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Investigation of Brain Natriuretic Peptide, Total Antioxidant Capacity and Creatine Kinase-MB in Males with Ischemic Heart Diseases

A thesis

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

By

Amjad Kareem Alwan

B.Sc. in Department of pathological analyses, Al-Isra University/ 2017

<u>Supervised By</u>

Prof. Dr. Fadhil Jawad Al-Tu'ma
PhD in Clinical Biochemistry
Department of Chemistry and Biochemistry

Prof. Dr. Ahmed Hussein AL Mayali
C.A.B.M, F.R.C.P
Consultant Physician
Intervention Cardiologist

College of Medicine - University of Kerbala

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Supervisors Certification

We certify that this M.Sc. thesis titled: -

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was prepared by (**Amjad Kareem Alwan**) under our supervision at the College of Medicine, University of Kerbala, as a partial fulfillment of the requirement for the Degree of Master in **(Clinical Chemistry).**

Prof. Dr. Fadhil Jawad Al-Tu'ma
Department of Chemistry and Biochemistry

Prof. Dr. Ahmed Hussein Al-Mayali
Department of Internal Medicine
Interventional Cardiologist

College of Medicine - University of Kerbala

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

Atheer

Assist. Prof. Dr. Atheer Hameed Al-Ghanmi
Head of Department of Chemistry and Biochemistry
College of Medicine / University of Kerbala

Examination Committee Certification

We the examining committee, certify that we have read this M.Sc. thesis entitled:

"Investigation of Brain Natriuretic Peptide, Total Antioxidant Capacity and Creatine Kinase-MB Isoenzyme in Males with Ischemic Heart Diseases"

we have examined the student **" Amjad Kareem Alwan"** in its content and in our opinion, it is adequate with (**Very Good**) and it meets the standard of thesis for the Master degree in **"Clinical Chemistry"**

Signature
Prof. Dr. Hala G. Mahmood
College of Medicine-University of Baghdad
8 / 9 /2024
(Chairman)

Signature
Prof. Dr. Rana F. Mousa
College of Veterinary Medicine
University of Kerbala
10 / 9 / 2024
(Member)

Signature
Assist. Prof. Dr. Maher A. Mukheef
College of Medicine
University of Kerbala
6 / 9 / 2024
(Member)

Signature
Prof. Dr. Fadhil J. Al-Tu'ma
College of Medicine - University of Kerbala
12 / 9 / 2024
(Member/Supervisor)

Signature
Prof. Dr. Ahmed H. Al-Mayali
College of Medicine - University of Kerbala
12 / 9 / 2024
(Member/Supervisor)

Approved by the College of Medicine - University of Kerbala

Signature:
Date: / / 2024
Prof. Dr. Riyadh Dayhood Al-Zubaidi
Dean of the College of Medicine

Dedication

With my thanks to God

I would like to dedicate my message to

The source of my ambition: My father

And to my source of inspiration: My mother

And to all my beloved family

To everyone who stood by me

And I dedicate this work

To every patient in the world

Amjd

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First of all, I thank God for the ability, strength and patience to accomplish this work. I would also like to extend special thanks to the owner of the perfume, for his history and enlightened thought, as he was the first credit in my life I obtained a higher education (my beloved father), may God prolong his life, and to the one who put me on the path of life, calmed me down, and took care of me until I grew up (my mother), may God prolong her life. And to my brother and sister who had a great impact on many obstacles and difficulties.

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Amjd

Summary

Background:

Ischemic heart disease is considered one of the most common diseases in the world, which classified to Acute coronary syndrome (ACS) (Unstable angina, Myocardial Infraction), coronary artery disease (CAD) (Chronic Coronary Syndrome), affecting males at a greater rate than females. Some special enzymes related to their work in the heart play a role and are more evident when the heart is stressed, including Brain natriuretic peptide (BNP) which is one of several proteins that help regulate blood circulation throughout the body. Although this protein is made by the heart, providers sometimes call it the "brain" natriuretic peptide because it was first detected in brain tissue.

Total anti-oxidant capacity (TAC) which Antioxidants protect against free radical damage by providing electrons to neutralize them. have a very effective effect in the event of its deficiency, as it is a protective factor against the oxidants to which the body is exposed daily for several reasons, including what is the product of electronic leakage, the electron transport chain, including exposure to radiation, smoking, and indigestible molecules.

Justification:

In an attempt to find a new diagnostic methods and early diagnostic markers that helps clinicians for diagnosis heart attack and angina patients. Such methods should be characterized by accuracy and speed, in addition to being financially inexpensive and easy to apply in the laboratory.

Aim of the study:

The study aims to investigate the association between plasma B-Type Natriuretic Peptide (BNP) concentrations and long-term cardiovascular health, exploring whether this relationship persists or diminishes over time after BNP

measurement. Additionally, it examines lipid profile levels in patients with ischemic heart disease compared to a healthy control group, and analyzes the correlation between BNP levels, total antioxidant capacity (TAC), and their impact on long-term outcomes in ischemic heart disease.

Patients and Methods:

This study, which is a case-control study, was conducted on 120 participant with age ranged between (41-70 years), 60 of whom suffered from ischemic heart disease (patient group) and 60 were apparently healthy (control group),who attended the cardiac care unit at the Kerbala Heart Center for the period from December 2022 until July 2023 and who were diagnosed clinically and laboratory according to the standards used for diagnosing acute coronary syndrome and chronic coronary syndrome, and all information was recorded, according to questionnaire paper.

Results:

The results showed that the mean level of BNP (pg/ml) was increased significantly as compared with control group in ACS and chronic CAD with mean ± SD level of 71.42 ±15.56, 55.45±18.21 ACS group and chronic CAD and mean ± SD level of 30.85 ±18.21control group respectively whereas TAC was decreased significantly as compared control group with mean ± SD level of 3.12±0.46, 3.21 ±0.34 in ACS group and chronic CAD and mean ± SD level of 3.47±0.68 in control group respectively.

Conclusion: The study showed that elevated BNP level was an early sign of ACS with higher sensitivity and specificity, while TAC was a protective factor, but with lower sensitivity and specificity. The study showed that CK-MB enzyme activity as a relative indicator (CK-MB RI) of STEMI myocardial infarction was the highest among patients and was followed by NSTEMI then unstable angina (UA) and the lowest was stable angina (SA).

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List of Abbreviations

Abbreviations	Description
ACAT	Acyl coenzyme cholesterol acyltranseferase
ACS	Acute coronary syndromes
AMI	Acute myocardial infarction
AUC	Area under the curve
BMI	Body mass index
BNP	Brain natriuretic peptide
CAD	Coronary artery disease
CCU	Coronary care unit
CHE	Cholesterol esterase
CK	Creatine kinase
CK-MB	Creatine kinase isoenzyme
CVD	Cardiovascular disease
D.M	Diabetes mellitus
DBP	Diastolic blood pressure
ECG	Electrocardiography
ELISA	Enzyme-linked immunosorbent assay
FH	familial hypercholesterolemia
G-Px	Glutathione peroxidase
HDL-C	High-density lipoprotein cholesterol
hs-cTn	High sensitive cardiac troponin
IHD	Ischemic heart disease
IL	Interleukin
LDL-C	Low-density lipoprotein cholesterol
MI	Myocardial infarction
NO	Nitric oxide
NSTEMI	Non-ST-segment elevation myocardial infarction
ROS	Reactive oxygen species
SA	Stable angina
SBP	Systolic blood pressure
SOD	Superoxide dismutase
STEMI	ST-segment elevation myocardial infarction
TAC	Total Antioxidant capacity
TC	Total cholesterol
TF	Tissue factor procoagulant
TG	Triglyceride
TNI	Troponin I
TNT	Troponin T
UA	Unstable angina
VLDL	Very -low density lipoprotein

Chapter One

Introduction and Literature Review

1. Introduction

1.1. Ischemic Heart Diseases

Ischemic heart diseases (IHD) occur when a portion of the heart muscle does not receive enough blood, causing recurrent chest pain or discomfort. A bodily component that is "ischemic" does not receive enough oxygen-rich blood flow . One of the main causes of death worldwide, ischemic heart disease, often known as coronary heart disease, is prevalent in Iraq**(Shi, Xia et al. 2024).**

Narrowing of the coronary arteries is a major cause of various cardiac conditions. When the arteries become constricted, the delivery of blood and oxygen to the heart muscle decreases, leading to coronary artery disease (CAD), also known as coronary heart disease (CHD). This restriction can result in myocardial infarction (MI), commonly referred to as a heart attack. The underlying cause of this narrowing is often atherosclerosis, characterized by the accumulation of cholesterol and plaque within the arterial walls, which restricts blood flow and reduces oxygen supply to the myocardium Fig. (1-1) **(Ferreir, Kumar *et al.* 2023).**

Ischemic heart disease (IHD), or CAD, can manifest as angina pectoris or myocardial infarction. The latter occurs when there is a sudden and complete obstruction of blood flow, causing myocardial cell death. Acute Coronary Syndrome (ACS) is a broader term encompassing the progression from unstable angina to MI, either with ST-segment elevation (STEMI) or without (NSTEMI). This condition is often precipitated by the rupture of an atherosclerotic plaque, which results in arterial blockage **(Bakhtiyor and Saloxiddinovich 2023).**

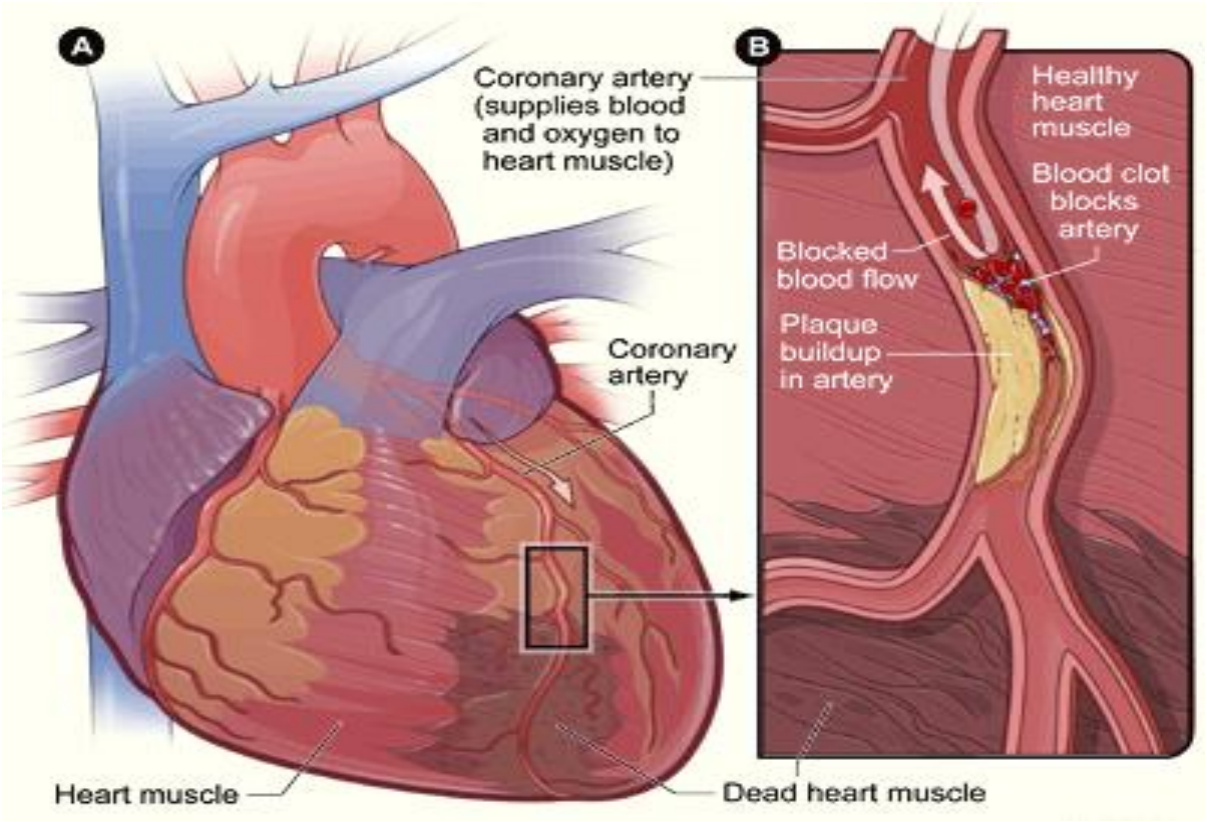

Fig. (1-1): An illustration of Acute Coronary Syndrome (ACS), showing the heart and coronary artery with damage, highlighting areas of dead heart muscle due to a heart attack (Concistrè 2023).

1.2. Signs and Symptoms of Ischemic Heart Disease

The disease's signs and symptoms may appear gradually as the arteries get blocked by plaques. However, if an artery suddenly becomes clogged, the symptoms may not always appear right once. Chest pain or discomfort is a typical symptom that might radiate to the shoulder, arm, back, neck, or jaw. It could occasionally feel like heartburn. Stable angina is linked to the narrowing of the coronary arteries. Symptoms typically worsen with exertion or emotional stress, last for a few minutes, and improve with rest or during predictable conditions. Patients often describe chest heaviness, pressure, numbness, fullness, or squeezing **(Aldiwani, Zaya *et al*. 2020) .**

Unstable angina is defined as varying in intensity, type, or frequency. Myocardial infarction may be preceded by unstable angina. About 30% of

persons who visit the emergency room with pain that is not clearly related to another condition have coronary artery disease. The symptoms of a heart attack, or myocardial infarction, include angina, shortness of breath, perspiration, nausea or vomiting, and dizziness. Receiving emergency medical attention is vital **(Macvanin, Gluvic et al. 2024) ;** as shown in Fig. (1-2).

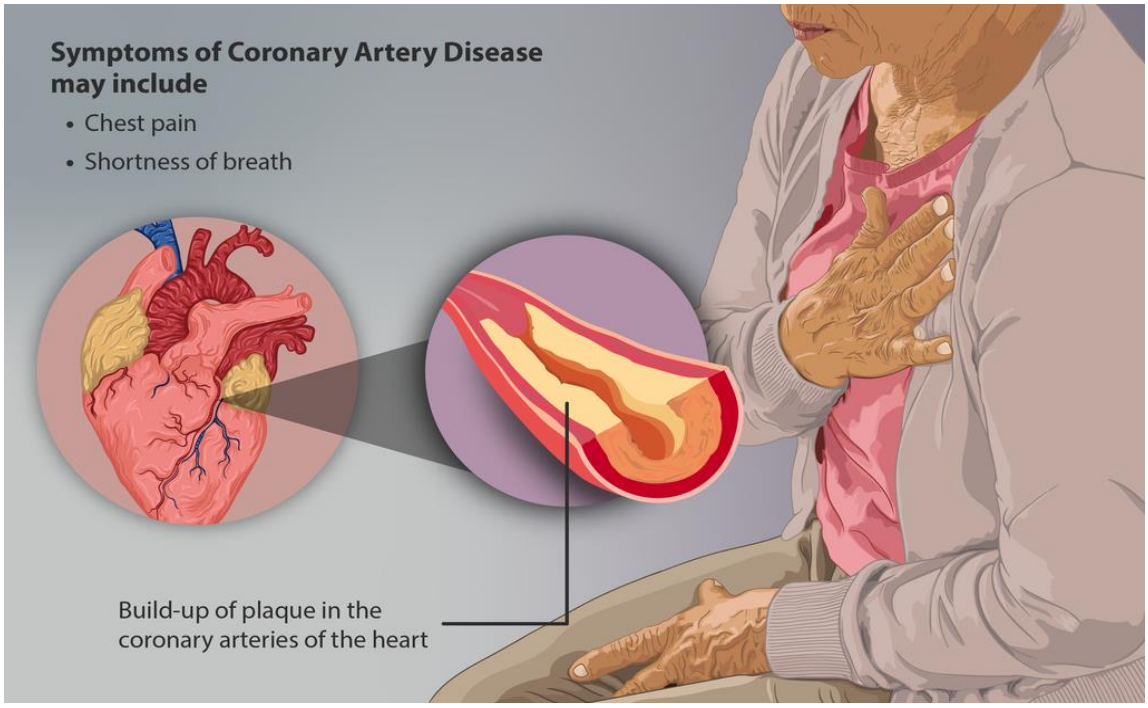

Fig. (1-2): Clogged Artery (Hicks, Veith *et al.* 2024).

