within (61-71) years old , as in a study done by (Kim *et al* .,2019) who reported the prevalence and incidence of hypertension in the study showed slightly different trends in terms of age and sex. For example, the age group with the highest prevalence was 65 and older for hypertension . In contract, Wang *et al* .,(2020) had proved

hypertension was association with a higher risk of CAD ,the association was higher within younger age .

(Li *et al* .,2024) found that the prevalence of hypertension in young CAD patients is 50.83% compared with older CAD patients. There are some potential explanations. First, hypertension is generally recognized as a complex disease that arises from both genetic predisposition . Existing evidence suggested that early-onset hypertension has been found to be more affected by hereditary susceptibility. On the other hand, younger-onset hypertension patients have the increased risk of left ventricular hypertrophy, coronary calcification, and multiple target organ damage including proteinuria , which would consequently increase the risk of CAD and all-cause mortality .

4.3.2 Distribution of hypertension in cases according to BMI categories

The present study showed a significant difference (*p= 0.0018*) regarding BMI, as shown in figure (3-6). The highest proportions of patients were within normal weight (45.90%), then (22.20%) of the patients within over weight ,and (25%) of the patients were within obesity. The study disagrees with a study of Qiao *et al*. (2021) and Li *et al*. (2024) whose found an association between BMI and CAD, and suggested that early intervention and treatment of hypertensive patients with highly BMI are be necessary to reduce risk of CAD outcome .

4.3.3. Distribution of hypertension in cases according to diet state

The study shows non-significant relation (*p=0.4756*) record to the hypertensive patients according to dietary intake, as present in figure (3-7) . This study was in the same line with the finding by Mousavi *et al* .,(2020) who proved dietary protein intake from different source had no-significant association with increased risk of the disease. Reynolds *et al* . (2022) had observed a higher dietary fiber intakes have demonstrated in the prevention

of premature mortality of CVD. The beneficial effects of dietary fiber intake, as the improvements in blood pressure, blood lipids, and body weight would be effected to reduce the progression of the CAD .

4.3.4. Distribution of hypertension in cases according to smoking habit

The study shows no significant relationship between smoking and hypertensive patients ,There were 30% of patients smokers ,and the remaining 70% were not smokers, as found in figure (3-8). There is less information about how smoking affects CAD in high blood pressure patients .It had been shows that smoking less daily for a prolonged duration is much riskier than smoking much daily for a shorter time (Lubin *et al*., 2016). Grubb *et al* .,(2020) shows heavy smoking increase risk of CAD in patients with high blood pressure versus light or moderate smokers. Another study had Paquett *et al* .,(2020) reported a strong effect of smoking in raising the incidence of CAD in hypertensive patients. Smoking increases the severity of CAD by contributing to the narrowing and blockage of coronary arteries. Cigarettes and nicotine damage the coronary vascular endothelium, increase sympathetic tone ,and cause vasospasm, which leads to reduced blood flow to the heart. Nicotine also promotes myocardial necrosis, vascular inflammation, and calcium deposition in arteries (atheroma), accelerating the development of atherosclerosis.

4.4 Evaluation of immunological and laboratory markers in patients and control

4.4.1 Evaluation of Serum levels IL-32 for Patients and control

There were a significant differences (*P value* 0.004) between IL32 levels within the study groups as clarify in table (3-2), which proved an elevation in the serum level of the patients compared to healthy control. Tomasi *et al.*, (2023) demonstrated this by showing an elevation between

circulating IL32 levels and by revealing that circulating IL32 levels are associated with impaired blood pressure regulation in individuals at risk of cardiovascular disease. In addition, Kaymaz *et al.*, (2025) found a noticeable rising in IL32 serum levels were mentioned in Behçet's disease patients. Such an increase might be connected to vascular involvement. Moreover, IL-32 have a role in other disease, Choi *et al* (2019) had revealed that Serum IL-32 levels are elevated in patients with endometriosis. furthermore , Yao *et al* , showed that IL-32 mRNA expression in Graves Disease group was positively associated , suggesting that IL-32 was related to the occurrence of Graves Disease and function of thyroid. which indicated that IL-32 is related to the pathogenesis of Graves Disease. However, the specific mechanism of IL-32 in GD is still unclear.