1.3. Classification of Ischemic Heart Disease

1.3.1. Stable Coronary Artery Disease (Chronic Coronary Syndromes)

The chronic form of ischemic heart disease often remains stable over many years because the coronary arteries gradually narrow, reducing the heart's oxygen supply over time. Although the patient may experience symptoms, they can typically manage the condition on a day-to-day basis. **(Chakraborty 2024).**

It is the most typical sign. Angina stable refers to sporadic, regular episodes of chest pain or discomfort. This is often observed when engaging in physical exercise or experiencing mental anguish. Resting or taking nitroglycerin, an angina medication, relieves it **(Malik, Mir *et al.* 2023).**

1.3.2. Acute Coronary Syndrome

is one of the most dangerous types of ischemic heart disease (IHD) ACS represents a life- threatening manifestation; like cardiac arrest, electrical or hemodynamic instability with cardiogenic shock (CS) due to ongoing ischemia or mechanical complications such as severe mitral regurgitation, The final diagnosis is made on the basis of clinical presentation, ECG changes, and measurement of cardiac biomarkers **(Kawai, Finn *et al.* 2023).**

1.3.2.1. Unstable angina

Unstable angina occurs when there is a sudden and severe reduction in blood flow to the heart, primarily caused by intraluminal thrombosis, vasospasm, increased blood pressure, or intraluminal plaque formation. This reduction often results from the rupture of atherosclerotic plaques in the coronary arteries, leading to the formation of a blood clot (thrombosis). The condition is considered a medical emergency due to the high risk of progression to myocardial infarction. Unstable angina represents a critical form of ischemic heart disease, requiring immediate intervention to prevent fatal outcomes. **(Abbas, Raza *et al.* 2023)**. Also possible side effects from platelet-derived products include coronary artery spasm. The pain may worsen while at rest and becomes more frequent and persistent. The capacity to precisely forecast discomfort in relation to a specific exertion level is lost. A cardiac attack is highly likely. A partial or total blockage of the coronary arteries **(Tajabadi, Orimi et al. 2022)** .

1.3.2.2. Myocardial Infarction

MI causes the cardiac muscle to irreversibly deteriorate from a shortage of oxygen. A MI may cause systolic and diastolic function to be compromised and increase the patient's risk of arrhythmias. A MI can also result in a number of very dangerous side effects. Reperfusion and reestablishing blood flow to the

heart are crucial. The prognosis improves with earlier treatment—less than six hours after the onset of symptoms. **(Khan 2023).**

A- STEMI (ST- elevation myocardial infarction): Is an event in which trans mural myocardial ischemia results in myocardial injury or necrosis **(Vogel, Claessen *et al.* 2019).**

B- Non STEMI (Non ST- elevation myocardial infarction): Is part of the ACS spectrum. Usually caused by a partial or near-complete occlusion of a coronary artery resulting in compromised blood flow to myocardium with subsequent myocardial injury or called infarction Fig.(1-3) **(Karin 2020).**

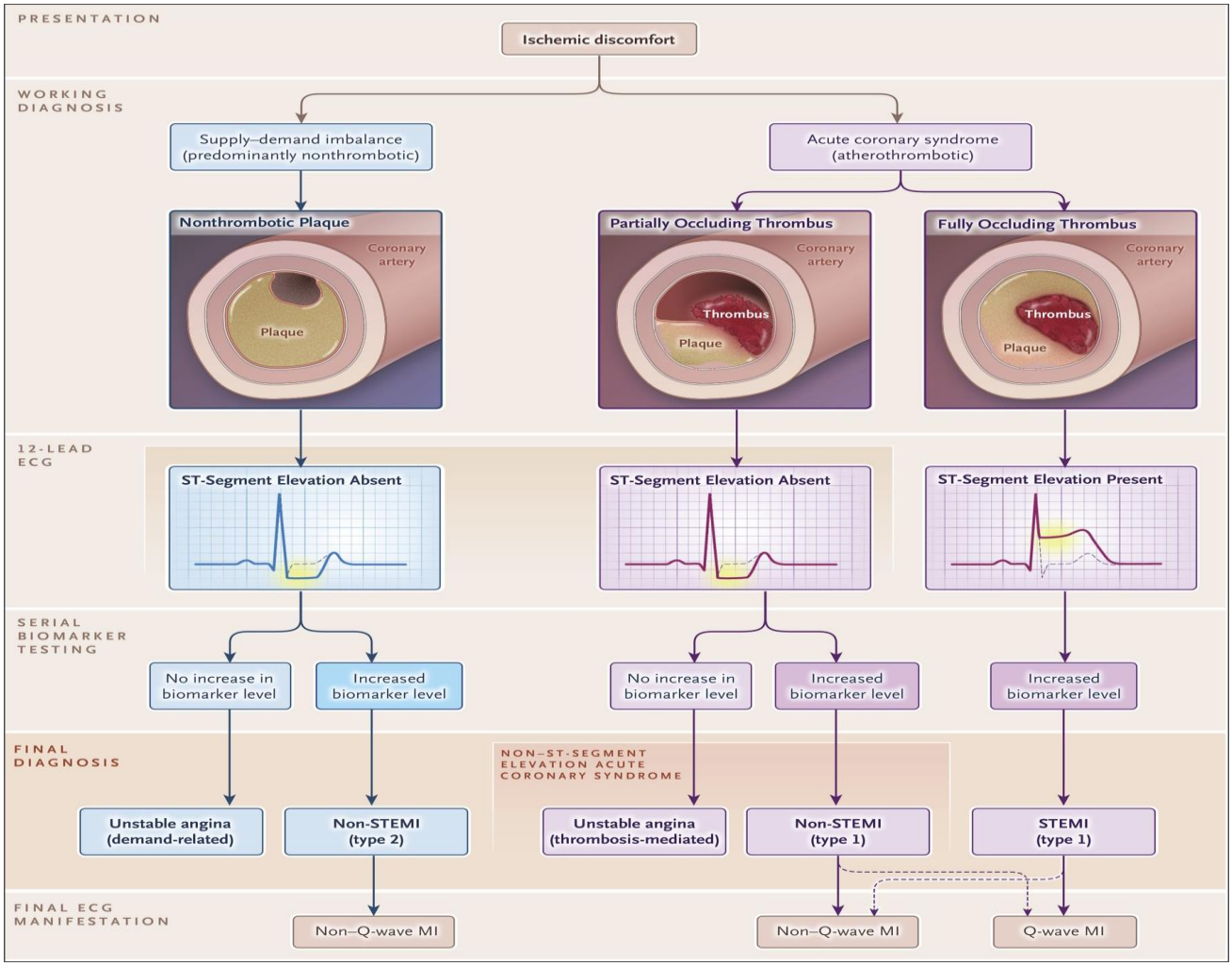

Fig.(1-3): Spectrum of Pathologic and Clinical ST-Segment Elevation Acute Myocardial Infarction (STEMI) and Non-STEMI Acute Coronary Syndromes ECG denotes electrocardiogram, and MI myocardial infarction (Damluji, Forman *et al.* 2023).

1.4. Causes Coronary Artery Diseases

Coronary artery disease (CAD) is primarily caused by atherosclerosis, a progressive condition characterized by the buildup of plaque within the arterial walls. This plaque consists of lipids, cholesterol, calcium, and cellular waste products, along with fibrin, a protein involved in blood clotting. As plaque accumulates, the arteries harden and narrow, limiting blood flow to the heart muscle and increasing the risk of ischemic events such as angina or myocardial infarction (Libby et al., 2019; Lusis, 2020). Over time, the impaired blood flow caused by plaque buildup leads to CAD, which is a leading cause of morbidity and mortality globally**(Caplan 2024).**

Atherosclerosis is a chronic, inflammatory process influenced by various factors, including high cholesterol levels, hypertension, smoking, and diabetes. These risk factors contribute to endothelial dysfunction, which promotes the adhesion of inflammatory cells and lipid accumulation, further driving plaque formation **(Djuraeva, Gaybullayev et al. 2023).**

Angina pectoris and the risk of a heart attack can be caused by the buildup of plaque in the coronary arteries, which reduces blood flow to the heart muscle and prevents it from receiving the oxygen and nutrients it needs to function normally. This condition is known as myocardial ischemia **(Severino, D'Amato et al. 2020).**

1.5. Epidemiology of Ischemic Heart Disease

Ischemic heart disease (IHD) remains the leading cause of death worldwide, accounting for approximately 16% of all deaths globally. According to the World Health Organization (WHO), IHD caused an estimated 9 million deaths globally in 2020. The prevalence of IHD increases significantly with age, and men are more commonly affected than women. In Iraq, IHD was responsible for over 19,000 deaths in 2020, making it the primary cause of

death for both men and women. This aligns with global trends, where IHD disproportionately affects older adults, and the risk triples every ten years of life **(World Health Organization 2018).**

1.6. Risk Factors of Ischemic Heart Disease

1.6.1. Smoking

Smoking damages the lining of the arteries, leading to a buildup of fatty material (atherosclerosis) that narrows the artery. This can cause angina, a heart attack, or a stroke. The carbon monoxide in tobacco smoke reduces the amount of oxygen in the blood. This means that the heart is pumping harder to provide the body with the oxygen it **needs (Virani, Alonso et al. 2020)**.

1.6.2. Hypertension

is a chronic medical condition characterized by consistently elevated blood pressure in the arteries.**(Narita, Hoshide *et al*. 2023)**.Prolonged increase of blood pressure is a substantial risk factor for CAD. **(Parati, Bilo *et al*. 2023).** Blood pressure is classified using the systolic and diastolic pressures, which are the greatest and lowest pressures, respectively. Most persons have normal blood pressure at rest, which ranges from 60 to 80 mmHg diastolic and 100 to 130 mmHg systolic.

1.6.3. Dyslipidemia

A blood concentration of lipids or lipoproteins that is abnormally high is known as hyperlipidemia. Diabetes, familial hypercholesterolemia (FH), obesity, dietary issues, and other medical disorders can all contribute to it. diabetic complications **(Wodaje 2023).** Patients with hyperlipidemia are about twice as likely to develop cardiovascular disease (CVD). Excess cholesterol raises the risk of heart disease and other cardiovascular disorders by causing plaque to accumulate between the layers of artery walls, which makes it more difficult for the heart to pump blood **(Henein, Vancheri *et al.* 2022).**

Although hyperlipidemia is well recognized to exacerbate blood vessel atherosclerosis, numerous studies have shown that it can also have an immediate impact on the heart, increasing the risk of ischemia/reperfusion and decreasing the effectiveness of cardiac prophylaxis. Procedures like preconditioning for ischemia **(Miao, Zang et al. 2020).**

There is a relationship between cholesterol levels and the chance of coronary heart disease. This is caused by a fatty deposit known as plaque under the blood vessel's endothelium, or inner lining. The plaque becomes larger and cracks or ulcerates on the outside. **(Reiter, Sharma et al. 2024).**

Platelets are now drawn to the damaged endothelium, and clot formation begins. Numerous vascular disease symptoms are brought on by this process, which causes a partial or complete obstruction of the vessel's flow. This include stroke and associated events in addition to angina and heart attacks **(Flora and Nayak 2019).**

Based on their origin and lipoprotein content, the different types of lipoprotein are classified as HDL, LDL, VLDL, IDL, or chylomicrons. One type of high cholesterol is brought on by a genetic mutation that destroys LDL receptors, preventing LDL from entering cells. This is recognized as a family history of elevated cholesterol **(Kumaran, Devadarshini et al. 2024)** The HDL type of cholesterol: HDL Hepatocytes and intestinal mucosal cells produce the smallest, densest, and most soluble lipoproteins, along with VLDL and chylomicron production. High density lipoprotein (HDL) transports cholesterol from tissues to the liver **(Sánchez-Ortiz, Mateo-Sanz et al. 2024).**

Mostly there is a 2% to 3% reduction in the risk of developing cardiovascular disease for every 1 mg/dL increase in HDL cholesterol. According to current standards, patients with angiographically confirmed coronary artery disease are more likely to have high levels of LDL and low levels of HDL. Additionally, HDL may contain antioxidant enzymes that lower

oxidized phospholipid levels in athermanous lesions, thereby accelerating thermogenesis **(Balzarotti 2018).**

The evidence for LDL cholesterol as a cause of coronary heart disease is strong.

Elevated cholesterol levels in human populations are consistently linked to an increased risk of cardiovascular events in the future. Large-scale clinical trials have shown a decrease in cardiovascular events when LDL cholesterol levels are lowered through the use of statins, intestinal bypass surgery, bile acid-binding resins, and other therapies. Hence, LDL cholesterol satisfies the requirements of the modified Koch's postulates and is one of the agents that causes atherosclerosis **(Gupta, Kushwah et al. 2024).**

The plasma atherosclerosis index (AIP) is a new index consisting of triglycerides and high-density lipoprotein cholesterol. It has been used to measure blood lipid levels and is commonly used as an optimal indicator of dyslipidemia and associated diseases (eg, cardiovascular disease).

The lipid profile of atherosclerosis is defined as an increase in total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), and a decrease in high-density lipoprotein cholesterol (HDL-C). Some studies have reported that HDL cholesterol levels are lower in patients with CD than in healthy people. Other studies have reported that the Castelli risk index I and II (TG/HDL-C and LDL-C/HDL-C, respectively), the plasma arterial stiffness index (PAI; logarithm of TG/HDL-C), and the non-HDL-C index (TG). -HDL-C), and indices of atherosclerosis coefficient (AC; non-HDL-C/HDL-C) are more sensitive in predicting atherosclerotic cardiovascular disease risk.

Triglyceride-rich lipoproteins (TGs) and the Risk of Heart Disease Hypertriglyceridemia, or an excess of triglycerides in plasma, is linked to coronary artery disease. It is carried by the chylomicron lipoprotein particles.

Triglycerides (85–92% of the total), phospholipids (6–12%), cholesterol (1-3%), and proteins (1-2%) are among these particles(Malick, Waksman et al. 2023). They work to transport dietary lipids from the intestines throughout the body. An elevated plasma TG level alone is a risk factor for coronary heart disease (CHD), even though hypertriglyceridemia (HTG) is frequently linked to insulin resistance in obese individuals **(He, Ding et al. 2024).**

High blood cholesterol levels are another name for dyslipidemia. Every cell membrane contains cholesterol, a waxy, white, fatty substance. A essential component of numerous bodily processes, bile acids are also made of it, as are other significant compounds as erect hormones, estrogen, and testosterone. Both animal meals and bodily synthesis are sources of this substance **(Ofori 2023)**.

One kind of lipid that is present in every human cell is cholesterol. Hyperlipidemia and other lipid disorders, however, can be brought on by an excess of lipids and other fatty substances in the blood. Atherosclerosis and heart disease are significantly more likely to develop in those with hyperlipidemia **(Yu, Ding et al. 2023).**

The circulatory system consists of blood arteries, the heart, and blood. Throughout the body, blood is responsible for carrying out numerous vital functions, such as distributing hormones, nutrients, carbon dioxide, and oxygen. Red blood cells, white blood cells, platelets, and nutrients are all found in blood. Additionally, cholesterol moves through the bloodstream, the "bad cholesterol," or LDL, and the "good cholesterol," or HDL, are the two common types of cholesterol. When blood LDL levels are higher than recommended, it is referred to as hyperlipidemia **(Upadhyay 2023)**.

Plaque is a substance that forms in the blood vessels when cholesterol and other fatty compounds mix in the bloodstream. Over time, plaques resulting from an increase in lipids may enlarge and impede blood flow Even in the absence of evident coronary artery stenosis, long-term hyperlipidemia disrupts

cardiac function and electrical activity and induces fat buildup in the heart **(Das and Ingole 2023)** .

1.6.4. Body Mass Index and Obesity

Atherosclerosis and obese share causes. In both cases, lipids, oxidized LDL, and free fatty acids cause inflammation and disease. From early endothelial dysfunction to consequences, inflammation produces atherosclerosis and is linked to obesity, insulin resistance, and diabetes **(Malekmohammad, Bezsonov et al. 2021).** BMI is considered an estimate of body fat and a good gauge of your risk for diseases that can occur with more body fat. The higher BMI, the higher your risk for certain diseases such as heart disease, high blood pressure, type 2 diabetes, both overweight and obesity can increase the risk of developing coronary artery disease. Maintaining a healthy diet and a moderate weight can help decrease the chance of this and other heart conditions. can be classify BMI Categories as the following: Underweight = <18.5, Normal weight = 18.5–24.9, Overweight = 25–29.9, Obesity = BMI of 30 or greater.

1.6.5. Diabetes Mellitus

The hallmark of diabetes is consistently high blood sugar levels. When cells do not respond to insulin type II or when the pancreas does not produce enough insulin type I, the result is diabetes. Severe diseases, such as acute coronary syndromes (ACS), thrombolysis, percutaneous coronary intervention (PCI), and ST-segment elevation myocardial infarction (STEMI), are associated with an increased risk of mortality in hospitalized patients with high blood sugar levels **(Babes, Bustea et al. 2022).**This linear association between blood glucose and mortality seems to hold true even after controlling for diabetes; the probability of death increases as blood glucose levels rise **(Lin, He et al. 2020)**.

1.6.6. Chronic Stress

Adversity causes chronic stress, which can cause arterial plaque. Chronic

stress affects AS through various pathways that are still unknown. Chronic stress-induced autonomic problems may increase AS risk. Experimental stress-induced hyperlipidemia may also be involved **(Popovic, Bjelobrk et al. 2022).**

1.6.7. Aging

Cardiovascular function diminishes with age, increasing CVD risk in elderly people. CVD risk factors include obesity, diabetes, and hypertension. Oxidative stress increases with aging, causing functional and electrical disturbances that cause CVD, ROS and inflammatory signal molecules from oxidative stress cause AF and HF Frailty, obesity, and diabetes increase with age. Figure 1-4 shows that these parameters, along with TNFα and CRP, are independent risk factors for CVD **(Abd-Elmoniem, Ishaq *et al.* 2024).**

1.7. Pathophysiology

CAD is characterized by atherosclerotic plaque. Fatty plaque narrows channel lumens and restricts blood flow **(Komilovich and PRACTICE 2023)**. The first step in the process is the formation of a "fatty streak." Fatty streak is formed by subendothelial deposition of lipid-laden macrophages, also called foam cells. When a vascular insult occurs, the intima layer breaks, and monocytes migrate into the subendothelial space where they become macrophages. These macrophages take up oxidized low-density lipoprotein (LDL) particles, and foam cells are formed. T cells get activated, which releases cytokines only to aid in the pathologic process. Growth factors released activate smooth muscles, which also take up oxidized LDL particles and collagen and deposit along with activated macrophages and increase the population of foam cells. This process leads to the formation of subendothelial plaque **(Kovanen 2019).**

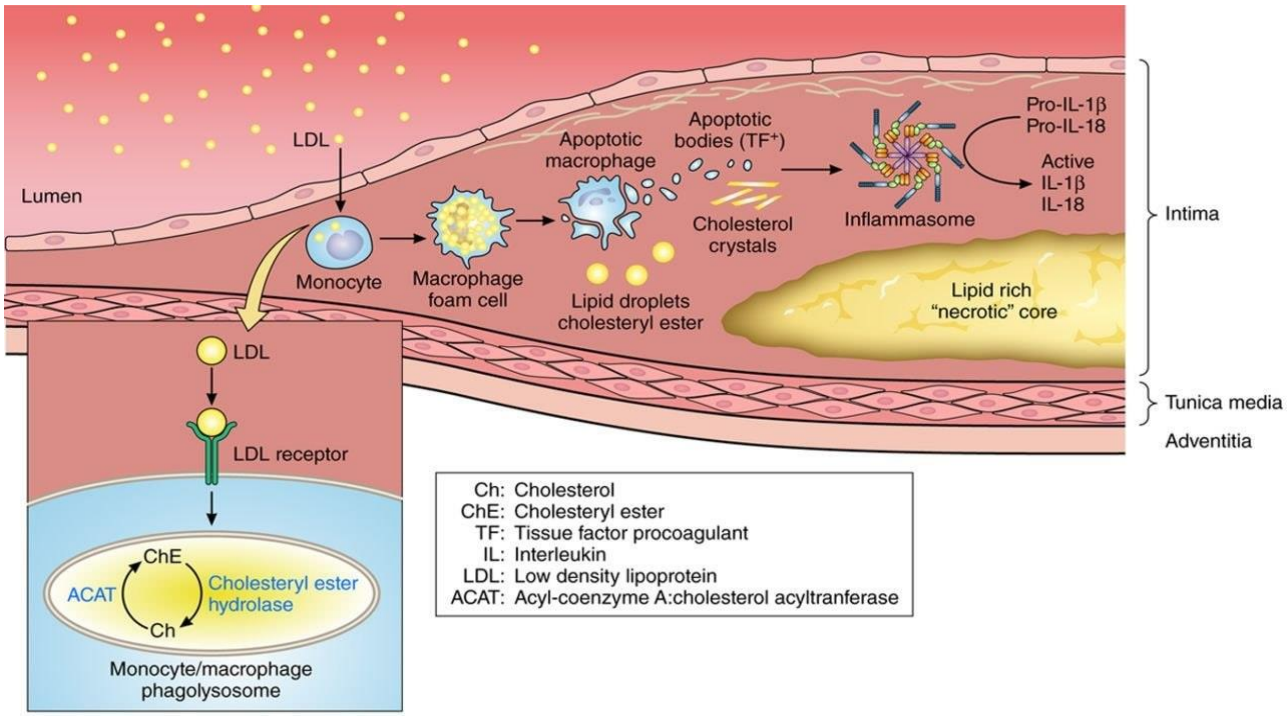

Fig. (1-4): Cholesterol crystals activate local innate immune pathways in the atherosclerotic plaque (Schelemei, Wagner *et al*. 2024).

If the endothelium is not further insulted, this plaque may develop or become stable. If stable, a fibrous cap will form and calcify the lesion. Time can make the lesion hemodynamically severe enough to prevent blood from reaching cardiac tissue at higher demands, causing angina. At rest, oxygen requirements drop, reducing discomfort. A 90% stenosis lesion causes resting angina **(Shahjehan and Bhutta 2020).** Some plaques burst, exposing tissue factor and causing thrombosis. Depending on the level of injury, this thrombosis could produce subtotal or total lumen blockage and acute coronary syndrome (ACS) as unstable angina, NSTEMI, or STEMI **(Baaten, Nagy *et al.* 2024).**

1.8. Diagnosis of Ischemic Heart Disease

1.8.1. Clinical Diagnosis of Ischemic Heart Disease

Depends largely on the nature of the symptoms, there are a number of investigations that can be done to confirm the diagnosis depends on the clinical presentations and suspected pathology like:

a. Baseline electrocardiography (ECG),

b. Exercise ECG – Stress test

c. Exercise radioisotope test (nuclear stress test, myocardial scintigraphy)

d. Echocardiography (including stress echocardiography). Coronary angiography

e. Intravascular ultrasound

f. Chest x ray

g. Magnetic resonance imaging **(Beltrame, Tavella *et al*. 2021)**.

1.9. Oxidants and Anti-oxidants

Electrons in an atom or molecule occupy orbitals. The outermost orbital contains two electrons, each spinning in opposite direction. The chemical covalent bond consists of a pair of electrons, each component of the bond donating on electron each. A free radical, in contrast, is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital(Mahmoudi, Dehkordi et al. 2021). Free radical is generally represented by a superscript dot (R•), Fig. 1-5.

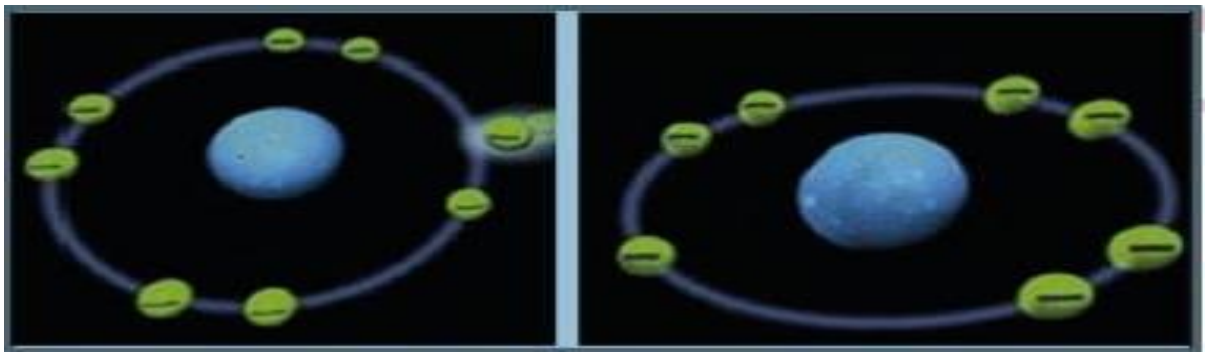

Fig. (1-5): Left side = normal oxygen atom with all paired electrons; one electron is in the process of jumping out. Right side = free radical, with an unpaired electron (Mizuno, Matsuoka *et al.* 2024).

Oxidation reactions totally convert molecular oxygen to water. Partial reduction of oxygen produces extremely reactive chemicals that damage living systems. ROS are reactive oxygen species (Adetuyi, Adebayo et al. 2022). The

following are members of this group as shown in Fig. (1-6):

1. Superoxide anion radical (O_2•–)
2. Hydroperoxyl radical (HOO.)

Free radicals oxygen and nitrogen have unpaired electrons. Oxygen-based ROS includes **H_2O_2,** the singlet oxygen, the supercharged oxide ions (O_2•−), hydroxyl radicals (HO•), and nitric oxide (•NO). Normal metabolism produces ROS **(Martemucci, Portincasa *et al*. 2023)**. Pathological ROS can overwhelm cellular defenses and kill cells. ROS decrease and oxidize. O_2•−, H_2O_2, HO•, and lipid peroxides have a vital role in biological processes such a process called aging, inflammatory processes, repairs to tissues, and cellular messenger systems **(Sanabria-Castro, Alape-Girón *et al.* 2024).**

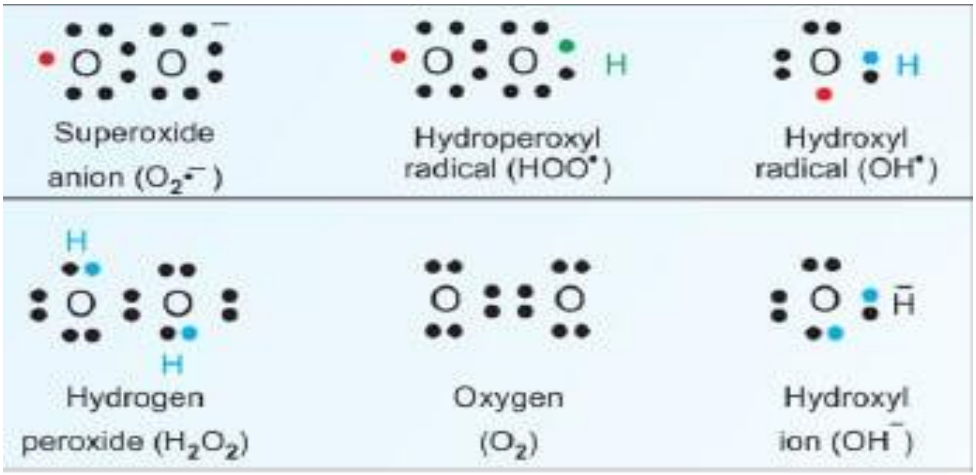

Fig. (1-6): Some free radicals. Please compare hydroxyl radical (free radical) with hydroxyl ion, which is not a free radical. Also compare oxygen with superoxide anion(Wu, Li *et al*. 2024).

1.10. Antioxidants

Antioxidants donate electrons to fight free radicals and reduce damage. Antioxidants scavenge free radicals to protect cells **(Asfaw 2022).** Before damaging important molecules, low-molecular-weight antioxidants safely battle free radicals and stop chain reactions. Metabolism produces glutathione, ubiquinol, and uric acid. Other diet antioxidants are moderate. Various enzyme

systems in the body fight free radicals, but the main antioxidant are the antioxidant vitamins E (α-tocopherol), C (ascorbic acid), and β-carotene. The body cannot produce these tiny nutrients, thus they must be ate **(Chauhan, Chandel et al. 2022)**.

Radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, co-antioxidants, gene expression modulation, and metal-chelating agents are Intracellular and extracellular antioxidants detoxify ROS enzymatically and nonenzymatically **(Chaudhary, Janmeda et al. 2023)**.

A chemical reaction that produces free radicals (typically autoxidation). Organic substances, including living things, degrade by autoxidation. To lengthen their lifespans, polymers, fuels, and lubricants are often treated with antioxidants **(Çelebi, Koç et al. 2024)**.Antioxidants protect foods from rancidity, especially oils and fats. Glutathione, as well as superoxide dismutase, can protect cells from oxidative stress. Dietary antioxidants or exogenous antioxidants include vitamins A, C, selenium and E, although many additional substances have antioxidant effects only in vitro and minimal evidence in vivo. Human studies have demonstrated that antioxidant supplementation do not prevent disease **(Mota, Almeida *et al.* 2023).**

1.10.1. Enzymatic Antioxidants

Endogenous antioxidants include enzymatic antioxidants such as SOD, CAT, G-Px, G-Red, and NADH–metHb reductase which inhibit erythrocyte oxidative denaturation. Antioxidant enzyme SOD catalyzed the dismutase reaction (O_2•− + 2H+ ----------► H_2O_2 + O_2). Superoxide anion is transformed to O_2 and H_2O_2 by the ubiquitous metal-containing enzyme SOD. Depending on the metal cofactor, superoxide dismutases can bind copper and zinc, iron or manganese, or nickel. SOD isoenzymes are found in several cell compartments

in higher plants. The Mn-SOD resides in mitochondria and peroxisomes **(Islam, Rauf *et al.* 2022)**.

1.10.2. Non-Enzymatic Endogenous Antioxidants

The two steps of endogenous non-enzymatic antioxidants are:

a. Fat-soluble (vitamin E, carotenoids, ubiquinone, melatonin)
b. Hydrophilic or water-soluble (vitamin C, glutathione, uric acid, ceruloplasmin, transferrin, haptoglobulin) **(Chakraborty 2023)**.

Antioxidant vitamins A, C, and E protect against oxidative damage. Vitamin E is a chain-breaking antioxidant in the lipid phase and vitamin C in the aqueous phase. Vitamin C lowers O_2•− and lipid peroxyl radicals and works synergistically with vitamin E. It is the enolate anion at physiological pH. GSH-dependent dehydroascorbate reductase recycles dehydroascorbate from a second reduction or dismutation step **(Gulcin 2020)**. Dehydroascorbyl radical can dismutate to ascorbate and dehydroascorbate. It prevents t-butylhydroperoxide from peroxidizing erythrocyte membrane lipids and tocopherols, preserving lipids by 92% and vitamin E by 50% and 65%. Vitamin E is nature's most abundant antioxidant. Vitamin E contributes electrons to lipid peroxyl radicals to create resonance-stabilized free radicals **(Burdon 1999).**

1.11. Oxidative Stress

Oxidative stress favors prooxidants over antioxidants **(Donn, Seyyedi-Mansour et al. 2024).** This imbalance can cause macro-molecular damage like DNA strand breakage, damage to membrane ion transport systems, enzymes, and other proteins, and lipid peroxidation, or oxidative stress, which damages a wide range of molecular species. Trauma, infection, heat injury, hypertoxia, toxins, and extreme exercise can cause short-term oxidative stress **(Rizvi, Zaidi *et al.* 2020)**. Injured tissues create excess ROS due to phagocyte activation, release of free iron and copper ions, or disruption of oxidative phosphorylation

electron transport chains. The imbalance between ROS and the antioxidant defense system has been associated to cancer initiation, development, and progression, as well as radiation and chemotherapy adverse effects , ROS are linked to diabetes, age-related eye disease, and neurological diseases including Parkinson's **(Leyane, Jere *et al*. 2022**).

Its arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of biomolecules **(Engwa, Nweke et al. 2022)**. Lipoprotein particles or membranes characteristically undergo the process of lipid peroxidation, giving rise to a variety of products including short chain aldehydes such as malondialdehyde or 4-hydroxynonenal, alkanes, and alkenes, conjugated dienes, and a variety of hydroxides and hydroperoxides. Oxidative damage to proteins and nucleic acids similarly gives rise to a variety of specific damage products as a result of modifications of amino acids or nucleotide. Such oxidative damage might also lead to cellular dysfunction which might contribute to the pathophysiology of a wide variety of diseases**(Kumar and Gupta 2022)**.

1.12. Biomarkers of Ischemic Heart Disease

Clinicians can now identify myocardial infarction (MI) in about one third of patients who would not have met criteria before the availability of blood cardiac markers with much increased sensitivity for myocardial damage **(Clerico, Zaninotto et al. 2021)**. The development of new standards has been prompted by the growing use of biomarkers that are more sensitive and by the advent of more accurate imaging techniques **(Achenbach, Fuchs et al. 2022)**.