4.4.2 Evaluation of laboratory Parameters in CAD patients

The laboratory tests that had done to patients group described in figure (3-9). The study shows noticeable rising in some biological parameters mean such as: ESR =17.06, CRP=7.202, Neutrophils=5.1478. However, Monocyte=0.5156, Eosinophil=0.128 and Basophil=0.0744 were at lower levels. This finding was in the same line with Matei *et al*.,(2022) which reported a significant elevation in ESR levels within CAD patients. As ESR is not a disease-specific marker, increased values of this parameter maybe observed in several conditions, such as inflammatory diseases, infections or tumors .Additionally, Matei observed un elevation in CRP levels as un indicator of early inflammation, the role of CRP in cardiovascular events and as a marker of chronic inflammation is well known .Due to the association of high CRP levels with the development of atherosclerotic lesions and because CRP mediates tissue fibrosis in several cardiovascular diseases. Moreover, Wang *et al*, (2023) reported a

significant increased in neutrophil counts in CAD patients. This finding agrees with present study results.

4.5 Multivariable analysis for immunological and laboratory markers in cases

4.5.1 Impact of age categories on studied markers in patients group

The current study had shown non-significant relationship to the distribution of biological markers regard age category as displayed in table (3-3). ESR,CRP were the only markers that had shown noticeable rises within 60-70 age category (*p=0.002* and *0.000*, respectively) . Dugani *et al* (2021) mentioned that all the inflammatory biomarkers examined showed positive associations (by approximately 1.2 to 1.8-fold per SD) with incident CAD, as the incidence rates of CAD increased with age and were approximately 10-fold higher for CAD onset at age 75 years or older vs younger than 55 years, consistent with age being a substantial risk factor.

4.5.2 Impact of BMI on studied markers in cases

Statistically, there were non-significant differences *(p>0.05*) in distribution of the studied biomarkers regard BMI categories as declared in table (3-4). Buschmann *et al*., (2020) reported neither the BMI and nor the biomarkers level are relevant as the systemic inflammation markers that are probably responsible for the worse prognosis and high cardiovascular event rate.

4.5.3 Impact of Diet on studied markers in Cases

The study revealed non-significant (*p>0.05*) differences in the distribution of inflammatory biomarkers regarding to the diet in CAD patients as observed in table (3-5). A study accomplished by Ghanavati *et*

al., (2021), observed non- significant difference in concentration of CRP within study groups according to the diet.

4.5.4 Impact of Hypertension on studied markers in Cases

The current study finding was reported that patients with hypertensive had no significant relationship regard inflammatory markers. This result was in agreement with a study done by Bisaria *et al.*, (2020) that had Shown non-significant statistical difference between CRP level and hypertension among patients group. There was a significant increased in eosinophil levels. This result was contrary to what Gao *et al.*, (2019) who reported a significant lowering in eosinophil levels. The results show CAD patients exhibited lower eosinophils than non-CAD patients. This result may attributed to the extensive thrombus formation could have induced the decreased eosinophil count. A study of Wang *et al.*, (2023) had supported this idea by proven the same results. In contrast, Tanaka *et al.*, (2012) found a significant increase in the number of eosinophils in peripheral blood in patients with unstable angina pectoris compared with that in control, due to Eosinophils synthesize and release bioactive mediators such as leukotriene C4, a potent stimulant of vasoactivity and smooth muscle contraction.