1.12.1. Cardiac-Specific Troponin

Three subunits make up the troponin complex, which controls the contractility of striated muscle in response to calcium. One of them is troponin C (TnC), which binds calcium ions; another is troponin I (TnI), which

suppresses connections between actin and myosin; and finally, troponin T (TnT) binds tropomyosin, allowing the troponin complex to be attached to the thin filament. The cytosolic pool and the structural (myofilament-bound) pool are the two locations from which cTnT and cTnI are released following myocyte injury **(Keyt, Duran et al. 2022).** Cardiac and skeletal muscle TnT and TnI are encoded by separate genes, which enables the development of quantitative test antibodies that are unique to the heart In order to diagnose MI, the new criteria center on measuring cTnT or cTnI. Assays for cTnT and cTnI were examined **(Clerico, Zaninotto *et al.* 2024).**

Subtypes of troponin (cardiac I and T) are sensitive and specific indicators of heart muscle damage (myocardium). They are measured in the blood to differentiate between unstable angina and myocardial infarction (heart attack) in people with chest pain or acute coronary syndrome. A person who recently had a myocardial infarction has areas of damaged heart muscle and elevated cardiac troponin levels in the blood.(Sari and Nugraheni 2024)This can also occur in people with coronary vasospasm, a type of myocardial infarction involving severe constriction of the cardiac blood vessels. After a myocardial infarction troponins may remain high for up to 2 weeks **(Qaddoori, Mohammed et al.).**

1.12.2. Creatine Kinase

Creatine kinase (CK) is an enzyme that produces and uses phosphocreatine to transfer energy for maintaining tissue and cellular energy homeostasis, being considered the main controller of cellular energy homeostasis. Creatine kinase is mainly exists in heart and skeletal muscle, with small amounts in the brain. The cells in skeletal, heart muscles or brain release creatine kinase into blood stream when they are damaged **(Liu, Ullahkhan *et al.*, 2024).**

Its activity in plasma/serum has been commonly used to evaluate tissue damage, since CK is released into the bloodstream when tissue damage occurring. The small amount of CK that's normally found in blood mainly comes from skeletal muscles (the muscles that are attached to bones and tendons). Any condition such as injury causes muscle damage and/or interferes with muscle energy production increases levels of serum CK activity, for example, intense exercise can increase CK levels. Muscle diseases (myopathies) such as muscular dystrophy can also increase serum CK activity levels **(Gropp, Bolon *et. al*., 2024).**

1.12.2.1. Biochemical Roles of Creatine Kinase

Creatine kinase is an extremely important enzyme in tissues with large and variable energy demands, like the brain, heart and muscle**.** Creatine kinase catalyzed the following reaction:

MgADP + Phosphocreatine + H^+ ◄=====► MgATP + Creatine

This reaction adds a natural phosphate group to muscle creatine producing high-energy molecule phosphocreatine, which used to generate energy. CK gets into bloodstream when the muscles, heart or brain experience acute damage or chronic degeneration **(Syed, Khan, *et. al*., 2024).** When the muscles are damaged, the cells break down, and their contents, including creatine kinase, leak into bloodstream.

The enzymatic activity of CK is an important biological reaction in the formation of adenosine triphosphate (ATP), which is necessary for energy-demanding processes in human and animal cells, especially muscle contractions **(Jurjiu, Damian *et. al*., 2021)**.

CK is present at high levels in the cytoplasm and mitochondria of tissues with high energy demands, and is highly compartmentalized, with isoenzymes

in cytoplasmic (cytosolic CK) and mitochondrial (mitochondrial CK) subcellular locations **(Can, Inel *et. al*., 2023).**

1.12.2.2. Creatine Kinase Isoenzymes

. There are typical three isoenzymes of CK identified in the cytoplasm of various tissues and organs, namely CK-3 or CK-MM; the hybrid form CK-2 or CK-MB; and CK-1 or CK-BB isoenzyme which differs in their subunit constituents. CK-MM is the predominant isoenzyme found in normal serum, and it is primarily located in the skeletal muscle and to a lesser amount in the heart muscle or myocardium **(Parsanathan, *et al*., 2020).** CK-MB isoenzyme is mostly present in cardiac muscle and contributes to approximately 25–46% of the total CK activity in the myocardium, with minor amounts found in skeletal muscle**.** CK-BB is mainly expressed in the brain tissues, as well as the it was found in prostate, bladder, smooth muscle, uterine, liver, kidney and gastrointestinal tract **(Jurjiu, *et al*., 2021).**

1.12.2.3. Roles of Creatine Kinase and its Isoenzymes in Clinical Diagnosis

Creatine kinase (CK) with its isoenzymes have been widely used as a indices in clinical diagnosis to identify skeletal muscle, myocardium, prostate, spleen and brain disorders and other health conditions. Their activity levels hold significant diagnostic value, appearing in distinct patterns across various tissues. Muscular activity, especially long-term and intense exercise and eccentric muscular training, may increase serum activity level of CK markedly **(Infusino, *et. al*., 2024),** while leisure physical exercise increases its activity level modestly; i.e., approximately 5% **(Stamatakis, *et al*., 2024).** In addition to physiological elevation of CK activity, population studies have identified that a slightly increased in serum CK activity as a possible cardiovascular disease (CVD) risk factor which include hypertension, obesity, and metabolic syndrome **(Rafaqat, *et al*., 2024).**

1.12.2.4. Creatine Kinase – MB isoenzymes in Heart Diseases

Following acute myocardial infarction, total CK and CK-MB levels begin to rise 5 to 6 hours after the onset of chest pain. The serial profile of the rise and fall of both activities is nearly always indicative of AMI. The recent increase in the use of thrombolytic agents in an attempt to attain reperfusion of occluded coronary arteries alters the enzyme profiles observed in blood after AMI **(Al-Toma and Al-Mudhaffar, 1990 ; Motamed, *et al*., 2023).** The level of CK-MB in the serum will be rapidly increased in response to the damage to either cardiogenic or non-cardiogenic tissue, with the release of large amounts of muscle enzymes into the bloodstream **(Wu, *et. al*., 2021).** Therefore, a marked elevation of CK-MB levels in serum or plasma is a crucial diagnostic indicator of coronary syndrome especially acute myocardial infarction (AMI) **(Al-Mudhaffa and Al-Toma,1992),** therefore, CK-MB isoenzyme activity becomes a specific and good sensitive biomarker for myocardial injury. CK-MB activity levels begin to rise 4-6 hours after the onset of chest pain due to acute myocardial injury and returns to baseline level after 36-48 hours **(Aslam Hayat, *et. al*., 2022).** Despite less sensitivity and specificity for acute myocardial infarction compared to troponin, CK-MB is still a useful cardiac marker. CK-MB rises and returns to baseline more rapidly, which make it a preferred marker for reinfarction. Moreover, CK-MB can be used to indicate successful reperfusion after fibrinolysis, to estimate the infarct size, and to predict infarct-related mortality.

Therefore, CK-MB is a useful marker not only for acute myocardial injury but also can be elevated in sera of various disorders such as (acute myocardial infarction, myocarditis, cardiac trauma or contusion, cardiac surgery, endomyocardial biopsy and defibrillation or cardioversion) **(Komarova, *et. al*, 2022).**

1.12.2.5. CK-MB Isoenzyme and other Biomarkers in Coronary Artery Diseases

Cardiac biomarkers such as creatine kinase-MB (CK-MB) isoenzyme, troponin I, and lipid profile are important in the evaluation of hypertensive subjects. Creatine kinase-MB has a sensitivity and specificity of >90% for the detection of myocardial injury. The CK-MB is the heart specific isoenzyme and used to be the reference method for the diagnosis of acute myocardial infarction in most laboratories and increased levels are frequently interpreted as objective evidence of myocardial injury clinically **(Abhra Ghosh, *et. al*., 2023).** Due to the greater cardio-specifity of cardiac troponin I (cTnI) and cardiac troponin T (cTnT), compared to CK-MB, troponins are increasingly used as diagnostic markers for cardiac injury or infarction **(Savonnet, *et. al*., 2021).** It was suggested that high CK activity in black population may be responsible for increased risk of hypertension, since the enzyme aids highly energy-demanding processes in the body such as sodium retention, cardiovascular contractility, and modulation of arteries **(Kumar and Cannon, 2009).** Consequently, high CK activity especially in resistance arteries may enhance pressure responses and increase blood pressure.

Troponin remains in circulation for a longer duration when compared to CK-MB. In conditions where reinfarction is suspected, CK-MB may be useful to classify a new event due to its shorter duration of elevation at detectable levels in plasma**.** However, after the advent of troponin and the current aggressive interventional approach to AMI, and due to a lack of literature comparing CK-MB against troponin in the diagnosis of reinfarction, the use of CK-MB has declined **(Savonnet*, et. al*., 2021).**

1.12.2.6. A CK-MB relative index

A CK-MB relative index < 3% is consistent with the skeletal muscle source, whereas a relative index > 5% is consistent with the cardiac source of

CK-MB**.** However, prior studies in patients with trauma and patients with chronic skeletal muscle abnormalities have demonstrated the failure of the CK-MB relative index in differentiating skeletal muscle sources of CK-MB from myocardial cell death **(Lloyd-Jones, *et. al.*, 1999).** Hence in patients with clear evidence of lack of trauma, chronic skeletal muscle abnormalities, and a high index of suspicion for AMI, the use of CK-MB RI can increase the specificity of CK-MB testing. Miscellaneous causes include hypothyroidism, renal failure, alcohol intoxication, pregnancy, and certain types of malignancies.

1.12.3. Natriuretic Peptide

Natriuretic peptides (NPs) are peptide hormones synthesized by the heart, brain, and other organs. The release of these peptides by the heart is stimulated by atrial and ventricular dilatation, as well as by neurohormonal stimuli, usually in response to heart failure. The main physiological effects of natriuretic peptides are to reduce arterial pressure by decreasing blood volume and systemic vascular resistance. It is considered Natriuretic peptides are a family of hormones that play important roles in salt and water balance. Human natriuretic peptides include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodelatin. Most natriuretic peptides are produced primarily in the heart but are also released in other tissues, including the kidneys, lungs, and central nervous system.

1.12.3.1. B-Type Natriuretic Peptide

B-type Natriuretic Peptide (BNP) is one of several proteins that help regulate blood circulation throughout the body. Although this protein is made by the heart, providers sometimes call it the "brain" natriuretic peptide because it was first detected in brain tissue **(Sarzani, Allevi *et al*. 2022).**

In the circulatory system, the heart and blood vessels supply oxygen-rich blood to muscles, organs, and tissues. The left ventricle pumps a lot of blood

through the heart and body which is increased as a result of myocardial stress of the ventricular wall and tissue hypoxia, Rather than infecting cells, it has been shown to be predictive Index is independent of hemodynamics and other variables and Biochemical markers in patients with known acute coronary artery disease **(Kim 2022)**.

To improve its utility in therapeutic contexts, additional data on BNP's prognostic marker efficacy in both the general population and patients with ischemic heart disease is required **(Zhao, Gao *et al.* 2020).**

1.12.4. Total Antioxidant capacity

Antioxidants protect against free radical damage by providing electrons to neutralize them. Use antioxidants to shield cells from free radicals **(Sibanda 2021).** Protect critical molecules from free radicals with low-molecular-weight antioxidants. The body produces antioxidants such glutathione, ubiquinol, and uric acid during metabolism.Food contains softer antioxidants. Primary antioxidants in the body include α-tocopherol, ascorbic acid, and β-carotene, along with several enzyme systems that fight free radicals. The body can't make some micronutrients, thus you must eat them (Pisoschi and Pop 2015).

“Antioxidants” are natural or manufactured compounds that may prevent or postpone oxidative cell damage produced by physiological “oxidants” with positive reduction potentials, such as ROS/RNS and free radicals **(Acworth, McCabe et al. 2017)**. The phrases “oxidant” and “antioxidant” are complementary since they neutralize each other. However, a “prooxidant” may not have a large reduction potential but may cause oxidative damage to DNA, lipids, and proteins. Transition metal ions at lower oxidation states are not oxidants, although they can generate ROS/RNS with hydrogen peroxide or molecular oxygen, making them prooxidants **(Yordi, Pérez et al. 2012)**. When present at relatively low quantities compared to the oxidizable substrates, antioxidants delay or prevent oxidation **(Olatunde, Ogunro *et al.* 2024)**. . The

phrase "oxidizable substrate" refers to biomacromolecules like lipid, protein, and DNA in general **(Zhu, Wang *et al.* 2024)**. This description stresses the damage target and ROS/RNS source utilized to assess antioxidants and their effects. However, Finley et al. note that the term "antioxidant" may have different connotations to different audiences, such as the ability to quench metabolically generated ROS (to biochemists and nutritionists), retard food oxidation (to food scientists), or yield high TAC values in ET- and HAT-based in vitro assays. Secondary or preventative antioxidants (typically transition metal ion chelation) and primary or chain-breaking antioxidants (primarily ROS/RNS scavenging) have been traditionally split for convenience **(Sies, Belousov *et al.* 2022**).Thus, antioxidants can directly scavenge or prevent reactive species. It may also indirectly boost endogenous antioxidant defenses. This study examines antioxidant capacity/activity assay techniques that measure chain-breaking or preventative antioxidant ability**(Singh 2024)**.

1.13. Relationship between Biomarkers in Ischemic Heart Disease

1.13.1. Role of Beta Natriuretic Peptide

Stressing heart releases beta natriuretic peptide (BNP) and N-proBNP. The commercial assay detects BNP and N-proBNP in suspected heart failure patients. Detect and treat heart failure with N-pro BNP and BNP. the growing involvement of BNP and N-proBNP in acute coronary syndromes. Experimental studies show rapid BNP release in response to myocardial ischemia, but many other disorders cause small BNP elevations, rendering BNP unreliable for diagnosis. A highly predictive BNP was observed in ACS patients. High BNP readings predict poor clinical outcomes and indicate clinical factors, biomarkers, and LVEF. The therapeutic effects of BNP rise in ACS patients need further study **(Weber and Hamm 2006, 2021).**

1.13.2. Chemistry of Beta Natriuretic Peptide

A physiologically inactive prohormone, NT-proBNP (BNPT), has a 32-amino-acid polypeptide BNP connected to a 76-amino-acid N-terminal fragment After release, B-type natriuretic peptide (BNP) binds to and activates the atrial natriuretic factor receptors NPRA and NPRB, similar to ANP but with a tenfold lower affinity. NT-proBNP has an even longer biological half-life than BNP, which is twice as long as ANP. Therefore, these peptides are better diagnostic blood test targets than ANP, see Fig. (1-7) **(Fu, Ping et al. 2018, Franceschini, Crosta et al. 2022).**

	Antibody	
Assay group	Capture	Detection
Group A: Alere/Beckman	Ring	N-terminus
Group B: Siemens/Bayer	Ring	C-terminus
Group C: Abbott	N-terminus	C-terminus

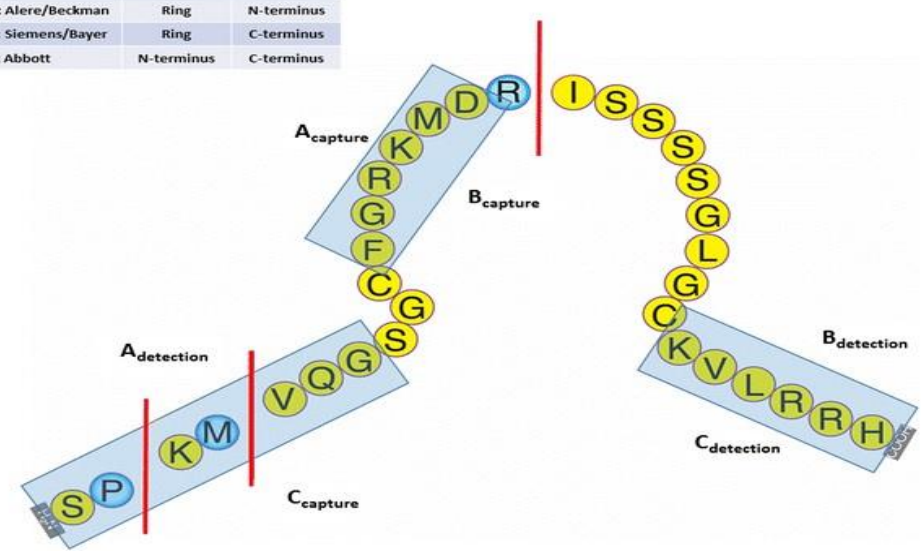

Fig. (1-7): The structure of BNP, the zones bound by typical immunoassays used clinically for BNP (Bhandari and Cunningham 2020)

1.13.3. Relationship between Total Antioxidant Capacity and CAD

Many studies found that TAC and prediabetes are adversely linked with LV geometry, whereas IHD and dilated LV were associated with reduced TAC. TAC can be utilized as an extra marker in HFrEF patients to indicate the severity of the condition. Interventions aiming at regulating oxidative stress

may be beneficial in HFrEF patients, reducing oxidative stress while improving LV geometry and quality of **life (Kasap, Gönenç et al. 2007).**

Previous studies showed that plasma total antioxidant capacity (TAC) was significantly reduced in patients with coronary artery diseases (CAD) as compared with healthy subjects but the TAC level not to be an independent coronary risk factor Some studies reported that the DNA damage was increased in patients with CAD However, little is known whether there is a relationship between the DNA damage and TAC in patients with CAD **(Demirbag, Yilmaz et al. 2005).**

1.13.4. Relationship between Total Antioxidant Capacity and NT-proBNP in CAD

This trial revealed interesting findings. Finding that typical IPF patients had raised NT-proBNP and that lower plasma TAC and increased urine isoprostanes after low-intensity exercise are related with hypoxemia is relevant. Such data could test the hypothesis that systemic oxidant stress is caused by cellular hypoxia and that exercise-induced oxidant stress affects IPF patients' endurance. These findings indicate that IPF patients develop hypoxemia and oxidant stress at low activity levels and that isoprostanes may increase pulmonary vascular resistance. suggest reinfarction or extension. Small skeletal muscle B-subunit. Muscle breakdown raises CK-MB and total. CK-MB may rise 3–30% post-MI. The bulk of CK-MB is myocardial. Thus, CK-MB can detect acute myocardial injury in sera from infarction, myocarditis, cardiac trauma or contusion, surgery, endomyocardial biopsy, defibrillation, and cardioversion. recommend reinfarction or extension. Small skeletal muscle B-subunit. Muscle breakdown elevates CK-MB and total. 3-5% to 15-30% post-MI CK-MB may increase. The majority of CK-MB is myocardial. Thus, CK-MB can detect acute myocardial injury in sera from infarction, myocarditis,

cardiac trauma or contusion, surgery, endomyocardial biopsy, defibrillation, and cardioversion.