4.6. Molecular analysis

4.6.1. *IL-32* genotypes and allele frequency in patients and control

According to the *IL32* gene and allele frequency in study groups the allele and genotype frequency of *IL-32* gene polymorphism is presented in table (3-7). Patients with CC genotype of rs4786370 were 27 (54%) represented a greater frequency of genotypes compared with healthy control with a significant different *p-value* of *(0.0455*). This study shows that the CC genotype was associated with increased risk of coronary artery

diseases, as in the study of Jin *et al* ., (2022) ,which proved that the CC genotype was more accuracy in patients with CAD. Another study done by Dos Santos *et al* .,(2020). who proved the *IL32* rs4786370 genetic variant was associated with American Tegumentary Leishmaniasis, where a CC genotype of the promoter SNP (rs4786370) trends to have higher expression than a CT ,TT ,genotypes .

In the present study patients with CT genotype of rs4786370 were 15 (30%) , while, patients with TT allele of rs4786370 were 8 (16%) .In contract, Mazlum *et al* ., (2021) who found the frequencies of CT, TT genotypes, and T allele were shown to be higher in preeclampsia patients.

4.6.2. The distribution of serum level of IL-32 in study population according to genotypes of *IL-32* gene

The current study shows the distribution of IL32 level according to the *IL32* genotypes within study groups , which recorded non-significant relationship within patients, as demonstrated in table (3-8). As the mean of IL32 serum level was a highly associated with CC genotypes, that were predominant within CAD patients, suggested increasing the progression risk of the disease in CAD patients. Damen *et al* ., (2018) had supported this finding by proving that, the CC genotype of rs4786370 would be associated with slightly increased expression of IL-32 in patients with rheumatoid arthritis. Damen *et al*., (2017), previously, reported that; the C allele of rs4786370 was linked to an increase in HDL-C levels, which could affect and possibly lowering the CAD risk in rheumatoid arthritis patients. This study was inconstant with present study. likely, due to differences in ethnicity, sample size, patient selection, clinical heterogeneity, or a combination of these factors.

4.7. Percentage Distribution for Genotypes of *IL-32* gene by sex in patients

The present study had shown the distribution of *IL32* genotypes were significantly (*p=0.0001*) increased the CC genotypes within female group. while, the TT genotype increased within male group with a significant relationship (*p=0.0001*). This study is the first of its kind to demonstrate the relationship between *IL-32* genotypes and the sex, which indicates that females are more susceptible than males for this disease due to the elevation of CC genotypes in females. Therefore, there must be other studies in this field to proven this result.

4.8 Receiver Operative Characteristic Curve Analysis of IL32

The ROC (Receiver Operating Characteristic) curve analysis of IL-32 in CAD patients revealed a promising Area Under the Curve (AUC) of 0.786, indicating good diagnostic accuracy. A sensitivity of 0.60 and a specificity of 0.88 at a cut-off value of 20.71 suggest that IL-32 is moderately sensitive but highly specific in distinguishing CAD patients from healthy controls. The p-value (0.000) indicates statistical significance, reinforcing IL-32's potential as a diagnostic biomarker. A study conducted by Mohmmad-Rezaei *et al*.,(2021) investigated serum IL-32 levels in patients with CAD. Who found that IL-32 levels were significantly elevated in patients with obstructive CAD compared to those without. The study used ROC analysis and reported comparable AUC values, confirming that IL-32 has strong discriminative power for CAD diagnosis.

Conclusions

&

Recommendations

Conclusions

The study has reached to the following Conclusion:

1. Age is a well-established independent risk factor for CAD, indicating a progressive increase in disease prevalence with advancing age.
2. Urban area exhibited the highest prevalence of the disease.
3. IL-32 has a potential as an early diagnostic marker for CAD.
4. Females are more susceptible to the CAD than males. Therefore, further improvements may be needed to improve that.
5. The "CC" genotype of the *IL-32* gene polymorphism were associated with the pathogenesis of the CAD.

Recommendations

It is suggested that:

1. A study can be conducted involving larger sample size to investigate the relationship between IL32 and other inflammatory cytokine in the development and progression of the disease.
2. A further research is needed to evaluate the impact of BMI on the pathogenesis and clinical outcomes of the disease.
3. A study can be conducted to explore the sex-related differences in IL32 level and their correlation with the risk and progression of the disease.
4. Further research can be done of more SNPs of IL32 gene to detect which one is more prevalence and susceptible to the disease .
5. Additional study can be done to measure IL32 level in a wide range of CAD patients from different regions to clarify the influence of environment on IL32 serum level.

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Appendix

Appendix I: Coronary artery disease patients and healthy control questionnaires.

Data:

1-age

2-Sex

- male
- female

3-Body mass index (BMI) (BMI was defined as weight (kilograms)/height (meters)

4- Duration of disease

6- Diet

- Vegetarian
- non Vegetarian

7- Family history of coronary artery disease

8- Smoking

- yes
- no

9- Education status

- Graduate or above
- Secondary school or less
- Illiterate

10- Economic status

- Higher class
- Middle class
- Lower class

11- Habitat status,

- Urban

- Sub-urban
- Rural

12- Diabetes

- yes
- no

13- Hypertension

- yes
- no

Lab. tests:

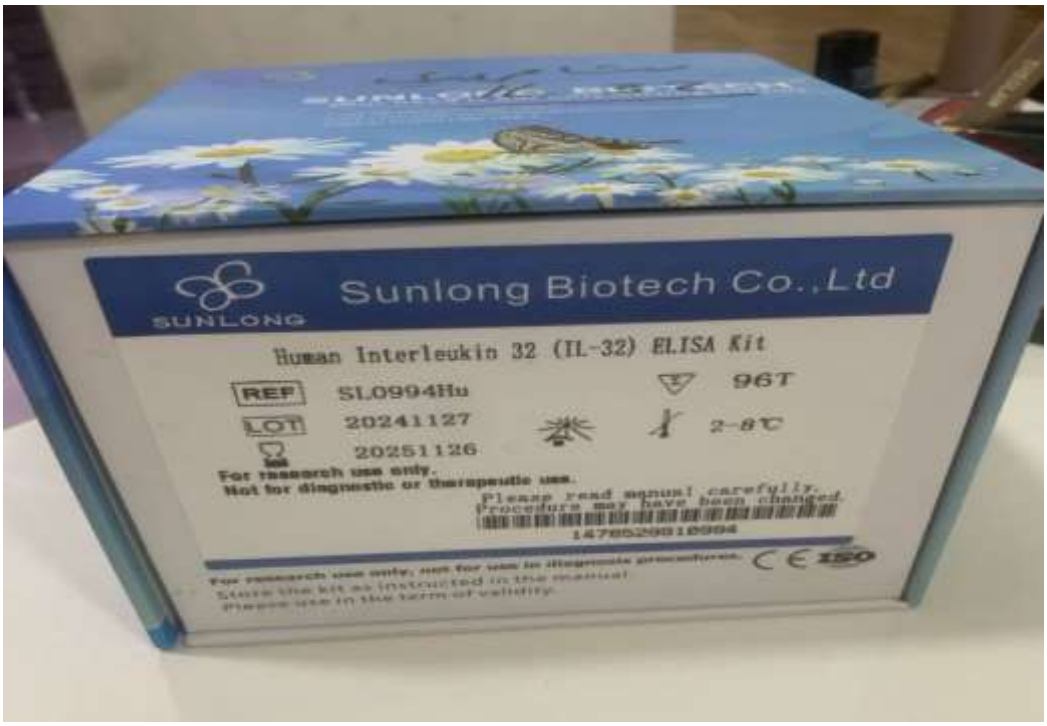
1- CBC

2- ESR

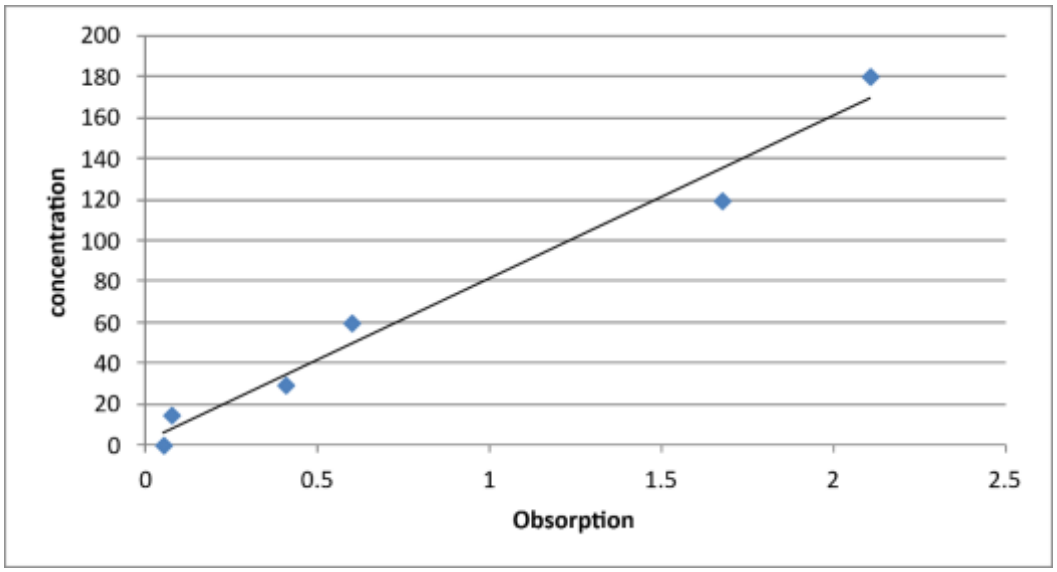
3- CRP

Appendix II: ELISA instrument (Reader)

Appendix III : ELISA Kit for IL32 serum level



Appendix IV: ELISA curve for IL32



Appendix V : (A) PCR instrument, (B) Gel electrophoresis and (C) High-speed Refrigerated Centrifuge

(A):PCR instrument

(B) Gel electrophoresis

(C) High-speed Refrigerated Centrifuge

الخلاصة