1.14. Aim of Study:

This study aimed to:

1. Study the association between plasma BNP concentration and coronary disease.
2. Determination of lipid profiles in patients and control group.
3. Study of the correlations between B-Type Natriuretic Peptide; Total Antioxidant Capacity and Creatine Kinase-MB Isoenzyme activity levels with various parameters studied in sera of each Ischemic Heart Diseases and CAD.

Chapter Two

Material and Methods

2. Material and Methods

2.1. Subjects and Study Design

A case-control study design was conducted on 120 participants of males aged between (41-70) years, 60 of whom suffered from ischemic heart disease (patient group) and 60 were apparently healthy (control group), and were conducted during the period from December 2022 until July 2023. Patients suffering from typical chest pain were presented to Coronary Care Unit (CCU) at Kerbala Cardiac Center / Kerbala Health Directorate. Diagnosis is based on clinical history and presentation confirmed by electrocardiography (ECG) and various investigations of cardiac biomarkers. Family history, smoking, blood pressure, height, and weight were taken from each subject in this study.

2.1.1. Patients Groups:

In the study patients are divided into:

1. Acute coronary syndrome patients: 34 patients classify clinically into STEMI (N=12), Non-STEMI (N=12), unstable angina (N=10).
2. Chronic Coronary Syndromes (Stable Coronary Artery Disease) patient stable angina (N= 26) patient.

2.1.1.1. Inclusion Criteria of patient

Every patient underwent a comprehensive evaluation, including a thorough review of their medical history, a detailed physical examination, and appropriate laboratory tests.The diagnosis of Acute Coronary Syndrome and Chronic Coronary Artery Disease clinical conditions was made based on the most recent clinical practice guidelines provided by the American College of Cardiology (ACC) and the European Society of Cardiology (ESC). The aetiology of instances was determined by analysing signs and symptoms, evaluating ECG readings, and doing laboratory investigations. So Criteria was included Patients who have myocardial infarction, unstable angina, whether they have diabetes or

not, or have high blood pressure or not, and who have a family history of heart disease or not, and the age groups are males between 40 and 70 years of age.

2.1.1.2. Exclusion Criteria of patient

Sever renal or liver disease, Chemotherapy-induced cardiotoxicity, Cardiac surgery and failed.

2.1.2. Control Group

Sixty subjects were selected from apparently healthy as a control group. Those subjects were either apparently healthy subjects or with no recent complain of cardiac problems.

2.1.2.1. Inclusion criteria of control group

This group includes apparently healthy individuals only.

2.1.3. Ethical Committee Approval

As a mandatory step, this study was approved by the ethical committees that include: Kerbala University College of Medicine, Committee of Kerbala Centre for Cardiac Diseases and Surgery, Kerbala Health Directorate / Kerbala-Iraq.

2.1.4. Blood Sampling

About 5 ml of venous blood sample were drawn from each patient during 24hrs after admission to the CCU. The same quantity of blood was drawn from control group. Sample was transferred into gel tube at room temperature and left to stand for thirty minutes for clotting then centrifuged at 3000 rpm for 10 minutes. Serum was separated and divided into small aliquots. Serum kept frozen at -20 °C until the time of assays, it was used to perform the following analysis, B-type Natriuretic Peptide (BNP), total antioxidant capacity , troponin, creatine kinase-MB isoenzyme and lipid profile.

2.1.5. Collected Data Using a Questionnaire Form

This study analyses a number of clinical parameters of the cases (patients and controls). Some of the parameters are collected from a number of blood tests, while others are collected from the case directly (either a patient or control). In the following subsection, these parameters are discussed. Furthermore, Appendix A includes the questionnaire form on which all the collected data are registered for each case.

2.1.6. Personal Data

The collected personal data does not break person anonymity. It contains a number that identify the case (sample no.), gender, age and smoking status, family history regarding to IHD, and the duration of IHD disease (in case of patient). Furthermore, the collection of date is also registered.

2.2. Materials

2.2.1. Instruments and Tools

All instrument used in this study are listed in the Table (2-1).

Table (2-1): Instrument and their suppliers.

Instruments	Suppliers
Deep freezer	Kryolab /Italy
High speed centrifuge	Kokusan /Japan
Huma Reader HS ELISA system	Germany
Pipette	Slamed
SMART -150 Chemistry Analyzer	USA
Vortex shaker mixture	Stuart scientific England

2.2.2. Chemicals and Kit

The chemicals with their suppliers used in this study are listed in the table (2-2).

Table (2-2): Chemicals and Kit with their suppliers

Chemicals	Source (company)
cholesterol kit	BIOLABO / France
Glucose HK Kit	ROCHE Germany
HDL-cholesterol kit	BIOLABO / France
Human B-type natriuretic peptide (BNP) ELISA Kit	Sun Long Biotech Co /China
Human Creatine Kinase MB isoenzyme,CK-MB ELISA Kit	Sun Long Biotech Co /China
Human Total antioxidant capacity	Nanjing Pars Biochem
LDL-cholesterol kit	BIOLABO / France
Triglyceride kit	BIOLABO / France

2.3. Methods

2.3.1. Calculation of Body Mass Index

The body mass index (BMI) is a value derived from the mass (weight) and height of an individual. BMI was calculated by dividing the body weight (kg) by the square of height (m^2) according to the following equation **(Liu, Zhao *et al.* 2024)** BMI, kg/ m^2 = Weight (kg) / Square Height (m^2).

Table: Obesity Classification (World Health Organization, 2023)

Category	Body Mass Index (BMI) (kg/m^2)
Underweight	<18.5
Normal weight	18.5 to <24.9
Overweight	25 to <29.9
Obese Class I	30 to <34.9
Obese Class II	35 to <39.9
Obese Class III (Morbid)	≥40

2.3.2. Determination of Serum Lipid Profile

2.3.2.1. Determination of Serum Total Cholesterol Concentration

Cholesterase breaks down esterified cholesterol into fatty acids. Cholesterol oxidase converts free cholesterol to 4-Cholesten-3-one and H_2O_2. Peroxidase (POD), hydrogen peroxide, phenol, and 4-aminoantipyrine (4-AAP) form a cholesterol-dependent colored complex. A schematic representation of the reaction is shown in following equations:

Cholesterol ester + Cholesterol Esterase ----------► Cholesterol + fatty acids

Cholesterol + Cholesterol Oxidase ----------► 4-Cholesten-3-one + H_2O_2

H_2O_2 + Phenol + 4-Amino Antipyrine + Peroxidase --------► Colored complex + H2O

Reagents

Table (2-4): Reagents used for total cholesterol assay.

	Buffer	mmol/l
Reagent A (100 ml)	4-AAP	1 mmol/l
	CHE	300 U/l
	CHOD	300 U/l
	POD	1500 U/l
	Derivative of phenol	1 mmol/l

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase; POD, peroxidase

Procedure

Total cholesterol concentrations were measured by SMART-150 chemical analyzers. See the sample, standard, and blank measurements in Tables (2-5). The combination was left to incubate at 37°C for 5 minutes. At 510 nm, the absorbances of Ax and As were measured in comparison to a blank reagent at a ratio of 1:100.

Table (2-5): Procedure of total cholesterol assessment

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	1000	1000	1000
Water	10		
Sample		10	
Standard			10

Cholesterol (mg/dl) = Ax/As × 200 (standard value)

2.3.2.2. Measurement of Serum Triglyceride Concentration

The free circulation of triglycerides in plasma is limited to protein-bonded lipoproteins. Triglyceride detection methods often begin with enzymatic hydrolysis to glycerol and free fatty acids. Enzymes measure liberated glycerol. Triglyceride hydrolysis by lipase produces glycerol and free fatty acids. H2O2 is created when glycerol participates in a chain of linked enzymatic processes

involving GK and GPO. The sample's triglycerides are proportional to the absorbance of the colorful complex created by H2O2 reacting with TOOS and 4-AAP.

A schematic representation of the reaction is shown in following equations:

Triglyceride + **LPL** --------► Glycerol + Fatty acids

Glycerol + ATP + **GK** --------► Glycerol-1-phosphate + ADP

Glycerol-1-phosphate + O_2 **GPO** ------► Dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-AAP + TOOS + **POD** ---------► Colored complex + H_2O

Reagents

Table (2-6): Reagents used for triglycerides assay

Reagent	Content	Concentration
Reagent A	Good buffer	100 mmol/L
	Magnesium chloride	15 mmol/L
	ATP	4 mmol/L
	4-AAP	1 mmol/L
	TOOS	0.1 mmol/L
	LPL	2500 U/L
	POD	1800 U/L
	GK	1000 U/L
	GPO	5500 U/L
Standard	Glycerol	200 mg/dl

ATP, adenosine triphosphate; 4-AAP, 4-aminoantipyrine; LPL, lipoprotein lipase; POD, peroxidase; GK, glycerol kinase; GPO, glycerol phosphate oxidase.

Procedure

Table (2-7) displays the measurements of the blank, standard, and sample triglycerides as determined by the SMART-150 chemistry analyzer. The combination was left to incubate at 37°C for 5 minutes. At 510 nm, the absorbances of Ax and As were measured in comparison to a blank reagent at a ratio of 1:100.

Table (2-7): Procedure of triglycerides assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	1000	1000	1000
Water	10		
Sample		10	
Standard			10

Triglycerides (mg/dl) = Ax/As × 200 (standard value

2.3.2.3. Measurement of Serum High-Density Lipoprotein Cholesterol

Most cholesterol in the blood is transported by HDL, LDL, and VLDL. HDL has the largest protein-to-lipid ratio of any lipoprotein class, slightly more than 50%, and is the densest and smallest. Indeed, high LDL and low HDL levels greatly raise the risk of unfavorable cardiovascular events. In the first phase, particular polianions block LDL, VLDL, and chylomicrons (CM), and in the second phase, a surface-active agent prevents their coloring. Color intensity is proportional to sample HDL-C**(Hoang, Cao et al. 2023, Soo 2024).**

A schematic representation of the reaction is shown in following equations:

HDL + LDL + VLDL + CM ----------► HDL + (LDL+VLDL+CM)

HDL **CHE,D** ----------► Fatty acids +H_2O_2

H_2O_2 + 4-AAP + HDAOS ----------► Colored complex +H_2O

Reagents

Table (2-8): Reagents used for high density lipoprotein cholesterol assay.

Reagent	Content	Concentration
Reagent A	Good Buffer	100 mmol/L
	Polianions	1 mmol/l
	4-AAP	4 mmol/l
Reagent B	CHE	800 U/l
	CHOD	500 U/l
	Peroxidase	1500 U/l
	HDAOS	1 mmol/l
	Detergent	4 mmol/l

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase.

Procedure

The absorbance of the blank sample (Abx) was measured at 600 nm compared to the blank reagent after incubation at 37 °C for 5 minutes. Mixing and incubating at 37°C for 5 minutes followed the addition of reagent B. The absorbance of the sample (Ax) and the standard (As) were measured in comparison to a blank reagent. Table (2-9) displays the method.

Table (2-9): Procedure of high-density lipoprotein cholesterol assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	300	300	300
Water	4		
Sample		4	
Standard			4
Reagent (B)	100	100	100

HDL (mg/dl) = (Ax – Abx)/ (As – Abs) × (Standard Value)

Low-density lipoprotein cholesterol (LDL) was calculated as:

LDL (mg/dl) = Total Cholesterol – HDL – (Triglycerides/5)

2.3.3. Determination of Fasting Serum Glucose Concentration

Principle

Outside the body, glucose makes up most blood carbs. Cells derive most energy from glucose oxidation. The liver and adipose tissue convert glucose into glycogen and fatty acids. Blood glucose is strictly regulated. hexokinase is an enzymatic glucose reference. ATP-induced glucose phosphorylation by hexokinase produces glucose 6 phosphate and ADP. G6PDH converts glucose 6 phosphate to NADPH when exposed to NADP+ **(Brameld, Parr et al. 2023)** .

Glucose + ATP + **HK** --------► Glucose-6-phosphate + ADP

Glucose-6-phosphate + $NADP^+$ + **G6PDH** --------► 6-phosphogluconate + NADPH +H^+

The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the absorbance at 340 nm.

Reagents

Table (2-10): Reagents used for glucose assay.

R1	**TRIS buffer, pH 7.8** **Mg^{2+}** **ATP** **$NADP^{+}$**	**100 mmol/L** **4 mmol/L** **1.7 mmol/L** **1 mmol/L**
SR	HEPES buffer, pH 7.0 Mg^{2+} HK (yeast) G6PDH (microbial)	30 mmol/L 4 mmol/L ≥ 130 µkat/L ≥ 250 µkat/L

2.3.4. Determination of B-Type Natriuretic Peptide

Principle of ELISA

This kit uses Sandwich-ELISA. BNP antibody pre-coats this kit's Microelisa stripplate. In Microelisa stripplate wells, standards or samples are mixed with the antibody. Each Microelisa stripplate well receives HPP-conjugated BNP antibody. Freebies wash away. TMB substrate goes to each well. Only BNP and HRP-conjugated BNP antibody wells shift blue to yellow with stop solution. Optical density is measured using 450 nm spectroscopy. BNP concentration is O.D. Measure BNP concentration by comparing sample OD to standard curve.

Table (2-11): Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T
4	Microelisa stripplate	1	2-8℃
5	Standard：225pg/ml	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution	A 6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X)×1bottle	2-8℃

Sample preparation

1. Serum preparation

Once the whole blood has been collected, it is important to let it clot undisturbed at room temperature. Typically, this process requires 10-20 minutes. Centrifuge the sample at 2,000-3,000 rpm for 20 minutes to remove the clot. If there are any precipitates during the reservation process, it is recommended to centrifuge the sample again.

Procedure of ELISA

1. **Dilution of Standards B-Type Natriuretic Peptide.**

   The standard was diluted using microtubes first, then a 50-µl volume of each tube was pipetted into a microwell plate, each tube using two wells,

150pg/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
100pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
50pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
25pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
12.5pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

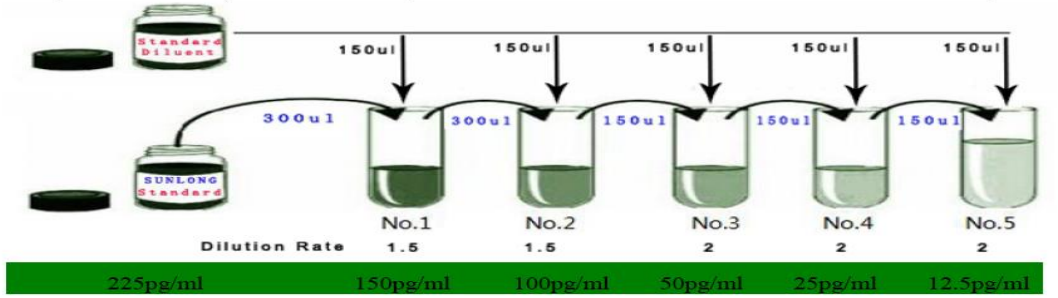

Fig. (2-1): Preparation of standard of B-type natriuretic peptide(BNP) and their concentration.

2. In the Microelisa stripplate, an empty well as blank control was leaved. In sample wells, 40µl. Sample dilution buffer and 10µl sample are added (dilution factor is 5).
3. Samples had been loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
4. The membrane of the closing plate securely was closed and it was left to incubate for 30 minutes at 37°C.