تعدّ أمراض الشرايين التاجية (CAD) السبب الأكثر شيوعًا للوفاة في عموم السكان، وتتميز بنقص التروية الدموية والأوكسجين إلى عضلة القلب . يُعدّ الإنترلوكين-32 (IL-32) سايتوكينًا التهابيًا معززًا، يعمل على تقوية الاستجابة الالتهابية من خلال تحفيز إفراز مجموعة من السايتوكينات الالتهابية الأخرى. وقد تبين أن هناك تعدد شكلٍ نكليوتيديًا منفردًا (SNP) في منطقة المحفز لجين IL32 يؤثر في التعبير الجيني لأنواعه في الخلايا أحادية النواة في الدم المحيطي، مما يشير إلى أن الطفرات في هذا الجين قد تسهم في تطور أمراض التهابية متعددة مثل مرض الشريان التاجي.

شملت الدراسة جمع 100 عينة دم ومصل من الذكور والإناث (48 ذكرًا و52 أنثى) ممن تراوحت أعمارهم بين 40 إلى 70 عامًا، من مراجعي مركز الشهيد المحراب لقسطرة القلب وجراحة القلب ومستشفى الإمام الصادق في محافظة بابل، العراق، وذلك خلال الفترة الممتدة من أكتوبر 2024 إلى يناير 2025. تم جمع عينات الدم في أنابيب تحتوي على مادة EDTA وأنابيب جل، حيث خُصصت 50 عينة لمرضى الشريان التاجي و50 عينة لأشخاص أصحاء كمجموعة ضابطة. وتم الحصول على عينات الدم والمصل من جميع المشاركين لغرض قياس مستويات IL-32 في المصل باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA)، في حين تم تحديد تعدد الأشكال الجينية لجين IL32 باستخدام تقنية تفاعل البوليميراز المتسلسل (PCR) بطريقة الأليل المحدد.