5. The concentrated washing solution Diluted with distilled water. For 120T, diluted it 30 times. For 60T, diluted it 20 times.
6. Washing: the closure plate membrane, aspirate was removed, and the wash solution replaced with care. A short break of 30 seconds was taken and the washing solution was discarded. It was washed 5 times.
7. Fifty µl of HRP-Conjugate reagent in all wells except the blank control.
8. Incubation, just like in Step 3. Washing, just like in Step 5.
9. To achieve optimal staining, 50 µl of chromogen solutions A and B were combined into each well. The mixture was gently shaken and left at 37°C for 15 minutes.
10. The reaction was stopped by adding 50 µl of stop solution to each well. The color changed from blue to yellow.
11. A Microtiter Plate Reader Utilized to measure the absorbance at 450nm O.D.

2.3.5. Determination of Total antioxidant capacity.

Principle of the assay

The group measured human T-AOC levels by coating the wells of a microtiter plate with purified antibody, adding T-AOC, and combining the antibody-antigen complex and the HRP-labeled antibody. Washing well completes the process. Addition of TMB substrate solution turned it blue. Sulfuric acid terminated the reactions catalyzed by the HRP enzyme, and the color change was detected spectrophotometrically at 450 nm. Samples are compared to a standard curve to determine the T-AOC concentration.

Table (2-12) Materials provided with the TAC kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T
4	Microelisa stripplate	1	2-8°C

5	Standard：225pg/ml	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution	A 6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X)×1bottle	2-8℃

Assay procedure

1. The sample was mixed with the diluted standard. 100 µl of standard and 50 µl of dilution were plated into 10 wells on coated ELISA plates. Then 100 µl from each of the first and second wells were added to the third and fourth wells separately. 50εl of standard dilution was added to the wells, mixed, scooped out, and added to the fifth and sixth wells. Mix, scoop out, and add it to the seventh and eighth wells. mixed, then 50εl were added from these wells to t. Place 50 µL of the standard dilution into wells 9 and 10, mix, and then throw away. 50 µl of sample was added to each well (density 6, 4, 2, 1, 0.5 units/ml) After thawing. The sample was placed and blank reference wells were made (no HRP-Conjugate reagent, other steps are the same). Samples were scanned. 40 µL of sample reduction and 10 µL of test sample were placed in each well. Then, fill the wells
2. After placing the membrane on top of the dish, incubated it at 37°C for 30 minutes.
3. Add 30 times (or 20 times) pure water to the washing solution and leave it aside.
4. The sealing plate membrane was removed, the liquid was poured out, and the plate was dried by oscillating it. Then, wash buffer was added to each well, waited 30 s, filtered five times, and the plate dried by patting. To

add the enzyme, add 50µl of HRP-Conjugate solution to all but one of the wells.

5. Incubation was done as in 3
6. Cleaning was done as in (5)
7. Fifty µL of chromogen solutions A and B were added to each well. The dishes were kept away from light for 15 minutes at a temperature of 37°C. Added 50µl of Stop Solution into each well to stop the reaction. The color will change from blue to yellow.
8. Assay: the blank was Used well as a reference point and the absorbance has been read at 450 nm within 15 minutes of adding the Stop Solution.

2.3.6. Determination of Creatine Kinase-MB Isoenzyme Activity

Principle

This kit utilizes the Sandwich-ELISA technique. The Microelisa stripplate from Kit is pre-coated with CK-MB antibody. Standards or samples are combined with the specific antibody in Microelisa stripplate wells in a highly skilled manner.

Every well in the Microelisa stripplate is treated with HPP-conjugated CK-MB antibody and incubated. Parts wash off for free. Every well is provided with TMB substrate. Only certain wells will exhibit a blue and yellow glow after the addition of the stop solution. Optical density (OD) can be measured using spectrophotometry at 450 nm. Regarding CK-MB concentration, OD. By comparing the sample OD to the standard curve, the concentration of CK-MB can be determined.

Table (2-13) Materials provided with the CK-MB kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8℃
5	Standard: 27 ng/ml	0.5ml×1 bottle	2-8℃

6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution A	6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	Wash Solution	20ml (30X)×1bottle	2-8℃

Procedure

1. The standard was It was diluted using microtubes first, then a 50-µl volume of each tube was pipetted into a microwell plate, each tube using two wells, a total of ten wells.

18 ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
12 ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
6 ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
3 ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
1.5 ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

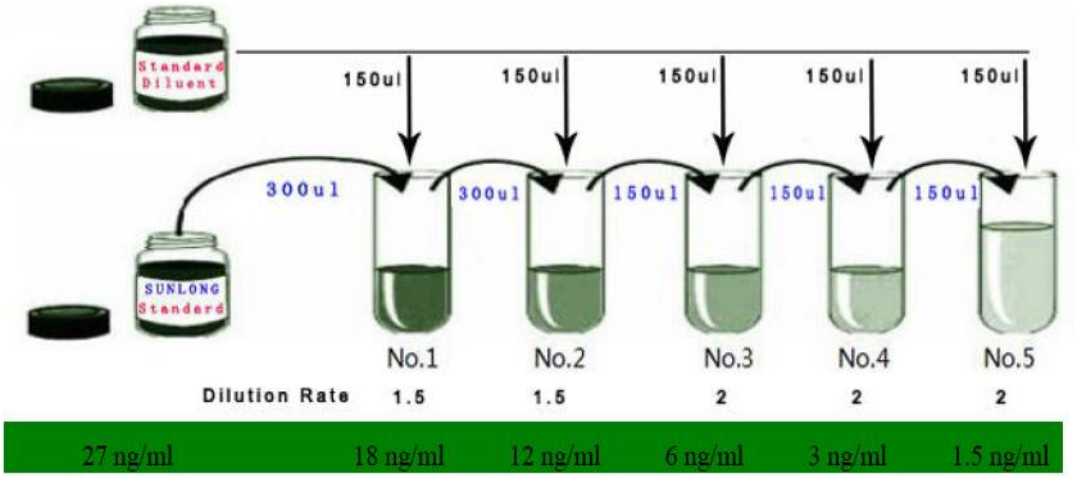

Figure (2-2): Preparation of standard of Human Creatine Kinase MB Isoenzyme and their concentration.

1. In the Microelisa stripplate, leave an empty well as blank control. In sample wells, 40µl. Sample dilution buffer and 10µl sample are added (dilution factor is 5).
2. Samples had been loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. The membrane of the closing plate securely was closed and it was left to incubate for 30 minutes at 37°C.

4. The concentrated washing solution Diluted with distilled water. For 120T, diluted it 30 times. For 60T, diluted it 20 times.
5. Washing: the closure plate membrane, aspirate was removed, and the wash solution replaced with care. A short break of 30 seconds was taken and the washing solution was discarded. It was washed 5 times.
6. Fifty µl of HRP-Conjugate reagent in all wells except the blank control.
7. Incubation, just like in Step 3. Washing, just like in Step 5.
8. To achieve optimal staining, 50 µl of chromogen solutions A and B were combined into each well. The mixture was gently shaken and left at 37°C for 15 minutes.
9. The reaction was stopped by adding 50 µl of stop solution to each well. The color changed from blue to yellow.
10. A Microtiter Plate Reader Utilized to measure the absorbance at 450nm O.D.

2.3.7. CK-MB relative index (CK-MB RI)

calculate the CK-MB relative index (CK-MB RI) by using the below formula.CK-MB RI = CK-MB (ng/mL) x 100/CK (IU/L)

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test and Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability. Estimate of correlation coefficient between variables in this study.

The ROC curve analysis is conducted using a range of statistical applications, including commercial software products like IBM SPSS, MedCalc, Stata, and NCSS, as well as open-source software such as R. The majority of statistical analysis software products offer fundamental functionality for ROC analysis. Nevertheless, there are modest variations in the functions offered by each software application. IBM SPSS, a highly prevalent commercial software, offers essential statistical analysis capabilities for ROC curves. These capabilities include the ability to generate ROC curves, compute the Area Under the Curve (AUC), and determine confidence intervals (CIs) with statistical significance. Nevertheless, IBM SPSS lacks a range of functions for determining suitable cut-off values and does not offer a facility for calculating sample sizes. Stata offers a range of functions for doing ROC curve analysis, encompassing the pAUC analysis, multiple comparisons of ROC curves, determination of ideal cut-off values using Youden's index, and the utilization of different performance measurements. MedCalc is a software application that has been specifically designed for the purpose of doing medical research. MedCalc offers a means of estimating the sample size for a singular diagnostic test and incorporates a range of analytical methodologies to ascertain the most suitable threshold value. However, it does not provide a function for computing the pAUC.

The data sheet was utilized to record the information obtained from the questionnaire and the test findings obtained from the study group samples. The

present study employed the Statistical Package for the Social Sciences software, specifically version 28.0 (IBM, SPSS, Chicago, Illinois, USA), and the Real Statistics Resource Pack program for Mac (Release 7.2) from the Excel 2016 resource pack to conduct the data analysis.

The data of each group was subjected to descriptive statistics analysis. Categorical values were represented by n (%). Normal data was represented by mean ± 2 standard deviation for scale variables, whereas non-normal data was represented by mean for continuous variables. The Shapiro-Wilk test was employed to evaluate the normality of the data distribution.

The Spearman rank test was employed to assess the association between biomarkers in the case study. Analytical statistical tests were conducted to confirm the presence of significant variations in categorical variables among the parameters. All hypothesis tests that had p-values less than 0.05 (two-sided) were deemed to possess statistical significance.

The Fisher's LSD technique was used to calculate the simultaneous confidence level for each of the confidence intervals. The concurrent confidence level represents the likelihood that all confidence intervals encompass the actual disparity. In the analysis of variance (ANOVA), Fisher's least significant difference (LSD) method was utilized to generate confidence intervals for the pairwise differences observed between biomarkers and study groups. An ideal threshold that exhibits both high specificity and sensitivity for the cases under examination. Receiver operating characteristic (ROC) analysis was employed to detect the phenomenon.

Chapter Three

Results

3. Results

3.1. Demographic and Clinical Characteristics

The base line characteristics of the study groups are presented in Table (3.1) The total of samples were 120 samples and subject groups consisted of 60 patients with ischemic heart disease, and 60 apparently healthy adults as a control group to compared with patients groups. Patients with ischemic heart disease were included in this study 34 with ACS and 26 with Chronic CAD. Distribution of samples studied was performed according to different factors as summarized in table.(1-3)

Table (3-1): Demographic data of studies groups using Frequency Table

Factors		Patients N=60		Healthy control N=60		P-value
		No.	(%)	No.	(%)	
Age group	(41-50)	18	30.0%	15	25.0%	0.824
	(51-60)	21	35.0%	22	36.7%	
	(61-70)	21	35.0%	23	38.3%	
BMI	Normal	15	25.0%	60	100.0%	0.0001**
	Overweight	27	45.0%	0	0.0%	
	Obese	18	30.0%	0	0.0%	
Family History	No	43	71.7%	60	100.0%	0.0001**
	Yes	17	28.3%	0	0.0%	
HT	No	37	61.7%	60	100.0%	0.0001**
	Yes	23	38.3%	0	0.0%	
Hyper Cholesterolemia	Yes	54	90.0%	0	0.0%	0.0001**
	No	6	10.0%	60	100.0%	

*** (P≤0.05), ** (P≤0.01). HT: Hypertension, BMI: Body Mass Index, N: Number of subject.**

3.2. Correlation Studies

3.2.1. Comparison of Age, Waist and BMI between Patient and Control

In table 3-2 it was shown the mean level of age, waist and BMI was increased and statically significant except age was no significant as compared between patient and control with mean level of 57.82±8.00 , 106.23 ±6.66 , 27.40 ±3.75 in ACS and mean level of 54.08 ±8.71 , 96.85 ±11.82 , 23.70 ±1.80 in control respectively and also was shown the mean level of age, waist and BMI was increased and statically significant except age was no significant as compared between patient and control with mean level of 56.31 ±7.91 ,105.70 ±6.26 ,28.90 ±4.11 in chronic CAD and mean level of 54.08 ±8.71 , 96.85 ±11.82 , 23.70 ±1.80 in control respectively.

Table (3-2): Comparison of Age, Waist and BMI between patient and control

Groups	Mean ± SD		
	Age (year) Mean ± SD	**Waist (cm) Mean ± SD**	**BMI (kg/m^2) Mean ± SD**
ACS N=34	57.82 ±8.00 **a**	106.23±6.66 **a**	27.40 ±3.75 **a**
Chronic CAD N=26	56.31 ±7.91 **ab**	105.70 ±6.26 **a**	28.90±4.11 **a**
Healthy Control N=60	54.08 ±8.71 **b**	96.85 ±11.82 **b**	23.70 ±1.80 **b**
P-value	0.106	0.0001 **[S]**	0.0001 **[S]**

ANOVA test was used N: number of cases; SD: standard deviation; S: significant; NS= Non-significant (P≤0.01), means having the different letters in the same column represent significantly while means having the same letters in the same column represent non significantly.**

3.2.2. Comparison of Lipid Profile between Patients and Control

In table 3-3 it was shown that the mean level of TC, TG, HDL-C, LDL-C, VLDL-C was increased and statically significant except HDL-C was decreased in ACS and Chronic CAD as compared between patient and control with mean level of **(** 247.38 ±44.36 , 221.29 ±82.93, 34.44 ±2.48 , 170.58 ±39.97 , 42.07 ±17.75 **)** in ACS and and mean level of **(** 241.03 ±39.29 ,191.07 ±82.65 ,35.03 ±3.14 ,158.88 ±33.09 ,39.65 ±16.77 **)** in Chronic CAD as compared with control and mean level of 176.50 ±14.50 ,128.53 ±29.67 ,53.85 ±7.52 ,98.03 ±42.80 ,21.70 ±5.76 **)** in control.

Table (3-3): Comparison of Lipid Profile between patient and control

Groups	Mean ± SD				
	TC (mg/dl)	**TG (mg/dl)**	**HDL-C (mg/dl)**	**LDL-C (mg/dl)**	**VLDL-C (mg/dl)**
ACS N=34	247.38 ±44.36 **a**	221.29 ±82.93 **a**	34.44 ±2.48 **a**	170.58 ±39.97 **a**	42.07 ±17.75 **a**
Chronic CAD N=26	241.03 ±39.29 **a**	191.07 ±82.65 **a**	35.03 ±3.14 **a**	158.88 ±33.09 **a**	39.65 ±16.77 **a**
Healthy Control N=60	176.50 ±14.50 **b**	128.53 ±29.67 **b**	53.85 ±7.52 **b**	98.03 ±42.80 **b**	21.70 ±5.76 **b**
P-value	0.0001**[S]**	0.0001**[S]**	0.0001**[S]**	0.0001**[S]**	0.0001**[S]**

ANOVA test was used N: number of cases; SD: standard deviation; S: significant; NS= Non-significant (P≤0.01), means having the different letters in the same column represent significantly while means having the same letters in the same column represent non significantly TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol, LDLC: Low density lipoprotein-cholesterol, VLDL-C: Very Low-Density Lipoprotein- Cholesterol, N: Number of subject.**

3.2.3. Comparison of BNP and TAC between Patients and Control

In Table 3-4, the mean BNP levels (pg/ml) were significantly elevated in both the ACS and Chronic CAD groups compared to the control group, with mean values of 71.42 ± 15.56 pg/ml and 55.45 ± 18.21 pg/ml, respectively. These increases were statistically significant ($P < 0.01$), indicating a strong association between elevated BNP levels and the presence of cardiovascular disease. Similarly, Total Antioxidant Capacity (TAC) was significantly reduced in both patient groups, with mean values of 3.12 ± 0.46 U/ml in ACS and 3.21 ± 0.34 U/ml in Chronic CAD, compared to 3.47 ± 0.68 U/ml in the control group.

Table (3-4): Comparison of BNP and TAC levels between patient and control

Groups	Mean ± SD	
	BNP (pg/ml)	**TAC (U/ml)**
ACS	71.42 ±15.56 **a**	3.12±0.46 **a**
Chronic CAD	55.45 ±18.21 **b**	3.21±0.34 **ab**
Healthy Control	30.85±18.21 **c**	3.47±0.68 **b**
P-value	0.0001**[S]**	0.011**[S]**
ANOVA test was used, N: number of cases; SD: standard deviation;, BNP: Brain natriuretic peptide, TAC: Total Antioxidant capacity S: significant; NS= Non-significant (P≤0.01).**		

3.3. Correlation Coefficient of Study Group in Acute Coronary Syndrome and Chronic Coronary Artery Disease

3.3.1. Correlation coefficient of BNP with Parameters in Patient Groups

BNP displayed one significant negative correlations BMI (r= -0.47, p= 0.005) in ACS and two significant negative correlations Waist (r= -0.62, p= 0.001), BMI (r= -0.62, p= 0. 0.001), and one significant positive correlations with Age (r= 0.84, p= 0.0001) in Chronic CAD. On the other hand, other BNP no significant negative correlations with waist, TC, HDL-C, and no significant positive correlation with age, TG and LDL-C in ACS while no significant positive correlation with TG in Chronic CAD.