أظهرت نتائج الدراسة الحالية عدم وجود فروق ذات دلالة إحصائية ($p>0.05$) فيما يخص العمر، الفئات العمرية، والجنس بين المجموعتين. في المقابل، أظهرت التحليلات الإحصائية للوضع الاقتصادي وجود فروق ذات دلالة إحصائية بين المرضى والأصحاء على التوالي ($p=0.0001$) و($p=0.0015$). كما بيّنت النتائج وجود فروق معنوية ($p=0.0455$) فيما يتعلق بتاريخ العائلة، حيث تبين أن المرضى الذين لا يملكون تاريخًا عائليًا للإصابة بأمراض القلب أظهروا فروقًا ذات دلالة.

وفيما يتعلق بمؤشر كتلة الجسم (BMI)، فقد كان 74% من المرضى و86% من الأصحاء ضمن الفئة الطبيعية، في حين كانت النسب 18% و14% على التوالي في الفئة الزائدة الوزن، بينما

بلغت نسبة السمنة 8% لدى مجموعة المرضى فقط. كما أظهرت الدراسة أن أعلى نسبة للإصابة بارتفاع ضغط الدم كانت ضمن الفئة العمرية من 61 إلى 71 عامًا، حيث بلغت 50%.

من جهة أخرى، أظهرت الدراسة وجود فرق معنوي ($p=0.004$) في مستوى IL-32 في مصل الدم بين مجموعة المرضى والمجموعة الضابطة. كما سُجل ارتفاع في مستويات بروتين سي التفاعلي (CRP) بمعدل (7.202) ومعدل ترسيب كريات الدم الحمراء (ESR) بمعدل (17.06) في مجموعة المرضى.

علاوة على ذلك، أظهرت نتائج تعدد الأشكال الجيني (rs4786370) لجين IL32 وجود فروق معنوية ($p=0.0455$)، بنسبة أرجحية (OR) بلغت 2.2788، وبفاصل ثقة (CI) يتراوح بين (1.0165 – 5.1085). وقد ارتفعت نسبة النمط الوراثي CC في مجموعة المرضى (54.0%) مقارنةً بالمجموعة الضابطة (34.0%)، بينما زادت نسبة النمط TT في المجموعة الضابطة (38.0%) مقارنة بالمرضى (16.0%).

وفيما يخص تحليل تردد الأليلات، كانت النتائج معنوية أيضًا ($p=0.0028$)، بنسبة أرجحية بلغت 2.4113 وبفاصل ثقة (1.3535 – 4.2959). إذ لوحظ ارتفاع في تردد الأليل C في مجموعة المرضى (69%) مقارنةً بالأصحاء (48%)، بينما ارتفع تردد الأليل T في مجموعة الأصحاء (52%) مقارنةً بالمرضى (31%).

كما أظهرت الدراسة نتائج ذات دلالة معنوية عند إجراء تحليل منحنى العامل التشغيلي للمستقبل (ROC) لمستويات IL-32، حيث بلغت مساحة تحت المنحنى 0.786 (AUC)، مع حساسية 0.60 ونوعية 0.88، وبقيمة إحصائية ($p=0.000$).

أظهرت نتائج هذه الدراسة ارتفاعًا في مستوى IL-32 في مصل دم مرضى الشريان التاجي مقارنةً بالأفراد الأصحاء، ما يشير إلى إمكانية استخدام هذا السايتوكين كمؤشر تنبؤي لتشخيص المرض. كما أظهرت الدراسة أن النمط الوراثي CC كان أكثر شيوعًا بين المرضى، في حين كان النمط TT أكثر انتشارًا بين الأصحاء، مما قد يدل على أن النمط CC يرتبط بزيادة خطر الإصابة بأمراض الشريان التاجي، بينما قد يكون للنمط TT دور وقائي ضد هذا المرض.

جمهورية العرا ق

وزارة التعليم العالي والبحث العلمي

جامعة كربلا ء

كلية الطب

فرع الأحياء المجهرية

العلاقة بين تعدد اشكال جين 32(rs 4786370) و مستوى المصل مع مرض الشريان التاجي

رسالة

مقدمة الى مجلس كلية الطب /جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية

من قبل الطالب

رشا مهدي خليفة سليمان

بكالوريوس علوم حياة / كلية العلوم / جامعة بابل/ 2010

بأشراف

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2025 م

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