Table (3-5): Correlation coefficient of BNP with Parameters in patient groups

Parameters	Acute Coronary Syndrome (ACS)		Chronic CAD	
	R	P	R	P
TAC	-0.34	0.051	-0.14	0.487
Age, (year)	0.49	0.789	0.84**	0.0001**[S]**
Waist, (cm)	-0.19	0.291	-0.62**	0.001**[S]**
BMI, kg/m^2	-0.47*	0.005**[S]**	-0.62*	0.001**[S]**
TC, (mg/dl)	-0.49	0.789	-0.08	0.673
TG, (mg/dl)	0.02	0.915	0.006	0.975
HDL-C, (mg/dl)	-0.15	0.386	-0.15	0.470
LDL-C, (mg/dl)	0.02	0.904	-0.02	0.942
VLDL-C, (mg/dl)	-0.18	0.317	-0.13	0.523

Pearson correlation coefficient test was used, N: number of cases; SD: standard deviation; S: significant; NS= Non-significant (P≤0.01), , BMI: Body Mass Index TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol, LDLC: Low density lipoprotein-cholesterol, VLDL-C: Very Low-Density Lipoprotein- Cholesterol, TAC: Total antioxidant capacity, r= Pearson correlation coefficient.**

3.3.2. Correlation Coefficient between TAC with Parameters in Patient's Groups

TAC displayed no significant negative correlations Waist (r= -0.08, p= 0.664), TG (r= -0.14, p= 0.551), HDL-C (r= -0.11, p= 0.539), VLDL-C (r= -0.05, p= 0.673), BNP (r= -0.38, p= 0.051) in ACS and one significant negative correlations TC (r= -0.44, p= 0.025), and no significant negative correlations Age (r= -0.24, p= 0.244), Waist (r= -0.06, p= 0.786), HDL-C (r= -0.23, p= 0.255), VLDL-C (r= -0.38, p= 0.168), BNP (r= -0.14, p= 0.487) in Chronic CAD. On the other hand, TAC displayed no significant positive correlations Age, BMI, TC, LDL-C in ACS and no significant positive correlations BMI, TG, LDL-C in Chronic CAD. Table (3-6).

Table (3-6): Correlation coefficient between TAC with Parameters in patient's groups.

Parameters	Acute Coronary Syndrome (ACS)		Chronic CAD	
	R	P	R	P
BNP, (ng/ml)	-0.38	0.051	-0.14	0.487
Age, (year)	0.12	0.499	-0.24	0.244
Waist, (cm)	-0.08	0.664	-0.06	0.786
BMI, (kg/m^2)	0.16	0.379	0.17	0.412
TC, (mg/dl)	0.06	0.755	-0.44	0.025**[S]**
TG, (mg/dl)	-0.14	0.551	0.07	0.717
HDL-C, (mg/dl)	-0.11	0.539	-0.23	0.255
LDL-C, (mg/dl)	0.11	0.793	0.20	0.058
VLDL-C, (mg/dl)	-0.05	0.673	-0.38	0.168

Pearson correlation coefficient test was used, N: number of cases; SD: standard deviation; S: significant; NS= Non-significant (P≤0.01), BMI: Body Mass Index TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol, LDLC: Low density lipoprotein-cholesterol, VLDL-C: Very Low-Density Lipoprotein-Cholesterol, BNP: Brain natriuretic peptide, r= Pearson correlation coefficient.**

3.4. CK-MB Isoenzyme in Ischemic Heart Disease

In a clinical context, the CK-MB relative index is used to determine whether elevated CK-MB levels are due to heart muscle damage (as seen in conditions like ACS) or from skeletal muscle damage. This index is calculated as the ratio of CK-MB to total CK (Creatine Kinase) and is expressed as a percentage.

Normal or Control Levels: In healthy individuals (as shown in Table 3-7), the CK-MB relative index is around 1% ± 0.2, which is typical of a normal, healthy population without cardiac damage.

Chronic CAD: In patients with Chronic CAD, the index is close to 2 ± 0.8. This value is still below the critical threshold of 3%, suggesting that while these patients have some underlying cardiovascular issues, there is no significant acute cardiac injury or myocardial infarction.

ACS Patients: The CK-MB relative index for ACS patients is significantly elevated at 45 ± 3.2. This high value may be reflects acute cardiac muscle damage, as seen in myocardial infarction (MI), making CK-MB a reliable marker in these situations.

Table 3-7. Comparison of A CK-MB relative index levels between patient and control

Groups	A CK-MB relative index
ACS N=34	45±3.2
Chronic CAD N=26	2±0.8
Healthy Control N=60	1±0.2
P-value	0,002
T test was used N: number of cases; SD: standard deviation; S: significant; NS= Non-significant** (P≤0.05),	

3.5. ROC Analysis

3.5.1. ROC Analysis in Patients with ACS

The ROC curve analysis for BNP and TAC in ACS patients demonstrated that BNP is an excellent diagnostic marker with an AUC of 0.997, indicating high accuracy. At a cut-off point of 42.22 pg/ml, BNP showed a sensitivity of 0.97 and specificity of 0.98, meaning it can reliably differentiate ACS patients from non-ACS individuals. In contrast, TAC had a much lower AUC of 0.322, indicating poor diagnostic value. Despite a high sensitivity of 0.94 at a cut-off of 2.74 U/ml, TAC's specificity was only 0.13, limiting its effectiveness in accurately distinguishing ACS patients. Therefore, BNP is a far superior biomarker for diagnosing ACS compared to TAC.

Table (3-8): AUC, optimal threshold, sensitivity, and specificity of BNP and TAC obtained by ROC curve in patients with ACS.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CL	
BNP (pg/ml)	42.22	0.97	0.98	0.997	0.0001	0.991	1.000
TAC (U/ml)	2.74	0.94	0.13	0.322	0.004	0.214	0.430

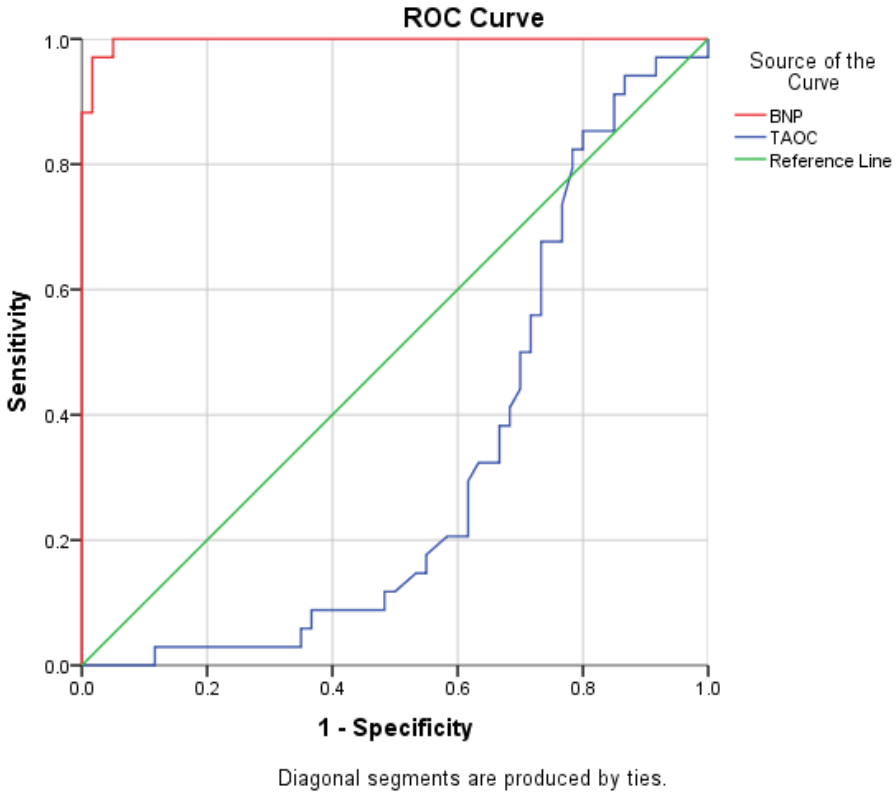

Figure (3-1): Receiver Operating Characteristic (ROC) curve of serum BNP and TAC levels as discriminators of patients with ACS.

3.5.2. ROC Analysis in Patients with Chronic CAD.

In receiver operating characteristic (ROC) for BNP and TAC patients group had AUC which was 0.896 [95%CI (confidence interval) = 0.824-0.964, 2.96 [95%CI (confidence interval) = 0.244-0.473, sensitivity= 0.98, 0.96 , specificity =0.65, 0.24 ,Cut-off point =41.06] 2.96] respectively .

Table (3-9): AUC, optimal threshold, sensitivity, and specificity of BNP and TAC obtained by ROC curve in patients with Chronic CAD.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CL	
BNP (pg/ml)	41.06	0.98	0.65	0.896	0.0001	0.824	0.964

TAC (U/ml)	2.96	0.96	0.24	0.358	0.038	0.244	0.473

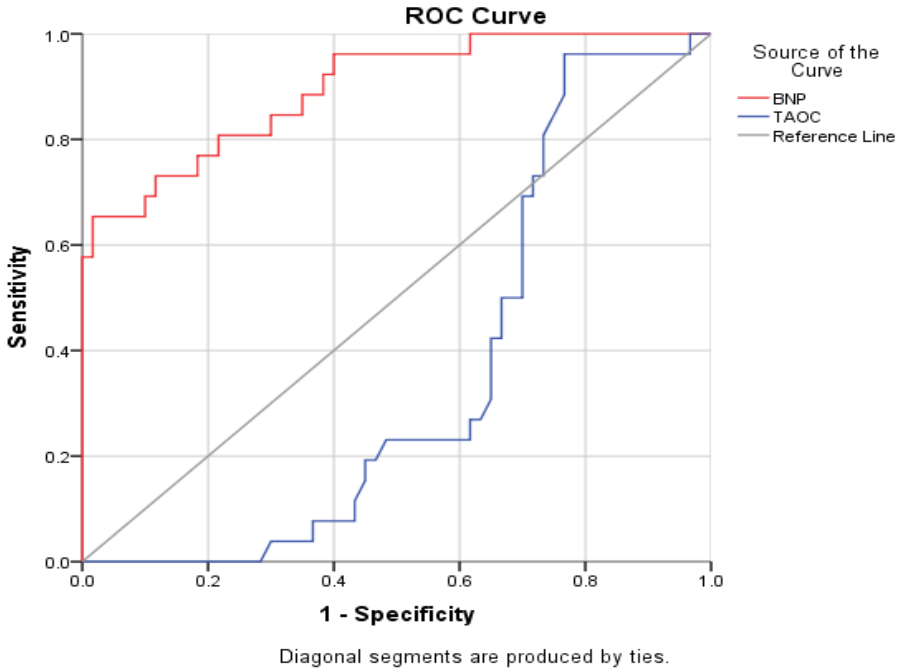

Figure (3-2): Receiver Operating Characteristic (ROC) curve of serum BNP and TAC levels as discriminators of patients with Chronic CAD

Chapter Four

Discussion

4. Discussion:

4.1. Demographic and Clinical Characteristics

The present study disclosed that all patients with ACS and chronic CAD were significantly of higher waist and BMI while age was non significant of ASC than control group as presented in table (3-2), these results are agreement with. **(Kim, Di Giovanna *et al*. 2024).** People who are 65 years old or older have higher risk of experiencing a heart attack, having a stroke, or developing coronary heart disease (also known as heart disease) and heart failure compared to younger people **(Wierzbicki 2024).**

Additionally, the changes that take place as a person ages may make them more susceptible to developing heart disease. The accumulation of fatty deposits in the walls of the arteries over a period of many years is one of the primary factors that contribute to the development of heart disease **(Kalisz, Navin *et al.* 2024).**

It is also considered to be the most common changes that occur with aging, which is an increase in the hardening of the large arteries, which is known as atherosclerosis. This causes high blood pressure, which is more common as one gets older. Because there are many risk factors that can be modified, atherosclerosis is not necessarily a normal part of the aging process. Plaque accumulates within the walls of your arteries, and as time passes, your arteries become more rigid and constricted, so restricting the transfer of oxygen-rich blood to organs and other regions of the body.**(Kawai, Finn *et al.* 2024).**

A high body mass index (BMI) indicates obesity, which is known to increase the likelihood of coronary heart disease (CHD).Obesity also increases the risk of cardiovascular disease by increasing the likelihood of hypertension and dyslipidemia, two well-known risk factors **(Luo, Zhan *et al.* 2024)**.

4.2. Lipid Profile

In the current study, the interpretation of the results of the lipid profile biomarkers as follows

HDL in ACS and Chronic CAD was significantly lower than controls groups as in table (3-3). Similar results were obtained by **(Arumugam, Yellurkar et al. 2024)** who demonstrated that lipid profile of patients with AMI correlated with systemic inflammation.

Atherosclerotic disease risk is inversely proportional to HDL serum levels. Because they can counteract oxidation, inflammation, and thrombosis pathways and promote reverse cholesterol transfer, HDL particles are thought to be antiatherogenic. Consistent with previous research, this study **(Arumugam, Yellurkar *et al*. 2024).**

The severity of coronary artery disease (CAD), acute myocardial infarction (ACS), and chronic coronary disease (CCD) in hypertensive patients is strongly indicated by a high TG level (≥150 mg/dl), even when other conventional risk variables are unaccounted for.**(Yamada, Horikoshi *et al.* 2024).**

Among the many risk factors for acute coronary syndrome (ACS), dyslipidemia—characterized by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) and decreased high density lipoprotein cholesterol (HDL-C) is responsible for over half of the population's attributable risk. The leading indicator of mortality while hospitalized is untreated dyslipidemia. The present study is in agreement with **(Pan, Jiang *et al*. 2024).**

4.3. Comparison between BNP and TAC in patient and control

There are many diverse and recent studies that confirmed high levels of BNP in patients with cardiovascular disease compared to healthy people as in table (4-3), and this is consistent with the results of this study**(Zhang, Li et al. 2024, Zhu, Zhang et al. 2024).**

It was originally introduced as a prognostic marker for patients with ischemic heart disease, but its diagnostic and prognostic utility was later expanded to include patients with stable CAD and a wide range of acute coronary syndromes (ACS). The ventricles secrete natriuretic hormone (BNP) in response to volume and pressure overload. Patients with acute coronary syndromes and stable coronary artery disease may have increased BNP levels because ischemia-induced transitory ventricular dysfunction can cause BNP production on its own, even if there is no obvious myocardial necrosis. **(Lee, Sriram *et al*. 2024).** Following a myocardial infarction (MI), blood flow increases quickly for the first 24 hours before leveling off; patients who suffered a severe infarct may see a second surge about five days following the initial one. **(Vogel, Claessen *et al.* 2019).**

In patients with acute coronary syndromes (ACS) and stable coronary artery disease (CAD), elevated Brain Natriuretic Peptide (BNP) levels are commonly observed. This increase is due to heightened myocardial wall stress, ischemia, and left ventricular dysfunction. In ACS, BNP levels tend to rise rapidly due to acute myocardial injury, while in stable CAD, chronic ischemia and ventricular strain cause moderate BNP elevation. BNP serves as a useful biomarker for diagnosing heart failure and gauging the severity of ischemic heart disease**(De Lemos, Morrow et al. 2001, Pagano, Corallo et al. 2024).**

Myocardial necrosis is not always necessary for BNP levels to reflect the magnitude of an ischemia insult. A number of findings lend credence to this theory. To begin, both infarcted and non-infarcted tissues show increased BNP production in experimental acute MI. Additionally, it has been demonstrated that BNP levels may temporarily rise following simple percutaneous transluminal coronary angioplasty (PTCA), even if the pressures within the heart do not alter. Lastly, BNP increases after exercise in coronary disease patients, but only temporarily; the increase is related to the size of the ischemic territory **(Muluhie, Castiglioni *et al*. 2024).**

Taken together, these results point to the following: temporary ischemia raises wall stress, and BNP production and release are induced by ischemia insults that rise in proportion to their severity. In addition, the quick increase in BNP levels following PTCA and exercise-induced ischemia implies that the release of stored BNP, rather than de novo synthesis, could play a more significant role in the reaction to hemodynamic stress and ischemia **(Ahmad, Dhar *et al*.).**

The ventricular wall stress may rise after a myocardial infarction. This shift in intracardiac transmural pressures might serve as a key trigger for BNP production. The findings corroborate those of experimental models of MI that included synthetic BNP. A rise in both the infarcted and non-infarcted muscles in the area. Myocardial ischemia, even in the absence of necrosis, activates the cardiac hormonal system, and hypoxia is an independent trigger for BNP production **(Kiseleva, Kirichenko *et al*. 2024).**

Recently, however, there is a growing body of data on the importance of BNP in CAD. The pathophysiological process underlying increased BNP levels may be ventricular systolic or diastolic dysfunction due to myocardial ischemia resulting in increased wall stress **(Casper, El Wakeel *et al.* 2024).**

However, data from experimental studies suggest that there is a direct release of BNP from cardiac myocytes in response to myocardial ischemia, independent of ventricular wall stress. In agreement with these experimental results, BNP levels have been shown to increase even after myocardial ischemia **(Shimohata, Usui *et al.* 2024).**

It seems that increasing wall stress triggers the production of BNP, according to multiple studies. Cardiac systolic and diastolic dysfunction can be caused by ischemia, however it is temporary. We therefore hypothesise that exercise-induced ischemia causes an increase in left ventricular end-diastolic wall strain, which in turn causes the ventricle to produce brain-derived neurotrophic factor (BNP). Plasma levels of BNP are increased in patients with ventricular dysfunction and seem to have high sensitivity and specificity for identifying ventricular dysfunction in patients with symptoms of heart failure. However, BNP is not an accurate test for ventricular dysfunction among subjects who do not have overt symptoms of heart failure, especially those with underlying coronary disease. These observations suggest that BNP elevations may be associated with cardiac processes other than ventricular dysfunction. One potential explanation is that elevations of BNP may be the result of ischemia in patients with stable coronary disease. BNP is known to be elevated in acute coronary syndromes and is a powerful predictor of short- and long-term mortality, independent of ventricular function**(Abubakar, Irfan et al. 2024)**.

Patients with stable CAD can have an unpredictable clinical trajectory; thus, additional tools to prognosticate risk in this cohort are warranted. In recent years, a wide range of biomarkers has been recognized for their diagnostic capabilities in patients with stable CAD, identifying those with obstructive disease who may require more intensive preventive therapies or even consideration of percutaneous coronary intervention in some circumstances. In addition, a multiple-biomarker approach may identify stable CAD patients at

highest risk for future major adverse cardiac events**(Kotecha, Flather et al. 2019).** One study found an association between resting BNP and ischemia in a large sample of outpatients with stable coronary artery disease. These findings suggest that study participants with evoked ischemia may also have been experiencing ischemia in their daily lives. This daily ischemia may increase ventricular volume and wall stress, leading to elevations in BNP. Alternatively, it is possible that elevations in BNP are part of a process leading to ischemia, with elevated BNP reflecting increased ventricular filling pressures that may lead to increased demand and myocardial ischemia. This is consistent with our study. also Elevated BNP levels are independently associated with induced ischemia among outpatients with stable coronary artery disease, particularly among those with a history of myocardial infarction. The observed association between BNP levels and ischemia may explain why BNP tests are not specific for ventricular dysfunction among patients with coronary artery disease**(Bibbins-Domingo, Ansari et al. 2003)**.

In patients with acute coronary syndromes (ACS) and stable coronary artery disease (CAD), reduced Total Antioxidant Capacity (TAC) levels are frequently observed. This decrease is due to increased oxidative stress caused by ischemic events, which leads to an imbalance between free radical production and antioxidant defenses. In ACS, the rapid onset of ischemia triggers excessive reactive oxygen species (ROS) production, leading to lower TAC. In stable CAD, chronic oxidative stress also contributes to reduced TAC, reflecting a compromised antioxidant system **(Guo, Ji *et al*. 2024)**.

A study suggests that patients with cardiovascular disease have lower levels of total antioxidant capacity (TAC), poorer antioxidant system performance, and lower amounts of antioxidants overall compared to healthy controls. Thus, in order to decrease oxidant status in these patients, it may be therapeutically beneficial to apply exogenous antioxidants. There is still a lack

of research into other important mechanisms that contribute to atherogenesis, such as those linked to pro-inflammatory indicators **(Vona, Pallotta *et al*. 2021).** Given the significant correlation between GPx and TAC and their decreased levels in ACS diseases, because they have the same mechanism of action as antioxidants, they are included in the discussion here **(Sugamura and Keaney Jr 2011, Jakubczyk, Drużga et al. 2020, Bil-Lula, Kuliczkowski et al. 2024, Tonin, Dolžan et al. 2024).**

The current study found that people with ACS had lower TAC activity. According to a study that compared healthy and sick persons, erythrocyte GPx also reduces **(Bastani, Rajabi *et al*.).** Evidently, the generated oxidative stress that went hand-in-hand with the decreased erythrocyte GPx and TAC activity in the patient groups led to substantial oxidative damage and rendered the erythrocyte membrane more susceptible to this oxidant scenario.The number**(Bastani, Rajabi *et al*. 2018).** The results of this prior research suggest that GPx level may be increased during ACS in reaction to changes in oxidative stress. **(Liang, Xu *et al*. 2024).**

4.4. Correlation between BNP, TAC and parameters studied in ACS and CAD patients

In patients with Acute Coronary Syndrome (ACS) and Chronic Coronary Artery Disease (CAD), the relationship between Brain Natriuretic Peptide (BNP) and factors like age, Body Mass Index (BMI), and waist circumference is significant. BNP levels tend to increase with age, reflecting age-related cardiac stress and declining heart function. Similarly, elevated BMI and waist circumference are associated with higher BNP levels due to the increased cardiac workload and volume overload in obese patients. This suggests that age, obesity, and abdominal fat accumulation contribute to cardiac stress and BNP elevation in cardiovascular disease **(Lavall, Bonfanti et al. 2016).**

There is a significant inverse correlation between Total Antioxidant Capacity (TAC) and Triglyceride (TG) levels. Elevated TG levels, often linked to dyslipidemia and metabolic syndrome, are associated with increased oxidative stress, which depletes the body's antioxidant reserves. As a result, higher TG levels are commonly accompanied by lower TAC levels in ACS and CAD patients. This relationship underscores the role of oxidative stress and lipid metabolism in the progression of cardiovascular diseases **(González-Pacheco, Amezcua-Guerra et al. 2015).**

While present study sheds light on the association between BNP, TAC and the oxidative status of affected patients. The results compared to a study on CAD patients with indicate that the chronic form of disease is more adapted to oxidative stress in comparison to the acute form, indicating the need to reduce oxidative status in ACS patients. However, in both ACS and chronic CAD patients, high erythrocyte membrane susceptibility **(Pokharel, Garcia-Flores et *al.* 2024)**. low antioxidant capacity and decreased function of antioxidative systems were detected compared with the healthy controls (Kul and Ozturk Kurt 2024). Exogenous antioxidants may help reduce oxidant status in these patients, which could lead to therapeutic advantages. **(Rojas-Solé, Pinilla-González *et al*. 2024)**. Further research into other important mechanisms related with atherogenesis, such as those involving pro-inflammatory signals, is necessary. **(Zhang and Dhalla 2024).**

The study showed the inverse correlation between BNP and TAC Since most modern studies emphasize the positive correlation between TAC and GPX .It is very likely that the stress of the heart, which was the result of a lack of antioxidants, was busy trying to modify the state of oxidative stress resulting from the activity of free radicals and their work in lipid peroxidation and endothelial dysfunction. Thus, the injury to the heart muscle by stress releases

proportions of BNP that are proportional to the state of decline in antioxidants **(Romuk, Wojciechowska et al. 2019).**

4.5. Receiver Operating Characteristic

Receiver Operating Characteristic (ROC) curve that gave us high sensitivity and privacy values, area under the curve for the BNP with the TAC. It can be used in studies as a way to predict or monitor the development of patients with ACS. Previous studies provided confusing results on the relationship of the presence of cardiovascular diseases to TAC. Several studies found significantly lower blood antioxidants and TAC in patients with CHD. This is consistent with the results of this study. It has been suggested that higher antioxidant potential can protect the organism against undesirable ROS activity and thus prevent disease incidence.

4.6. CK-MB relative index

The significant difference in A CK-MB relative index between patients with Acute Coronary Syndrome (ACS), Chronic Coronary Artery Disease (CAD), and healthy controls. The ACS group had a dramatically elevated CK-MB index (45 ± 3.2), indicating substantial myocardial damage, as CK-MB is primarily released from the heart muscle during ischemia or infarction. In contrast, the Chronic CAD group had a CK-MB index of 2 ± 0.8, indicating less acute myocardial stress, while the control group showed a normal index of 1 ± 0.2, suggesting no myocardial injury.

The A CK-MB relative index serves as a critical biomarker for differentiating between various degrees of myocardial injury. CK-MB (Creatine Kinase-MB) is a myocardial enzyme that is released during heart muscle injury. The relative index is calculated as the ratio of CK-MB to total CK (Creatine Kinase), expressed as a percentage **(Munir 2023).**

In Acute Coronary Syndrome (ACS) patients, elevated CK-MB values indicate significant myocardial necrosis, supporting its diagnostic use in identifying acute myocardial infarction (MI). The high CK-MB levels in ACS highlight its role in early detection and confirm the acute nature of cardiac damage. The rapid elevation of CK-MB typically peaks within 4-6 hours after an ischemic event, making it a useful marker for early MI diagnosis. This characteristic has been supported by multiple studies, including those by Antman et al. (2000), who showed its efficacy in early MI detection. However, for patients with Chronic Coronary Artery Disease (CAD), the CK-MB relative index (2 ± 0.8) remains moderately elevated, suggesting ongoing but less severe myocardial stress. In these cases, CK-MB is not as sharply elevated as in ACS patients due to the chronicity of the disease. This reflects milder, persistent ischemia without acute infarction. Healthy controls exhibit a normal CK-MB relative index (1 ± 0.2), indicating no myocardial injury or stress. This low value aligns with the absence of cardiovascular disease**(Tan, Liu et al. 2023).**

Supporting Studies: Parikh and Jeremias 2010 showed that CK-MB is a reliable biomarker for early myocardial infarction detection, with its peak levels occurring within hours of infarction, making it highly relevant for early diagnostic decisions **(Parikh and Jeremias 2010).**Apple & Wu (2001) reinforced CK-MB's role in early detection but also highlighted the increasing use of troponin due to its superior sensitivity in detecting smaller degrees of myocardial injury **(Apple and Wu 2001).** Wang 2011 suggested that while CK-MB is still useful, troponins are more specific for long-term monitoring of cardiac injury, especially in chronic conditions like CAD **(Wang 2011).**

Finally, it must be mentioned that because there is a direct relationship between high CK-MB and CK-MB relative index, an elevation in CK-MB levels generally increases the A CK-MB relative index. The A CK-MB relative index is calculated as the ratio of CK-MB to total CK (Creatine Kinase) and is

expressed as a percentage. When myocardial injury occurs, such as in a heart attack, CK-MB is released into the bloodstream, raising both the absolute CK-MB level and the relative index. This increase in the relative index helps differentiate cardiac muscle injury from skeletal muscle injury, especially when CK-MB rises significantly above total CK levels **(Kurapati and Soos 2020).**

Chapter Five

Conclusions and Recommendations

Conclusions and Recommendations

5.1. Conclusions

1. Direct relationship between BNP and coronary artery disease more in ACS than Chronic CAD versus control group. (showed significantly higher level of BNP in ACS more than Chronic CAD Compare with control group).

2. Inverse relationship between TAC and Chronic artery disease (both ACS and Chronic CAD) versus control group.

3. The study showed that the level of CK-MB relative index is higher in ACS more than Chronic CAD Compare with control group.

5.2. Recommendations and Future Works

1. BNP and TAC is useful biomarker to study the severity the extent of coronary artery disease involvement in acute coronary syndrome patient.
2. Study new and novel genetic biomarker in sera of ischemic heart diseases.
3. Study the effect of various risk factors on biomarkers in sera of ischemic heart diseases.
4. Study the effect of angiography and stent intervention as an advance treatment method on serum biomarkers of patients with ischemic heart diseases.

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الخلاصـــة

يعتبر مرض القلب الإقفاري من أكثر الأمراض شيوعًا في العالم، ويصنف إلى متلازمة الشريان الذبحة الصدرية غير المستقرة، احتشاء عضلة القلب)، مرض الشريان (ACS) (التاجي الحاد متلازمة الشريان التاجي المزمنة)، والتي تصيب الذكور بنسبة أكبر من الإناث. (CAD التاجي تلعب بعض الإنزيمات الخاصة المرتبطة بعملها في القلب دورًا وتكون أكثر وضوحًا عندما يتعرض ، وهو أحد البروتينات العديدة التي (BNP)القلب للإجهاد، بما في ذلك ببتيد الدماغ المدر للصوديوم تساعد على تنظيم الدورة الدموية في جميع أنحاء الجسم. على الرغم من أن هذا البروتين يصنعه القلب، إلا أن مقدمي الخدمة يطلقون عليه أحيانًا اسم الببتيد المدر للصوديوم "الدماغي" لأنه تم وهو من مضادات الأكسدة يحمي من أضرار TAC اكتشافه لأول مرة في أنسجة المخ. وبالمثل، فإن الجذور الحرة عن طريق توفير الإلكترونات لتحييدها. له تأثير سلبي فعال جداً في حالة نقصه، فهو عامل وقائي ضد المواد المؤكسدة التي يتعرض لها الجسم يومياً لعدة أسباب منها ما هو نتاج التسرب .الإلكتروني، سلسلة نقل الإلكترون، ومنها التعرض للإشعاع والتدخين والجزيئات غير القابلة للهضم

المبرر:

في محاولة لإيجاد طرق تشخيصية جديدة وعلامات تشخيصية مبكرة تساعد الأطباء في تشخيص النوبات القلبية والذبحة الصدرية. ويجب أن تتميز هذه الطرق بالدقة والسرعة، بالإضافة إلى كونها غير مكلفة مالياً وسهلة التطبيق في المختبر.

هدف الدراسة:

هدفت الدراسة إلى تقصي دورBNP و TAC والتحقق من الارتباط بين هذه المؤشرات الحيوية والمعلمات قيد الدراسة ومحاولة ربط حركية إنزيم CK-MB بالمؤشر النسبي لتلك المعلمات وفهم ارتباطها بأمراض نقص التروية في كلا النوعين.

المرضى وطرائق العمل:

أجريت هذه الدراسة، وهي دراسة الحالات والشواهد، على 120 مشاركاً من الذكور تراوحت أعمارهم بين (41-70 سنة)، 60 منهم يعانون من أمراض القلب الإقفارية (مجموعة المرضى) و60 من الأصحاء ظاهرياً (المجموعة الضابطة)، والذين حضر وحدة العناية القلبية في مركز قلب كربلاء للفترة من ديسمبر 2022 حتى يوليو 2023 والذين تم تشخيصهم سريريا ومخبريا وفق المعايير المستخدمة لتشخيص متلازمة الشريان التاجي الحادة ومتلازمة الشريان التاجي المزمنة وتم تسجيل جميع المعلومات الخاصة. بالمرضى في ورقة الاستبيان،

النتائج:

أظهرت النتائج أن متوسط مستوى BNP (pg/ml) ارتفع بشكل ملحوظ مقارنة بمجموعة التحكم في ACS و CAD المزمن بمتوسط ± انحراف معياري 71.42 ± 15.56 و 55.45 ± 18.21 في مجموعة ACS و CAD المزمن ومتوسط ± انحراف معياري 30.85 ± 18.21 في مجموعة التحكم على التوالي بينما انخفض TAC بشكل ملحوظ مقارنة بمجموعة التحكم بمتوسط ± انحراف معياري 3.12 ± 0.46 و 3.21 ± 0.34 في مجموعة ACS و CAD المزمن ومتوسط ± انحراف معياري 3.47 ± 0.68 في مجموعة التحكم على التوالي.

الخلاصة:

أظهرت الدراسة أن ارتفاع مستوى BNP كان علامة مبكرة على ACS بحساسية وخصوصية أعلى، بينما كان TAC عاملاً وقائيًا، ولكن بحساسية وخصوصية أقل. وأظهرت الدراسة أن نشاط إنزيم CK-MB كمؤشر نسبي (CK-MB RI) لنوبة قلبية STEMI كان الأعلى بين المرضى وتبعه احتشاء عضلة القلب NSTEMI ثم الذبحة الصدرية غير المستقرة (UA) وكان الأدنى هو الذبحة الصدرية المستقرة (SA).

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

استقصاء ببتيد الدماغ المدر للصوديوم والقدرة الاجمالية لمضادات الاكسدة و الكرياتين كاينيز MB لدى الذكور المصابين بأمراض القلب الاقفارية

رسالة ماجستير

مقدمة الى مجلس كلية الطب في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في [الكيمياء السريرية]

من قبل

أمجد كريم علوان

بكالوريوس تقنيات طبية – تحليلات مرضية (2017)

بأشراف كل من

أ. د. فاضل جواد ال طعمة
دكتوراه كيمياء حياتية سريرية
فرع الكيمياء والكيمياء الحياتية

أ. د. احمد حسين الميالي
طبيب اختصاص بالطب الباطني - أمراض القلب والشرايين

كليـــة الطـــب – جامعـــة كـــربلاء

2024 م

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